University
of Gdańsk

FOOD CHEMISTRY AND ANALYSIS

LABORATORY INSTRUCTIONS
(EXPERIMENTAL PART)

Gdańsk, 2024

Exercise 1

Water content in wheat flour

Topics to study:

— *physicochemical properties of water in various states of aggregation*

— *water as a solvent (bonds and chemical interactions)*

— *types of water found in food (free water, bound water)*

— *water and dry substance content in food products*

— *methods of water content determination in food*

AIM

The purpose of this exercise is to get familiar with the methodology for the determination of water and dry matter (mass) in a selected food product (flour).

METHOD

The method involves evaporating water from a product sample during the drying process and determining the residue (dry substance) by weight.

INSTRUCTIONS

Weigh a weighing vessel on a technical balance with an accuracy of 0.01 g. Transfer approximately 10 g of the flour sample into the weighing cup, re-weigh (sample + vessel), and then place in the oven at 130 ºC for 1 hour. Cool the sample in a vacuum desiccator (1 hour), and then weigh again. Perform in three repetitions.

RESULTS

Calculate the percentage dry matter and water content of the flour tested. Compare the result obtained with the standards. The upper limit of moisture content for all types of flour is 15%.

Exercise 2

Elements of sensory analysis

Topics to study:

— *Classification of methods of laboratory sensory analysis (Differential methods, Sequence (serialization) method, Scaling methods, Quantitative Descriptive Analysis (QDA) method, Methods of determining changes in the intensity of sensory impressions over time (TimeIntensity - T-I).*

— *Bring a bottle of water for yourself*

AIM

The purpose of the exercise is to perform four tasks to test the sensory sensitivity of subjects, during which students will learn the basic principles of the three sensory tests.

METHOD

Students perform the exercises in pairs: the first person in the pair gives the other person (the test subject) samples to evaluate according to the principles given in the methods, and then after evaluation decodes the samples and checks the results (the codes and random presentation of the samples are known only to the person giving the samples for evaluation). Assessment form for the methods used in the exercises are provided at the end of this exercise.

INSTRUCTIONS

a) **TEST 1 -** Determining the ability to recognize three of the four basic tastes

Consists of assessing the ability to recognize and define three of the four basic tastes (sweet, salty, sour, and bitter) prepared from sucrose, sodium chloride, and citric acid, respectively, present in small, suprathreshold concentrations.

Prepare three coded disposable cups and pour approximately 20 mL each of the appropriate flavor type into the cups. The testee is given a set of three coded cups, given in random order, identifies the type of taste experienced, and the tester fills out a results sheet according to the information received from the teste. Water is used to neutralize the taste of the samples.

b) **TEST 2** - Evaluation of the sweet taste of aqueous solutions of sucrose and citric acid by the even-numbered method (binary method).

Compare samples in pairs (one or more pairs of samples) to determine the difference in sensory quality of a particular characteristic.

<u>Solutions:</u>

$\rightarrow$ Binary aqueous solution containing sucrose (1.0 g/100 mL) and citric acid (0.04 g/100 mL).

$\rightarrow$ Binary aqueous solution containing sucrose (0.6 g/100 mL) and citric acid (0.04 g/100 mL).

Prepare four coded disposable cups, pour approximately 20 mL each of the above solutions into the cups. The test subject is given a set of two pairs of coded cups, given in random order, indicates the sample with the more intense sweet taste in each pair, and the test person fills out an evaluation sheet. Water is used to neutralize the taste of the samples.

c) **TEST 3** - Evaluation of the intensity of salty taste of aqueous solutions of sodium chloride by the serialization method

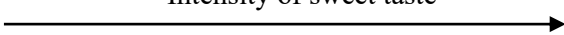It involves ordering several samples in terms of the intensity of a certain quality characteristic.

