Laboratory analysis of selected plant foodstuffs commodities with theoretical backgrounds

Table of Contents

Introduction

Laboratory analysis of plant foodstuffs commodities represents a complex issue from the point of view of sample preparation, interactions between compounds in complex food matrix, the correct detection and calculation, and the choose of correct techniques both for the sample preparation and the analysis of the sample.

The following issues were emphasized in the written texts:

- The main techniques used in the chemical laboratory
  The students have to be introduced with the laboratory, to learn the main laboratory glasses used in the chemical laboratory, to set up titration equipment and to perform titration technique. The calculation needed for the quantification of analyzed compounds is also the part of the instructions where calibration curve calculation is explained and given as an example.
- Chromatographic techniques
  The main chromatographic techniques are described, including: thin layer chromatography, high pressure liquid chromatography and gas chromatography. The principals of different chromatographic techniques are described (stationary and mobile phases, the main detectors used in liquid and gas chromatography are described too. The main terms used in chromatography are explained too. Practical examples of thin layer chromatography (the determination of synthetic dyes) and high pressure liquid chromatography (the determination of malondialdehyde) are given too.
- Spectrophotometric analysis

The main principals of spectrophotometric analysis are described, same as the main types of spectrophotometer that can be used in the analysis of food commodities. Practically is given the experimentally procedure for the spectrophotometric determination of L-ascorbic acid.

- Viscosity, polarimetry and textural properties of food commodities
  The part is focused on the explanation of the viscosity and textural properties of food commodities importance in the evaluation of food quality or monitoring the quality of food commodities during ripening or storage periods under different conditions. The part is containing also experimental examples of textural determination of edible/biodegradable packaging.
- Salt and sugar determination in food commodities
  The part is supported with the theoretical (salt content in food commodities and sugar content determination issues) and practical parts explaining some main possibilities for the determination of sugar and salt in different food commodities.

The introduction of the laboratory equipment

The aim of the first chapter is to meet students with an ordinary work in the laboratory by assembling the titration apparatus, practicing titration, calculating simple equations, making calibration curves, and understanding the quantification possibility of compound detection by the calibration curve. The students are introduced with the laboratory equipment safety precautions and the correct laboratory practice.

The practical work:

1. The assemble the titration apparatus. The following steps should be performed:
   - Fill the burette with 0.1 M NaOH solution.
   - Pipette 10 ml of the prepared HCl solution into a titration flask and add 3 drops of phenolphthalein.

   Titrate to the equivalence point
   - Calculate the concentration of the HCl solution.
   - Conduct the NaOH standardization (Why this is done?
     Solid NaOH is highly hygroscopic (it absorbs easily water from the environment), meaing that NaOH cannot be accurately weighed (the procedure is called standardization of the NaOH solution). When the standardization is done the NaOH solution serves as a standard for further work.
   - The formula of the reaction is the following:
     $HCl + NaOH \rightarrow NaCl + H_2O$

The calculation should be done according to the following formula:

$$(n_A = \frac{a}{b} \cdot nB)$$

Where:

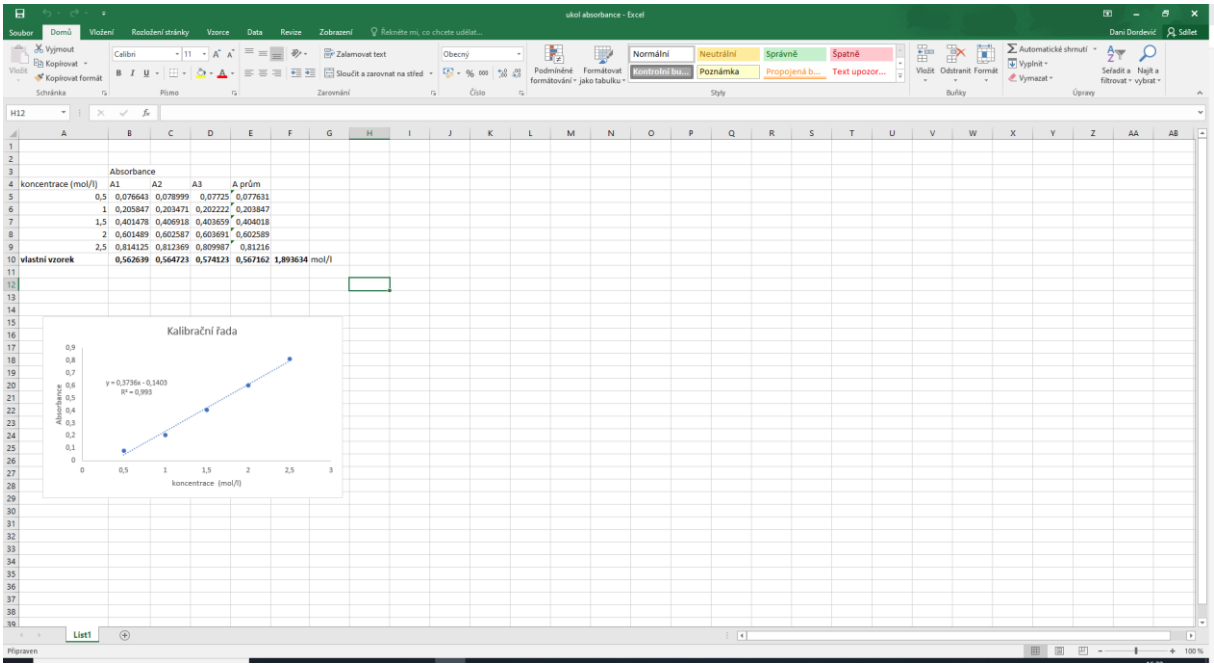
n_A = substance quantities of the compound A
a = stoichiometric coefficient
b = stoichiometric coefficient
n_B = substance quantities of the compound B

2. Calibration curve calculation

Theoretical problem. From the absorbance values of the standard, construct a calibration curve and calculate the concentration of the sample itself according to the regression equation.

Students have to work with the excel document and conduct the following operations:

- The calculation of the mean/average,
- The calculation of the standard deviations,
- The production of the regression line,
- The production of the regression equation
- The production of the coefficient of determination R^2



Picture 1. The example of the calibration curve calculation in the excel document

3. Task
   Prepare a calibration series of sodium chloride concentrations

Table 1. Sodium chloride concentrations for the preparations

Sodium chloride concentrations				
0.05 mol/l	0.1 mol/l	0.15 mol/l	0.2 mol/l	0.25 mol/l

The calculations have to be conducted according to the following information:

Mr(NaCl)=58,44 g/mol

The following formula has to be used:
(c = n/V; n=m/Mr)

Chromatography, TLC, HPLC, GC

This part is describing the basic of chromatographic techniques, including thin layer chromatography (TLC), high pressure liquid chromatography (HPLC) and gas chromatography (GC). The main principals are described, the main detectors and important terminology used in chromatographic techniques.

TLC – thin layer chromatography

The application of partition chromatography started with paper chromatography, meaning thin layer chromatography. It started in the 1940s. Thin layer chromatography (TLC) than started to be the most used chromatography techniques. In comparison with column chromatography, TLC can be defined as:

- Quick
- Sensitive
- Inexpensive technique
- It requires only a small amount of sample (usually a few grams)

TLC is usually used for the following:

- To find the number of compounds in a mixture
- Verification of the identity and purity of a compound in a mixture
- Monitoring the reaction progress
- Solvent composition determination for preparative separations
- Fractions analysis obtained by column chromatography.

As in other chromatography techniques, TLC also consists of mobile phase and stationary phase (molecules equilibrium, defined as a dynamic and rapid, between two phases). The main difference between TLC and other chromatographic techniques is the existence of gas phase. It is called chamber saturation between mobile phase and its vapor. During the development of TLC analysis, molecules are moving continuously back and forth (free and adsorbed states). Intermolecular forces are determining the equilibrium position and the capability of the solvent to move the solute through the plate. Figures 1 and 2 are showing schematically the development chamber for conventional TLC and the example of the separation, respectively.

