PRACTICAL EXERCISES FOR THE COURSE OF FOOD ANALYTICS

Dr. Nikolett Czipa, associate professor

University of Debrecen

Faculty of Agricultural and Food Sciences, and Environmental Management

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Content

Exercise 1: Safety training 3
Exercise 2: Determination of dry matter content, ash content and electrical conductivity 4
Exercise 3: Determination of fat content (Soxhlet method) 7
Exercise 4: Determination of protein content (Kjeldhal method) 8
Exercise 5: Determination of total carbohydrate content 11
Exercise 6: Determination of total dietary fibre content 13
Exercise 7: Determination of vitamin C 16
Exercise 8: Determination of diastase activity 19
Exercise 9: Determination of total phenolic and flavonoid content 21
Exercise 10: Determination of proline content 25
Exercise 11: Determination of acid content, acidity and pH 27
Exercise 12: Determination of physicochemical parameters of alcoholic beverages 31
Exercise 13: Sensory analysis 33
References 34

Exercise 1: Safety training

Purpose of the exercise: Students will be able to work safely in the laboratory

Required knowledge: None

Exercise 2: Determination of dry matter content, ash content and electrical conductivity

Purpose of the exercise: Obtaining laboratory practice
Required knowledge: Knowledge of lecture materials

Exercise: Determine the moisture, dry matter and ash content of chosen food and determine the EC of a chosen honey sample based on the following methods.

Determination of dry matter and moisture content

Principle of the method: Water evaporates from the sample on a temperature above 100°C. It is influenced by many factors, e.g. particle size and distribution, surface and layer thickness. Advantages of this method are: simple, standardized and reliable; disadvantages of this method are: other volatile compounds can leave the sample (but this loss is usually negligible) and in certain cases thermal decomposition may happen.

Test procedure:
Dry the Petri dish at 103±1°C in oven for 30 minutes, then leave to cool it in a desiccator and weight it with analytical balance (four decimal places). Weight 5 g homogenized sample in a Petri dish (four decimal places). Place the Petri dish containing the sample into the oven and dry it for four hours from the reach of corresponding temperature. Then place the Petri dish containing the sample into desiccator and leave to cool it for room temperature. After cooling, weight it (Petri dish and dried sample) with analytical balance (four decimal places).
Calculate the dry matter content by the following formula:

$$Dry\ matter\ content\ (\%) = \frac{m2 - m0}{m1 - m0} \times 100$$

where:
m0: weight of empty dried Petri dish (g)
m1: weight of Petri dish containing the sample (g)
m2: weigh of Petri dish containing the dried sample (g)

From the calculated dry matter content, the moisture content can be calculated with the following equation:

$$Moisture\ content\ (\%) = 100 - dry\ matter\ content$$

If the sample is liquid or has high moisture content, it should be mixed with sand.

Determination of ash content

Raw ash is a generic term for non-combustible materials in the examined sample, i.e. total mineral content of the sample. Mineral content in various type of food is very different. In case of plant origin food this content is between wide ranges because the agricultural area and soil properties have important effect on this parameter. Micro and macro elements may be differentiated based on their concentration in the body. Minerals may be essential and nonessential. The most important macro elements are Na, K, Mg, Ca, P, S and Cl, and the most important micro elements are Fe, F, Zn, Si, Cu, V, Se, Mn, I, Ni, Mo, Cr and Co. Nonessential elements are e.g. Pb, As, Hg and Cd.
Incineration (ashing): A procedure where water, volatile compounds and organic compounds are removed from the sample.

Principle of method: Sample is ashed on high temperature (550°C) and the residue is weighed. If the temperature is higher than 900°C, platinum crucible should be used. Ash content of food is influenced by many factors, e.g. soil, climate, cultivation conditions, manufacturing defect, inadequate handling. Advantages of this method are: safe, few reagents are required, many samples can be analysed simultaneously. Disadvantages of this method are: long time (12-24 hours), volatile minerals (e.g. Hg, Pb, Ni, Zn) can be lost.

Test procedure:
Ash the crucible at 550°C, cool it in a desiccator and weight it (four decimal places). Weight 5 g sample in the crucible and weight the combined mass of the crucible and the sample. Place the crucible containing the sample in the furnace which is heated progressively until the sample is charred. Then carry out the incineration at 550°C for at least 3 hours. Place the crucible containing the sample in desiccator, leave to cool it for room temperature and weight it.

Calculate ash content by the following formula:

$$\text{Ash content (\%)} = \frac{m_1 - m_0}{m_2 - m_0} \times 100$$

where:

m_0: weight of empty crucible (g)

m_1: weight of crucible containing the sample (g)

m_2: weight of crucible containing the ashed sample (g)

Determination of electrical conductivity (EC) of honey samples

EC of honey is defined as of a 20% weight to volume solution in water at 20°C, where 20% refers to honey dry matter.

Principle of method: EC of a honey solution of 20 g dry matter of honey in 100 ml distilled water is measured using an EC cell. The determination of EC is based on the measurement of the electrical resistance, of which the EC is the reciprocal. Results are expressed in mS/cm.

Sample preparation: Dissolve an amount of honey, equivalent of 20 g anhydrous honey in about 50 ml distilled water. Transfer this solution quantitatively in a 100 ml volumetric flask and make up to volume with distilled water.

Test procedure:

Transfer the solution into a 200 ml baker, place it on magnetic stirrer. Immerse the electrode in the solution, stir and read the EC of the sample from the EC meter. If the meter does not calculate with temperature, correction factor should be used for calculation of the value of 20°C:

For temperature above 20°C: subtract 3.2% of the value per °C.