Prepare five coded disposable cups, pour into each about 20 mL of the corresponding sodium chloride solution with the following concentrations: 0.20 g/100 mL, 0.35 g/100 mL, 0.50 g/100 mL, 0.70 g/100 mL (NaCl solution of the same concentration pour into two coded cups). The test subject is given five coded cups, given in random order. The test person ranks the samples from most salty to least salty and fills in the evaluation sheet. Water is used to neutralize the taste of the samples.

d) **TEST 4** - Evaluation of the sweet taste intensity of the modified orange solution by scaling method

The principle of the method is to evaluate the intensity of the analyzed quality characteristic(s) on a structural scale.

Prepare five coded disposable cups, pour into them about 20 mL each of prepared beverage samples (without and with sucrose: commercial orange drink with sucrose to the concentration: 0.5 g/100 mL, 1.0 g/100 mL, 2.0 g/100 mL, 3.0 g/100 mL). The subject is given five coded containers, given in random order. The test person's task is to evaluate the sweetness intensity of the orange drink samples. The test person marks the perceived impressions in a table. Water is used to neutralize the taste of the samples.

SENSORY EVALUATION CARD

Person tested:

<table>
<tr><td colspan="3">Task 1: Determining the ability to recognize three of the four basic tastes</td></tr>
<tr><td>Sample code</td><td>Flavor</td><td>Identification [yes/no]</td></tr>
<tr><td></td><td></td><td></td></tr>
<tr><td></td><td></td><td></td></tr>
<tr><td></td><td></td><td></td></tr>
</table>

<table>
<tr><td colspan="4">Task 2: Evaluation of the sweet taste of aqueous solutions of sucrose and citric acid by the even-numbered method (binary method).</td></tr>
<tr><td>Pair number</td><td>Sample code</td><td>The intensity of the sweet flavor [weaker/more intense]</td><td>Identification [yes/no]</td></tr>
<tr><td rowspan="2">1</td><td></td><td></td><td></td></tr>
<tr><td></td><td></td><td></td></tr>
<tr><td rowspan="2">2</td><td></td><td></td><td></td></tr>
<tr><td></td><td></td><td></td></tr>
</table>

<table>
<tr><td colspan="6">Task 3: Evaluation of the intensity of salty taste of aqueous solutions of NaCl by the serialization method</td></tr>
<tr><td>Salt concentration</td><td>0,2 g/100 mL</td><td>0,35 g/100 mL</td><td>0,35 g/100 mL</td><td>0,5 g/100 mL</td><td>0,7 g/100 mL</td></tr>
<tr><td>Sample code</td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>Identification [yes/no]</td><td></td><td></td><td></td><td></td><td></td></tr>
</table>

<table>
<tr><td colspan="4">Task 4: Evaluation of the sweet taste intensity of the modified orange solution by scaling method</td></tr>
<tr><td>Sample type</td><td>Sample code</td><td>Intensity of sweet taste →</td><td>Identification [yes/no]</td></tr>
<tr><td>Pure juice</td><td></td><td></td><td></td></tr>
<tr><td>+ 0,5 g/100 mL</td><td></td><td></td><td></td></tr>
<tr><td>+ 1 g/100 mL</td><td></td><td></td><td></td></tr>
<tr><td>+ 2 g/100 mL</td><td></td><td></td><td></td></tr>
<tr><td>+ 3 g/100 mL</td><td></td><td></td><td></td></tr>
</table>

Gdańsk, 2024

Exercise 3

Determination of the content of "sugars in total" in hard candy using Bertrand method

Topic to study:

— *Reactions occurring on the carbonyl group and anomeric carbon atom, reactions of hydroxyl groups, glycosidic bond reactions;*

— *Sugar transformations in alkaline and acidic environment;*

— *Reducing properties of sugars;*

— *Sugars as sweeteners - properties, saccharides occurring in food, functions of saccharides in the human body*

— *Characteristics of methods for the determination of digestible saccharides (physical (densimetric, refractometric, polarimetric), chemical (Fehling method, Lane-Eynon method, Bertrand method, Luff-Schoorl method), physicochemical (anthrone method, resorcinol method, hexacyanoferrate method), chromatographic (thin-layer or paper chromatography, column chromatography, gas chromatography, high-performance liquid chromatography) and enzymatic methods).*

AIM

The purpose of this exercise is to determine the total sugar content of candies by the Bertrand method.

