Essential Oils from Steam Distillation

Learning Objectives:
- Enhance participant understanding of plant organic chemistry.
- Enhance participant understanding of high-value uses for biomass.
- Provide opportunity for participants to gain hands-on experience with steam distillation equipment and essential oils.

Learning Outcomes:
Upon completion of this lab, participants will be able to:
- Explain the difference between primary and secondary plant metabolites.
- List examples of secondary metabolites, terpenes and terpenoids.
- Describe secondary metabolite extraction methods.
- Extract essential oils from biomass using steam distillation equipment.

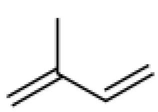
Pre-Lab

Background

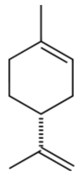
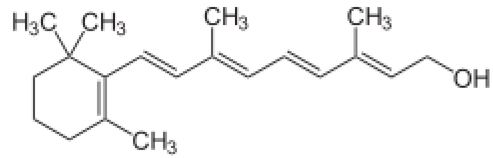
Secondary Metabolites

The primary goal of plants, like all organisms, is to grow and reproduce. Most of the metabolites produced by plants, therefore, are polysaccharides and proteins that give the plants structure and function. Plants also produce very small amounts of secondary metabolites: compounds that are not directly related to growth or reproduction. Many of these secondary metabolites are very commercially valuable and some have very complex chemistry. Most of the plant compounds used in perfumes, flavors, and natural medicines are secondary metabolites.

Terpenes and Terpenoids



One of the key secondary metabolite building blocks is a five carbon molecule called isoprene (or, more officially, 2-methyl-1,3-butadiene). Isoprene is used by plants to produce terpenes, molecules made from 2 or more isoprenes, and terpenoids, terpenes that have slight chemical modifications, especially terpene alcohols. Terpenes and terpenoids are the chemical compounds responsible for many plant smells and flavors. Pine trees produce a large number of terpenes and the solvent turpentine was originally derived from pine tree resin.

D-limonene farnesol retinol

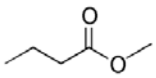
Terpenes may be classified by the number of isoprene units in the molecule. *Monoterpenes* consist of two isoprene units and have the molecular formula $C_{10}H_{16}$. Limonene, the smell of citrus, is a monoterpene. *Sesquiterpenes* consist of three isoprene units and have the molecular formula $C_{15}H_{24}$. Farnesol, is an alcohol sesquiterpenoid that is responsible for floral smells like roses. *Diterpenes* consist of four isoprene units and are precursors for many important biological molecules with anti-inflammatory, antimicrobial and anti-cancer properties. Retinol, aka Vitamin A, is a diterpenoid. *Triterpenes* consist of six isoprene units. Squalene is a triterpene that organisms use to make cycloartenol, the precursor to steroids. *Tetraterpenes* contain eight isoprene units and include antioxidants lycopene and beta-carotene. *Polyterpenes* are even longer chains of isoprene units. Natural rubber is a polyterpene.

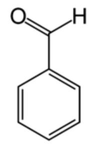
Extraction Methods

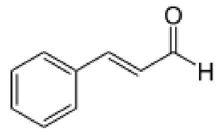
Since plants only make small amounts of secondary metabolites, a great deal of plant material is needed to produce a useable amount of a certain product. Over millennia, people have developed several methods to extract and concentrate desired compounds from plants. One well-known example is hot-water extraction used to make coffee, teas, and soup stocks. Solvents such as alcohol can also be used such as to make liquid extracts used in food flavorings and perfumes. Cold-pressing with fats and waxes can be used for compounds sensitive to heat such as jasmine. The best extraction method to use depends on the volatility (ease of evaporating) and polarity (hydrophilicity or hydrophobicity) of the desired compounds. Steam distillation, the method used in this lab for essential oil extraction, takes advantage of the volatility of a compound to evaporate when heated with steam and the hydrophobicity of the compound to separate into an oil phase during condensation.

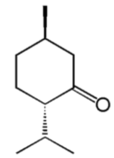
Essential Oils

Essential oils are the collection of hydrophobic secondary metabolites that can be extracted from plants and are used in perfumes, flavorings and alternative medicine techniques such as aromatherapy. While a single compound may have a distinct smell, most essential oils are actually hundreds of compounds that, when combined, create the smell associated with that particular plant. Below are some examples of compounds found in many essential oils in various concentrations.