For temperature below 20°C: add 3.2% of the value per °C.

Exercise 3: Determination of fat content (Soxhlet method)

Purpose of the exercise: Obtaining laboratory practice

Required knowledge: Knowledge of lecture materials

Exercise: Determine the fat content of chosen food based on the following method

Extraction: This is a separation process where components are extracted from solid or liquid phase with the application of corresponding solvent (e.g. petroleum ether, hexane). The speed of the process is influenced by many factors, e.g. temperature, particle size of the sample, specific surface area of the sample, purity of the solvent and flow of the solvent. The most common and widespread extraction process is Soxhlet extraction, which is a generally accepted standard method in food analytics.

Test procedure:

Weigh two-three grams of the homogenized sample with analytical balance (four decimal places) in a fat-free extraction thimble and close it with a cotton plug. Place the thimble in the middle part of extractor and connect this part to a 250 ml round bottom flask and water cooler. Before connection, place four pumice stones into the 250 ml flask and weigh it, then fill it with 150 ml petroleum ether. After assembling the extractor, start the water cooling and heating (sand bad). The boiling point of petroleum ether is 40-60°C. The extraction process requires about two and eight hours which depends on the type of sample. At the end of process, take the thimble out from the middle part of the extractor and distil the petroleum ether. Dry the flask containing fat at 98±2°C to constant weight in oven, then cool it in desiccator for room temperature, then weight it (four decimal places).

Calculate dry matter content with the following formula:

$$Fat\ content\ (\%) = \frac{m1 - m2}{m0} \times 100$$

where:

m1: the weight of flask, pumice stones and the extract (g)

m2: the weight of flask and pumice stones (g)

m0: the weight of the sample (g)

Exercise 4: Determination of protein content (Kjeldhal method)

Purpose of the exercise: Obtaining laboratory practice
Required knowledge: Knowledge of lecture materials

Exercise: Determine the nitrogen content and calculate the protein content of chosen food based on the following method

Kjeldhal method is a generally accepted standard method for the protein content determination of different organic samples (food and feed). The advantage of this method is that it can be used for all kinds of food samples, it gives suitable and accurate results and it is not too expensive. Its disadvantage is the usage of harmful chemicals (for human health and environment), and it is a time-consuming method.

Principle of the method: Nitrogen content of the sample is formed by boiling with sulphuric acid to ammonium salts and the ammonia is released with base. Then it is distilled into boric acid solution and the nitrogen content is determined by titration with sulphuric acid.

Sample preparation:
Weight 1 gram homogenized sample in nitrogen-free paper and take it into a digestion tube. Add two catalyst tablets (Se) and 14 ml sulphuric acid, then boil it on 420-430°C in a heating unit (start the water suction pump). Time of the digestion is about two hours; samples should be colourless. A blank sample is necessary where only nitrogen-free paper is taken into the digestion tube (the other steps are the same as in case of the samples).

Test procedure:
Place the digestion tube containing cold sample into the appliance and start the distillation with 33% sodium hydroxide solution. Distillation is happening into a 250 ml Erlenmeyer flask containing 30 ml 4% boric acid solution. Boric acid solution contains mixed indicator (methyl red and bromocresol green). Original colour of this solution is red; however it will be green due to the distillation (nitrogen). Titrate the sample with 0.1 M sulphuric acid until a pink colouration occurs.

Calculate nitrogen content with the following formula:

$$Nitrogen\ content\ (\%) = \frac{(S_m - S_v) \times f_{H_2SO_4} \times 0.0014007}{b} \times 100$$

where:

Sm: the volume of sulphuric acid consumed in the titration of the sample solution (ml)

Sv: the volume of sulphuric acid consumed in the titration of the blank solution (ml)

f_{H2SO4}: the factor of sulphuric acid in the titration

b: the weight of the sample

From the calculated nitrogen content, protein content can be calculated with the following equation:

$$\text{Protein content } (\%) = \text{Nitrogen content } (\%) \times \text{F}$$

where F is a conversion factor. It is usually 6.25 because the mean nitrogen content of proteins is 16% (100 / 16 = 6.25). However, in certain cases the factor can be different, e.g. wheat = 5.7; soy = 5.71; rice = 5.95; sunflower seed = 5.3.

Preparation of solutions:

- 33% sodium hydroxide solution: Dissolve 1000 g NaOH in 1000 ml distilled water then quantitatively transfer it into a 3000 ml volumetric flask and make up to volume with distilled water
- 4% boric acid solution: Dissolve 200 g boric acid in 3000 ml hot distilled water. Add another 1500 ml distilled water and cool it. Dissolve 50 mg bromochresol green indicator in 50 ml 96% ethyl alcohol and dissolve 50 mg methyl red indicator in 50 ml 96% ethyl alcohol. Add 50 ml bromochresol indicator and 35 ml methyl red indicator to the cooled boric acid solution. Transfer it quantitatively into a 500 ml volumetric flask and make up to volume with distilled water.
- 0.1 M sulphuric acid solution (the density of sulphuric acid is 1.84 g/ml; 96% (m/m); the molar mass of sulphuric acid is 98.078 g/mol). Fill 500 ml distilled water into a 1000 ml

volumetric flask and add 5.55 ml sulphuric acid, then make up to volume with distilled water

- Determination of 0.1 M sulphuric acid factor using the following equation:

$$factor = \frac{weight\ of\ sodium\ carbonate(g) \times content\ of\ sodium\ carbonate\ (\%) \times 1000}{53{,}0\ (mg/ml) \times volume\ of\ sulphuric\ acid\ consumed(ml) \times 100}$$

Dissolve 0.2 g dried anhydrous sodium carbonate (dry it on 300°C for two hours) in 50 ml distilled water, then add 0.1 ml methyl orange indicator. Titrate it with 0.1 M sulphuric acid until a reddish-yellow colouration occurs. Boil this solution for two minutes (its colour will turn into yellow). After cooling the solution, continue the titration until a reddish-yellow colouration occurs. 10.6 mg sodium carbonate is equivalent to 1.0 ml 0.1 M sulphuric acid.