INSTRUCTIONS

Preparation of the stock solution

Crush candies (2-4 pieces) in a dry mortar, then weigh about 4 g of crushed candy on a technical balance to the nearest ± 0.01 g. Transfer to a beaker (250 mL) and dissolve in about 100 mL of distilled water. Neutralize with 0.1 M NaOH solution in the presence of phenolphthalein until slightly pink, transfer quantitatively to a volumetric flask (capacity 250 mL) and make up to the mark with distilled water.

Hydrolysis (inversion) of the test product

Prepare a water bath with water at 80ºC. Into 250 mL conical flask introduce 50 mL of candy stock solution, then pipette 5 mL of concentrated sulfuric acid (carefully), mix thoroughly. Place the flask in an 80°C water bath, bring the temperature of the solution in the flask to 68-

71°C within 2 to 3 minutes and maintain it for another 5 minutes. Cool the flask, then neutralize the solution with 10% NaOH solution in the presence of methyl orange. Quantitatively transfer the contents of the conical flask into a 250 mL volumetric flask and make up to the mark with distilled water. The determination should be performed very carefully, because the amount of sulfuric acid varies it can cause incomplete decomposition of polysaccharides and oligosaccharides (mainly sucrose) to glucose and fructose, while prolonging the reaction time or increasing the temperature, it can decompose the hydrolysis products!

Determination of total saccharides by the Bertrand method

For the determination of total saccharides by the Bertrand method, use 5 mL of invert solution and 15 mL of water. Introduce 20 mL of the above solution into the flask, then pipette 20 mL of Bertrand solution I and 20 mL of Bertrand solution II. Mix the contents of the flask thoroughly, then place the flask on a heating mantle. Bring the solution to a boil and maintain it at this temperature for 3 minutes, then allow it to cool. **Adjust the flask so that the precipitate of oxygen copper (I) is constantly covered by the solution!**

The supernatant liquid should have a clear blue color, indicating an excess of reduced copper salt. If, for example, the solution turns dull green when heated, this would indicate that the amount of saccharides in the sample exceeds the reducing capacity of the Bertrand I and II reagents; then the test solution must be diluted and the determination repeated.

Decant the liquid from above the precipitate into a Schott funnel so as not to expose the surface of the Cu2O precipitate, either in the flask or on the funnel. Then quantitatively transfer the precipitate from the flask with hot water to the Schott funnel, followed by rinsing the sides of the flask with hot water and transferring the solution to the funnel (repeat the procedure several times). Rinse the sediment on the funnel with hot water until the blue coloration of the filtrate disappears. Filtration and rinsing should be done as soon as possible to prevent oxidation of some of the precipitate.

Place a Schott funnel with the copper (I) oxide precipitate in a clean flask. Dissolve the precipitate in 20 mL of Bertrand III solution and filter into the flask. Make sure that all the precipitate on the funnel is completely dissolved. Usually several (2-3) smaller aliquots of Bertrand III solution are used. If the precipitate does not dissolve completely, add a small amount of Bertrand III solution. Finally, rinse the filter several times with hot water until the acidic pH disappears.

Titrate the filtrate in the flask with 0.005 M potassium manganate (VII) solution until a light pink coloration appears for 30 seconds. The color change from yellow-greenish to pink occurs very clearly from one drop of excess potassium manganate solution.

