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To bee or not to bee: the chemistry of honey

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Learn about a variety of biochemical aspects of honey through a series of simple experiments using the sugary product of bees.

Bees have repeatedly attracted media attention in recent years. Documentaries such as *More than Honey*[1] and alarming figures on bee mortality have helped the honeybee to take on a new role as a flagship species for biodiversity.

On the list of the world's most frequently adulterated foods, honey ranks third (after olive oil and milk). Its definition is simple and beautiful: honey is the sweet substance that bees produce by ingesting nectar or honeydew, enriching it with their own substances, changing it in their bodies, storing it in honeycombs, and allowing it to ripen. Nothing shall be added to or removed from it.

In 2021, EU Member States imported 173 400 tonnes of natural honey from non-EU countries, worth €405.9 million, whereas 25 500 tonnes were exported by EU Member States outside the EU worth, €146.6 million.[2] Honey is the most expensive form of sugar, and that's what makes honey fraud so lucrative. Fake or adulterated honey includes incorrect declaration of origin; bulking out honey with sugar syrup from corn, sugarcane, and rice; or completely artificial products.

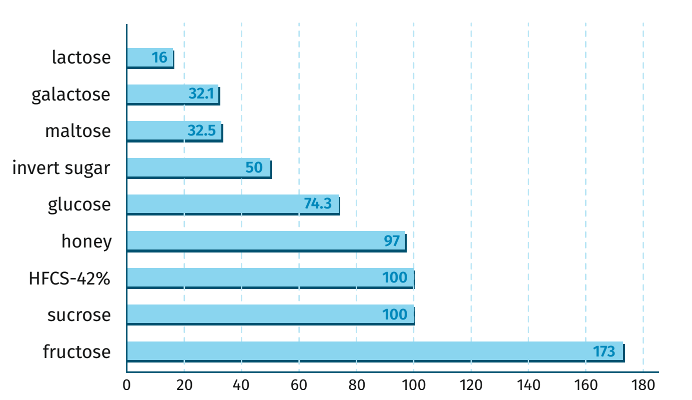
Chemically, honey consists of different types of sugars. These can be mixed and produced artificially. In terms of biology, enzymes and a lot of other molecules are added in the stomach of the bee. This makes honey a fantastic starting point for learning about biochemistry.

In a series of experiments, students learn methods to distinguish real honey from artificial honey, and they investigate different aspects and phenomena of honey and bees. The activities are suitable for secondary school students.

Activity 1: Production of artificial honey

If we talk about sugar in our daily lives, we normally refer to sucrose, but in nature we find a big variety of mono- and disaccharides. Sucrose is a disaccharide, in which one molecule of glucose is linked to one molecule of fructose. In the stomach of bees, there is an enzyme (invertase) that can break down sucrose ingested with nectar. Therefore, honey contains high amounts of glucose and fructose. Such a catalytic decomposition of sucrose can be easily carried out outside the bee's stomach using acid instead of an enzyme, and so-called invert sugar is obtained. This can be used as artificial honey, or in the further production of jams, or sweets like gummy bears.

This activity takes about 20 min.



The relative sweetness of sugars and sweeteners (high-fructose corn syrup (HFCS) is a sweetener made from corn starch; HFCS 42 refers to a dry-weight fructose composition of 42%, with glucose as the remainder)

Image: Christina Hof, Science in Schools; based on Yang Zhao/Wikimedia, CC BY-SA 3.0

Safety notes

- Heated sugar solution must be handled with care, as there may be boiling delays.
- Safety goggles should be worn to perform the Fehling test.
- As long as clean, uncontaminated lab equipment is used, students may taste the artificial honey.