Exercise 5: Determination of total carbohydrate content

Purpose of the exercise: Obtaining laboratory practice
Required knowledge: Knowledge of lecture materials

Exercise: Determine the total carbohydrate content of chosen food based on the following method

Determination of total carbohydrate content with phenol sulphuric method

Principle of the method: In hot acidic medium glucose is dehydrated to hydroxymethyl furfural. The solution turns into a yellow-orange colour as a result of the interaction between the carbohydrates and phenol. Absorption maximum of this solution is at 490 nm. This is a widely used method to determine the total concentration of carbohydrates in foods. This is an easy, rapid, sensitive method, reagents are cheap easily accessible. Inorganic ions, alcohols, organic acids and amino acids do not affect the determination.

Sample preparation: Weight 1 g sample into a 200 ml flask and add 10 ml sulphuric acid (72%). Let the sample swell, then make up to volume with distilled water. Place it in 100°C water bath for 4 hours, then leave it to cool. Add 3 ml Carrez I solution and 3 ml Carrez II solution (proteins will be coagulated), then filter it through a filter paper. Dilute the solution to 1/10. Prepare a blank solution with distilled water too.
Preparation of the calibration solutions: Dissolve 10 g saccharose in 100 ml distilled water (stock solution). Pipette into five 10 ml test tubes 1, 2, 3, 4 and 5 ml stock solution and make up to volume with distilled water.

Test procedure:
Pipette into seven 10 ml test tubes 1 ml sample solution, calibration solution and blank solution. Add 1 ml 5% phenolic solution to each tube. Then add 5 ml concentrated sulphuric acid to each tube through the side of the tubes without disturbing the tube. Leave it for 10 minutes then place it in water bath (25-30°C) for 20 minutes. Determine the absorbance at 490 nm against a blank solution in a 1 cm cell.

Absorbance values should be plotted versus concentrations. The X axis represents the concentrations and the Y axis represents the absorbance. Draw a regression line through the points and read the concentration of the sample versus absorbance.

Exercise 6: Determination of total dietary fibre content

Purpose of the exercise: Obtaining laboratory practice
Required knowledge: Knowledge of lecture materials

Exercise: Determine the TDF content of chosen food based on the following method

Determination of total dietary fibre (TDF) content with enzymatic-gravimetric method from prepared sample

Principle of the method: TDF is determined on duplicated samples of dried or defatted (if the fat content is higher than 10%). Starches are broken down with heat-resistant α-amylase enzyme, then proteins are hydrolysed by protease enzyme. Residues of carbohydrates are hydrolysed by amyloglucosidase enzyme into simple sugars. Soluble fibre, depolymerised protein and glucose (from starch) are precipitated with four volumes of ethyl alcohol. The residue is filtered, dried and weighed. One duplicate is analysed for protein and the other is incubated on 525°C to determine ash. TDF is the weight of the filtered and dried residue less the weight of protein and ash (TDF = weight residue – weight (protein + ash)).

Sample preparation: Dry the homogenised sample overnight at 70°C in oven, cool it in desiccator, then mill to 0.3-0.5 mm mesh. If the sample should not be heated, freeze-dry it before milling. If the fat content of the sample is higher than 10%, defat it with petroleum ether (25 ml/g; three times). In this case, determine the weight loss which should be considered during the calculation of TDF.

Test procedure:
Weight 1 g sample into two 400 ml tall-form beakers, then add 50 ml pH 6.0 phosphate buffer and 0.1 ml Termamyl solution. Cover it with aluminium foil and place it in boiling water bath for 15 minutes (temperature should be kept between 95°C and 100°C for 15 minutes). Cool the solutions to room temperature.

Adjust pH 7.2±0.2 with 10 ml 0.275 M NaOH. Add 5 mg protease or pipette 0.1 ml protease solution (50 mg protease in 1 ml phosphate buffer) to each sample. Cover it with aluminium foil and incubate at 60°C for 30 minutes while stirring, then cool the solutions.

Adjust pH 4.0-4.6 with 10 ml 0.325 M HCl. Add 0.3 ml amyloglucosidase to each sample. Cover it with aluminium foil and incubate at 60°C for 30 minutes while stirring. Add 280 ml 95% ethyl alcohol preheated to 60°C and let the solutions stay on room temperature for 60 minutes.

Weight the crucibles containing Celite, then wet Celite beds with 78% ethyl alcohol and apply suction to draw it onto fritted glass as even mat. Maintain suction and quantitatively transfer the precipitate of enzyme digest to crucible.

Wash the residue with 20 ml 78% ethyl alcohol (three times) and 10 ml 95% ethanol (twice) and 10 ml acetone (twice). Dry the crucibles containing the residue overnight in 70°C oven. Cool the crucibles in desiccator and weight them. Subtract weight of the crucible and Celite to determine the weight of the residue. Determine the protein content of one residue with Kjeldhal method. Determine the ash content of other residues on 525°C for 5 hours.

