Hort4EUGreen

Strategic Partnership No. 2020-1-RO01-KA203-080398

NUTRITIONAL QUALITY OF HORTICULTURAL PRODUCTS

Laboratory methods for nutritional quality analysis. Practical training module.

University of Agronomic Sciences and Veterinary Medicine of Bucharest
Лесотехнически университет
MATE

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"Enhancing practical skills of horticulture specialists to better address the demands of the European Green Deal"

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Laboratory methods for nutritional quality analysis.

Practical training module

Summary

The Practical training module is intended to provide the underlying principles, and practical training in the physicochemical analysis of food samples. The module will consist of practical laboratory operations and onsite question and answer sessions to cover the following topics: sample preparation and treatment, sample analysis, and data treatment. The course is comprised of simple (usual) and more advanced analytical methods which will develop the skills of the working understanding of MS and PhD students.

Learning outcome descriptors

By the end of the Module, the trainee should be able to:

General and transferable skills

1	Prepare a working station for specific analytical method
2	Work in the laboratory independently or with a minimal guidance where appropriate
3	Work in team with minimal guidance where appropriate
4	Show good laboratory skills
5	Demonstrate the capacity to understand the obtained results in order interpret the information from a variety of analytical methods

Knowledge, understanding and professional skills

1	Select the appropriate analytical method for determination of nutritional quality of different type of fruits
2	Gain working knowledge in laboratory approach using different methods of characterization.
3	Interpretation of a result based on the obtained data.

Unit 1. Determination of dry matter

Ioana Cătuneanu,
Andreea Barbu

1.1. Method principle

This method determines the moisture as a percentage mass loss of a sample, by heating under certain conditions depending on the nature of the matrix. The difference in mass of a sample quantity before and after the drying process is a measure of the amount of dry matter and water.

1.2. Apparatus and reactive

Method 1

- Laboratory mill;
- Oven with thermostat and forced air ventilation, capable of maintaining a temperature between 70 °C and 130 °C ± 5 °C;
- Desiccator with an active dehydrating agent;
- Analytical balance, to the nearest ± 1 mg;
- Glass pans with a sealed lid so that they do not absorbmoisture, with a capacity of 25 mL to 100 mL;

Method 2

- Laboratory mill;
- Thermobalance;
- Drying trays made of a non-absorbent material such as glass or aluminum.

1.3 Sample preparation

- Avoid leaving the sample in prolonged contact with the surrounding atmosphere;
- Remove large foreign bodies from the sample;
- Homogenize the laboratory sample well before taking the working sample.

Samples that *do not require* grinding:

It is not necessary to grind the products that have a particle size distribution similar to the ones shown in **Table 1.**

Particle size (mm)	Ratio %
< 1.7	100
< 1.0	70
< 0.5	20

Table 1. Particle size distribution of products that do not require grinding

Samples that *require* grinding:

If the samples do not meet the particle size characteristics listed in **Table 1**, they must be grinded.

Grinding

Quickly grind a quantity of the laboratory sample by adjusting the mill to obtain particles of the dimensions indicated in Table 1.

1.4. Sample analysis

Weighing pan conditioning

Before drying, the pan is brought to a constant mass together with the lid in which the sample whose humidity we want to determine is inserted. This is done by keeping it in the oven at 105 °C (fruits and vegetables) or 130 °C (for cereals and food samples) for 1 hour, followed by storage in the desiccator for 30 min and weighing with an accuracy of 0.001 g.

Note the mass of the capsule and the lid. The procedure is repeated, and the mass is considered to be constant, if the difference between two successive readings differs only to the third decimal place. All weights are noted in the weighing books.

Method 1 - Method using oven drying

→ Weigh quickly a quantity of 5 ± 0.001 g of the laboratory sample into the conditioned weighing pan (which was previously weight). Note the mass of the working sample and the pan with the lid.