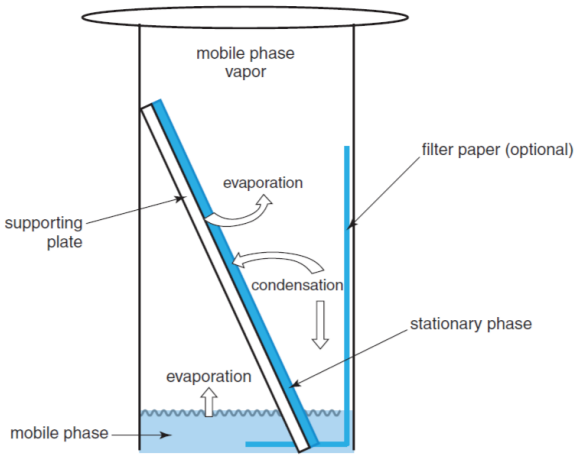


Figure 1. Schematic overview of development chamber for conventional TLC (Cai, 2014)

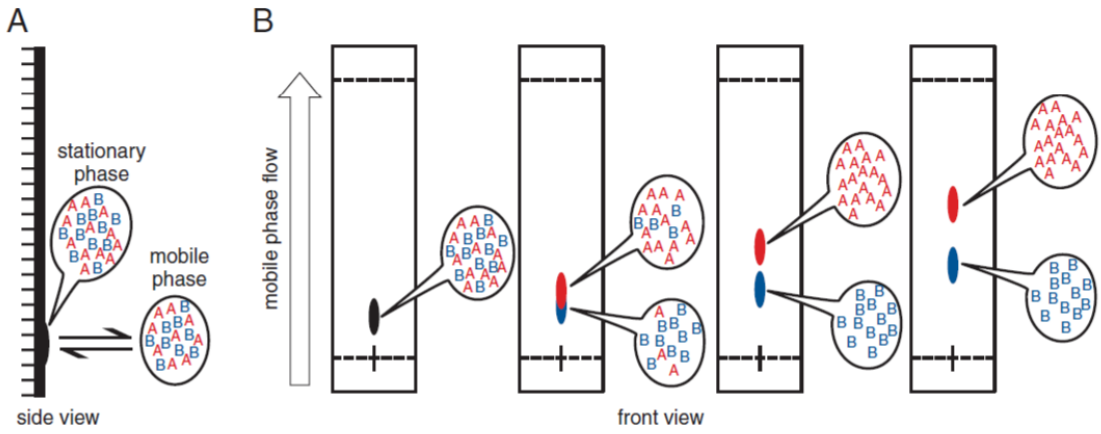


Figure 2. The separation example for conventional TLC (Cai, 2014)

The issues should be solved before TLC analysis conduction:

- TLC plate type, coating material
- The best separation and resolution can be achieved with which solvent
- How TLC plate will be handled and developed

- The visualization of compounds in the sample
- The prediction of possible problems during TLC experiments
- Tips and special techniques for a successful TLC (Cai, 2014).

Addressing and solving these mentioned issues are supporting the success of TLC analysis, same as the overall time required for the successful TLC analysis conduction (Cai, 2004).

Precoated TLC plates

TLC plates can be precoated with glass, aluminum, and plastic. Glass TLC plates can be defined as very robust, since it is rigid, transparent, it has a high chemical resistance, and it is heat stabile. The advanceable side of glass plates is also that it is economical since glass plates are reusable. The disadvantage of glass plates is that they are relatively heavy and thick, also glass plates are not easy to cut to desired size. A potential safety issue can relate to glass plates, since glass plates are fragile, and they can break easily (Cai, 2014).

Aluminum foil coated TLC plates, in comparison with glass TLC plates, are easy to handle, they are thin and lightweight. Oppositely from glass TLC plates, aluminum foil coated TLC plates can be easily cut (usually with scissors) to desired size. Since aluminum plates possess a strong adsorber layer adherence, they are good to be used with eluents that contain a high-water concentration. Otherwise, aluminum plates are not so good chemical resistant as glass plates, especially toward reagents containg strong acids, iodine or concentrated ammonia Cai, 2014).

Plastic (PET: polyethylene terephthalate film) plates for TLC analysis have been used less frequently, recently. Advantage sides of plastic plates are the same as for aluminum plates, since plastic plates are also lightweight, thin, easy to cut, and they are easy to handle too. Disadvantage sides of plastic TLC plates are their flexibility (they can easily crack) and they are not heat stabile (Cai, 2014).

The most often used TLC plate is silica coating, but for substances that are very sensitive aluminum oxide is preferred, due to sample decomposition prevention. At the beginning of TLC application were also used cellulose, polyamide and magnesium silicate (Cai, 2014).

Mobile phase (Solvent system)

A suitable mobile phase is the most difficult part in the performance of TLC experiments since solvent system influences the most TLC results. Mobile phase or solvent system is very rarely consisting out of only one component, up to five components in the mixture are used for the mobile phase. The most important thing is that no matter how many components are included in the mobile phase, mobile phase must be a homogenous system without cloudiness. There are three main criteria for a solvent system:

- Solubility (the greatest solubility must be provided with the separation achievment).
- Affinity (the most important factor for the resolution – affinity between solvent, stationary phase)
- Resolution (directly in correlation with the affinity and solubility) (Cai, 2014).

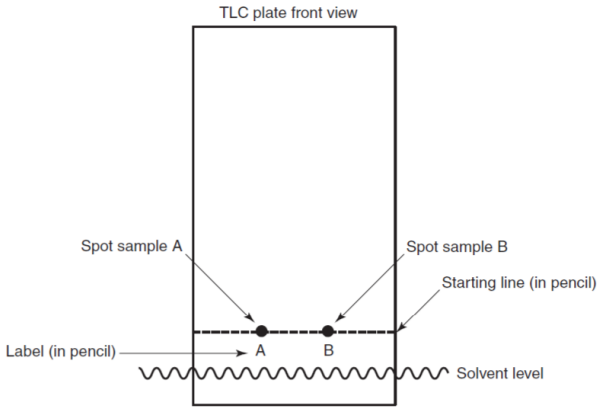


Figure 3. The example of TLC plate spotting (Cai, 2014)

The example how spotting/marking of TLC plate should be done is shown in Figure 3.

The most common solvents used in TLC analysis, their polarity and elution power are shown in Figure 4 (Hahn-Deinstrop, 2006).

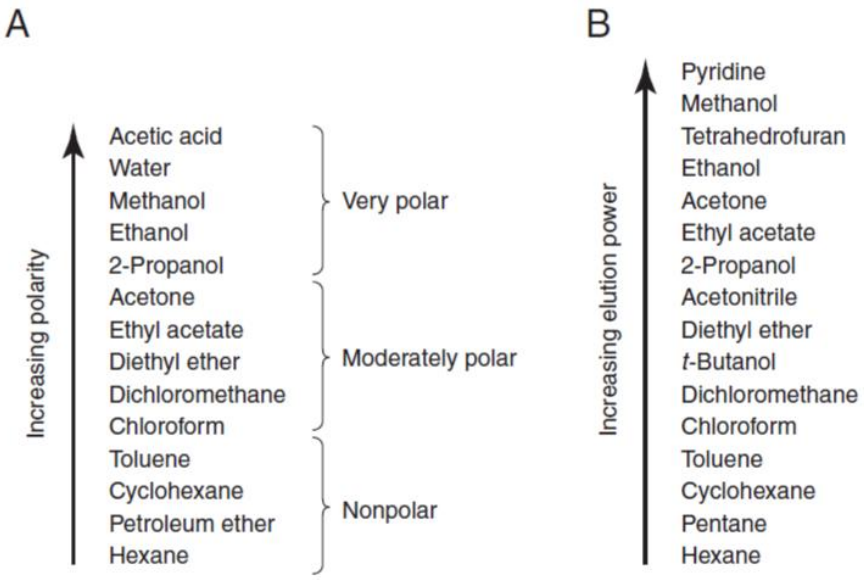


Figure 4. Common mobile phase solvents listed according to polarity

Safety considerations in TLC performance

These are safety considerations that should be taken into considerations:

- Cutting glass TLC plates can result in sharp edges that can cause cuts to the hands.
- Silica gel (especially dust form) is dangerous to inhale since it can cause severe lung irritation (long-term exposure can cause the lung disease silicosis). It is recommended to use a safety mask.
- Organic solvents used in TLC analysis are often flammable and combustible. The inhalation is to be avoided too. Some organic solvents are also carcinogenic (such as benzene, chloroform and dichloromethane).
- Reagents used in TLC analysis are often toxic and the handling must be done with care. The following protection should be used: disposal gloves, masks, and safety goggles) (Cai, 2014).

Retention factor (Rf)

As all chromatography techniques, TLC can be used for qualitative and quantitative determinations. Qualitative determinations in TLC analysis is done by simple localization of substances. The localization of the substances on the TLC plate is done with the use of parallel runs with reference compounds (the compound with similar structure with analyzed compound in the sample). Retention factor or Rf value is an important factor for qualitative analysis. The principal is explained as the following: if two spots have the same Rf value (two spots travel the same distance), then two components are probably the same molecule. Certainly that identical Rf values do not mean identical molecules. The reproducibility of Rf values are secured by the following conditions: TLC plates are run under identical conditions, meaning the same chamber saturation, solvents composition, temperature, etc (Cai, 2014).