Materials

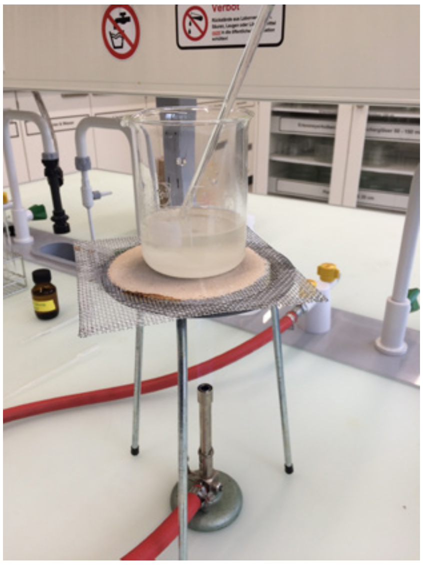
- Heat-resistant beaker (500 ml)
- Sucrose
- Lactic acid (4 drops) or fresh lemon juice (10 ml)
- Bunsen burner with tripod or magnetic stirrer with hot plate
- Distilled water (80 ml)
- Tablespoon
- Glass rod

Optional

- Test tubes
- Fehling solution I ($CuSO_4 \cdot 5H_2O$)
- Fehling solution II ($KNaC_4H_4O_6 + 4H_2O$)
- Pipette (1 ml)
- Test tube rack
- Safety goggles

Procedure

1) Mix 80 ml of water in the beaker with eight tablespoons of sucrose and add four drops of lactic acid or 10 ml of fresh lemon juice.
2) Remove 1 ml of the mixture for the Fehling test (see step 7).
3) Heat the mixture, stirring regularly (be careful not to burn the sugar).



Cooking the sucrose solution together with lemon juice

Image courtesy of the author

4) Reduce the heat shortly before the boiling point and continue heating without stirring until the solution boils. Leave the glass rod in the beaker. It will act as a boiling chip.
5) Continue boiling until about 1/3 of the water has evaporated.
6) Then slowly cool the solution.

Optional: Perform a Fehling test before and after the experiment

7) Mix 2 ml of each of the two Fehling solutions in two test tubes.
8) Add 1 ml of the artificial honey mixture to one test tube at the beginning of the experiment (step 1) and 1 ml of the artificial honey mixture to the other test tube at the end of the experiment (step 6).
9) The Fehling test can be tried with real honey too: add this to a third test tube containing 2 ml of each of the two Fehling solutions.
10) Now heat the test tube briefly in a Bunsen burner flame (or wait a couple of minutes).

Optional: Add colour and flavouring to make the fake honey more believable

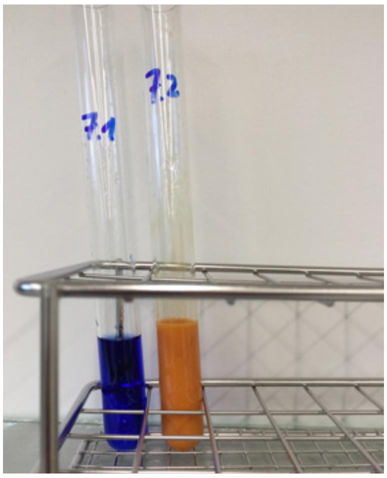
11) Add yellow (or a mix) food colouring to give a honey colour.
12) Dandelions or lime (linden) blossom tea can give a more complex flavour. Commercial honey flavouring will, of course, also work.
13) Experiment with different combinations to make a believable artificial honey.

Results

The result is a viscous, transparent mass, which tastes very sweet. Yellow food colouring and flavour compounds can then be added to make artificial honey. Commercially used honey flavour is a white crystalline powder, which gives the same taste and aroma as honey when it is diluted with other ingredients. It is basically dried honey produced from natural honey.

The Fehling test turns orange in the presence of mono- and disaccharides. If the Fehling solution stays blue, none of these sugars are present. Sucrose is an exception to the rule: 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 beginning of the experiment when sucrose is the only sugar present. The catalytically acting protons of the lactic acid cause the hydrolytic cleavage of sucrose into glucose and fructose. The invert sugar at the end of the experiment reacts positively to the Fehling test and indicates that sucrose has been broken down into the two monosaccharides.