- Place the open vial containing the working sample, together with its lid, in the oven and leave for 2 h at 105°C (fruits and vegetables) or 130 °C (for cereals and food samples) time required for the sample to dry.
- Quickly remove the vial from the oven with a pair of pliers or cotton gloves, place the lid on and place in the desiccator. Expect to reach ambient temperature (about 30 - 45 minutes).
- After cooling, weigh the vial containing the sample, together with its lid, to the nearest 0.001 g. Note the mass of the dry working sample and the vial with the lid.
- The procedure is repeated, and the mass is considered to be constant, if the difference between two successive readings differs only to the third decimal place.

Note: *Do not open the oven door during drying and do not put wet products in the oven before removing the dry work samples, as this will lead to partial rehydration of the samples.*

Moisture (M%) is expressed as g/100 g of sample and is given by the equation:

$$M\% = \frac{m_i - m_f}{m_i} \text{ x } 100 \quad (1)$$

where:
M% – moisture
mi – initial mass of wet sample (g);
mf – final mass of dried sample (g);

Method 2 - Method using thermobalance

- Open up the thermobalance from the on/off button;
- Set the drying temperature 105 °C (fruits and vegetables) or 130 °C (for cereals and food samples);
- Place the conditioned aluminum tray in the specially provided place and tare.
- Place at least 1g of the ground sample as scattered as possible in the aluminum tray, note the weighted mass and start the analysis (the analysis time will be determined automatically by the thermal balance depending on the humidity of the sample);
- At the end of the analysis the results (mass of the dry sample expressed in grams, humidity expressed as a percentage and dry matter expressed as a percentage) will be automatically displayed. Note the results in the books.

Unit 1.2. Determination of leaf physiological parameters using multiparameter portable equipment LC Pro-SD

Liliana BĂDULESCU,
Monica BADEA

2.1. Method principles

a. The photosynthesis intensity is determined based on CO_2 quantity consumed by a given leaf area during analysis, using non-dispersive infrared gas analyzer.

b. The transpiration intensity is determined based on H_2O quantity eliminated by a given leaf area,using non-dispersive infrared gas analyzer.

c. The respiration intensity is determined based on CO_2 quantity produced by a given leaf area, in dark conditions, using non-dispersive infrared gas analyzer.

2.2. Apparatus and reactive

- analysis chamber with known area (usually 6,25 cm^2);
- multiparameter portable equipment LC Pro-SD;

LC Pro-SD is portable and it is recommended to determine directly in the field several physiological indicators. The results are stored in the equipment computer and stored on a 256 KB floppy disk, too.

Different analysis chambers can be connected to the analyzer, depending on the type of leaves. For large area leaves, connect the chamber with an area of 6.25 cm^2. The analysis chamber delimits a space so that air is taken from both sides of the leaf with a fun from the base which introduces it into the analyser.

The analyser has a sensor for light intensity, one for temperature, and another for humidity. The software of multiparameter analyser LC Pro-SD calculate and display more than 18 parameters and measurements could be read or memorize into the disk.

2.3 Sample analysis

In order to determine the leaf physiological parameters check if the equipment is connected with appropriate analysis chamber and let the equipment to stabilize around 5 min (it will signal when stable). The device is set with certain coefficients that allow it to automatically calculate the physiological parameters (**A = Intensity of photosynthesis**; **E = Intensity of transpiration)**.

Turn on the appliance and leave it to warm up for about 5 minutes (until a squeak is heard). Choose one leaf undetached from the plant and introduce it in the reading chamber.

The temperature of the leaf (Tleaf = ˚C), the air flow (ml min-1), as well as the light intensity (Qleaf = µmol m-2s-1) are measured instantly. Wait few minutes in order to adapt the leaf to the desired light intensity before to aquire data.

Also, on the screen of the device, in addition to these values are shown: the intensity of the transpiration process (E = mmol H_2O m-2s-1), the intensity of the photosynthesis process (A = µmol CO_2 m-2s-1) and the stomatal conductance (gs). The minus value for photosynthesis means that less CO_2 is consumed in photosynthesis, compared to the amount of CO_2 released into the respiration.

To determine the intensity of the respiration, the analysis chamber with undetached leaf is inserted into a black cover to prevent light from penetrating and thus to suppress photosynthesis. The characteristic value is displayed on the screen as a negative value.