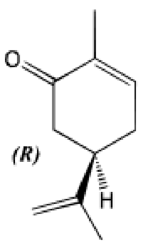

methyl butyrate

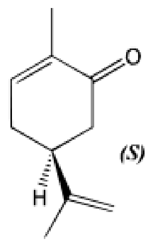

benzaldehyde

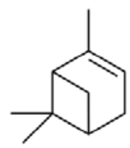

cinnamaldehyde

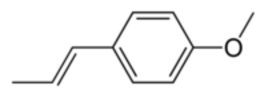

methone

Methyl butyrate is the fruity smell of apples and pineapple. Benzaldehyde is associated with almonds. Cinnamaldehyde, as the name implies, is the smell of cinnamon. Methone is one of several compounds associated with minty smells.


R-carvone


S-carvone


pinene


anethone

The R and S entiatiomers of carvone are associated with the smell of spearmint and caraway, respectively. Pinene is the smell of pine and rosemary. Anethole is responsible for the distinctive smell of anise and black licorice.

<u>Pre-Lab Tasks</u>

1. Brainstorm reasons why secondary metabolites are generally higher-value products from biomass than primary metabolites, especially polysaccharides.
2. Choose a biomass from the list in the background section of the lab procedure and find at least one of the chemical structures responsible for the smell/flavor (not including the ones listed above).
3. List and describe biomass extraction method you use on a daily or monthly basis. What kind of products are you obtaining from biomass?
4. Many of the earliest synthetically produced pharmaceuticals were based on plant products. Research and summarize the discovery of aspirin. What are some other drugs do we have that are based on plant molecules?
5. Make up and draw your own sesquiterpene or diterpene. Describe the bonds you chose to use. Use that molecule to draw a related terpenoid.

Steam Distillation of Biomass

Safety Checklist

- o Proper attire is worn (long pants and closed-toe shoes).
- o Food and drinks are stored and consumed outside the laboratory.
- o Lab coat and safety glass are worn.
- o Latex or nitrile gloves are used when handling samples and chemicals.
- o Insulated gloves are used when handling hot materials.
- o Ear protection is used when a procedure involves loud noises.

Problem Statement

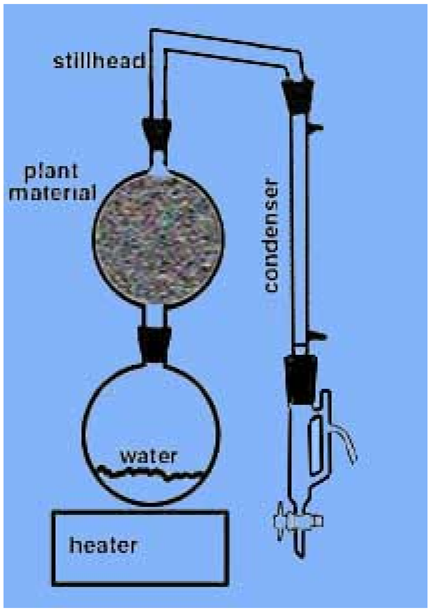
A founder of a soap production company wants to make a new product line of “natural” soaps using plant-based surfactants and essential oils. Your team has been asked to determine how much plant material is needed to produce sufficient essential oils using steam distillation. As a bonus, the citrus essential oil production includes co-product fruit juices suitable for making refreshing summer beverages.

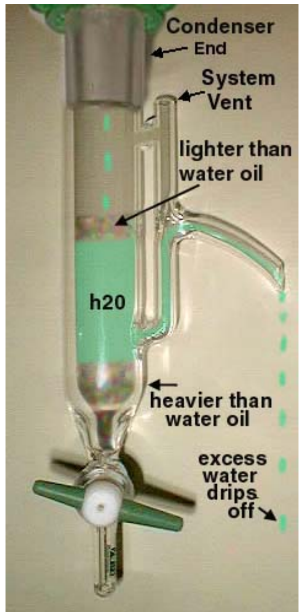
Steam Distillation

Background

This all-glass, vertical steam distillation unit, consisting of a hot plate, boiling flask, biomass flask, still head, condenser and receiver, is used for “dry steam” distillation of plant material (see equipment diagram below). Steam is produced in the boiling by heating distilled water with the hot plate. This steam travels upward into the biomass flask where essential oils and water-soluble plant compounds are removed into the vapor stream. The vapor stream travels through the still head, condenses in the water-cooled condenser, and collects in the receiver, where the essential oil layer phase separates. The receiver is specially designed to retain both heavier-than-water oils and lighter-than-water oils, while allowing excess water, containing the water-soluble compounds, to be drained out and collected separately (see diagram below). In this way, the essential oils are condensed. Dry steam distillation is different from “wet” steam distillation because the biomass does not directly contact the water and the vertical design allows any water that condensed on the biomass to drain back into the boiling flask.