Fehling test before (left, blue) and after (right, orange) the production of invert sugar
Image courtesy of the author

Some of the following questions can be discussed with the students:

- How can the difference in the outcome of the Fehling test before and after boiling be explained?
- Could you tell the difference between real and fake honey in a blind taste test?
- Does artificial honey taste sweeter than real honey or vice versa?
- Beekeepers feed their bees after the honey harvest with invert sugar. Why don't they use sucrose? Answer: the splitting of sucrose into glucose and fructose is an energy-intensive task for the bees. If the sugar is already inverted, i.e., split, the bees can store the food more easily.

Activity 2: Biochemical analysis of real honey and artificial honey

Honey contains about 80% sugar. Water, another main ingredient, contributes about 17%. In addition, there are over 100 other components, such as amino acids, minerals, vitamins, small amounts of fatty acids, flavourings, 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.

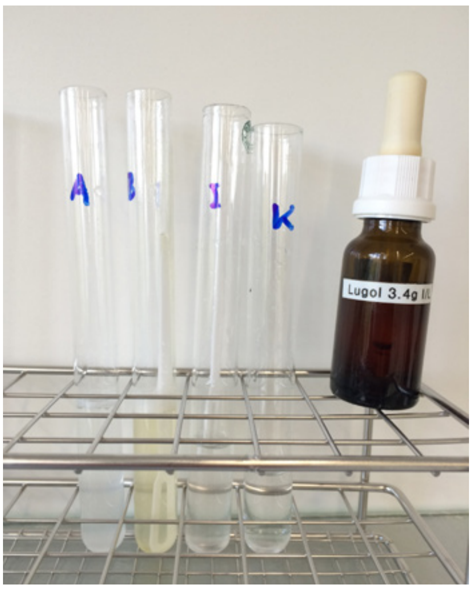
The activity takes about 45 min (including 30 min of waiting time).

Materials

- Real honey
- Fake honey from Activity 1
- Dextrin solution (1%)
- Lugol solution
- Water bath or incubator
- Distilled water
- 4 test tubes
- Test tube rack
- Pipettes
- Glass rod
- Amylase (or use your own spit)
- Beaker

Procedure

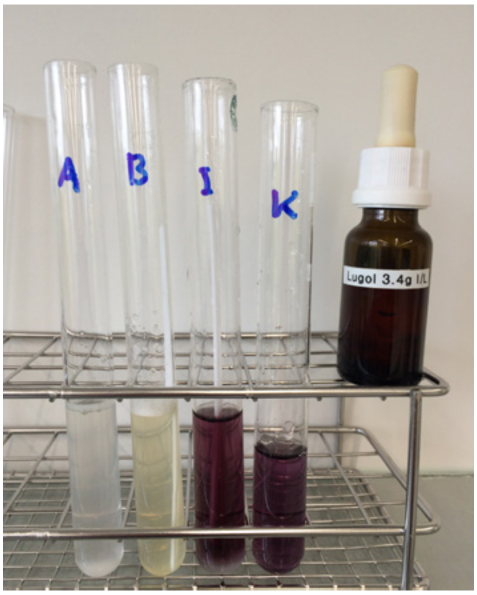
1) Label four test tubes (e.g., 1 to 4).
2) Add 1 ml of dextrin solution and 5 ml of water to each test tube.
3) Add a spatula tip of amylase to test tube 1 (alternatively: spit into a small beaker and add 1 ml of it to test tube 1).
4) Add some real honey to test tube 2: dip a glass rod about 1 cm deep into the honey and transfer it to the test tube. Mix well.
5) Add some of the artificial honey to test tube 3: dip a glass rod into the artificial honey and transfer it into the test tube. Mix well.
6) Test tube 4 serves as a control. Nothing else is added to it.
7) Shake the contents of the test tubes well and then put them into a water bath (or, if available, into an incubator) at 37°C.
8) After 30 min, add one drop of Lugol solution to each test tube.



Start of the experiment on the biochemical analysis of real honey and artificial honey
Image courtesy of the author

Results

Dextrin (also called maltodextrin) is a degradation product of starch and lies between oligosaccharides and starch in its molecular size. Starch and dextrin discolour with Lugol solution (iodine solution). Iodine is deposited in the tunnel-like starch molecules and causes a colour change from orange to dark violet or black by changing the light-absorption spectrum. In test tube 1, amylase has digested dextrin, and the Lugol test is therefore negative. In real honey, various enzymes are present, including amylase. This is why the Lugol test in test tube 2 will also be negative. In test tubes 3 and 4, iodine will interact with dextrin, which is still present because of the lack of amylase.