Unit 1.3. Determination of respiration rate for fruits and vegetables

Liliana BĂDULESCU, Monica BADEA,
Ioana CĂTUNEANU

3.1. Method principles

The intensity of respiration for fruits and vegetables is determined based on measurement of the amount of carbon dioxide released in one hour by the plant material into a container of known volume, using infrared technology.

3.2. Apparatus and reagents

- Sealed containers of known volume;
- Clamp;
- Weighing trays;
- Volumetric cylinder, 1000 mL;
- CO_2 monitoring equipment - Lambda T NDIR;
- Analytical balance - Radwag AS60/220.R2;

3.3 Sample analysis

Equipment preparation

Check that the device is working and records the initial CO_2 value between 400 - 600 ppm.

Preparation of samples for analysis (raw material)

Choose 3 fruits / vegetables from the same sample and place them in the dark sealed containers. For berries and plums, the intensity of the respiration rate is measured after 10 minutes; for apples the respiration rate is measured after 30 minutes.

After the mention time has passed, first measure CO_2 concentration in the air (CO_2i) and afterword measure the concentration of CO_2 inside the sealed container containing the fruits/vegetables (CO_f).

After measuring the CO_2 concentration of the fruits/vegetables, weigh the sample using the analytical balance and then measure the sample volume in a 1000 mL graduated cylinder using water.

Calculation of results

The values read with the Lambda T NDIR Monitor equipment are entered in an excel table and calculated according to the following formula:

$$R_{rate} = \frac{\frac{CO_{2f} - CO_{2i}}{10000} \times V \times 18.4 \times 60}{m} / t \quad (2)$$

R_{rate} – respiration rate (mg CO_2/Kg/h)
V (mL) – sample volume (mL)
CO_{2f} - sample CO_2 concentration at the end of experiment
CO_{2i} - air CO_2 concentration at the beginning of experiment
m – sample mass (g)
t – time of analysis (min)

Unit 1.4. Determination of transpiration rate using weighing method

Liliana BĂDULESCU, Monica BADEA,
Ioana CĂTUNEANU

4.1. Method principle

The purpose of this method is to determine the intensity of transpiration rate of fruits and vegetables. The method is based on weighing the sample, aftera specific period of time (ex., 10 minute after). Based on mass differences the transpiration rate is calculated.

4.2. Apparatus and reagents

- Weighing trays;
- Analytical balance;
- Timer;

4.3 Sample analysis

Choose 3 fruits / vegetables from the same sample and place them on the weighing trays. For berries and plums chose a larger sample (6-10 fruits). Note the initial weight (w_i), and start the timer for 10 min, and after the time has passed weight the samples again (w_f) .

Calculation of results

$$T_{rate} = \frac{w_i - w_f}{w_i} \times 100/t \quad (3)$$

where:
T_{rate} = transpiration rate (g H_2O/100 g sample/h).
w_i - initial weight (g)
w_f- initial weight (g)
t – time (h)

Unit 1.5. Determination of assimilating pigments (chlorophylls and carotenoids)

Aurora Dobrin, Monica Badea, Liliana Bădulescu

5.1. Method principle

This method is based on the extraction of pigments in an organic solvent, for example 80% acetone, and then measuring the extract absorbance using a spectrophotometer, at the wavelengths of 470 nm, 646 nm, and 663 nm.

5.2. Apparatus and reage

Reagents

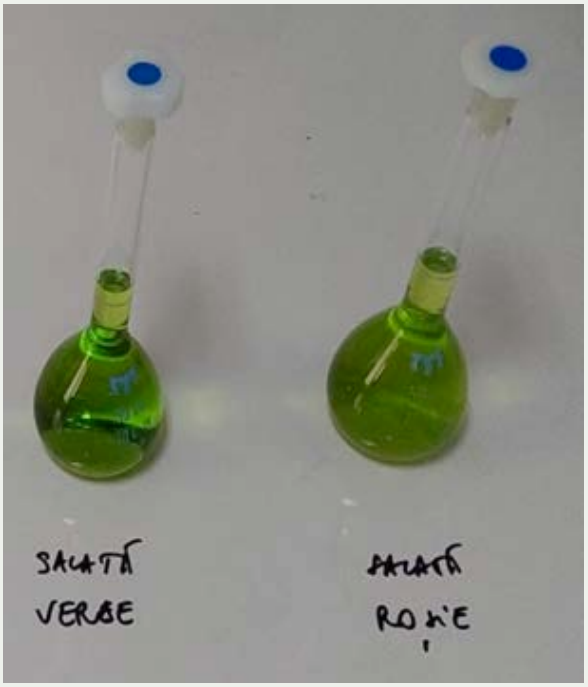
- Ultrapure water;
- Acetone;
- Quart sand;