Prepare two blank samples too!

Determination of blank sample:

$$m_{blank} = m_p - m_{bpr} - m_{ba}$$

where:

m_{bp}: average of residue weights (mg) of the parallel blank determinations

m_{bpr}: weights (mg) of protein determined in the first and second blank residues

m_{ba}: weights (mg) of ash determined in the first and second blank residues

Calculate TDF with the following formula:

$$Total\ dietary\ fibre\ (TDF)(\%) = \frac{m_p - m_{pr} - m_a - m_{blank}}{m_s} \times 100$$

where:

m_p: average of residue weights (mg) of the parallel sample determinations

m_{pr}: weights (mg) of protein determined in the first and second sample residues

m_a: weights (mg) of ash determined in the first and second sample residues

m_s: average of sample weights (mg)

Preparation of solutions:

- 78% ethyl alcohol solution: Place 207 ml distilled water into 1000 ml volumetric flask and make up to volume with 95% ethyl alcohol
- 0.08 M phosphate buffer (pH 6.00): Dissolve 1.4 g anhydrous sodium phosphate dibastic and 9.68 g sodium phosphate monobastic monohydrate in 700 ml distilled water and quantitatively transfer it into a 1000 ml volumetric flask and make up to volume with distilled water. (Check pH with pH meter)
- 0.275 M sodium hydroxide solution: Dissolve 11 g sodium hydroxide in 700 ml distilled water, quantitatively transfer it into a 1000 ml volumetric flask and make up to volume with distilled water
- 0.325 M hydrochloric acid solution: Pipette 18.5 ml hydrochloric acid (36.3% m/m; density: 1.762 g/cm^3) into a 1000 ml volumetric flask containing 500 ml distilled water and make up to volume with distilled water

Exercise 7: Determination of vitamin C

Purpose of the exercise: Obtaining laboratory practice
Required knowledge: Knowledge of lecture materials

Exercise: Determine vitamin C content of chosen food based on the following methods

Metaphosphoric acid method

Sample preparation: Weight 5 g sample into a 250 ml beaker, add 100 ml 3% metaphosphoric acid solution, then mix it (at least 30 seconds). After homogenization, quantitatively transfer the solution into a 250 ml volumetric flask and make up to volume with 3% metaphosphoric acid solution. Filter this solution through a filter paper into a 250 ml Erlenmeyer flask.

Test procedure:
Pipette 50 ml sample solution into a titration flask and add 5 ml 2% hydrochloric acid solution, 5 ml 1% potassium iodide solution and 1 ml 1% starch solution. Titrate it with 0.004 N potassium iodate solution until the blue colour appears.

Calculate vitamin C content with the following formula:

$$Vitamin\ C\ (mg/100g) = \frac{V \times 0{,}3522 \times k \times 100}{n}$$

V: ml of 0,004 N KIO_3 solution used for the titration
k: dilution
n: weight of the sample (g)

Preparation of solutions:

- 3% metaphosphoric acid: Dissolve 30 g crystallized metaphosphoric acid in 1000 ml distilled water.
- 2% HCl solution: Weight 4.7 ml HCl (36.3%; ρ=1,18 g/cm^3) into a 100 ml volumetric flask containing about 40 ml distilled water, then make up to volume with distilled water

- 1% KI solution: Dissolve 1 g crystallized KI in 100 ml distilled water
- 1% starch solution: Dissolve 1 g anhydrous starch in 100 ml distilled water and boil it for 2 minutes. Then quantitatively transfer the solution into a 100 ml volumetric flask and make up to volume with distilled water
- 0.004 N KIO_3 solution: Dissolve 0.856 g KIO_3 in 500 ml distilled water, then quantitatively transfer it into a 1000 ml volumetric flask and make up to volume with distilled water.

α-α-dipiridil method:

Principle of the method: Ascorbic acid reduces Fe^{3+} ions to Fe^{2+} ions in acidic medium. Fe^{2+} ions react with α-α-dipiridil reagent and they form a Fe(II)-dipiridil complex. This complex dissolves in the examined solution with red colour. Absorbance of this solution is proportionate to the quantity of ascorbic acid.

Sample preparation: Pipette 2 ml sample into a 200 ml volumetric flask and make up to volume with distilled water. Pipette 4 ml of the sample solution into a 100 ml volumetric flask and add 0.6 ml 40% phosphoric acid solution, 1.0 ml 1% ferric chloride solution and 2.5 ml 1% α-α-dipiridil solution. Place the solution in the dark for 30 minutes, then make up to volume with distilled water and shake it. Prepare also a blank sample that contains 2 ml distilled water instead of sample.

Preparation of calibration solutions: Ascorbic acid stock solution (20% m/m) should be used for the preparation of calibration solutions. Pipette into seven 100 ml volumetric flask 1, 2, 3, 4, 5, 7.5 and 10 ml of the ascorbic acid stock solution and make up to volume with distilled water. Add 0.6 ml 40% phosphoric acid solution, 1.0 ml 1% ferric chloride solution and 2.5 ml 1% α-α-dipiridil solution. Place the solution in the dark for 30 minutes then make up to volume with distilled water and shake it. These solutions contain respectively 0,2 mg; 0,4 mg; 0,6 mg; 0,8 mg; 1,0 mg; 1,5 mg and 2,0 mg ascorbic acid per litre.