Result of the experiment on the biochemical analysis of real honey and artificial honey
Image courtesy of the author

Some of the following questions can be discussed with the students:

- Most honeys will crystallize over time. To liquefy it, honey may be heated in a water bath. Why should the temperature not exceed 40°C?
- Apart from the enzymes, which other compounds are missing in artificial honey?
- Not only chemically produced invert sugar is considered to be fake honey. What other ways are there to adulterate real honey? Answer: honey from different regions is often mixed. There are expensive honeys, such as mountain honey or lavender honey. These can be mixed with cheap honey from other regions or countries, and thus, adulterated. In addition, honey can also be diluted with water or syrup.

Activity 3: Measuring the water and sugar contents of honey

Honey should not exceed a certain water content. If you measure a water content above 20%, it might have been diluted with water or syrup, and it might ferment over time.

A refractometer determines the refractive index, according to how strongly the honey deflects a light beam. In other words, it indicates the sugar content or, indirectly, the water content of the honey. Since honey contains different sugars (mainly mono- and disaccharides), which have different effects on the refractive index, this does not determine the true water content. However, it is a standardized procedure in the quality guidelines of beekeepers, and the resulting error is accepted.

In this activity, a refractometer is used to measure the water and sugar contents of fake and real honeys. The activity takes about 15 min.

Materials

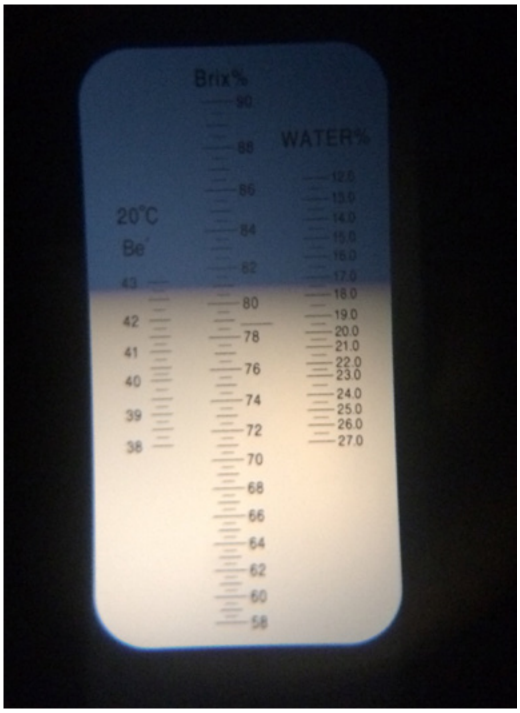
- Different types of honey, e.g., lavender honey, acacia honey (needs to be liquid)
- Fake honey from Activity 1
- Refractometer
- Pipette or spoon

Procedure

1) If the honey is crystallized, it needs to be warmed in a water bath at about 40°C.
2) Since the measuring instruments are normally calibrated at 20°C, the honey should also be brought to this approximate room temperature.
3) Using a pipette or a spoon, place a few drops of honey on the measuring window of the refractometer. Close the lid and make sure that the liquid is evenly distributed (no air bubbles or dry spots).

Preparing the refractometer
Image courtesy of the author

4) Hold the refractometer against the light and look through the eyepiece (the scale may need to be focused).
5) Read the value on the scale (= border between the blue and white zones).



Reading the scale of the refractometer
Image courtesy of the author

6) Clean the refractometer with a wet soft cloth.

Results

The unit of measurement is Brix degrees (symbol °Bx) and corresponds to the relative density of liquids. One degree Brix corresponds to 1 g of sucrose in 100 g of solution. The unit is mainly used in fruit, grape, and honey processing. Since these liquids contain various sugars (mainly glucose, fructose, and sucrose) in addition to water, the density is also used to indicate the approximate sugar or water contents. Honey should not exceed a water content of 18–20%. Should honey have a water content above 20%, it might have been diluted with water or syrup, and it might ferment over time.

