Honey predominantly contains gluconic acid, acetic acid, formic acid, butonic acid, citric acid, lactic acid, maleic acid and oxalic acid. A relatively high concentration of formic acid indicates a treatment against Varroa mites while flowers were in bloom. The concentration of free acid in honey has to be analysed according to the regulations on honey. This is to prevent honey which has already started to ferment (but whose process of fermentation may have been interrupted) from entering the market. During fermentation the concentration of acetic acid and lactic acid increases. According to a recommendation of the European Honey Commission the acidity of honey shouldn't exceed 50 mmol/kg.

### Basic principle of analysing free acid in honey

The concentration of free acid is analysed through titration with soda lye, titrating the honey to a pH value of 8.3. Acid titration of honey isn't clearly defined as the end point of titration keeps shifting slightly because of hydrolysing lactones. The method of analysis is adequately exact if the process of titration is accomplished within the recommended short period of time. The scale of the burette should have an accuracy of ± 0.1 ml.

The amount of free acid can be calculated using the amount of lye used for the titration.

### Calculating the amount free acid

The consumption (ml) of NaOH (0.1 mol/l) x 10 is equivalent to the amount of free acid in mmol/kg honey.

### Preparations for the experiment

Before each application the pH-meter has to be calibrated using different buffer solutions, mostly with a buffer pH = 4 and a buffer pH = 7.[[1]](#footnote-1)

## Experiment

Materials Scales (accuracy ideally 1 mg); measuring cylinder, beaker (250 ml), pH-meter, funnel, stirring rod (glass), burette, stand with muffle and clamp, magnetic mixer

Chemicals distilled water, soda lye, c(NaOH) = 0,1 mol/L, various honey samples

GHS-Piktogramme - ÄtzendGHS-Piktogramme - ReizendHazards Soda lye

1. 10g of honey are weighed in a 250 ml beaker.
2. Add 75 ml of distilled water (using the measuring cylinder) to the honey sample.
3. Mix with the magnetic mixer until the solution is clear.
4. Fill the burette with soda lye and measure the amount of lye exactly (mind the meniscus!).
5. Put the pH-meter into the solution and start titrating with soda lye while stirring continuously with the magnetic mixer until you reach a pH-value of 8.3. This should be accomplished within 120 seconds.
6. After the first run: Rinse the glass electrode with distilled water.
7. The experiment has to be repeated once with every sample.
8. After the analysis, the glass electrode is kept in a watering cap which is filled with a potassium chloride solution [c(KCl) = 3 mol/l].

## Measurements / Observations

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | Use of NaOH | | Average of the assays | Acid concentration in mmol/kg |
| Measurement 1 | Measurement 2 |
| Honey from the eHive |  |  |  |  |
| Blossom honey 1 |  |  |  |  |
| Blossom honey 2 |  |  |  |  |
| Forest honey 1 |  |  |  |  |
| Forest honey 2 |  |  |  |  |

## Interpretation

1. Explain possible reasons for the varying measurements.
2. The introduction tells you why free acid in honey is examined. Explain why honey which has completed fermentation shouldn’t be sold. Research the reasons on the internet.
3. Discuss whether forest honey can be distinguished from blossom honey according to the amount of free acid they contain.
4. Discuss the results with your partner in your group and write them down.

## Literature

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1. The pH value depends on the temperature. Most devices can compensate varying temperatures. The temperature of the sample shouldn't be above 30° C because the measurements aren't precise enough (even with temperature compensation). [↑](#footnote-ref-1)