Steam distillation of biomass generally yields two products: a relatively high purity essential oil and an aqueous condensate called a hydrosol. The oil consists of hydrophobic, often aromatic compounds that are produced in very small concentrations as secondary metabolites in the plants. The hydrosol also contains secondary metabolites but these compounds are more hydrophilic. Plant parts that can be used to produce essential oil are berries (anise, juniper), seeds (almond, nutmeg, cumin), bark (cinnamon, sassafras), wood (cedar, rosewood, sandalwood), rhizome (ginger), leaves (basil, bay leaf, sage, eucalyptus, oregano, peppermint, pine, rosemary, spearmint, tea tree, thyme, wintergreen, lemon grass), resin (frankincense, myrrh), flowers (chamomile, clove, geranium, hops, jasmine, lavender, marjoram, rose), peels (orange, lemon, lime, grapefruit), and roots (valerian).





Pre-experiment Checklist

The following checklist is to be performed each time before setting up the distillation.

- Cold water for the condenser(s) is available at the sink.
- Hot plate surface is clean and dry. Power cord is in good condition.
- Biomass is available and prepared. Essential oils are best extracted from fresh biomass shortly after harvesting. If fresh biomass needs to be stored between harvest and extraction, store in a labeled plastic bag or sealed container in the refrigerator. Dried biomass can also be used. To prepare, remove as much of the non-oil containing plant parts as possible (steams, fruit pieces, etc.) and break/cut biomass to be extracted into ~1" wide pieces. In general, the smaller and thinner the biomass pieces (but not powder), the more efficient the extraction is.
- All the distiller pieces (boiling flask, boiling flask stopper, biomass flask, metal screen, still head, condenser, condenser tubing, receiver, large (red) and small (green) plastic glassware connector clamps, metal vertical support rod, beaker to collect hydrosol, and two metal tube clamps) are present and clean.
- Silicon joint lubricant is on hand for distiller assembly.

Distiller Set-Up

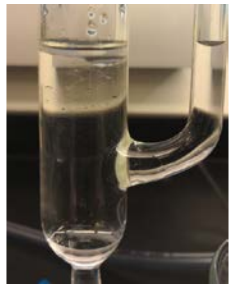
1. Check all glassware for chips or cracks and discard any broken pieces in broken glass container. Even small cracks can become big problems when heated. See lab supervisors for replacements.

2. Fill boiling flask with approximately 1.25 L of distilled water such that flask is between 1/2 and 2/3 full. Place boiling flask on top of hot plate and secure in place with tube clamp around the neck of the flask. (Tube clamps should already be in the correct orientations and therefore should not need to be adjusted much aside from closing and opening the “pincher”.)
3. Coat boiling flask stopper ground glass joint with thin layer of silicon lubricant and place in boiling flask. (Keeping ground glass joints lubricated is critical to preventing joints from “freezing” and breaking during disassembly.)
4. Secure condenser in place using second metal tube clamp such that the male end is facing downwards and tube clamp is attached slightly above water inlet connection (water tubes will likely already be connected to inlet and outlet). Coat male end of condenser with thin layer of silicon lubricant.
5. Attach receiver to bottom of condenser and secure in place with green plastic connector clamp. (Condenser height may need to be adjusted to accommodate receiver).
6. Make sure stopcock on receiver is in closed position and fill receiver with distilled water such that the water level is at least as high as the top of the lower outlet (see above diagram of how receiver works). The metal heat shield should be between receiver and hot plate.
7. Carefully insert metal screen (folded part ups) into the male end of the biomass flask such that bottom (open part) of screen is flush with the bottom of the ground glass joint.
8. Record wet weight of biomass to be used.
9. Fill biomass flask through the top with prepared biomass. Coat male end ground glass joint of biomass flask with thin layer of silicon lubricant and set flask into top of boiling flask.
10. Coat both ground glass joints of still head with thin layer of silicon lubricant. Simultaneously connect still head to top of biomass flask and condenser. If the height/orientation of the condenser needs be adjusted, set the still head on the counter before making the adjustments to prevent dropping or knocking over any of the glassware (another set of hands is useful here). Secure still head to condenser using red plastic glassware connection clamp.
11. Verify that condenser tubing is connected. Cold water should enter in the bottom and exit out of the top.
12. Connect inlet condenser tubing to sink cold water tap using cream-colored faucet adapter. Make sure that outlet condenser tubing is drained into the sink, or connected to inlet of second condenser if running multiple distillations in a daisy-chain set-up.