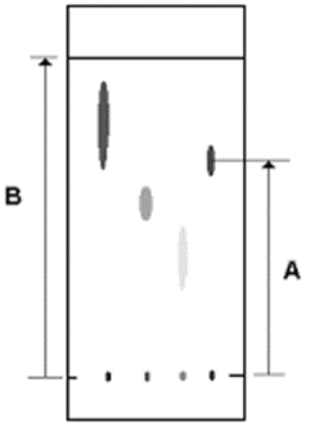
Practical work

Handle the TLC plate in such a way that do not damage the silica gel layer and that it is not splashed with unknown solutions!

Carefully draw the starting line on the chromatographic plate about 15 mm from the lower edge so as not to break the silica gel layer. We then apply a number of methanolic and chloroform standard solutions to the starting line using capillaries so that we do not damage the silica gel layer and the stain is as small as possible. The outermost spots should be about 1 cm from the edge of the plate. The gaps between the individual samples and the standards should be between 5-10 mm. If we have enough space on the plate, the gaps can be even bigger. Samples and standards should be applied in sufficient concentration, ie samples and standards can be layered on top of each other by applying another layer after the first layer has dried using a capillary. It should be borne in mind that a clean capillary must be used for each sample / standard.

After the samples and standard have dried, carefully place the TLC plate in the developing chamber, which contains a mixture of propanol and water (80:20). We insert the TLC plate into the developing chamber with the starting line down. The amount of mobile phase in the developing chamber should be such that its level does not touch the applied samples, washing could occur. Close the development chamber with the lid. After 1-2 hours, when the mobile phase reaches about 1 cm from the edge of the foil, remove the foil from the chamber and immediately mark the front of the solvent (ie the place where the mobile phase rises) with a pencil or spatula and let the chromatogram dry.

Recommendation: To orient yourself, draw a diagram of your TLC plate on the backing paper and mark the order in which you apply the samples. Evaluation:



A: the distance start – the middle part of the dot
B: the distance start – the head of the solvent
Figure 5. The evaluation of the thin layer chromatography

The position of the spot, i.e. the ratio of its distance from the start (A) to the distance of the forehead from the start of the division (B), (the graphical explanation is given in Figure 5) is constant for the given solvent and the given divided component under constant conditions and is called RF (retardation factor):

Rf=A/B

Conclusion:
Identify the composition of the unknown sample. Discuss the causes of the failures.

High pressure liquid Chromatography (HPLC)

The current food laboratory analysis is conducting in the way that more visualization and characterization are necessary since there are ever increasing number of compounds that

need to be identified. The overall principal of high-pressure liquid chromatography (HPLC) technology is to offer numerous methods and analytical capabilities (Ahuja, 2006).

High pressure liquid chromatography (HPLC) types

Liquid chromatography can be classified in many ways.
Firstly, it can be identified three modes according to the nature of the stationary phase and the separation process:

- Adsorption chromatography (an adsorbent is the stationary phase, such as silica gel or some other silica based packings). The separation is done by the repeated adsorption-desorption steps.
- Ion-exchange chromatography (the stationary bed and the sample have opposite ionic charge). This type of chromatography is mainly used with ionic or ionizable samples.
- Size exclusion chromatography (precisely controlled pore sizes represent the material for the column filling). The whole principal is based on the fact that larger molecules are washed (elute) faster through the column, while smaller molecules elute later. Reasonably, this technique is called gel filtration or gel permeation chromatography (at the present time, the stationary phase is made from other materials too, not only from gel) (Ahuja, 2006).

Adsorption chromatography and ion-exchange chromatography can be divided according to the relative polarity of two phases (mobile and stationary phases):

- Normal phase chromatography (the stationary phase/bed is polar in nature, such as silica and gel; the mobile phase in non-polar: n-hexane or tetrahydrofuran). The principal is that polar samples elute longer than less polar materials.
- Reversed phase chromatography (opposite principal from the normal phase chromatography). The stationary phase is non polar (hydrophobic), the mobile phase is polar (usually mixtures of water, acetonitrile and methanol). In reversed phase chromatography non polar samples elute longer.

Eluent polarity in the most important factor of HPLC analysis. There are two types of elution:

- Isocratic elution (the constant eluent composition is flowed/pumped through the system/column during the analysis).
- Gradient elution (eluent composition is changed during the analysis or also in chromatography called the run) (Ahuja, 2006).

Mobile Phases in high pressure liquid chromatography

The type of mobile phase is one of the very important variable that significantly affect the chromatographic separation. Certainly, each analysis conducted by HPLC acquire its own requirements, but acceptable properties of mobile phase are (Ahuja, 2006):

- The purity
- Detector compatibility
- The sample solubility
- Low viscosity (means lower pressure in the chromatographic system)
- Chemical inertness
- Affordability, reasonable price (Ahuja, 2006)

Stationary phase (adsorbents)

Separations in the stationary phases of HPLC are done according to the surface interactions, meaning that separations are dependent on adsorption site types (Ahuja, 2006). This principal can be defined with the term surface chemistry. Beside the main characteristics of the modern HPLC adsorbents to contain small rigid porous particles with high surface area, the main properties of adsorbent/column are:

- Particle size: 3 to 10 µm
- Particle size distribution (as narrow a possible)
- Pore size should be from 70 Å to 300 Å
- Surface area should be from 50 m^2/g to 250 m^2/g
- Bonding phase density: 1 to 5 per 1 nm^2
- Adsorbent/column surface chemistry, it depends on the ligand type attached to the surface: normal phase (OH, NH_2) and reversed phase (C8, C18, Phenyl)

Detectors used in the high-pressure liquid chromatography

There are many different types of detectors that are used for a wide or specific HPLC analysis. The detector in the HPLC system (the same detectors in other chromatographic techniques) has a role to turn a physical or chemical attribute to a measurable signal, the signal corresponding to concentration or identity (quality or quantitative method). In the beginning of chromatographic use, the detection was conducted in the way that fractions were collected and then they were analyzed of-line. The first HPLC detectors were discovered and introduced in 1940's and 1950's. Certainly, the introduction of HPLC detectors led to more sensitive and rapid analysis (Swartz, 2010).

A major breakthrough in the development of modern liquid chromatography is the invention of the flow-through cell (firstly done in 1940, in Sweden). The modern detectors used in the liquid chromatography are performing, on the same instrument, both analytical and preparative scale runs. One of the biggest advance side of modern detectors is their high sensitivity (they are capable to detect compounds in nano grams), though it is dependable on properties of the device as the whole system. The flexibility of the detectors is also good property of the detector, since it is allowing a rapid conversion of mobile phase and mode to another. The modern detectors are almost all on-stream or on-line monitors (the detection is monitorial during the analysis). Certainly, the most sensitive detector is mass

spectrometer (MS), and it is also an on-line HPLC detector. MS detector used in high pressure liquid chromatography is defined as the most sensitive, selective and also the most universal detector (on the other side MS detector is the most expensive detector used in high pressure liquid chromatography).

The most common refractive index detectors are:

- UV/VIS detector
  This is the most common HPLC detector used today due to the fact that many compounds absorb in the visible region, from 190 nm to 600 nm (Shwartz, 2010).
- Fixed wavelength
- Variable wavelength
- Diode array
- Fluorescence
  Fluorescence detectors can be very sensitive for substances with native fluorescence or which fluorescence is made by derivatization. Fluorescence detector can be up to 100 times more sensitive than detectors such as UV, meaning that fluorescence detector is useful for trace analyses, limited samples or in the case of low sample concentrations situations. Certainly, sensitivity of fluorescence detector can be influence by mobile phase, buffers and solvents that are not properly degassed (Shwartz, 2010).
- Refractive index detection
  The refractive index detector is considered to be the oldest LC detector; it has a universal bulk property. The refractive index detector is measuring the difference in optical refractive index between the sample and mobile phase. Due to these properties of the refractive index detector, the detector is usually used for the analysis of suagars, organic acids and triglycerides (Shwartz, 2010).

Less common detectors used in high pressure liquid chromatography are:

- Conductivity
  It is a bulk property detector, measuring the conductivity of the mobile phase. In the case when the analyte does not have a UV chromophore the conductivity detector is the right choice (Swartz, 2010). An unsaturated group that absorbs and reflects light at specific angle, giving the hue. Is called chromophore (Chakraborty, 2015). The determination of organic acids is often done with the use of the conductive detector (Swartz, 2010).
- Mass-spectrometric
- Evaporate light scattering
  Evaporative light scattering detector (ELSD) is operating on the principle of mobile phase evaporation (nebulization), measuring the light scattered by the resulting particles (Swartz, 2010).

Important terms used in chromatography:

Eluent, eluate, and effluent?

Eluent (it is spelled in some literature as eluant): it is synonymous for the mobile phase. Simply, eluent is the solvent or solvent mixture in elution chromatography (Majors and Hinsaw, 2013).