Apparatus

- Mortar;
- Volumetric flask, 50 ml;
- Vacuum filtration unit;
- Filter paper;
- Funnel;
- Analytical balance;
- Mortar;

5.3 Sample preparation

Weigh 1 g of fresh fruit/vegetable material, triturate in presents of quart sand, until a homogenous paste is formed. Add 5 mL of acetone keep triturating, and then pass the extract in the vacuum filtration unit, which has a filter paper. Wash the mortar several times with acetone, until there is no more extraction of chlorophyll, and then pass the extract in the 50 mL volumetric flask, which already has 10 mL of ultrapure water. Complete the flask to mark with acetone.



5.4. Sample analysis

After the spectrophotometer has achieved the use parameters, the filtered extract is transferred in a quartz cuvee with a 1 cm path length. Use as reference acetone 80%. Set the wavelength to 470 nm, 646 nm, and 663 nm, and read the sample using the equipment software.

Calculation of results

Knowing the sample absorbance at all the wavelengths you can calculate the quantity of the pigments using the formulas by Lichtenthaler and Wellburn (1983):

$$C_a = 12.21A_{663} - 2.81A_{646}\ \mu g/mL\ (4)$$

$$C_b = 20.13A_{646} - 5.03A_{663}\ \mu g/mL\ (5)$$

$$C_{x+c} = \frac{1000\ A_{470} - 3.27C_a - 104\ C_b}{229}\ \mu g/mL\ (6)$$

The results can be further calculated for mass and final extraction volume and the final results expressed as mg/g.

REFERENCE

Lichtenthaler, H. K., & Wellburn A. R. (1983). Determinations of total carotenoids and chlorophylls a and b in leaf extracts in different solvents. Biochem.Soc. Trans. 11, 591-592.

Delian, E., Burzo, I., Voican, A., Dobrescu, A., Bădulescu, L. & Mihailescu, D. (2003). Fiziologia plantelor, Lucrări Practice, Atelierul de multiplicat cursuri.

Ion, V.A., Nicolau, F., Petre, A., Bujor, O.C. & Bădulescu, L. (2020). Variation of bioactive compounds in organic Ocimum basilicum L. during freeze-drying processing, Scientific Papers. Series B, Horticulture. Vol. LXIV, No. 1, 397 – 404.

Unit 1.6. Determination of total polyphenol content using Folin-Ciocâlteu method

Oana Crina Bujor, Violeta
Alexandra Ion, Aurora Dobrin

6.1 Method principle

The Folin–Ciocalteu method is widely used to determine the total phenolic content of plant and food extract samples. The principle of this method is the reduction of the reagent in the presence of phenols resulting in the production of molybdenum–tungsten blue that is measured spectrophotometric at 760 nm and the intensity increases linearly with the concentration of phenols in the reaction medium.

6.2. Apparatus and reactive

- Centrifuge tubes 15, 50 mL;
- 10 mL vials;
- Analytical balance;
- Centrifuge with cooling membrane;
- Orbital shaker;
- Folin - Ciocalteu reagent 0.2 N;
- Sodium carbonate solution 7.5 %;
- Water bath;
- Timer;
- Spectrophotometer

6.3 Sample preparation

Extraction of polyphenols from fresh samples will be based on the method described by Stan et al. (2021). To 1 g of fresh sample add 10 mL of 70 % aqueous methanol and incubate in the dark overnight at room temperature. The next day, the extracts are shaken at 500 rpm for 1 h and then centrifuged at 5000 rpm, 4 °C, for 10 min. Recover the supernatant in a 50 mL centrifuge tube and re-extract the residue two more times with 10 mL of 70% aqueous methanol. All three supernatants will be combined and the volume of each sample will be adjusted to 30 mL with 70% aqueous methanol.