13. Turn on cold water such that there is a gentle flow of water through the condenser. The outlet temperature of this water will be monitored throughout the distillation to make adjustments to the flow rate.
14. Place beaker (400 ml or larger) underneath overflow spout on receiver to catch hydrosol.

Distillation Procedure

1. Double-check all ground glass connections to make sure there is a good seal and that joints are lubricated.
2. Plug in hot plate and turn on to high setting.
3. Water in boiling flask should gradually begin to boil and travel upward into biomass flask. Steam will appear and start to condense in the still head after about 30 minutes of heating. Some water will also condense in biomass flask and flow back down into boiling flask which may cause the water in the boiling flask to change color; this is normal (think tea-making).
4. Make note of the time when the first drops run down the condenser and into the receiver.
5. Begin periodically checking the temperature of the water draining out of the condenser outlet tube into the sink. Water should be cool and at most, lukewarm; if water is warm, gradually increase the flow rate of the cold water until the outlet water is cool.
6. Watch liquid continue to collect in the receiver. An oil layer should begin to form on top of the hydrosol. Most of essential oil will collect in the first 10-20 minutes, but more will come off in the next 1-2 hours.



7. Continue the distillation until essential oil layer has not grown in the last ½ hour or until new hydrosol collecting in the receiver no longer has any smell (this can be checked by collecting a couple drops from the overflow spout in a small beaker). When the distillation is finished, the biomass should look “spent”.
8. To stop the distillation, turn off and unplug the hot plate. Let the entire set-up cool until the biomass flask is cool enough to handle (takes about 30-40 minutes).
9. Disconnect receiver from condenser and use stopcock to carefully drain remaining hydrosol into overflow beaker, leaving the essential oil layer. If desired, save a sample of the hydrosol in a labeled vial.
10. Label and weigh an empty vial for the essential oil. Wait about 5 minutes and then drain out the essential oil layer into vial.
11. Record weight of full vial and cover vial with a lid.

Disassembly and Clean-up

1. Partially fill Rubbermaid tub with warm soapy water for washing glassware. Glassware should be washed in this tub rather than in an unlined sink to prevent breaking the glassware against the hard sides of the sink.
2. Set empty receiver into tub to soak.
3. Turn off water flowing to condenser.
4. Disconnect still head from condenser and carefully lift off condenser and biomass flask. Set still head into tub.
5. Left off biomass flask and carefully empty contents through top opening into non-hazardous waste can. If biomass gets stuck, gently pull it out and/or tap flask. Bits clinging to the side can be rinsed out with very small amount of water into garbage can. **Make sure to save metal screen.** Set biomass flask and metal screen into tub.
6. Using hot gloves, unclamp boiling flask and set on bench to cool further. Dump water left in boiling flask down the sink.
7. Wash all glassware except for condenser. Rinse thoroughly with tap water then distilled water before leaving to dry.
8. Disconnect condenser from stand (water inlet and outlet tubes do not need to be disconnected from condenser). Rinse condenser with warm tap water then distilled water and reconnect to stand to dry.

Data Analysis

1. Calculate the yield of essential oil based on as-received (wet) weight of the biomass.
2. Calculate how much biomass would be needed to produce 5 g of essential oil for your biomass. Comment on the resulting added cost to the batch of soap.
3. Sniff essential oils and make observations about the smells. (If the essential oil has a slightly "burnt" smell, it contains some hydrophobic phenolic compounds. This smell can be removed by letting the essential oil vial sit uncovered for a day or two; during this time, the phenolic compounds should evaporate off and the essential oil should smell better.)
4. If essential oils are most efficiently extracted from fresh biomass, what kind of time window is available to make essential oils?