Test procedure:

Measure the absorbance of the sample and calibration solutions against the blank solution at 496 nm in 1 cm cell. Absorbance values should be plotted versus concentrations. The X axis represents the concentrations and the Y axis represents the absorbance. Draw a regression line through the points and read the concentration of the sample versus absorbance.

Preparation of solutions:

- L-ascorbic acid stock solution: Weight 0.2 g L-ascorbic-acid into a 100 ml volumetric flask and add 0.5 ml acetic acid, then make up to volume with distilled water. Pipette 1 ml from this solution into a 100 ml volumetric flask and make up to volume with distilled water.
- 1% α-α-dipiridil solution: Dissolve 1 g α-α-dipiridil in 20 ml 95% ethyl alcohol. Quantitatively transfer it into a 100 ml volumetric flask and make up to volume with ethyl alcohol.
- 40% phosphoric acid solution: Weight 40.2 ml phosphoric acid (85%; ρ=1,17 g/cm^3) in a 100 ml volumetric flask containing 40 ml distilled water and make up to volume with distilled water.
- 1% ferric chloride solution: Dissolve 1 g Fe(III)-chloride in 40 ml distilled water, quantitatively transfer it into a 100 ml volumetric flask and make up to volume with distilled water.

Exercise 8: Determination of diastase activity

Purpose of the exercise: Obtaining laboratory practice
Required knowledge: Knowledge of lecture materials

Exercise: Determine the diastase activity of chosen honey sample based on the following method

The unit of diastase activity (DA) (the Gothe unit) is defined as that amount of enzyme which will convert 0.01 g of starch to the prescribed end-point in one hour at 40°C under the condition of test. Results are expressed in diastase number per gram of honey.

Sample preparation: Weight 10 g homogenized honey into a 100 ml baker, add 5.0 ml acetate buffer and 20 ml distilled water and solve it. Quantitatively transfer it into a 50 ml volumetric flask containing 3.0 ml sodium chloride solution and make up to volume with distilled water.

Preparation of starch solution: Weight into a 150 ml baker the amount of starch which is equivalent to 2.0 g anhydrous starch (or use anhydrous starch). Add 90 ml distilled water and mix it. Boil this solution for 3 minutes and quantitatively transfer the hot starch solution in a 100 ml volumetric flask. Cool it down rapidly to room temperature in running cool water, then make up to volume with distilled water. Keep the starch solution in 40°C water bath.

Calibration of the starch solution: This procedure is carried out to determine the amount of water that has to be added to the reaction mixture, so that the absorbance range of the iodine starch solution is between 0.730 and 0.770. Pipette 5.0 ml starch solution into a test tube containing 10 ml distilled water. Mix it then pipette 1 ml from this solution into 10 ml 0.0035 M iodine solution and add 35 ml distilled water. Measure the absorbance of this solution at 660 nm against a water blank in a 1 cm cell. If the value is lower or higher than 0.75±0.02, the amount of added distilled water should be changed until the value is between 0.730 and 0.770. This amount of distilled water should be used for the determination of diastase activity of honey.

Test procedure:

Pipette 10 ml honey solution into a 50 ml flask and place it in a 40°C water bath. When the temperature of honey solution reaches 40°C, pipette 5 ml starch solution into the honey solution and start the timer. In every five minutes, pipette 1 ml of this solution into 35 ml 0.0035 M iodine solution and add the amount distilled water, mix and read the absorbance of this solution at 660 nm against a water blank in a 1 cm cell. This procedure should be repeated until the absorbance of the sample gets lower than 0.235. Absorbance values should be plotted versus time. The X axis represents the time and the Y axis represents the absorbance. Draw a regression line through the points (at least three points are necessary) and read the time (t) which belongs to the absorbance value of 0.235.

Calculate diastase activity with the following formula:

$$Diastase\ activity\ (DN) = \frac{300}{t}$$

Preparation of solutions:

- Iodine stock solution: Dissolve 4.4 g twice sublimated iodine and 11 g crystallized potassium iodide in 100 ml distilled water. Quantitatively transfer it into a 500 ml volumetric flask and make up to volume with distilled water.
- Iodine (diluted) solution: Dissolve 20 g potassium iodide in 40 ml distilled water and add 2 ml iodine stock solution. Quantitatively transfer it into a 500 ml volumetric flask and make up to volume with distilled water. Prepare fresh every day.
- Acetate buffer (pH 5.3): Dissolve 43.5 g crystallized sodium acetate in 40 ml distilled water and adjust the pH of this solution to 5.3 with glacial acetic. Quantitatively transfer it into a 100 ml volumetric flask and make up to volume with distilled water.
- 0.5 M sodium chloride solution: Dissolve 2.922 g NaCl in 20 ml distilled water and quantitatively transfer it into a 100 ml volumetric flask and make up to volume with distilled water.

Exercise 9: Determination of total phenolic and flavonoid content

Purpose of the exercise: Obtaining laboratory practice
Required knowledge: Knowledge of lecture materials

Exercise: Determine the TPC and flavonoid content of chosen fruit based on the following methods

Determination of total phenolic content (TPC)

Principle of the method: Folin-Ciocalteu reagent contains phosphor tungsten acid and phosphor molybdenum acid which oxidise the phenolic compounds of the sample and the solution turns into blue. Colour intensity is equivalent to the phenolic content of the sample.

Sample preparation: Dissolve 5 g homogenized sample in 50 ml MeOH:DW (80:20), then filter it through filter paper.