RESULTS

Calculate the copper titer ($T_{KMnO4/Cu}$) using the formula below:

$$T_{KMnO_4/Cu} = 5 \cdot c_m \cdot M_{Cu} \qquad [mg\ Cu/ml\ KMnO_4]$$

Where:

c_m – concentration of $KMnO_4$ [mol/L]

M_{Cu} – molar mass od copper [g/mol]

Using the determined copper titer, calculate the number of mg of copper corresponding to the volume of $KMnO_4$ used for the titration and then convert it (knowing that 20 mg of reduced copper corresponds to 9.7 mg of invert sugar) to the equivalent amount of mg of invert saccharide contained in 20 mL of the sample taken for the determination. Calculate the percentage sugar content of the candies, taking into account the dilutions used, rounded to the first decimal place.

Exercise 4

Determination of vitamin C content in sauerkraut juice and lemon juice titration method

Topic to study:

— *Water and fat soluble vitamins (physicochemical properties, role in organism, food sources)*

— *Determination of vitamin C (Tillmans method, liquid chromatography, fluorimetric method, spectrophotometric method)*

AIM

The aim of the exercise is practical acquaintance with the methodology for determining the vitamin C content (direct reductivity) in a selected food product (sauerkraut juice, lemon juice).

METHOD

The assay involves the reduction of a colored solution of 2,6-dichlorophenolindophenol (DCPIP) to a colorless leucoside by an acidic solution of ascorbic acid (vitamin C).

INSTRUCTION

Determination of the titer of 2,6-dichlorophenolindophenol (DCPIP) by titration with sodium thiosulfate solution

In a 50 mL conical flask with a ground stopper, dissolve 100 mg of potassium iodide in 5 mL of 1 mol/L sulfuric acid (VI), quickly add 10 mL of a solution of 2,6-dichlorophenolindophenol, seal the flask and leave for 10 min in a dark place. Titrate the separated iodine with sodium thiosulfate solution (0.001 mol/L), adding 1 mL of starch solution. Repeat the titration 3 times.

Determination of vitamin C in sauerkraut juice and lemon juice

Place 20 mL of cabbage juice and 20 mL of lemon juice in centrifuge tubes and centrifuge (8000 rpm, 10 min). Dilute 10 mL of the centrifuged lemon juice with 2% HCl in a 50 mL volumetric flask. Take 10 mL of the diluted lemon juice into three 25 mL conical flasks and titrate with Tillmans dye until slightly pink color persists for 10 s. Carry out the same procedure for the centrifuged sample. Perform a blank test with distilled water instead of the juice.

RESULTS

Calculate the dye titer according to the formula below:

$$C_M = \frac{(V_W - V_Z)C_T}{2V_b}$$

Where:

V_W - volume of sodium thiosulfate used to titrate the dye solution [mL],

V_Z - volume of sodium thiosulfate used for titration in the blank test [mL],

C_T - concentration of sodium thiosulfate solution [mol/L],

V_b - volume of dye solution [mL].

According to Figure 1, it follows that 1 mole of DCPIP dye reacts with 1 mole of ascorbic acid (molar mass is 176 g/mol). Based on the amount of dye used to titrate diluted juice, calculate the vitamin C content in the pure juice [mg/100 mL].
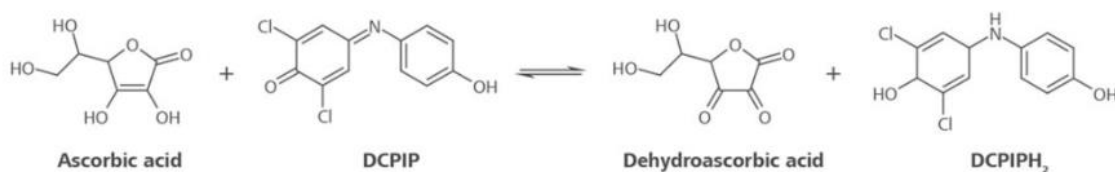

Figure 1. Reaction of ascorbic acid and 2,6-dichlorophenolindophenol (DCPIP).

