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To bee or not to bee: the biology of bees and the biochemistry of honey

Thomas Scheuber

Sweet understanding: learn about the science of honeybees and their sugary product through a series of hands-on activities.

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 fraud so lucrative. Fake or adulterated honey includes an incorrect declaration of origin; stretching honey with sugar syrup from corn, sugarcane, and rice; or completely artificial products.

In a series of experiments, students learn about bees, try a biological method for distinguishing real honey from artificial honey, and investigate different biological and biochemical aspects of honey. An article on the chemical aspects will follow in a later issue.

The activities are suitable for secondary school students aged 14 and older. They are related, so, ideally, they should all be done as part of a larger project on bees and honey, but the activities can also be carried out individually.

Activity 1: The fascinating world of bees

Before beginning the practical activity, it is nice to place the work on honey in context by learning about the fascinating biology of bees. Bees are also a really useful example to help put the curriculum topic of epigenetics into context, given the role this is known to play in the differentiation of queen bees from worker bees. This activity is flexible, and teachers can choose which parts to use, depending on how much time they have.

Materials

- Bee infosheet and/or the bee bonanza pages from Arizona State University; this short video on how bees make honey is also interesting and informative.
- Bee quiz
- Optional: video on epigenetics and royal jelly
- Optional: some dead bees (do not collect live ones and kill them)

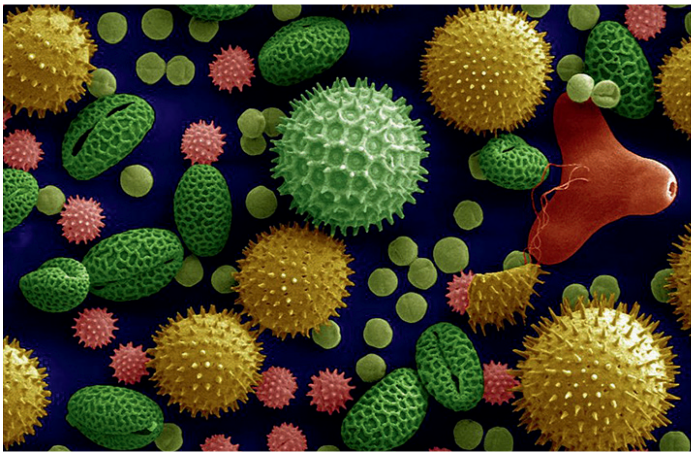
Procedure

1) Hand out the infosheet for students to read. The bee bonanza pages from Arizona State University are also an excellent resource.
2) Optional: watch the video on epigenetics and royal jelly. This is an excellent recap of gene expression and chromosomes, as well as an introduction to epigenetics (first half) and an interesting example of epigenetics at work (second half). This part can also be taken out and incorporated into genetics lessons.
3) Have students go through the bee quiz. Not all the answers are on the infosheet, so sometimes they will have to guess. The aim is to learn some fascinating bee facts; it's not a test!
4) Optional: examine the bees using hand lenses or dissecting microscopes and identify the external features. Can you identify the species? Perhaps also have students draw the feature they find most interesting.

Apis mellifera

Image: Luc Viatour/Wikimedia, CC BY-SA 3.0

Activity 2: Pollen analysis from different types of honey



Colorized scanning electron microscope image of pollen grains from a variety of common plants
Image: Dartmouth Electron Microscope Facility/Wikimedia, Public Domain

Honey is interesting to investigate with a microscope. Only real bee honey contains pollen grains, which are collected by the bees as larval food and transported to the hive on their legs. It is normally stored in separate cells of the honeycomb. However, in the case of real honey, some pollen always gets into the honey cells on a small scale. Pollen analysis can be used to check whether the honey is correctly labelled, since fake honey is a huge problem in some places. For this purpose, we analyse different types of honey. This will take approximately 45 min.

Materials

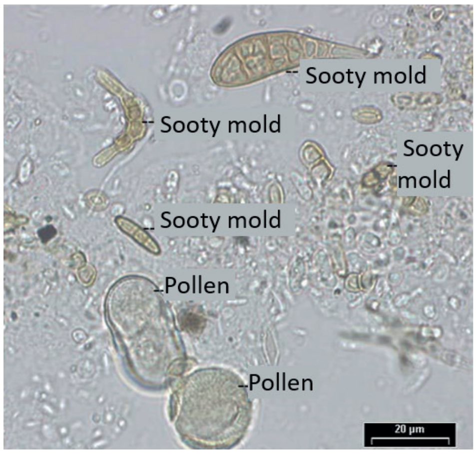
- Quick pollen guide (you might also find more detailed pollen guides online)
- Background information sheet on the characteristics of pollen
- Different types of honey, such as lavender honey or acacia honey
- Artificial honey (or inverted sugar syrup, which has a similar sugar composition to honey)
- Eppendorf tubes
- Small beaker
- Glass rod or spoon
- Eppendorf centrifuge
- Balance
- Distilled water
- Pipette (1 ml)
- Pasteur pipette
- Slides
- Coverslips
- Microscope
- Vortex mixer (if available)