Eluate: the mixture of solute and solvent exiting the column. Only during a separation, eluate exists (Majors and Hinsaw, 2013).

Effluent: the stream flowing out from the column. Actually, effluent is synonymous with eluate (Majors and Hinsaw, 2013).

Eluite: it is a more precise term for the chromatographic analyte or solute. Eluite is referring to the eluted sample component. The term eluite can be considered as a rare term (Majors and Hinshaw, 2013).

Dead volume: it is an unswept volume that is exposed to the mobile phase flow. Additional peak broadening is caused by excessive dead volume. It can be explained as extra volume caused by solutes passing through a system or chromatographic system (Cai, 2014).

Retention time: the time at which in the chromatogram the peak is obtained. The retention time is the crucial element for the compound recognition (Poole and Poole, 2009).

Peak area: it is an area under the specific peak obtained at the specific retention time. The calculation of the peak area is the crucial element for the compound quantification (Papai and Pap, 2002).

Theoretical part is concerning the following issues:

- Chromatography - what is it, what is its purpose and what are its benefits.
- The principal of thin layer chromatography, high pressure liquid chromatography, gas chromatography and the description of main detectors used in the mentioned types of chromatography systems.
- Explaining the terms mobile phase and stationary phase
- The types of chromatography systems according to different important factors related with mobile and stationary phases, particle sizes, detection limits etc.
- Explaining the terms: dead volume, retention time, eluent, eluate etc.
- Thin layer chromatography (TLC): principles, evaluation, types of mobile and stationary phases.

Practical part:

Identification of synthetic food dyes by TLC method

Material:

Food coloring standards

TLC plates

Capillaries

Development chamber
Mobile phase: propanol: water = 80:20

Determination of malondialdehyde by HPLC

Extract preparation:
Pipette 3 ml of the oil sample into the centrifuge tubes. Then add 6 ml of 20% TCA. Next, incubate the samples in a water bath at 95 ° C for 20 min. Samples must be protected from light. The samples are then cooled in a water bath to about 5 ° C and then centrifuged for 15 minutes. It is necessary to place samples of very close weight in the centrifuge opposite each other. Then take 2 ml of the supernatant into 10 ml tubes and add 1 ml of TBA solution. The samples are then incubated in a water bath at 95 ° C for 60 min. Samples must be protected from light. The samples are then cooled in a water bath to about 5 ° C and then centrifuged for 15 minutes. The bottom layer (pink) - 850 µl is filtered through a microfilter into vials and carefully closed.

Evaluation:
Based on the malondialdehyde calibration series, each student determines the analyte content of the sample. The student attaches the data and chromatograms as part of the protocol.
Conclusion:
Discuss the content and causes of malondialdehyde in the oil.

Spectrophotometry

Spectrophotometric techniques and the interpretations of data obtained by spectrophotometric analysis are the part of important analytical laboratories, including laboratories for food analysis. Spectrophotometric analyzer, this term relates to various spectrophotometric sensors, instruments, devices, testers, and probes using spectrophotometric techniques for analysis of different samples, including food and beverages.

The heart of any such spectrophotometric analyzer is the spectrophotometric transducer. The spectrophotometric transducer is converting an optical signal into the raw data of sequence. The most often used wavelengths are the following:

- 200 – 300 nm middle ultraviolet radiation
- 300 – 380 nm near ultraviolet radiation
- 380 – 750 nm visible radiation
- 750 – 2500 nm near infrared radiation
- 2.5 – 10 µm middle infrared radiation

Out of mentioned spectrophotometry techniques the most useful technique for food analysis can be marked Near infrared spectrometry (NIR) since it is evaluating overlapping absorption peaks that correspond with C-H, O-H and N-H chemical bonds. The simplicity and speed are the main advantage of NIR spectrometry since usually there is no need for the sample preparation and the time necessary for the analysis is usually less than 1 minute. Another advantage of NIR spectrometry is that it allows several identifications of constituents concurrently. NIR spectrometry is also good option for the analysis of high moisture food products (Morawski, 2008)

Practical work:

Principle of light absorption, UV-VIS spectrophotometry

Material and devices:

- Sodium carbonate solution c = 0,005 M
- Congo standard (stock) solution red (10 mg in 100 ml), unknown sample
- 10 ml volumetric flasks
- Automatic pipette 100-1000 µ
- Automatic pipette tips
- Cuvettes
- UV-VIS spectrophotometer
- Pasteur pipettes, cellulose wadding, waste beaker, syringe with distilled water and ethanol

The aim of the practical work is the following:
Understanding the principle of UV-VIS spectrophotometry, determination of the absorption maximum of a standard sample (λmax), creation of a calibration curve at a specified absorption maximum, determination of a certain compound concentration in an unknown sample, concentration calculations.

Procedure:

The determination of absorption maximum:

First, turn on the UV-VIS spectrophotometer to warm up. Usually it is common to turn on the spectrophotometer at least 30 minutes before measuring due to the warming of lamps. Rinse the prepared cuvettes thoroughly with distilled water and a small amount of sodium carbonate solution. Then fill them with sodium carbonate solution to ¾ volume and carefully clean the outer surface with distilled water (ethanol) and cellulose wadding. We make sure that we do not touch the area through which the beam will pass. Leaving fingertip mark on the cuvette can intertwined and influence the results. Place the clean and dry cuvettes correctly in the spectrophotometer. Place the first cuvette in the reference position (that is the blank sample) and the second in the working position. We reset the spectrophotometer

to wavelengths of 450-550 nm. After resetting, remove the working cuvette and pour its contents into a waste beaker. Rinse the working cuvette with distilled water.

Then pipette 1 ml of the standard Congo solution into 10 ml volumetric flask and make up to the mark with carbonate solution. Rinse the working cuvette with a small amount of the prepared solution to prevent dilution. Then fill the cuvette with this solution to ¾ volume and carefully clean the outer surface with distilled water (ethanol) and cellulose wadding. Insert the working cuvette into the spectrophotometer and start the measurement. To evaluate the absorption maximum, we record the absorbance from 450 nm to 550 nm with an interval of 5 nm. Repeat the measurement 3 times. Then we select the wavelength of the absorption maximum from the measured absorbance. At this wavelength, we measure the rest of the samples.

Calculation:

Express the measured absorbances and wavelengths in tabular form. Make the mean and standard deviation of the recorded absorbances. Plot the averaged absorbances (y-axis) and their wavelengths (x-axis) in a graph (line type of graph). Determine at which wavelength the absorbance was the highest (absorption maximum λmax).

Calibration curve and determination of the concentration of the unknown sample:

Pipette from the standard (basic) solution into 10 ml volumetric flasks in volumes of 0,5 ml; 1.0 ml; 1.5 ml; 2.0 ml and 2.5 ml and make up to the mark with sodium carbonate solution. Then we measure the absorbance of each solution of the calibration series. Finally, we measure the absorbance of the unknown solution. We measure each solution 3 times. We measure the calibration series from the lowest concentration to the highest. Notice the color changes.

Calculation:

First calculate the concentrations of the standard calibration series in mg/ml. Assume the concentration of the standard stock solution and the volume pipetted into 10 ml volumetric flasks. Express the measured absorbance and the concentrations calculated for them in the form of a table. Calculate the mean and standard deviation from the measured absorbance of the calibration series. Plot the averaged absorbance of the calibration series in a scatter plot where the y-axis is the absorbance and the x-axis the concentration in mg/ml. Create a regression equation and a confidence factor. Calculate the concentration of the unknown sample from the regression equation. For each absorbance of the unknown sample, calculate the concentration and calculate the mean and standard deviation from the calculated concentrations.

Practical work:

Determination of L-ascorbic acid spectrophotometrically

Principal:

After extraction of the ascorbic acid from the test sample with a solution of metaphosphoric acid and acetic acid, the ascorbic acid is reduced with the dye 2,6-dichlorophenolindophenol. Subsequently, the excess color compound is extracted with xylene and its excess is determined spectrophotometrically, measuring at a wavelength of 500 nm.

Chemicals:

- Extraction solution: metaphosphoric acid and acetic acid solution at a concentration of 30 g/L
- 2,6-dichlorophenolindophenol solution with a concentration of 250 mg/L
- Buffer solution: sodium acetate/acetic acid pH 4.0
- Xylene
- For calibration: standard ascorbic acid solution at a concentration of 1 g/L

Procedure (own sample):

- Pipette 5 ml of the own sample into a 50 ml volumetric flask and make up to the mark with the extraction solution.
- Pipette 0.25 ml of the diluted sample into a plastic tube.
- Then 0.25 ml of buffer, 1.5 ml of 2,6-dichlorophenolindophenol and 5 ml of xylene are added.
- After adding xylene, the tubes are shaken vigorously for 10 seconds and then centrifuged (3 minutes, 3500 rpm).
- The absorbance of the upper xylene layer at 500 nm against xylene is then measured.