Preparation of calibration solutions: Prepare a gallic acid stock solution (100 mg/l). Prepare six 10 ml test tubes and make calibration solutions according to the followings:

- 0 mg/l: Pipette 10 ml MeOH:DV in the first test tube
- 5 mg/l: Pipette 0.5 ml stock solution in the second test tube and make up to volume with MeOH:DV
- 10 mg/l: Pipette 1.0 ml stock solution in the third test tube and make up to volume with MeOH:DV
- 20 mg/l: Pipette 2.0 ml stock solution in the fourth test tube and make up to volume with MeOH:DV
- 50 mg/l: Pipette 5.0 ml stock solution in the fifth test tube and make up to volume with MeOH:DV
- 100 mg/l: Pipette 10 ml stock solution in the last test tube.

Test procedure:

Pipette into seven 10 ml test tube 0.5 ml sample solution and calibration solutions and add 2.5 ml 0.2 N Folin-Ciocalteu reagent solution into each of tube and wait five minutes. Pipette 2.0 ml sodium carbonate solution (75g/l) into each tube and shake them. Leave them in the dark for 2 hours.

After 2 hours, measure the absorbance of solutions at 760 nm against a blank solution (MeOH:DV) in a 1 cm cell. Absorbance values should be plotted versus concentration. The X axis represents the concentrations and the Y axis represents the absorbance. Draw a regression line through the points and read the concentration of the sample versus absorbance. Results are expressed in mg GAE/100 g (mg gallic acid equivalent per 100 g).

Preparation of solutions:

- 0.2 N Folin-Ciocalteu reagent solution: Pipette 10 ml Folin-Ciocalteu reagnt into a 100 ml volumetric flask and make up to volume with distilled water
- 75 g/l sodium carbonate: Dissolve 7.5 g anhydrous sodium carbonate in 50 ml distilled water, quantitatively transfer it into a 100 ml volumetric flask and make up to volume with distilled water
- 100 mg/l gallic acid stock solution: Dissolve 10 mg gallic acid in 50 ml MeOH:DV (80:20), quantitatively transfer it into a 100 ml volumetric flask and make up to volume with distilled water

Determination of flavonoid content

Sample preparation: see in “Preparation of sample” for TPC. The preparation of a blank solution is necessary, which contains MeOH:DV (80:20) instead of the sample.

Preparation of calibration solutions: Prepare a catechin stock solution (200 mg/100 ml). Pipette 10 ml stock solution into a 100 ml volumetric flask and make up to volume with MeOH:DV. Prepare five 10 ml test tubes and make calibration solutions according to the followings:

- 20 mg/l: Pipette 1 ml catechin stock solution in the first test tube and make up to volume with MeOH:DV
- 40 mg/l: Pipette 2 ml catechin stock solution in the second test tube and make up to volume with MeOH:DV
- 60 mg/l: Pipette 3 ml catechin stock solution in the third test tube and make up to volume with MeOH:DV
- 80 mg/l: Pipette 4 ml catechin stock solution in the fourth test tube and make up to volume with MeOH:DV
- 100 mg/l: Pipette 5 ml catechin stock solution in the five test tube and make up to volume with MeOH:DV

Test procedure:

Pipette 4 ml distilled water in seven 10 ml test tubes. Pipette 1.0 ml sample solution, blank solution and calibration solutions in the test tubes and add 0.3 ml 5% sodium nitrite in each test tube. Wait 5 minutes, then pipette 0.3 ml 10% aluminium chloride in each test tube. Wait 1 minute, then pipette 2 ml 1 M sodium hydroxide in each test tube and make up to volume with distilled water.

Measure the absorbance of solutions at 510 nm against the blank solution in a 1 cm cell. Absorbance values should be plotted versus concentration. The X axis represents the concentrations and the Y axis represents the absorbance. Draw a regression line through the points and read the concentration of sample versus absorbance. Results are expressed in mg CE/100 g (mg catechin equivalent per 100 g).

Preparation of solutions:

- 5% sodium nitrite: Dissolve 5 g anhydrous sodium nitrite in 20 ml distilled water, quantitatively transfer it into a 100 ml volumetric flask and make up to volume with distilled water
- 10% aluminium chloride: Dissolve 10 g aluminium chloride in 20 ml distilled water, quantitatively transfer it into a 100 ml volumetric flask and make up to volume with distilled water

- 1 M sodium hydroxide: Dissolve 4 g sodium hydroxide in 50 ml distilled water, quantitatively transfer it into a 100 ml volumetric flask and make up to volume with distilled water
- catechin stock solution: Dissolve 0.1 g catechin in 20 ml MeOH:DV and quantitatively transfer it into a 50 ml volumetric flask and make up to volume with MeOH:DV

Exercise 10: Determination of proline content

Purpose of the exercise:

Required knowledge: Knowledge of lecture materials

Exercise: Determine the proline content of chosen honey sample based on the following method

Principle of the method: Proline and ninhydrin form a coloured complex. After adding 2-propanol, the extinction of the sample solution and a reference solution at a wavelength maximum is determined. Proline content is determined from the ratio.

Sample preparation: Dissolve 5 g homogenized honey in 50 ml distilled water, quantitatively transfer it into a 100 ml volumetric flask and make up to volume with distilled water.

Test procedure:

Pipette 0.5 ml honey solution into the first tube, 0.5 ml distilled water into the second tube and 0.5 ml proline standard solution into the third tube. Pipette 1.0 ml concentrated formic acid and 1.0 ml ninhydrin solution into each test tube. Cap the tubes and shake them for 15 minutes. Place the tubes in boiling water bath for 15 minutes, then transfer them into a water bath on 70°C for 10 minutes. Add 5.0 ml 2-porpanol-water solution in each tube and cap immediately. Leave to cool and determine the absorbance at 510 nm (45 minutes after removing from the 70°C water bath) using a 1 cm cell.