Procedure

1) Label an Eppendorf tube with the type of honey to be tested.
2) Weigh out 1 g of honey in a beaker.
3) Add 1 ml of distilled water and stir the mixture with the glass rod.
4) Pipette 1 ml of the mixture into the prepared Eppendorf tubes and mix well (vortex briefly).
5) Place the samples in the centrifuge. CAUTION: Make sure that the centrifuge is balanced. Centrifuge the samples for 10 min at a speed of 5000 rpm.
6) Using a Pasteur pipette, carefully remove the sediment from the bottom of the Eppendorf tube and place a drop of it on the slide. Cover the drop with a coverslip.
7) Examine your sample under the microscope.
8) Read the infosheet on the characteristics of pollen and use the quick pollen guide to try to identify the plant it came from.

Results

Different types of pollen grains can be found. In varietal honey, such as lavender honey, you should find mostly the corresponding type of pollen. Compare your results with reference pictures from Pollen Wikis.[3] It is not always possible to identify the pollen grains, but at least you can distinguish different species from each other.



Pollen against a dense background of small crystals, yeasts, and other fungi (e.g., sooty mould) is characteristic of forest honey, which is made from honeydew not nectar.
Image courtesy of Thomas Scheuber

Fake or highly processed honey has no pollen in it. Pollen is removed, including to prevent crystallisation, and to hide any traces of where the honey was produced. Pollen acts as a fingerprint and allows scientists to trace where the bees came from, proving the country of origin.

Discuss some of the following questions with your students:

- Which honey showed the biggest variety of pollen?
- Do you find the expected pollen in the corresponding honey?
- Which honey showed the highest amount of pollen?

Activity 3: The antibacterial effect of honey

Honey is known to contain preservative compounds and to have antimicrobial properties. There are a number of compounds that contribute to this effect (two are explored further in Activity 4). In this experiment, we start by testing the antibacterial effect of honey. The activity will take about 30 mins of active time (with the results evaluated a day or two later).

Safety notes

Wear safety goggles when you perform the experiment.

Materials

- Various types of honey
- Fake honey or inverted sugar syrup
- Petri dishes with lysogeny broth (LB) nutrient agar
- Bunsen burner
- Petri dish with alcohol
- *Escherichia coli* liquid culture (let it grow overnight to reach the exponential phase of growth)
- Micropipette (100 microliter)
- Drigalski spatula
- Parafilm

Procedure

In this experiment, it is very important to work under sterile conditions to avoid contamination of the Petri dishes. Therefore, the plates must not be left open, and they must be closed quickly and sealed with parafilm after inoculation with bacteria.

1) Place 100 microlitres of the E. coli liquid culture on the nutrient agar.
2) Disinfect a Drigalski spatula: dip it in alcohol, ignite it at a Bunsen burner flame, wait until the flame extinguishes by itself, and let the spatula cool down for a short time
3) Evenly spread the bacteria on the nutrient agar.
4) Put one drop of honey and another drop of artificial honey on the nutrient agar. Several types of honey can be tested on one Petri dish at the same time.
5) Seal the dishes with parafilm.
6) Incubate the Petri dishes for approximately 24–48 h at 37°C in an incubator (if no incubator is available, incubation can also be done at room temperature).
7) Evaluate the experiment by measuring and comparing the inhibition zones.

Discussion

The use of honey as a traditional remedy for bacterial infections has been known since ancient times. Scientific research studies focused on identifying so-called inhibines, with antibacterial effects (apart from hydrogen peroxide), have been undertaken. Microbiological assays can be used to test for the antibacterial effects of honey. Different diameters of the inhibition zones of different honeys show their different antibacterial potentials. As a positive control, you may include a conventional antibiotic, such as penicillin, and as a negative control, water on filter paper may serve.

Discuss some of the following questions with your students:

- Which honey had the biggest effect on the growth of the bacteria?
- What difficulties do you have to consider in this experiment?
- Some inhibines are light and temperature sensitive. How does this fact influence the traditional treatment of bacterial infections and the suitable storage of honey?