Gdańsk, 2024

Exercise 5

Determination of the protein content in milk using the spectrophotometric method

Topic to study:

— *Structure and properties of proteins*

— *Simple and complex proteins*

— *Classification of proteins due to biological functions*

— *Proteins in food products (mainly in milk)*

— *Methods for the determination of protein content (Kjeldahl's, dye incorporation, formol titration, spectrophotometric methods (especially the biuret method), immunological methods)*

— *UV-Vis spectrophotometry (absorption laws, possibilities and limitations of spectrophotometric quantitative analysis)*

— *Calibration curve method*

AIM

The purpose of this exercise is to determine the protein content of milk by spectrophotometric method.

METHOD

The Biuret method uses a reaction occurring in an alkaline environment between peptide bonds and copper ions (Cu^{2+}), resulting in the formation of colored complexes.

INSTRUCTIONS

Preparation of the standard curve

a) Prepare working solutions with a volume of 1 mL at ten concentration levels: 1 mg/mL, 2 mg/mL, 3 mg/mL, 4 mg/mL, 5 mg/mL, 6 mg/mL, 7 mg/mL, 8 mg/mL, 9 mg/mL and 10 mg/mL using 10 mg/mL casein stock solution;

b) Add 4 mL of copper reagent to each sample, mix and set aside for 30 minutes in room temperature;

c) Measure the absorbance at wavelength of $\lambda = 540$ nm against the blank sample (pure copper reagent).

Analysis of the milk sample (should be performed simultaneously with other samples)

    a) Dilute the tested milk 10 times;

    b) Add 4 mL of copper reagent to 1 mL of diluted milk, mix and set aside for 30 minutes at room temperature;

    c) Measure the absorbance at a wavelength of $\lambda = 540$ nm against the blank sample (pure copper reagent); if the sample is not clear, it should be centrifuged (10 minutes at 4,000 rpm) or filtered (using syringe filters) before analysis.

RESULTS

Determine the mean values of the three measured absorbance values of the standard solutions. Plot the relationship A = f (c) in Excel, find the equation of the calibration curve and coefficient of determination (R^2). Based on the equation of the calibration curve, determine the concentration of protein in milk [g/100 mL]. Give the average casein content of the test sample and report it along with the standard deviation.

Gdańsk, 2024

<div align="center">

Exercise 6

Determination of the caffeine content in tea

</div>

Topic to study:

— *structure, physicochemical properties, occurrence and biological activity of alkaloids;*

— *structure, physicochemical properties, biosynthesis, occurrence and effect of caffeine on the organism;*

— *calibration curve method;*

— *SPE technique – definition, stages*

— *HPLC-UV/Vis technique – structure, principle of operation, basic concepts and laws (e.g. gradient and isocratic elution, quantitative and qualitative analysis, retention parameters, Lambert-Beer law, etc.)*

AIM

The exercise involves the extraction and determination of caffeine from a tea sample by HPLC with a UV detector.

INSTRUCTIONS

Preparation of working solutions:

→ *Caffeine* standard solution obtained from the class instructor

→ *Washing solution* – a mixture for washing impurities from the SPE column, prepared in the volume ratio: NH_3 : H_2O : MeOH (2 : 8 : 1, v/v/v).

→ *Eluting solution* – mixture for eluting caffeine from the SPE column, prepared in the volume ratio: MeOH : H_2O : CH_3COOH (15 : 5 : 0.2, v/v/v).

→ *Mixture for preparing standard solutions* – prepared in the ratio by volume: MeOH : H_2O (1 : 4, v/v)

Preparation of calibration curve:

From a 1 mg/mL caffeine stock solution, prepare working solutions for the calibration curve at six concentration levels: 0.001 mg/mL, 0.0025 mg/mL, 0.005 mg/mL, 0.01 mg/mL, 0.025 mg/mL and 0.05 mg/mL

<div align="center">

Gdańsk, 2024

</div>

Extraction of caffeine from tea leaves:

→ weigh 0.1 g of tea and 0.4 g of MgO;

→ place the tea and MgO sample in a 250 mL flask, add 100 mL of boiling deionized water and wait 10 minutes;

→ leave the extract until the sediment settles;

→ filter the extract through a paper filter.