Procedure (calibration series):

- •Prepare a stock solution of L-ascorbic acid (1 g / l).
- In addition, a number of standards with a composition in the range of 0.1-0.6 mg / ml are prepared.
- Pipette 0.25 ml of standard, 0.25 ml of buffer, 1.5 ml of 2,6-dichlorophenolindophenol and 5 ml of xylene into plastic tubes.
- After adding xylene, the tubes are shaken vigorously for 10 seconds and then centrifuged (3 minutes, 3500 rpm).
- The absorbance of the upper xylene layer at 500 nm against xylene is then measured.

Calculation:

The L-ascorbic acid concentration is calculated from the regression equation of the calibration curve. The result is then multiplied by the dilution factor (x10). The composition of the calibration series is given in Table 2.

Chemicals preparations:

Metaphosphoric acid and acetic acid solution at a concentration of 30 g/L:

Dissolve 15 g of metaphosphoric acid in 200 ml of water and 40 ml of glacial acetic acid and make up to the mark with distilled water in a 500 ml volumetric flask. The solution is stored in the refrigerator.

2,6-dichlorophenolindophenol solution at a concentration of 250 mg/L

In a 100 ml volumetric flask, dissolve 25 mg of 2,6-dichlorophenolindophenol sodium in 75 ml of warm distilled water containing 21 mg of sodium hydroxide. Then make up to the mark with water. The solution is stored in the refrigerator and regularly prepared fresh.

Sodium acetate/acetic acid buffer pH 4.0

30 g of anhydrous sodium acetate are dissolved in 70 ml of distilled water and 100 ml of glacial acetic acid.

L-ascorbic acid stock solution with a concentration of 1 g/L

It is prepared by dissolving 100 mg of ascorbic acid in a 100 ml volumetric flask supplemented with extraction solution.

Table 2. The composition of the calibration series (in 10 ml volumetric flasks)

Concentration (mg/mL)	A stock solution (1g/L) in mL	Extraction solution in mL
0,1	1,0	9,0
0,2	2,0	8,0
0,3	3,0	7,0
0,4	4,0	6,0
0,5	5,0	5,0
0,6	6,0	4,0

Viscosity

Viscosity is an important property of food commodity since it can be connected even with food intake. According to o literature data, an increased food viscosity is reducing food intake and suppresses appetite. This factor is explained with the possibility that an increase food viscosity is delaying gastric emptying rate. Consequently, the feeling of satiety (the feeling of fulness) is prolonged with the delayed gastric emptying. Since an increase of food viscosity is affecting the feeling of fulness, it is also influencing or modulating postprandial glycemic and insulin response (Zhu et al., 2013).

The following issues have to be discussed with the students:

- What is viscosity? Viscosity and food science.
- What relationship equation) expresses the behavior of a viscous liquid?
- What types of viscosities do we have?
- What is Newtonian and non-Newtonian fluid, differences?

- How do we divide non-Newtonian substances?
- What types of viscometers do we have?

The possibility to produce edible packaging. The matrices that can be used to produce edible packaging.
What is the principal of Polarimetry?

Practical part:
Viscosity measurement - Newtonian and non-Newtonian fluid
Concentrated distilled water with sorghum flour - measure 3 g of loose raw material into 100 ml of distilled water and bring to a boil. Cook for 1 min, then remove the mixture and allow to cool to room temperature.
Measurement on a rotary viscometer:
After putting the rotary viscometer into operation record dynamic viscosity from the lowest speeds to the highest speeds. The speed is recorded and the corresponding dynamic viscosities (mPa.s). Each liquid has to be measured twice. The temperature of the fluid has to be measured too.

The results have to be processed in the following way:
From the measured viscosities for the corresponding speed, the average and the standard deviation are calculated. For each fluid, the graph is created in which are plotted the average values of measurements (y = dynamic viscosity, x = speed) and deviations. From the course of the function, it is determined is the measured liquid a Newtonian or non-Newtonian fluid. If it is a Newtonian fluid, its mean value of dynamic viscosity is determined. If it is a non-Newtonian fluid, from the course of the function it is determined whether it is a pseudoplastic or dilatant fluid.

From this equation we determine the value of n

$$\eta = mD^{n-1}$$

Where η is the apparent viscosity (mPa), m is a constant and D is the strain rate (rpm).
For n <1 the substance is pseudoplastic, in the case of n> 1 it is a dilatant liquid.

Textural properties of food and devices used for the food textural properties measurement

The quality and especially acceptability of food commodities, both fresh and processed commodities, are often connected with textural properties. Textural properties often

represent the key quality attributes. Texture attributes are used for the following estimation and monitoring:

- Food value
- Control quality
- Harvest readiness
- Postharvest handling impact
- Processing operation impact
- Shelf life
- Consumer preferences and acceptability (Chen and Opara, 2013).

A wide range of instrumental tests has been developed to evaluate different food commodities, serving as research instruments or to assess food texture during food industrial processing. Certainly, the choice of instrument is dependent on its cost and availability of persons capable to operate it (Chen and Opara, 2013). Food texture can be defined with rheological and structural attributes, including geometric and surface, perceived by mechanical, tactile, visual and auditory receptors (Lawless and Heymann, 1998).

Instrumental measurement of food texture has been developing also due to overcome the limitations of sensory analysis. The reason can be found also in the fact that food texture perceived by consumer is dependable on the mechanical behavior of food in the mouth. Instrumental textural measurement applied in experiments can be divided as the following:

- Fundamental tests
  They are developed by scientists and engineers that want to measure construction materials. These tests are usually not useful for the measurement of food senses in the mouth.
- Empirical and imitative instrumental tests
  These tests are developed and used in the quantification of food textural properties (Chen and Opara, 2013).

The minimal number of the samples that have to be analyzed, to provide as objective analysis as possible, are at least five repetitions. Among instrumental textural food analysis there is the group of methods belonging to destructive methods:

- Three-point bending test
  The method is applied in the way that the force is focused to the center of the sample till the fracture occurs. The method is applied on samples such as biscuits, potato chips and cornflakes (Chanier at al., 2007; Rojo and Vincent, 2009; James et al., 2011).
- Single-edge notched bend (SENB) test

The whole test is based on the sample between two supported anvils. The notch is made in the middle of the sample with a third anvil, until fracture occurs. The example of food commodities on which the test can be applied are biscuits and apples (Chen and Opara, 2013).

- Compression and puncture test
  This is the most common used food texture instrumental analysis since food samples can be solid or semi-solid. The analyzed samples can be gels, apple rings, cornflakes, cheese, bread crumb, carrot, kiwifruit, potato slices, cereal snacks etc. The texture test can be conducted on whole fruits or parts of fruits in dependence on the experiment type or samples quantities (Rolle et al., 2012). The probes, for both tests, are usually cylindrical shape. The difference is in the diameters of heads, in puncture tests the head diameter is usually under 11 mm, even 1 mm small (something like a needle). These methods are usually standard methods for the evaluation of fruit flesh firmness.
- Stress relaxation test
  This test is usually conducted for the purpose to analyze the viscoelastic attributes of semi-solid foods. The examples are fish, cheese, sausage, and flour dough. The principal of the method is that the sample is compressed (the compression lasts till the certain expected strain) under a certain speed, while the force is recorded during the relaxation (the time is ranging usually from 1 minute to 10 minutes).
- Warner-Bratzler shear force test (WBSFT)
  WBSFT is the most often used instrumental textural test for the evaluation of meat tenderness. The method was invented in 1930s and it is still widely in use on different textural devices (TAXT, Instron etc.) (Chen and Opara, 2013). The principal of the test is that the meat sample is sheared perpendicularly in muscle fiber orientation (Destefanis et al., 2008). The maximum shear force is the most considered parameter in Warner-Bratzler shear force test (Chen and Opara, 2013).
- The combination of mechanical and acoustic methods tests
  These tests are mainly used for hard, crispy and crunchy food commodities, where their brittle fracture behavior and sharp sound are measured. These attributes of food commodities are closely related with their textural properties. The above-described tests (compression, penetration and three-point bending tests) are combined with acoustic signal determination. The combination of mechanical and acoustic tests was also successfully used to measure the crispiness of apples too. These tests are also conducted with a probe inserted into a food sample and measuring the vibration (Taniwaki et al., 2006). The following food commodities are evaluated by this method: chips, pear, cabbage leaves, grape flesh, and persimmon (Chen and Opara, 2013).