6.4. Sample analysis

The total phenolic content of the extract solutions will be determined by the Folin - Ciocalteu spectrophotometric method described by George et al. (2005). Measure 0.5 mL of sample extract in a 10 mL vial, add 2.5 mL of Folin - Ciocalteu reagent and incubate for 2 min at room temperature.

After incubation add 2 mL of sodium carbonate solution (7.5%) and place the vial in a water bath heated at 50 °C, for 15 min. After the heating time has passed, cool the vial on water-ice bath.

After the spectrophotometer has achieved the use parameters, the cooled extract is transferred in a quart cuvee with a 1 cm path length. Use as reference methanol 70%. Set the wavelength to 760 nm and read the sample using the equipment software.

Calculation of results

The concentration of the extract (µg/mL) is calculated based on a calibration curve using gallic acid as analytical standard. After the concentration in the extract has been determined, the following step is to calculate the concentration of total polyphenols as follows:

$$C_{(mg\,GAE/100\,g)} = \frac{C_{(\mu g/mL)} * V_{(mL)} * D}{m_{(g)} * 10} \quad (7)$$

where:
C (mg GAE/100 g) – concentration of total phenolic content reported as mg Gallic acid equivalents (GAE) per100 g of sample;
C (µg/mL) – concentration of total polyphenols in sample extract;
V (mL) – total extract volume (30 mL);
m (g)- sample mass (1 g)
D - dilution of the extract

REFERENCE

Stan, A., Frîncu, M., Ion, V.A., & Bădulescu, L. (2021). Modified Atmosphere Influence In Organic 'Tita' Plums Quality, Scientific Papers. Series B, Horticulture. 65(2), 77-82.
Georgé, S., Brat, P., Alter, P. & Amiot, J.M. (2005). Rapid determination of polyphenols and vitamin C in plant-derived products. Journal of Agricultural and Food Chemistry, 53, 1370–1373.

Unit 1.7. Determination of vitamin C using HPLC-DAD

Andreea Barbu, Oana-Crina Bujor,
Violeta Alexandra Ion,
Liliana Bădulescu

7.1. Method principle

The method determines the ascorbic acid (vitamin C) from fresh fruits or vegetables by extracting the analyte with o-phosphoric acid. The vitamin C is identified and quantified using HPLC-DAD detector. The separation principle of HPLC is based on the distribution of the analyte (sample) between a mobile phase (eluent) and a stationary phase (packing material of the column). Depending on the chemical structure of the analyte, the molecules are retarded while passing the stationary phase.

7.2. Apparatus and reagents

Reagents

- Ultrapure water;
- Ortho - phosphoric acid;
- Formic acid;
- Ascorbic acid;
- Acetonitrile;

Apparatus

- Ultrapure water;
- 20 mL graduated cylinder;
- 20 mL graduated cylinder;
- 10 mL volumetric flask;
- 1000 mL volumetric flask;
- 15 mL centrifuge tubes;
- 1.5 mL vials;
- vial caps;
- Micropipette 10-100 µL;
- Micropipette 100-1000 µL;
- RC syringe filters;
- 2 mL syringe;
- Magnetic hob;
- Vortex;
- Analytical balance;
- centrifuge;
- HPLC-DAD system;
- C18 column (4.6 x 50 mm, 1.8 µm i.d.);
- analytical guard column C18 (4.6 x 12.5 mm, 5 µm i.d.);

7.3. Sample preparation

To 1 g of raw material will be added 2 mL of o-phosphoric acid (2 %, v/v) and triturated for 1 minute at room temperature. The mixture will be quantitatively passed into 15 mL centrifuge tubes, and brought to a final volume of 10 mL with o-phosphoric acid (2%, v/v).

After extraction, all samples will be centrifuged for 5 minutes with 7000 rpm and 4 °C, filtered and immediately analyzed by HPLC-DAD.