Calculate proline content with the following formula:

$$\text{Proline (mg/kg)} = \left(\frac{E_s}{E_a}\right) \times \left(\frac{E_1}{E_2}\right) \times 80$$

where:

E_s: absorbance of the sample solution

E_a: absorbance of the proline standard solution

E_1: mg proline taken for the standard solution

E_2: weight of the honey (g)

80: dilution factor

Preparation of solutions:

- 3% ninhydrin solution: Dissolve 3.0 g anhydrous ninhydrin in 20 ml ethylene glycol monomethyl ether, quantitatively transfer it into a 100 ml volumetric flask and make up to volume with ethylene glycol monomethyl ether
- 0.32 mg/ml L-proline stock solution: Dissolve 32 mg L-proline in 20 ml distilled water, transfer it quantitatively into a 100 ml volumetric flask and make up to volume with distilled water
- 0.032 mg/ml L-proline solution: Pipette 10 ml L-proline stock solution into a 100 ml volumetric flask and make up to volume with distilled water.

Exercise 11: Determination of acid content, acidity and pH

Purpose of the exercise: Obtaining laboratory practice
Required knowledge: Knowledge of lecture materials

Exercise: Determine the acid content of a chosen fruits based on the following method

Sample preparation: Weight 20 g homogenized sample into a 250 ml Erlenmeyer flask. Add 150 ml distilled water and mix it, then boil it in 85-95°C water bath for 30 minutes. Leave the sample to cool.

Test procedure:
Filter the solution through cotton wool into a 250 ml volumetric flask and make up to volume with distilled water. Transfer 25 ml solution into a 100 ml volumetric flask and make up to volume with distilled water. Transfer the whole solution into a 250 ml titration flask, add few drops of indicator and titrate it with 0.1 M sodium hydroxide until the pink colour appears.

Calculate total acid content with the following formula:

$$\text{total acid content (\%)} = \frac{V \times f_{0,1\ M\ NaOH} \times h \times T}{n} \times 100$$

where:

V: ml of 0,1 M NaOH solution used for the titration
$f_{0,1\ M\ NaOH}$: factor of 0,1 M NaOH solution
h: dilution
T: rate (in case of cherry is 0.0064 g anhydrous citric acid; in case of apple is 0.0067 g anhydrous malic acid
n: weight of sample (g)

Preparation of solutions:

- 0.1 M sodium hydroxide solution: Dissolve 0.4 g sodium solution in 20 ml distilled water, quantitatively transfer into a 100 ml volumetric flask and make up to volume with distilled water
- standardisation of 0.1 M sodium hydroxide solution: For this method, 0.1 M HCl solution with known concentration is necessary. Pour 300 ml distilled water into a 1000 ml volumetric flask, add 8.4 ml concentrated HCl, then make up to volume with distilled water. Exact concentration is expressed in sodium hydrogen carbonate. Reaction equation: $HCO^{-3} + H^{+} = H_2CO_3 \rightarrow H_2O + CO_2$.

  Dissolve 0.1 g $KHCO_3$ in 50 ml distilled water, add few drops of methyl red indicator and titrate it until the onion skin colour appears. Boil the solution (remove carbon dioxide), then cool it and continue the titration until the onion skin colour appears again. The amount of 0.1 M HCl solution used for the titration means the technical volume. Calculate theoretic volume with the following formula:

$$theoretic\ volume = \frac{1000\ cm^3 \times m_{KHCO_3}}{M_{KHCO_3} \times 0{,}1\ mol}$$

  As the molar mass of $KHCO_3$ is 100 g and the concentration of HCl is exactly 0.1 mol/L, therefore 10 ml HCl is consumed for 0.1 g $KHCO_3$.

  Calculate the factor with the following formula:

$$factor_{HCl} = \frac{theoretic\ volume}{technical\ volume}$$

  So, the exact concentration of 0.1 M HCl is known, and with this result, the factor of 0.1 M NaOH may be calculated. Reaction equation: $NaOH + HCl = NaCl + H_2O$

  Pour 20 ml 0.1 M NaOH solution into a titration flask and add 2-3 drops of methyl red indicator. Titrate it with 0.1M HCl solution until the pink colour disappears.

  Calculate the factor of 0.1 M NaOH with the following formula:

$$f_{NaOH} = \frac{volume\ of\ HCl\ (ml) \times factor_{HCl}}{weight_{NaOH}(ml)}$$

Exercise: Determine the acidity of a honey sample based on the following method

Principle of the method: The sample is dissolved in water, pH is measured and the solution is titrated with 0.1 M sodium hydroxide solution until pH 8.30.

Calibration of pH meter: The pH meter should be calibrated before the measurement at pH 4 and pH 9.

Sample preparation: Dissolve 10 g honey in 75 ml CO_2-free distilled water in a 250 ml baker.

Test procedure:

Place the beaker containing the honey sample on magnetic stirrer, immerse electrode of pH meter in the solution, stir and record the pH. Titrate with 0.05 N sodium hydroxide at a rate of 5 ml/min to pH 8.50. Record the volume of sodium hydroxide used for the titration. Pipette 10 ml 0.05 N sodium hydroxide in the honey solution immediately, and without delay, back-titrate it with 0.05 N hydrochloric acid solution to pH 8.30. Record the volume of hydrochloric acid used for the titration.

