Lab 6
Protein, Fats, Sugars and Starch

Plants are composed of a variety of compounds, many of which humans use for food, drugs, oils and cosmetics. Besides water, certain plant organs have high concentrations of carbohydrates, proteins and lipids that can vary in different organs in the plant and between species. In today’s lab we will be investigating this variation by using simple techniques in detecting and measuring levels of important plant constituents. We will focus on products used primarily as food or in food preparation. At the end of the laboratory exercise you should be able to discuss the difference in protein levels between different flours, the lipid content in nuts, degree of saturation in different plant derived oils, sugar content in fruits (and fruit juices) and the storage starch content in tubers, roots and fruit.

1. Variation in Plant Protein

The protein content of most plant parts is relatively low, usually ranging between 1-4%. One exception to this is plant seeds, which often store protein or contain protein rich components such as the embryo (germ) and the aleurone layer found in cereals. Humans are largely concerned with plant proteins for dietary concerns, and many crops are actively bred for increases in protein yield.

In this exercise you will be determining the relative amounts of soluble protein in flours produced by grinding the seed of several different crop species. Although the protein extraction will be an estimate, you should be able to determine the relative levels of protein found among flours commonly sold in groceries and consumed in a variety of different ways including breads, soups and porridges.

Procedure for Protein Analysis

Protein Extraction

1. use measured volume (1 level scoopful ~125 mg) of different flours
   a. wheat (white)
   b. wheat (whole)
   c. soy flour
   d. cornmeal
   e. rye or rice flour
2. combine the flour with 5 ml extraction buffer (Phosphate Buffer pH 7.2) in a clean test-tube
3. cover the test-tube with parafilm and shake continuously for 1 minute
4. let stand 5 minutes to incubate
5. repeat shaking for 1 minute, and again let test tube stand for 5 minutes (these steps extract the soluble protein from the flour)
6. using a clean disposable pipette, place 1 ml of supernatant in microfuge tube; cap the tube and centrifuge for 5 minutes (take care to balance the centrifuge with a water blank or another student’s sample)
7. after centrifugation, dilute your extract 1/100 by combining 0.1 ml of your extract with 9.9 ml of buffer solution in a clean test-tube
8. determine protein content using the Bradford Micro Assay

Protein Assay

9. Warm up the spectrophotometer for 15 min. before use
10. Prepare standards containing a range of 1 to 20 micrograms protein (albumin or gamma globulin are recommended) to a volume of 200 µl (this step may be performed for you before lab)
11. Using a clean pipette, place 200 µl of your protein dilution into a spectrophotometer cuvette.
12. Add 800 µl dye reagent to cuvette and mix by covering cuvette with a small piece of parafilm and gently inverting the cuvette several times
13. Let the cuvette with the mixed sample incubate for 5 min.
14. Measure the absorbance at 595 nm.
15. Determine protein content by comparing absorbance value with standard curve of known protein concentrations.
16. Calculate total protein extracted for each flour (protein in sample x dilution factor x 5 ml of original extraction = mg/scoop of flour)
17. Use the table below to organize your data and to record your results

<table>
<thead>
<tr>
<th>Flour type</th>
<th>Amount in 5 ml buffer</th>
<th>Dilution factor</th>
<th>abs</th>
<th>mg protein in sample</th>
<th>Total protein</th>
<th>% (based on 125 mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

2. Variation in Fats and Plant Oils

Much like proteins, lipids (fats and oils) are often concentrated in seeds and used as storage products. Although most plants use starch for storage, many including nuts, legumes and some mustards store appreciable amounts of lipids in their seed. Many plants with lipid rich seeds or fruits are used as a source of vegetable oil. These vegetable oils have been used in a number of diverse ways including as diesel fuels (bio-fuels), lubricants, cleansers, sealants, plastics, cooking and medicines. Lipids are a class
of organic molecules called triglycerides. A lipid is composed of a glycerol molecule with three fatty acid chains attached to it. The nature of the fatty acids (e.g. the length and number of carbon: carbon double bonds) determines the nature of the lipid. Monounsaturated and polyunsaturated oils are those with one or more C:C double bonds, respectively. Saturated fatty acids have no double bonds. The degree to which oil is unsaturated is of some important health considerations. Saturated lipids have been linked to cardiovascular disease.

In this exercise you will first use a fat soluble dye (Sudan IV) to tests for the presence and localization of fats in various plant seed. You will also determine the relative amount of saturation in several plant oils by using a simple iodine test. If iodine is added to an oil, it will attach chemically to the C:C bonds and will change from a reddish color to clear. The more unsaturated the oil, the more complete the color change.

Procedure for Lipid Staining

1. Sudan IV solution is only absorbed by lipids
2. Obtain seeds provided today in lab and, using a sharp razor blade (take care!), cut seeds in thin slices (as thin as possible) and float the slices in a small amount of water for 5-10 minutes to hydrate.
   a. peanuts
   b. soy
   c. walnut
   d. corn
   e. Brazil nut
   f. bean
3. remove the slices from the water and place them on a microscope slide
4. add a small drop of Sudan IV solution to your slice
5. after a few minutes, observe under scope (low power) for oils (they should turn red)
6. use the table below to record your observations

<table>
<thead>
<tr>
<th>Species:</th>
<th>Species:</th>
<th>Species:</th>
<th>Species:</th>
<th>Species:</th>
</tr>
</thead>
<tbody>
<tr>
<td>sketch</td>
<td>sketch</td>
<td>sketch</td>
<td>sketch</td>
<td>sketch</td>
</tr>
<tr>
<td>Oil content (hi,med,lo)</td>
<td>Oil content (hi,med,lo)</td>
<td>Oil content (hi,med,lo)</td>
<td>Oil content (hi,med,lo)</td>
<td>Oil content (hi,med,lo)</td>
</tr>
</tbody>
</table>
Procedure for Degree Unsaturated Oil content