7.4. Sample analysis

Ascorbic acid identification and quantification is realized through High Performance Liquid Chromatography (HPLC) using a DAD detector based on the method from Stan et al., (2020). Chromatographic separation of compounds will be performed using a C18 column and analytical guard column C18. The temperature of the column during analysis will be kept at 30 °C. The injection volume will be 2 µL, with a recommendation that the samples would be stored in the autosampler at a low temperature due to fast degradation of ascorbic acid. The mobile phase consists of 0.05% formic acid in water, v/v, using an isocratic elution at a flow rate of 0.5 mL/min. The ascorbic acid is determined at a wavelength of 244 nm.

Calculation of results

The concentration of ascorbic acid of the extract (µg/mL) is calculated based on a calibration curve (usually 0 - 200 µg/mL, exception for kiwi 0 - 400 µg/mL).

After the concentration in the extract has been determined, the following step is used to calculate the concentration of ascorbic acid in the sample as follows:

$$C_{(mg\,GAE/100\,g)} = \frac{C_{(\mu g/mL)} * V_{(mL)} * D}{m_{(g)} * 10} \quad (8)$$

where:
C (mg GAE/100 g) – concentration of total phenolic content reported as mg Gallic acid equivalents (GAE) per100 g of sample;
C (µg/mL) – concentration of total polyphenols in sample extract;
V (mL) – total extract volume (30 mL);
m (g)- sample mass (1 g)
D - dillution of the extract

REFERENCE

Stan, A., Bujor, O.C, Dobrin, A., Haida, G., Bădulescu, L. & Asănică, A. (2020). Monitoring the quality parameters for organic raspberries in order to determine the optimal storage method by packaging. Acta Hortic. 1277: 461-468, Proc. XII International Rubus and Ribes Symposium: Innovative Rubus and Ribes Production for High Quality Berries in Changing Environments, DOI 10.17660/ActaHortic.2020.1277.66

Unit 1.8. Determination of sugars using HPLC-RID

Andreea Barbu, Liliana Bădulescu

8.1. Method principle

The sugars from fresh fruits and vegetables are determined by extraction with 80 % ethanol solution, followed by concentration and filtration. The identification and quantification of sugars are done by HPLC -RID technique. The separation principle of HPLC is based on the distribution of the analyte (sample) between a mobile phase (eluent) and a stationary phase (packing material of the column). Depending on the chemical structure of the analyte, the molecules are retarded while passing the stationary phase.

8.2. Apparatus and reagents

Reagents

- Ultrapure water;
- Ethanol;
- Acetonitrile;
- Sugars (glucose, fructose, sucrose, others);

Apparatus

- RC syringe filters;
- 2 mL syringe;
- Erlenmeyer flask;
- 10 mL volumetric flask;
- 50 mL round-bottomed flasks;
- Pasteur pipettes;
- 1.5 mL vials;
- vial caps;
- weighing trays;
- centrifuge tubes15, 50 mL;
- Analytical balance;
- Rotary evaporator;
- HPLC-RID ;
- NH2 column, 4.6 x 250 mm, 5 µm ;
- NH2 guard cartridge, 4.6 x 12.5 mm, 5 µm;

8.3. Sample preparation

Weight 1 g of raw material, add 2 mL of ethanol (80%, v/v) and triturated for 1 minute at room temperature. The mixture will be quantitatively passed into an Erlenmeyer flask at a volume of 10 mL and extracted in an ultrasonic bath for 15 min at 80 °C. Supernatant will be collected in a 50 mL centrifuge tube and residues will be re-extracted with 10 mL of ethanol (50%, v/v) in an ultrasonic bath for 15 min at 80 °C.

The second supernatant will be collected in the same 50 mL centrifuge tube and residues will be re-extracted with 10 mL of water in an ultrasonic bath for 15 min at 80 °C. The third supernatant will be reunited with previous two supernatants, reaching a 30 mL final volume of extract.
The obtained extract (30 mL) will be passed in a 50 mL round-bottomed flasks and evaporated at a rotary evaporator to a volume below 10 mL.
After evaporation extract will be passed in a 10 mL volumetric flask and add ultrapure water to the mark. The extract will be filtered with a 2 mL syringe and a 0.45 µm RC filter, in a 1.5 mL vial and immediately analyzed by HPLC.