Materials used for Activity 3

Image courtesy of Thomas Scheuber

Activity 4: Glucose oxidase activity

An important enzyme in honey is glucose oxidase. It catalyses the oxidation of b-D-glucose. This reaction produces gluconic acid and hydrogen peroxide. Both compounds have preservative properties. Gluconic acid lowers the pH value. This provides protection for the honey, especially against fungi. Bee mites (especially the Varroa mite) are also driven away by an acidic environment, which is why beekeepers treat hives with formic acid and oxalic acid. Hydrogen peroxide, on the other hand, is characterised by its bacteriostatic effect. The activity will take about 60 min.

Image courtesy of Thomas Scheuber

Materials

- 3 beakers
- Thermometer
- Glass rod
- Spatula
- Honey
- Distilled water
- Hydrogen peroxide test strips
- Magnetic stirrer with heater

Procedure

1) Heat 100 ml of water in one beaker to 40°C.
2) Heat 100 ml of water in the second beaker to 80°C.
3) In the third beaker, leave 100 ml of water at room temperature.
4) When the temperatures are reached, add two teaspoons of honey to each and stir gently for 3–4 min.
5) Use the test strips to determine the hydrogen peroxide concentration in the three honey solutions.

Discussion

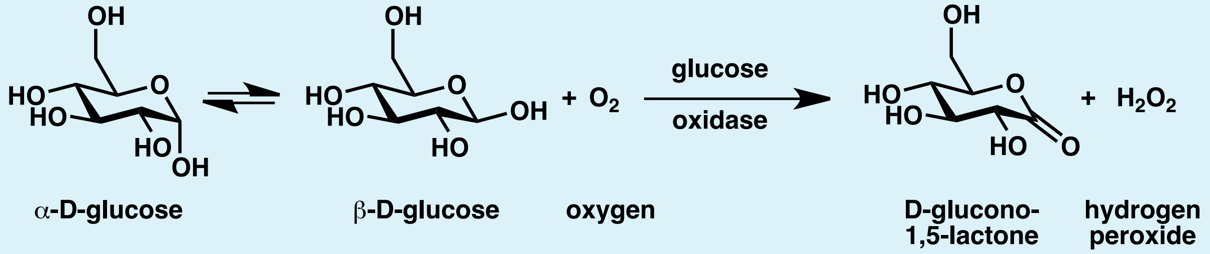
- Where do the two reactants (glucose and oxygen) and the catalyst (glucose oxidase) come from?
- What do you observe? What effect does temperature have on the hydrogen peroxide concentration?
- How would you explain this result (hint: think about what you know about the function of enzymes)?

Conclusion

Honey is an excellent starting point to explore a number of biological and chemical concepts. This article gives some biology examples, and another article with more of a chemistry focus will follow. If there is time, it is particularly beneficial

Glucose oxidase

- Glucose oxidase is an important enzyme in honey. It catalyses the oxidation of b-D-glucose, which produces gluconic acid and hydrogen peroxide. Both compounds have preservative properties.
- Gluconic acid lowers the pH value.
- Hydrogen peroxide, on the other hand, is particularly characterised by its bacteria-inhibiting effect.



Reaction catalysed by glucose oxidase

Image: Boghog/Wikimedia, Public Domain to try all the activities as part of a larger project, to show students that dividing science into biology/chemistry/physics is rather artificial: most research topics have multiple facets and require a multidisciplinary approach. These activities are intended as a starting point/inspiration for a project relating to bees; teachers can also bring in other aspects, such as the ecology of bees (e.g., their importance as pollinators) and the fascinating world of bee social behaviour. «

References

[1] The 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 digital pollen atlas (predominantly in German language): https://pollen.tstebler.ch/MediaWiki/index.php?title=Pollenatlas#gsc.tab=0

Resources

- Watch a video about epigenetics and royal jelly. The first half is a really good explanation of epigenetics, with excellent infographics. The half second places this in context using the example of royal jelly and bee development.
- Watch a short video on how bees make honey.
- Learn about the surprisingly sophisticated ways bees communicate and make decisions.
- For more fun facts about the fascinating world of honeybees, check out the bee bonanza pages from Arizona State University.
- Take a virtual tour of a beehive.
- Find another bee quiz that you can use in your class.
- Read about antibiotic resistance and drug development: Fernandez MD, Soler ML, Godinho T (2021) Microbiology: Discovering antibacterial agents. *Science in School* **55**.
- Discover the biochemistry of bananas: Glardon S, Scheuber T (2018) Go bananas for biochemistry. *Science in School* **44**: 28–33.
- Investigate the properties of so-called superfoods: Frerichs N, Ahmad S (2020) Are ‘superfoods’ really so super? *Science in School* **49**: 38–42.
- Explore food chemistry with mushrooms: Bunjes F et al. (2017) Natural experiments: chemistry with mushrooms. *Science in School* **42**: 36–41.

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.