1. measure out 5 ml of the different vegetable oils present in lab today into clean, labeled test-tubes
   a. olive
   b. corn
   c. peanut
   d. sunflower
   e. linseed
2. slowly add 10 drops of IKI solution to each tube
3. cover the tubes with parafilm and gently mix the solutions
4. place the tubes in a test-tube rack and mark the time
5. check every 30 minutes for any color change (unsaturated oils will become clearer with time)
6. Record your observations (relative color change from red to clear) in the table below

<table>
<thead>
<tr>
<th>oil</th>
<th>30 min</th>
<th>60 min</th>
<th>90 min</th>
<th>120 min</th>
<th>Saturated or Unsaturated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

3. Starch Content in Roots, Stems and Fruit

Most plants use starch as their storage product. Although starch can be found in most plant organs, it is often concentrated in organs dedicated for storage such as tubers and corms. In some cases, fruits can also be a major source of storage starch. Starch is stored as crystals in specialized cells called amyloplasts. Starch grains of different species can take on distinctive shapes and have been used in archaeology as identification tools.

In today’s lab, you will be roughly quantifying the volume of starch crystals stored in different plant organs of different agricultural species (banana, sweet potato and russet potatoes) and comparing the morphology of the different crystals.

Procedure for Starch Crystal Extraction

1. Collect potato, sweet potato and banana for starch extraction
2. Peel and cut about 50 grams of the structure into small pieces (less than 1 cm cubes).
3. Place the pieces in a hand blender and add 50 ml of 1% NaCl solution
4. Blend the mixture, to produce a loose slurry. Try blending for about 30 seconds, or more if needed.
5. Pour the slurry through two layers of muslin, stretched over the mouth of an appropriate sized beaker. Dispose of the solids left in the muslin.
6. Leave the filtrate in the beaker to stand for 15 minutes, to allow the grains of starch to form a solid sediment at the bottom of the beaker.
7. When the sediment has formed, carefully pour away the liquid above it, and re-suspend the sediment in 10 ml of fresh 1% NaCl.
8. Before the crystals can settle, pour the suspension into a calibrated test-tube. Rinse the beaker with a few more mls of NaCl solution and pour into the test-tube. Try to get as much of the white precipitate as possible.
9. Once again, allow time for the starch grains to settle to the bottom, now in test-tube, and pour off the top solution.
10. Pour into the test-tube 15 ml of fresh NaCl solution and re-suspend. Again allow time for the starch to settle and pour off the top solution.
11. Finally rinse the starch layer in a similar fashion, using 0.01 M NaOH.
12. Then assess the amount of sediment (containing the starch grains), by reading the volume of the starch crystals directly from the test-tube.
13. After recording the starch volume, pour off the solution into a waste beaker and take a small amount of the precipitated starch (with a needle probe) and spread it on a microscope slide. Add a drop of water and observe under the compound microscope to determine the shape of the crystals for each plant studied.
14. Record your observations in the table below.

<table>
<thead>
<tr>
<th>Species/organ:</th>
<th>Species/organ:</th>
<th>Species/organ:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume of crystals per gram</td>
<td>Volume of crystals per gram</td>
<td>Volume of crystals per gram</td>
</tr>
<tr>
<td>Sketch of Crystal (shape and size)</td>
<td>Sketch of Crystal (shape and size)</td>
<td>Sketch of Crystal (shape and size)</td>
</tr>
</tbody>
</table>

4. Sugars in Fruit and Fruit Juices

Sugars are used by plants as a primary energy source, and like animals, oxidize glucose during the process of respiration to make chemical energy. Unlike animals, plants produce their sugars through the process of photosynthesis and store them as starch, as we saw in the previous exercise. A few plants will store sucrose, such as sugarcane and sugar beets, but most plants do not store large concentrations of sugars in their vegetative bodies. The one exception is fleshy fruits. Presumably, plants use sugary fruit to attract animals to help them disperse their seed. After the plant seeds have matured fruit ripen and plants convert large amounts of starch into sucrose and fructose.
(and also frequently breakdown poisonous compounds) to make them more palatable to their animal dispersers. For millennia, humans have been selecting and breeding many crop species to enhance their production of sugary fruit. Fruit today are available in many forms: fresh in the market, frozen, dried and processed into jellies and juices.

*In this exercise, you will extract the juice from several fruits and measure their sucrose content with a brix refractometer. As a comparison, you will also measure the sucrose content of commercially available juices made from the same fruit.*

**Procedure for Determining Sugar Content**

1. Your TA will demonstrate the use of the brix refractometer (take care when using it)
2. Obtain small pieces of fresh fruit available in today’s lab.
   a. apple
   b. orange
   c. pineapple
   d. tomato
3. Place a small piece of tissue (size of a fingernail) between 2 glass slides and gently crush the tissue.
4. Carefully lift the daylight plate on the refractometer and place one or two drops of the juice squeezed from the tissue on the prism
5. Close the daylight plate and read the refractometer while looking toward the lab window.
6. The scale in the refractometer reads % sucrose in the sample.
7. Wipe the prism clean with a moist kimwipe (do not immerse the refractometer in water).
8. Repeat the procedure for each fruit and corresponding fruit juice.
9. Use the table below to organize your results

<table>
<thead>
<tr>
<th>species</th>
<th>tissue</th>
<th>juice</th>
<th>tissue</th>
<th>juice</th>
<th>tissue</th>
<th>juice</th>
</tr>
</thead>
<tbody>
<tr>
<td>% sucrose</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>