8.4. Sample analysis

Mobile phase should be ultrasounded for 5 minutes before system conditioning and analysis. The aqueous mobile phase shall not be used for more than 2-3 days. The sample should be prepared as soon as possible to avoid compounds denaturation/oxidation.

Chromatographic separation is performed using HPLC method, a RID detector and a NH_2 column (4.6 x 250 mm, 5µm i.d.). The mobile phase is a mixture of ACN with H2O (85:15) using an isocratic elution at a flow rate of 1.8 mL / min. The temperature of the column thermostat and the detector will be set at 30 °C, and the injection volume of the sample will be 30 µL.

Calculation of results

The concentration of sugar in of the extract (mg/mL) is calculated based on a calibration curve (0-10 mg/mL). After the concentration in the extract has been determined, the following step is used to calculate the concentration of ascorbic acid in the sample as follows:

$$C_{(mg\,GAE/100\,g)} = \frac{C_{(\mu g/mL)} * V_{(mL)} * D}{m_{(g)} * 10} \quad (9)$$

where:

C (mg GAE/100 g) – concentration of total phenolic content reported as mg Gallic acid equivalents (GAE) per100 g of sample;

C (µg/mL) – concentration of total polyphenols in sample extract;

V (mL) – total extract volume (30 mL);

m (g)- sample mass (1 g)

D - dillution of the extract

Unit 1.9. Determination of volatile compounds using GC-MS

Violeta Alexandra Ion,
Oana-Crina Bujor

9.1. Method principle

The volatile oils are extracted by hydro-distillation using a Neoclevenger apparatus. The obtained volatile oils are diluted in hexane and analyzed using GC-MS equipment. Results are expressed as a percentage of the identified compounds.

9.2. Apparatus and reagents

Reagents

- Hexane;
- Anhydrous sodium sulfate;
- Water;

Apparatus

- 10 mL graduated cylinder;
- Tubes;
- Micropipette 10-100 µL;
- Micropipette 100-1000 µL;
- Syringe filter PTFE 0.45 µm;
- Neoclevenger volatile oil extraction system;
- Analytical balance;
- Heating nest LabHeat;
- Freezer;
- GC- MS System;
- Chromatographic column DB 5 MS, length 30m 0.25x0.25x0.25 mm;

9.3. Sample preparation

A sufficient quantity of sample (around 300 g of fresh material) is spread on a clean filter paper and any other plant material that does not belong to the sample is removed.

With a technical balance weight the amount needed to perform the analysis which depends on the type of matrix. The weighted plant material is introduced into the 4 L round bottom flask which is placed in the heating nest. Add water over the sample from the 4 L flask (until its level slightly exceeds the level of the heating nest).

Position the **Neoclevenger system** over the balloon and attach it to the stand. Release the cooling water to cool the refrigerant. Close the tap of the Neoclevenger and fill it with distilled water, be careful the funnel must always have water in it. Plug in the heating nest and set it to maximum, initially on a heating speed of 10 and then, after boiling, on a speed of 5; then press the ON button.

From the moment the water with the sample starts boiling, the extraction lasts 3 hours (note in the notebook). At the end of the extraction, the total volume of oil on the Neoclevenger system is measured, then the water and oil are transferred to a test tube.

After transfer to the test tube, the oil is collected with a micropipette in a 1.5 mL Eppendorf tube and frozen at -20 °C until analysis.

The 4 L flask is allowed to drain and cool. Discard the plant residues from the flask and rinse it several times with water, ethanol and distilled water. The Neoclevenger will be rinsed with tap water, ethanol and distilled water.

REFERENCE

Ion, V.A., Nicolau, F., Petre, A., Bujor, O.C. & Bădulescu, L. (2020). Variation of bioactive compounds in organic *Ocimum basilicum* L. during freeze-drying processing, Scientific Papers. Series B, Horticulture. Vol. LXIV, No. 1, 397 – 404.