- Imitative methods
  Imitative methods also belong to destructive methods since they are mimicking the biting process during eating. These methods are often called "tooth methods" (Jiang et al., 2008). Tooth-like probes are often used to conduct these methods since the idea is to obtain the results correlating with the human perception in the mouth. Artificial saliva is also sometimes used to conduct these methods. The evaluated food samples are often starch based food commodities.
- Other destructive methods
  The following methods can be enlisted: probe tensile separation method (measuring the stickiness of fluid foods), cutting shear test (measuring cells being held degree and the cutting force of food, usually fresh food), traction test (measuring fundamental mechanical properties of food commodities during a time) (Chen and Opara, 2013).

Nondestructive methods testing fresh and processed food commodities are very important for monitoring and controlling the quality of products. These methods include mechanical techniques that are measuring quasi static force deformation and impact response (Chen and Opara, 2013).

Published experiments are indicating that there are two main instruments used to measured textural properties of different food commodities (including solid and semi solid food types):

- The Texture Analyzer (TA) (Stable Micro Systems Ltd.). The following models are used: TA-XT2i, TA-XT2 and TA.XT plus. TA.XT plus, according to literature data are the most popular one in food texture experiments.
- The Instron testing machine (Instron Ltd.)

TA.XT plus instrument, compared with the Instron , is more focused on food texture analysis and it is used both in academic and industrial areas. The Instron instrument can be defined as more general and professional instrument measuring mechanical properties of different materials (Chen and Opara, 2013).

Practical part:

Testing packaging material:

Remove the edible packaging from the petri dish using your hands and a spatula. Try not to break the packaging. Then cut out 3 strips measuring 1x5 cm from the package. When cutting, take care not to deform or stretch the packaging. Place the strips on the filter paper.

Repeat this procedure with other packaging materials. Then use a texturometer to measure the texture of each strip.

Polarimetry

The principal of the polarimetry is to measure the extent to which a certain substance is interacting with plane polarized light. The plane polarized light is defined as the light consisting of vibrating waves only in one plane. The quantification of different sugars, carbohydrates are often measured with the use of polarimetry (Caprita et al., 2014).

Practical work:

Polarimetric determination of starch according to Ewers
Method:
2.5 g of flour are placed in a 100 ml volumetric flask and 20 ml of 1.124% hydrochloric acid are added. The contents of the flask are mixed thoroughly and the walls are rinsed with another 25 mL of dilute hydrochloric acid. The flask is then placed in a boiling water bath for exactly 15 minutes and the contents of the flask are mixed every 3 minutes. After 15 minutes, the flask is removed from the water bath, another 20 mL of hydrochloric acid is added and cooled. The contents of the flask is clarified with 3 ml of Carrez reagent I and 3 ml of Carrez reagent II. After clarification, make up to the mark with water, mix thoroughly and filter.

Measurement on a polarizer:

- Put the polarimeter into operation at least 5 minutes before the measurement.
- Rinse the cuvette first with water and then with a small amount of sample. Then fill it with the sample to the brim (we use a Pasteur pipette for the final refill). Cover the cuvette with a slide and fasten the slide with a thread so that no liquid escapes. Clean the cuvette, slide and transfer the resulting air bubble to the spherical part of the cuvette.
- Before measuring on a polarimeter, set 0 ° with the dial. Then carefully insert the cuvette into the polarimeter. Slowly turn the adjusting dial and observe the circular field in the eyepiece. All 3 parts of the field of view are the same orange light. Then subtract the rotation on the scales on both sides of the polarimeter. The degrees read on both sides should match.
- During the next measurement, blur the polarimeter and again look for a position where all parts of the field of view are equally illuminated in orange. Measurement should be taken at least in 3 repetitions due to precision and statistical analysis.

Salt content in food commodities

The discovery to use salt in food preservation was the invention (5000 years ago) of Chinese people, according to the literature data. There is also an estimation that humans had been consuming lower than 0.25 g of salt per day for several million years. After the discovery that salt can be used effectively for food preservation, salt intake has been constantly increasing. According to literature data, the maximum level of sodium intake was achieved in the 1870s. The invention of refrigerators slightly decreased salt intake, but processed food (usually a high source of sodium) is highly acceptable to consumers that led again to the increase of salt intake. Certainly, there is a different salt sources in diets of western and Asian countries. The main source of sodium in western countries comes from processed food and restaurants, in Asian countries the main source of sodium comes from salt added during culinary food preparation at home. The overall salt daily intake is estimated to be from 9 g to 12 g (Brown et al., 2009; He and Macgregor, 2010; Leong et al., 2016; Rodrigues et al., 2016; Dordevic et al., 2018).

Sodium content determination

The following analysis are usually used for sodium content determination:

- Ion-selective electrode
- Mohr titration procedure
- Volhard titration procedure
- Test strips indicators
- Atomic absorption spectroscopy
- Inductively coupled plasma-optical emission spectroscopy

These methods are official methods for different commodities. Certainly, ion-selective electrode, Mohr titration, Volhard titration and test strips indicators are faster and less expensive analyses, though not so precise when atomic absorption spectroscopy and inductively coupled plasma-optical emission spectroscopy is applied for the direct estimation of sodium content (Nielsen, 2017).

The principal of Mohr method or Mohr titration is the quantification of chloride ions over which sodium ions are calculated. Silver nitrate is used for the titration of the sample containing chloride. Silver from silver nitrate is making complex with available chloride and then silver is reacting with chromate (that is added to the sample) forming orange-colored silver chromate. The volume of silver nitrate used till the formation of orange-colored silver chromate is used to calculate the sodium content in the sample (Nielsen, 2017).

During Mohr titration the following precautions should be taken into considerations:

- Using gloves and safety glasses
- Potassium chromate can be hazardous for sensitive skin
- Crystalline $AgNO_3$ and its solutions are making dark brown stains due to salt photodecomposition to metallic silver. The discoloration is ruining laboratory furniture and environment. The spill of the solution should be immediately sponged up and then sponge should be thoroughly rinse out at a sink. Area should be than rinsed and moped up at least 3-4 times with the clean sponge. All pipettes, burets, flasks, beakers, etc. should be rinsed after the experiments to assure that all residual $AgNO_3$ is removed.

The Volhard method is used to determine chloride anions in various food. The principal of the method is the addition of an excess of silver nitrate solution to an analyzed sample. Consequently, silver chloride is precipitated quantitatively. The back titration of silver ions excess is used to determine chloride concentrations. The back titration is done by thiocyanate solution, while the ferric alum is used as indicator (Haouet et al., 2006).

Practical work:

1. The principal of salt determination according to Mohr
2. The principal of salt determination according to Volhard

The Volhard method is an indirect or back titration method in which an excess of a standard solution of silver nitrate is added to a chloride containing sample solution. The excess silver is then back titrated using a standardized solution of potassium or ammonium thiocyanate with ferric ion as an indicator.

Determination of salt according to Mohr

Method

Samples: spice seasoning with high and low salt content

Weigh 1 g of the product sample into the beaker and quantitatively transfer to a 200 ml beaker with 100 ml of hot distilled water. Stir the product in the flask and allow to extract for 30 minutes. Then add 5 ml of Carrez reagent I and II and, after clarification, make up to the mark with water and filter through a pleated filter. Pipette 25 ml of the filtrate into a titration flask and add 50 ml of distilled water. Then add a few drops of phenoltalein to the flask and neutralize with 0.25M NaOH to a pH of about 7. Check the pH with litmus paper. Next, add 1 ml of 5% potassium chromate to the sample and titrate with a 0,05 M standard solution of silver nitrate to a permanent reddish color. Titrate in three replicates.

Calculation

Calculate the chloride content in terms of sodium chloride in % according to the formula:

$$x\ (\%) = \frac{v \cdot M \cdot c}{m} \cdot \frac{V_1}{V_2} * 0.1$$

where v is the volume of silver nitrate standard solution in ml; c is the concentration of the standard solution of silver nitrate in mol / l; M is the molar mass of NaCl (58.45 g / mol); V_1 is the volume of the aqueous extract of the sample in ml; V_2 is the volume of filtrate used for analysis in ml; m is the sample weight in g. Round the result and SD to two places after zero.

Determination of mustard acidity

Method

Full-fat and Kremž mustard

Using a syringe, weigh 20 g of the sample to the glass, add 100 ml of distilled water and homogenized, then transfer with additional 100 ml to 200 ml volumetric flasks and make up to the mark with distilled water. Then filter the contents of the flask, removing the first part of the filtrate and continue filtering. After filtration, measure 100 ml of filtrate into a beaker and titrate with a standard solution of 0,1 M NaOH using a pH meter to pH 8,1. Neglect the standardization of the measuring solution. Perform the determination in two replicates.

Calculation

The percentage of acetic acid in mustard is calculated using the relation:

$$x\ (\%) = \alpha \cdot 0{,}06005$$

where α is the consumption of standard sodium hydroxide solution. Round the result and SD to two places after zero.