Some of the following questions can be discussed with the students:

- Nectar and freshly stored honey in the beehive may show water contents above 20%. How might the bees lower that percentage? Answer: they ventilate the honey cells with their wings to evaporate water.
- Why will the water content of artificial honey from Activity 1 differ if you do a series of experiments? Answer: the water content will be dependent on time. The longer you cook the artificial honey, the lower the water content.

Image: coniferconifer/Wikimedia, CC BY 2.0

Extension activities: Discovering more about honey and bees

There are a number of additional experiments that can be carried out to learn more about the biology and chemistry of honey, as well as to link to other interdisciplinary topics.

For example:

- Ask students if they can think of any other ways to distinguish real from fake honey.
- Hint: where do bees get the nectar that they make honey from? What other things can you see in this image that the bee might carry back to the hive. The answer of course is pollen, which should be in most real honeys (except perhaps for honeydew honey)!
  A classroom investigation of pollen in honey is described in a previous article on the biology of bees and the biochemistry of honey.
- Visit a beekeeper and learn about the different honeycombs and behaviour of bees. The beekeeper can also provide pollen that bees have lost or is trapped at the entrance of the beehive.
- Find out about citizen science projects that investigate the diversity of pollen or pollinators.[3,4]
- Polarimetry extension activity: Measure the angle of rotation of sucrose and invert sugar with a polarimeter and learn about the chirality of molecules (experimental details and the chirality infosheet are given in the supporting material).
- Measure the activity of glucose oxidase in honey using test sticks to determine the hydrogen peroxide concentration. This is described in a previous article on the biology of bees and biochemistry of honey.
- Investigate the chemical aspects of beeswax (e.g., its melting point) and propolis and the hexagonal shape of the cells of the honeycomb.
- Learn about the (bio)chemistry of bee stings.

Acknowledgements

This activity was presented at the Science on Stage Festival 2022

References

[1] *More than Honey* movie (German language): http://www.morethanhoney.ch

[2] Data on the international trade of honey in 2021 on the Eurostat website: https://ec.europa.eu/eurostat/web/products-eurostat-news/-/edn-20220819-2

[3] The Citizen Scientist Investigation on pollen diversity in Europe: https://coloss.org/c-s-i-pollen/

[4] The Great Sunflower project, a citizen science project focused on pollinators: https://www.greatsunflower.org

Resources

- Read more about honey chemistry and bee venom in the Bee Culture magazine.
- Introduce the biology of bees and the biochemistry of honey with these teaching activities: Scheuber T (2023) To bee or not to bee: the biology of bees and the biochemistry of honey. *Science in School* **62**.
- Investigate the properties of so-called superfoods in your class: Frerichs N, Ahmad S (2020) Are 'superfoods' really so super? *Science in School* **49**: 38–42.
- Explore antibiotic resistance and drug development using honey and other foods: Deumal Fernandez M, Lladonosa Soler M, Godinho T (2021) Microbiology: Discovering antibacterial agents. *Science in School* **55**.
- Investigate food chemistry with mushrooms: Bunjes F et al. (2017) Natural experiments: chemistry with mushrooms. *Science in School* **42**: 36–41.
- Discover the biochemistry of bananas: Glardon S, Scheuber T (2018) Go bananas for biochemistry. *Science in School* **44**: 28–33.
- Explore the science of limonene: Butturini F, Fernández JJ (2022) Citrus science: learn with limonene. *Science in School* **58**.
- Learn how to spot pseudoscientific fake news in the media: Domenici V (2022) Fake news in chemistry and how to deal with it. *Science in School* **59**.

AUTHOR BIOGRAPHY

Thomas Scheuber is a biology teacher at the Gymnasium Kirschgarten in Basel, Switzerland. He represented Switzerland at the Science on Stage festival in Prague, Czech Republic, in 2022 with his experiments using honey, and he recently completed further training as a beekeeper.