Purification of caffeine extract using SPE (Solid Phase Extraction)

Column conditioning

→ place a 100 mL beaker under the column

→ wash the column with 5 mL of MeOH (**from this point do not allow the bed to dry!**)

→ wash the column with 5 mL H_2O

Sample application

→ apply 2 mL of filtered caffeine extract to the bed

Removal of impurities

→ wash the column with 2.5 mL of washing solution

→ repeat the operation using 2.5 mL of washing solution again

→ blow the column with air

Caffeine elution

→ place a volumetric flask (10 mL) under the column

→ apply 7.5 mL of the elution mixture to the column (in portions)

→ wait until the solvent completely drains from the column

→ fill the flask to the mark with the methanol:water mixture

Regeneration of the bed

→ place a 100 mL beaker under the column

→ wash the column with 5 mL of MeOH

→ wash the column with 5 mL H_2O

Gdańsk, 2024

HPLC analysis

Perform HPLC analysis of the obtained samples and standard solutions.

HPLC operating conditions:

→ HPLC column C18,

→ mobile phase flow 1 mL / min,

→ wavelength λ = 272 nm,

→ mobile phase methanol / water 30:70 (v/v).

RESULTS

Based on the results obtained during the analysis of standard solutions, plot a graph of the relationship of the detector's response and the concentration of caffeine in the solution (determine the coefficient of determination (R^2) and the equation of the line). Calculate the caffeine content in tea, express the result in mg of caffeine per 100 g of product (mg/100 g).

Exercise 7

Analysis of fatty acids in lard by gas chromatography

Topic to study:

— *definition and classification of lipids (simple, complex, secondary)*

— *structure and chemical properties of acylglycerols*

— *structure, nomenclature, physicochemical properties, methods analysis of fatty acids and related concepts (derivatization, transesterification, MUFA, PUFA, EFA, FAME, TG, etc.)*

— *internal normalization method*

— *GC-FID technique – structure, principle of operation, basic concepts and laws*

AIM

The aim of the exercise is to determine the content of fatty acids in lard using the gas chromatography technique with a flame ionization detector (GC-FID).

METHOD

This exercise provide the convert the triacylglycerols present in the lard sample to fatty acid methyl esters by the interesterification method (according to European standard EN ISO 5509) and then to perform a quantitative analysis of these esters by gas chromatography with a GC-FID flame ionization detector using the internal standardization method.

INSTRUCTIONS

Put 1 mL of lard solution into a graduated test tube (volume 10 mL), add 3 mL of isooctane and shake. Then, add 400 µL of methanolic potassium hydroxide solution (CAUTION!). Shake everything vigorously for about 30 seconds. After the initial turbidity, glycerol will be released and the reaction mixture will become clear. Then, to neutralize the potassium hydroxide, add approximately 1 g of sodium acid sulfate and shake vigorously. After the salt has settled, collect the upper layer containing methyl esters with a Pasteur pipet and transfer it to a clean 1.5 mL vial. The sample prepared in this way should be analyzed using the GC-FID technique. Also analyze a mixture of standard fatty acid methyl esters.

Analysis conditions with GC-FID:

Apparatus: Shimadzu GC-2025 gas chromatograph with flame ionization detector (Shimadzu, Kyoto, Japan)

Column: RTX-5 capillary column, 30 m, 0.25 mm internal diameter, 0.25 μm liquid phase film thickness (Phenomenex, Torrance, CA, USA).

Injector temperature: 300 °C

Detector temperature: 300 °C

Temperature program: from 120 °C to 220 °C, with an increase of 3 °C/min

Carrier gas: argon

Carrier gas flow: 1.5 mL/min

Injection volume: 1 μL

RESULTS

Based on the chromatogram of standard fatty acid methyl esters, determine the retention times of individual analytes. Using the internal normalization method, determine the percentage of each fatty acid in the tested lard.