The Honey Lab

*Reference:* T. Scheuber (2023), *To bee or not to bee: the chemistry of honey*, Science in School, Issue 65.

**Aim**: To investigate the difference between adulterated honey and unadulterated honey.

This session will involve 4 parts:

1. Production of artificial honey
2. Carry out the Fehlings test on artificial and natural honey
3. Carry out an amylase test with artificial and natural honey
4. Using microscopy to identify pollen found in natural honey

**Part 1: Production of artificial honey**

**Materials:**

|  |  |
| --- | --- |
| 50 cm3 glass beaker | Water |
| 14 g sucrose | Thermometer |
| 1 cm3 lemon juice | Glass rod |
| Magnetic stirrer and hotplate | Balance |
| Magnetic flea | Weigh boat |
| Spatula | 1 cm3 syringe |
| Test tube and rack | Marker pen |

**Method:**

1. To a beaker, add:
   1. 8 cm3 water
   2. 14 g sucrose.
   3. 1 cm3 fresh lemon juice
2. Heat the mixture, stirring regularly – use a hot plate rather than a Bunsen to allow control of the temperature. Collect a 2 cm3 sample of the “honey” at this point – this will be used in Part 2.
3. Reduce the heat before boiling point and continue heating, without stirring, until the solution boils. Leave the glass rod in the beaker to act as a boiling chip.
4. Continue boiling until about 1/3 of the water has evaporated.

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**Part 2: Fehling test**

**Materials**

|  |  |
| --- | --- |
| 1 cm3 of artificial honey | 3 test tubes |
| 1 cm3 real honey | Test tube rack |
| 6 cm3 Fehling I | 3x 1 cm3 syringes |
| 6 cm3 Fehling II | 3 cm3 plastic pipettes |
| Heat-proof mat | Bunsen burner |
| Tongs to hold a test tube |  |

**Method:**

1. Collect 3 test tubes and label appropriately.
2. Add 2 cm3 Fehling I and 2 cm3 Fehling II to each test tube.
3. Using a 1 cm3 syringe, add the following to each test tube:
   1. Test tube 1: 1 cm3 artificial honey mixture (from step 3 in Part 1 above)
   2. Test tube 2: 1 cm3 artificial honey mixture (from step 7).
   3. Test tube 3: 1 cm3 real honey.
4. Using the tongs, hold each test tube in a Bunsen burner flame for about 20 s. Record the colour of each sample.

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**Results**

|  |  |  |
| --- | --- | --- |
| **Sample** | **Colour of Fehling solution** | **Conclusion** |
| Artificial honey (step 3) |  |  |
| Artificial honey (step 7) |  |  |
| Real honey |  |  |

Fehling test turns orange in the presence of mono- and disaccharide carbohydrates. If the Fehling solution stays blue, none of these sugars are present. Sucrose is an exception – although sucrose is a disaccharide, the potential reactive aldehyde group of the molecule is “hidden” in the bond between glucose and fructose.

The Fehling test is negative at the start when sucrose is the only sugar.

The catalytically active protons of lemon juice cause the hydrolytic cleavage of sucrose into glucose and fructose. This results in a subsequent positive Fehling test.

Real honey contains a mixture of different sugars and should provide a positive Fehling test.

**Part 3: Amylase activity – a test for adulterated honey**

Honey contains about 80% sugar. Water, another main ingredient, contributes about 17%. In addition, there are over 100 other components, e.g. amino acids, minerals, vitamins, and small amounts of fatty acids, flavouring and enzymes. The last of these can be used to distinguish real honey from artificial honey. This investigation reveals the enzyme activity of amylase in real honey.

**Materials**:

|  |  |
| --- | --- |
| 1 cm3 real honey | 4x test tubes and rack |
| 1 cm3 artificial honey from Part 1 | 3 cm3 Pipettes |
| 4 cm3 1% starch suspension | Glass rod |
| 2 cm3 0.01 M iodine | 21 cm3 water |
| Thermotube block at 37 °C | 1 cm3 alpha-amylase |

**Method:**

1. Label 4 test tubes: “amylase”, “real”, “artificial”, “control”.
2. To each test tube, add:
   1. 1 cm3 1% starch suspension
   2. 5 cm3 water
3. Add the following:
   1. To test tube 1, add 1 cm3 alpha-amylase
   2. To test tube 2, add 1 cm3 real honey.
   3. To test tube 3, add 1 cm3 artificial honey
   4. To test tube 4, add 1 cm3 water (control).
4. Mix the contents of each test tube very well. Incubate in the waterbath at 37 °C for 30 minutes.
5. Add 3-4 drops of 0.01M iodine to each tube.

**A group of black objects on a rack

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**Results:**

|  |  |  |
| --- | --- | --- |
| **Solution** | **Colour** **of** **iodine** | **Presence/absence of active amylase** |
| Amylase |  |  |
| Real honey |  |  |
| Artificial honey |  |  |
| Water (control) |  |  |

**Part 4: Pollen Identification**

Aim: To explore the pollen diversity found in honey.

**Materials**:

|  |  |
| --- | --- |
| Microscope | Centrifuge |
| Microscope slide + coverslip | Centrifuge tube |
| Sample of real honey | Water |
| Pollen identification guide | Pipette |
| Test tube and rack | Spatula |

**Method:**

1. Add 10 cm3 water to a test tube.
2. Add a spatula of honey to the test tube and mix well to dissolve in the water.
3. Transfer the contents of the test tube to a centrifuge tube. Centrifuge at 6000 rpm for 5 minutes.

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1. Discard most of the supernatant using a pipette. Resuspend any pellet in the remaining volume of supernatant.
2. Transfer a drop of the material to a microscope slide and add a coverslip.
3. Use a microscope to identify any pollen grains found in the honey. This will vary based on type of honey, season and location in which the honey was produced.