Evaluation

State the acidity of mustard and compare the experimental results with the legislation.

Sugar content determination

Sugar content in food commodities can be determined by the application of different techniques. The following non-selective methods are usually used:

- Titration methods (used for the determination of reduced sugars, non-selective method)
- Refractometric methods (used for the estimation of the total amount of sugar, non-selective method)

Out of the selective methods used for the determination of different sugars the most often are used the following methods:

- Infrared spectroscopy (Ramasami et al., 2004)
- Liquid chromatography with the refractive index detector (Kamal and Klein, 2011) and mass spectrometry (Kubica et al., 2012)
- Gas chromatography with flame ionization detector (Adams et al., 1999) and mass spectrometry (Medeiros and Simoneit, 2007).

Certainly, the choose of techniques depends on many factors, including the laboratory equipment and employees' education.

Practical work:

The determination of reducing sugars according to Bertrand

Chemicals:

Fehling's solution I,
Fehling's solution II,
potassium permanganate (c = 0.02 mol/l),
sulfuric acid (c = 3 mol/l),
oxalic acid crystal. p.a. dihydrate, ferric sulphate solution.

Method:

First, the weight of oxalic acid dihydrate required to prepare 100 ml of a solution with a concentration of 0.05 mol/l is calculated. Weigh out the calculated quantity to four decimal places, transfer quantitatively to a 100 ml volumetric flask, dissolve and make up to the mark with water. Pipette 10 ml of this standard solution into a titration flask, acidify with 5 ml of sulfuric acid solution and add about 1 ml of the permanganate standard solution from the burette. The titration flask is heated to about 60 ˚C and, after the solution has decolorized, the titration is continued until the first pale pink color, which is stable for at least 30 seconds. The titration is performed three times.

Using the values of the stoichiometric reaction coefficients, express the ratio of the mass amounts of both reactants, n (KMnO4) / n ((COOH) 2) at the equivalence point and calculate the exact concentration of potassium permanganate. Determine the mean and standard deviation from the calculated concentrations.

Preparation of extracts

Samples: Linen cakes

Method:

A sufficient quantity of the sample is homogenized in a mortar and 5 g of the homogenized sample are weighed to 2-3 decimal places in a 250 ml beaker. The batch is then mixed with 100 ml of distilled water and the sample is ground using a mixer. The mixture is then filtered through a gauze folded several times.

Pipette 20 ml of Fehling's solution I and 20 ml of Fehling's solution II into a 100 ml Erlenmeyer flask, heat the mixture to about 60 °C, add 10 ml of filtrate and a magnetic stirrer, and heat the mixture further to boiling. The boiling should be mild and held for exactly 2 minutes. After boiling for 2 minutes, cool the flask with a stream of cold water. The contents of the flask were quantitatively filtered under reduced pressure through a Buchner funnel with filter paper with a wider diameter than the Buchner funnel so that the precipitate adhered only to the filter paper. Copper oxide should not be exposed to air to prevent oxidation. Finally, the precipitate is washed thoroughly with hot water. The precipitate filter was removed from

the Bucher funnel and placed in an Erlenmeyer flask (300 mL) containing 40 mL of ferric sulfate solution. The Erlenmeyer flask is then heated on a hot plate until the precipitate dissolves. The contents of the flask are then immediately titrated with a standard solution of potassium permanganate. Perform your own determination 2 times.

Calculation:
Consumption of 1 ml of potassium permanganate solution with a concentration of 0.020 mol/l corresponds to 3.315 mg of reducing sugars. Determine the percentage of reducing sugars in the product and the standard deviation. Determine the percentage of reducing sugars in the product.

Practical work:
Determination of diastatic power
Samples: wheat flour and rye flour

Method:
Amylase extraction: Weigh 10.00 g of the flour sample, mix it with 250 ml of distilled water at 37 °C and mix at third speed for three minutes. The resulting suspension was filtered and the filtrate was used for further determination.
Starch solution saccharification: Pipette 50 ml of starch solution into a 100 ml volumetric flask. Add 5 ml of buffer and heat for 10 minutes at 37 °C. 2.5 ml of the filtrate are then added to the flask and placed in a water bath heated to 37 °C. for 1 hour. After this time, the enzymatic activity is stopped by adding 2 ml of sodium hydroxide solution, the flask is made up to the mark with distilled water and the contents of the flask are mixed well.
Reduction of iodine by the resulting sugars: Pipette 50 ml of the solution from the 100 ml flask into a 300 ml Erlenmeyer flask and add 25 ml of volumetric iodine solution and 3 ml of sodium hydroxide solution. Close the flask and leave to stand for 15 minutes. The reaction is then quenched by the addition of 4.5 ml of sulfuric acid solution.
Titration of unused iodine: Titrate unused iodine immediately with a standard solution of sodium thiosulphate until the color of the solution changes from a bluish tone to a creamy yellow color which remains unchanged for at least one minute.
Blank test: Pipette 50 ml of starch solution into a 100 ml volumetric flask and heat in a thermostat for 10 minutes at 37 °C. Then add 2.5 ml of filtrate and 0.3 ml of sodium hydroxide solution to the flask and make up to the mark with distilled water.

Calculation:
The diastatic power de is calculated according to the relation:

$$DM = (a - b) \cdot 34{,}2 \cdot \frac{100}{100 - w}$$

where a is the consumption of volumetric thiosulphate solution in the blank test in ml, b is the consumption of volumetric thiosulphate solution in the self-determination in ml, w is the moisture content in percentage. The result is expressed as the whole number.

Practical work:

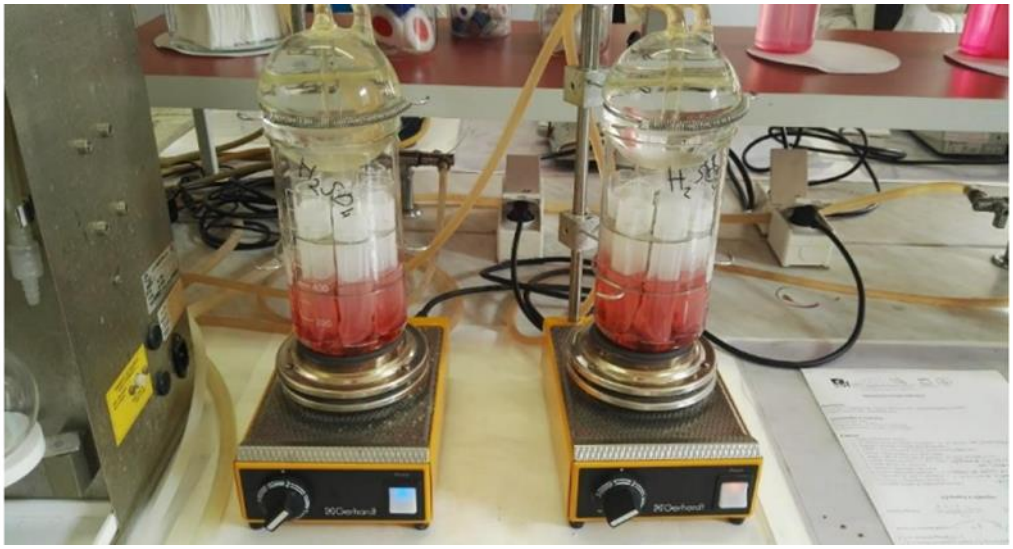
The determination of fiber

Samples: Dark/light toast bread, whole grain flour is included in the product.

Method:

The method is conducted in the following steps:

- It is first necessary to consider the weight of the filter bags (FiberBags). Then weigh a sample of 1 g into the FibreBag to the nearest 0.0001 g.
- Place the FibreBag samples in a carousel with spacers. Next, place the carousel in a 1-liter beaker without a spout with 360 ml of 0.13M sulfuric acid.
- Before heating, turn on the condenser, which will cool the sample beaker. Heat it for 30 minutes. This step is showed in the Picture 2.
- After this period of time (30 minutes), wash the residual sulfuric acid from the samples three times with boiling water.
- Then place the carousel in a 1-liter beaker with 360 ml of 0.23M potassium hydroxide.
- Heat the samples again for 30 minutes.
- At the end of 30 minutes time period, wash the residual potassium hydroxide from the samples three times with boiling water.
- Then place the carousel without spacers in the oven and dry at 105 °C overnight.
- After drying the FibreBag, the samples are placed in a desiccator for 30 minutes.
- Wegh FibreBag.
- Place FiberBags in ceramic cups and incinerate at 600 ° C for a total of 4 hours.
- Then place the samples from the oven in a desiccator and allow to cool to room temperature.
- Weigh the samples. Also determine a blank samples with an empty FiberBag.