Titration process should be carried out in two minutes!

Calculate free acidity, lactone and total acidity with the followings:

$$Free\ acidity = \frac{ml\ 0.05N\ NaOH\ from\ buret\ \times 50}{weight\ of\ sample}$$

$$Lactone = \frac{(10 - ml\ 0.05N\ HCl\ from\ buret) \times 50}{weight\ of\ sample}$$

$$Total\ acidity = free\ acidity + lactone$$

Results are expressed in milliequivalent/kg (meq/kg).

Exercise: Determine the pH of honey sample based on the following methods

Calibration of pH meter: The pH meter should be calibrated before the measurement at pH 2 and pH 7 because the pH value of honeys ranged between 2 and 5.

Sample preparation: Dissolve 10 g homogenized honey samples in 25 ml distilled water.

Test procedure: Place the beaker containing the sample on magnetic stirrer. Immerse the electrode in the solution, stir and read the pH value of the sample from the pH meter. The used digital pH meter measures the pH value of solution immediately, therefore correction is not necessary.

Exercise 12: Determination of physicochemical parameters of alcoholic beverages

Purpose of the exercise: Obtaining laboratory practice
Required knowledge: Knowledge of lecture materials

Exercise: Determine the colour intensity of a beer sample based on the following method:

Sample preparation: Measure 250 ml beer into a 500 ml beaker and place it in ultrasonic bath (degassing operation), then filter it through a filter paper.

Test procedure:
Fill the degassed beer into a 1 cm cuvette and measure its absorbance at 430 nm against blank (distilled water).
Calculate of colour intensity with the following formula:

$$Colour\ intensity = 10 \times A_{430nm}$$

Exercise: Determine the total acid content of a beer sample based on the following method:

Test procedure:
Fill 250 ml distilled water into a 500 ml flask and boil it. After two minutes of boiling, add 25 ml degassed beer and boil it for a further 30 seconds while stirring. Cool it under cool water and titrate it with 0.1 M sodium hydroxide solution in the presence of phenolphthalein indicator until the pink colour appears. Result is expressed in % of lactic acid. 1 ml 0.1 M sodium hydroxide solution is equivalent to 0.009 g lactic acid.
Calculate total acid content with the following formula:

$$Total\ acid\ content\ (lactic\ acid\ \%) = V \times 0.009$$

where:

V: 0,1 M NaOH solution from burette (ml)

Exercise: Determine the acidity of a beer sample based on the following method:

Acidity of beer is the quantity of NaOH that is required for the neutralization of 100 ml beer.

Test procedure:

Fill 50 ml degassed beer into a 100 ml beaker and place it on a magnetic stirrer. Immerse the electrode in the solution and titrate it with 0.1M sodium hydroxide solution to pH 9.

Calculate the acidity of beer with the following formula:

$$Acidity = V \times f \times 0.2$$

where:

V: 0,1 M sodium hydroxide solution from burette (ml)

f: factor of 0.1 M NaOH

Exercise: Determine the persisting foaming of a beer sample based on the following method:

Test procedure:

Fill 150 ml beer into a 1000 ml measuring cylinder (without foaming), then add 12 g silica sand from 30 cm height through glass funnel. This creates imbalance in the colloid system of beer and foam will be formed. Quantity of formed foam reaches its maximum at the end of the first minute, then it will gradually reduce. Read the quantity of foam at the end of first and tenth minute. The persistent foaming of beer depends on temperature, therefore measure the temperature of beer at the end of the tenth minute.

Calculate persistent foaming with the following formula:

$$Persistent\ foaming\ (H_{10}) = \frac{a^2 \times 20.5}{b \times (t + 10.5)}$$

where:

H_{10}: persistent foaming (ml) calculated on 10°C

a: quantity of foam (ml) at the end of first minute

b: difference of quantity of foam read at the end of the first and tenth minute

t: temperature of beer (°C)

Exercise 13: Sensory analysis

Purpose of the exercise: Test the students' sense of taste and colour vision

Required knowledge: None

On this training, previously prepared samples are used.

Sense of taste: Tastes are the following: sweet, salty, bitterness and sour.

- Sweet taste: Samples contain sucrose. The concentrations of the samples are: 12.0, 7.20, 4.32, 2.59, 1.56, 0.94 and 0.55 g/L
- Salty taste: Samples contain salt. The concentrations of the samples are: 2.00, 1.40, 0.98, 0.69, 0.48, 0.34 and 0.02 g/L
- Bitter taste: Samples contain caffeine. The concentrations of the samples are: 0.27, 0.22, 0.17, 0.14, 0.11, 0.09 and 0.07 g/L
- Sour taste: Samples contain citric acid. The concentrations of the samples are: 0.60, 0.48, 0.38, 0.31, 0.25, 0.20 and 0.16

Requirements:

- Taste the samples slowly
- Taste enough quantity (about 15 ml)
- Rinse the mouth with distilled water (after each sample)

Colour vision: Colours are the following:

- Red: Samples contain Azornbin colouring
- Green: Samples contain Lichtgrün colouring
- Yellow: Samples contain Chrysoin colouring

Concentration of test solutions are: 0.004, 0.0052, 0.0066, 0.0086, 0.0110, 0.0140, 0.018, 0.023, 0.0298 and 0.0384 g/100 ml.

Requirements:

- Separate the samples according their colours
- Align the same colour samples according to their colour density
- Use white paper

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