Picture 2. Heating FibreBag with samples in sulfuric acid (0.13M sulfuric acid)

Calculation:

The amount of crude fiber is determined on the basis of the following formula:

$$\text{Raw fiber} = \frac{((\chi - \alpha) - (\delta - \zeta)) * 100}{\beta} \quad [\%]$$

$$\text{Blank value } \zeta = \delta - \psi$$

Where:

α = the mass of the FibreBag in g,
β = the mass of the sample in g,
χ = the mass of the crucible and dried FibreBag after boiling in g,
δ = the mass of the crucible and ash in g,
ζ = the blank value of the empty FibreBag in g,
ψ = the mass of the crucible in g G.

References:

ADAMS, M.A., CHEN, Z., LANDMAN, P. and COLMER, T.D. Simultaneous determination by capillary gas chromatography of organic acids, sugars, and sugar alcohols in plant tissue extracts as their trimethylsilyl derivatives. *Analytical biochemistry*. 1999, **266**(1), 77-84.

AHUJA, S.,. High-pressure liquid chromatography. Comprehensive Analytical Chemistry, 47, pp.485-559.Borman, S., 1987. Eluent, Effluent, Eluate, and Eluite. *Analytical Chemistry*. 2006, **59**(2), 99A-99A.

BROWN I.J, TZOULAKI I, CANDEIAS V. and ELLIOTT, P. Salt intakes around the world: Implications for public health. *International Journal of Epidemiology*. 2009, **38**, 791–813.

CAI, L. Thin layer chromatography. *Current Protocols Essential Laboratory Techniques*. 2014, **8**(1), 3-6.

CAPRITA, R., CAPRITA, A. and CRETESCU, I. Determination of lactose concentration in milk serum by refractometry and polarimetry. *Scientific Papers: Animal Science and Biotechnologies*. 2014, **47**(1), 158-216.

CHAKRABORTY, J.N. ed. Fundamentals and practices in colouration of textiles. 2015, CRC Press.

CHAUNIER, L., DELLA VALLE, G. and LOURDIN, D. Relationships between texture, mechanical properties and structure of cornflakes. *Food Research International*. 2007, **40**, 493–503.

CHEN, L. and OPARA, U.L. Texture measurement approaches in fresh and processed foods—A review. *Food research international*. 2013, **51**(2), 823-835.

Dunnschicht-Chromatographie (HPTLC). Kontakte (Merck) 3:16-34.

DESTEFANIS, G., BRUGIAPAGLIA, A., BARGE, M. T. and DAL MOLIN, E. Relationship between beef consumer tenderness perception and Warner-Bratzler shear force. *Meat Science*. 2008, **78**(3), 153–156.

ĐORĐEVIĆ, Đ., BUCHTOVÁ, H. and MACHARÁČKOVÁ, B. Salt microspheres and potassium chloride usage for sodium reduction: Case study with sushi. *Food Science and Technology International*. 2018, 24(1), pp.3-14.

HAHN-DEINSTROP, E. Applied Thin-Layer Chromatography, 2nd ed. Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim, Germany. 2006.

HAOUET, M.N., ALTISSIMI, M.S., FRAMBOAS, M. and GALARINI, R.. Validation of the Volhard method for chloride determination in food. *Accreditation and Quality Assurance*. 2006, **11**(1), 23-28.

HE, F.J. and MACGREGOR G.A.. Reducing population salt intake worldwide: From evidence to implementation. *Progress in Cardiovascular Diseases*. 2010, **52**: 363–382.

JAMES, B., YOUNG, A., SMITH, B., KIM, E., WILSON, A. and MORGENSTERN, M.P.. Texture changes in bolus to the "point of swallow" — Fracture toughness and back extrusion to test start and end points. *Procedia Food Science*. 2011, **1**, 632–639

JIANG, M.K., WANG, Y.F., van SANTEN, E. and CHAPPELL, J.A. Evaluation of textural properties of channel catfish (Ictalurus punctatus Rafinesque) fillet with the natural contour method. *LWT- Food Science and Technology*. 2008, **41**, 1548–1554.

KAMAL, M.A. and KLEIN, P. Determination of sugars in honey by liquid chromatography. *Saudi journal of biological sciences*. 2011, **18**(1), 17-21.

KUBICA, P., KOT-WASIK, A., WASIK, A., NAMIEŚNIK, J. and LANDOWSKI, P.. Modern approach for determination of lactulose, mannitol and sucrose in human urine using HPLC–MS/MS for the studies of intestinal and upper digestive tract permeability. *Journal of Chromatography B*. 2012, 907, 34-40.

LAWLESS, H.T. and HEYMANN, H.H. Sensory evaluation of food: Principles and practices. New York, NY: Chapman and Hall. 1998.

LEONG, J., KASAMATSU C., ONG, E., HOI, J.T., and LOONG, M.N.. A study on sensory properties reduction and replacement in Asian food using difference-from – Control test. *Food Science and Nutrition*. 2016, **4**: 469–478.

MAJORS, R.E. and HINSHAW, J.. The chromatography and sample preparation terminology guide. *LC-GC North America*, 2013, **31**(10), S43-S43.

MEDEIROS, P.M. and SIMONEIT, B.R.. Analysis of sugars in environmental samples by gas chromatography–mass spectrometry. *Journal of Chromatography A*. 2007, **1141**(2), pp.271-278.

MORAWSKI, R.Z.. September. On food, spectrophotometry, and measurement data processing. In Proc. of 12th IMEKO TC1 & TC7 Joint Symposium'Man Science & Measurement. 2008, 7-20.

NIELSEN, S.S. Sodium determination using ion-selective electrodes, Mohr titration, and test strips. In Food analysis laboratory manual (pp. 161-170). Springer, Cham. of crisps from four potato varieties. *Engineering Failure Analysis*. 2017, **16**, 2698–2704

PÁPAI, Z. and PAP, T.L. Analysis of peak asymmetry in chromatography. *Journal of Chromatography A*. 2002, **953**(1-2), 31-38.

POOLE, C.F. and POOLE, S.K. Foundations of retention in partition chromatography. *Journal of Chromatography A*. 2009, **1216**(10), 1530-1550.

RAMASAMI, P., JHAUMEER-LAULLOO, S., RONDEAU, P., CADET, F., SEEPUJAK, H. and SEERUTTUN, A.. Quantification of sugars in soft drinks and fruit juices by density, refractometry, infrared spectroscopy and statistical methods. *South African Journal of Chemistry*. 2004, 57.

RODRIGUES, D.M., SOUZA, V.R., MENDES, J.F., NUNES, C.A. and Pinheiro A.C.M. Microparticulated mix: An alternative to reducing sodium in shoestring potatoes. *LWT—Food Science and Technology*. 2016, **69**, 390–399.

ROJO, F.J. and VINCENT, J.F. Objective and subjective measurement of the crispness of crisps from four potato varieties. *Engineering Failure Analysis*. 2009, **16**(8), 2698-2704.

ROLLE, L., SIRET, R., RÍO SEGADE, S., MAURY, C., GERBI, V. and JOURJON, F. Instrumental texture analysis parameters as markers of table-grape and winegrape quality: A review. *American Journal of Enology and Viticulture*. 2012, **63**, 11–28.

SWARTZ, M. HPLC detectors: a brief review. *Journal of Liquid Chromatography & Related Technologies*. 2010, **33**(9-12), 1130-1150.

TANIWAKI, M., HANADA, T. and SAKURAI, N. Device for acoustic measurement of food texture using a piezoelectric sensor. *Food Research International*. 2006, **39**, 1099–1105.

ZHU, Y., HSU, W.H. and HOLLIS, J.H. The impact of food viscosity on eating rate, subjective appetite, glycemic response and gastric emptying rate. *PLoS One*. 2013, **8**(6), 67482.

This work was co-funded by the Erasmus+ Programme of the European Union

Innovation of the structure and content of study programs profiling food study fields with a view to digitizing teaching

Táto publikácia bola spolufinancovaná programom Európskej Únie Erasmus+

Inovácia štruktúry a obsahového zamerania študijných programov profilujúcich potravinárske študijné odbory s ohľadom na digitalizáciu výučby

FOODINOVO | 2020-1-SK01-KA203-078333

Funded by the European Union. Views and opinions expressed are however those of the author(s) only and do not necessarily reflect those of the European Union or the European Education and Culture Executive Agency (EACEA). Neither the European Union nor EACEA can be held responsible for them.

Financované Európskou úniou. Vyjadrené názory a postoje sú názormi a vyhláseniami autora(-ov) a nemusia nevyhnutne odrážať názory a stanoviská Európskej únie alebo Európskej výkonnej agentúry pre vzdelávanie a kultúru (EACEA). Európska únia ani EACEA za ne nepreberajú žiadnu zodpovednosť.

FOODINOVO | 2020-1-SK01-KA203-078333