Unit 1.10. Determination of fatty acids (saturated and unsaturated) using GC-MS

Violeta Alexandra Ion,
Oana-Crina Bujor

10.1. Method principle

The fat is saponified, which liberates the fatty acids from triglycerides, phospholipids, etc. producing free fatty acids. These are trans-esterified to form fatty acid methyl esters. Matrices that are not pure fats and oils require an extraction step to liberate the fat for analysis. Fatty acids are analyzed by gas chromatography (GC-MS) after conversion to fatty acid methyl esters (FAMEs).

10.2. Apparatus and reagents

Reagents

- Mix of 37 components FAME;
- Ultrapure water;
- Hexane;
- Potassium methylate;
- Anhydrous sodium sulfate;

- Methanol;
- Hydrochloric acid;
- Potassium methylate solution 2 mole/L in methanol: 7.8 g of metallic potassium is dissolved in 100 mL of anhydrous methanol. It is prepared fresh on the day of use. Sodium methylate of the same concentration can also be used;
- Anhydrous methanol (molecular sieve): In 1 L of HPLC methanol add 2 fingers of molecular sieve. Shake and prepare at least 24 hours before use;p
- Hydrochloric acid methanol solution w (HCl) ~ 20% (mass fraction): Weigh 80 g of anhydrous methanol and add 54 g of 37% HCl over it, fuming;

Apparatus

- 10 mL graduated cylinder;
- 20 mL test tubes with septum and plug;
- 250 mL Erlenmeyer flask;
- Micropipette 10-100 µL;

Apparatus

- Micropipette 100-1000 µL;
- PTFE syringe filters;
- Magnetic hob IKA RH B2;
- Vortex Bio-Rad BR-2000;
- Refrigerator Bosch;
- Measuring equipment;
- Analytical balance Radwag AS60/220.R2;
- GC Agilent 6890N system;
- MS Agilent 5973N detector;
- Autosampler Agilent 7683 Injector;
- HP-5MS chromatographic column, length 30m, diameter 0.250 mm, film 0.25 µm;

10.3. Sample preparation

Weigh 50 ± 0.1 mg to 75 ± 0.1 mg of the sample for analysis in each of the reaction vials. The vials are treated at the same time as follows:

- Add 4 mL of n-hexane.

- Add about 75 mg of anhydrous sodium sulfate and dissolve the sample for analysis by shaking.
- Add 4 mL of potassium methylate, close the reaction vial and shake hermetically for 20 - 50 s. The solution immediately becomes opalescent due to the formation of glycerin which settles quickly.
- Add 2 mL of 20 % hydrochloric acid solution and a magnetic stir bar.
- Close the reaction vial and place in the heating block, previously brought to 50°C ± 3 °C/heated for 20 minutes under constant stirring. Shake the mixture manually several times during this period. This step can also be done at room temperature. Cool the vial with its contents at room temperature under a stream of cold tap water and shake vigorously. The upper layer containing the methyl esters is separated by decantation. Transfer the upper layer in a vial for GC injection. If the mass of the sample was between 50 mg and 75 mg, the concentration of methyl esters will be about 2% (mass fraction).

The solution is ready for GC-MS analysis. Dilutions of up to 1% are recommended if a split injector is used. Methyl ester solutions are suitable for immediate chromatographic analysis. If necessary, the methyl ester solution can be stored for a few weeks under inert gas at a temperature of 4 - 8 °C.

10.4. Sample analysis

The method of analysis for checking the chromatographic conditions is described below:

- Ultra-inert HP-5MS capillary column (30m x 0.25mm x 0.25 µm film thickness).
- Column oven temperature program: stationary at 50 °C for 4 min, heat from 50 °C to 150 °C at 4 °C/min, heat from 150 °C to 192 °C at 2 °C/min, stationary at 192 °C for 10 min, heat from 192 °C to 300°C at 2 °C/min.
- Helium carrier gas with a flow rate of 0.5 mL/min.
- Injection volume 2 µL, splitless.
-Ionization energy 70 eV, on the mass range 40 - 450 m/z at 2 scan/s.
-The temperatures for inlet, MS transfer line and ion source was 250 °C, 280 °C and 230 °C, respectively.

Results

The compounds will be identified based on retention time and MS spectra comparison with analytical standards. Each compound will be reported as a percentage of the total compounds.

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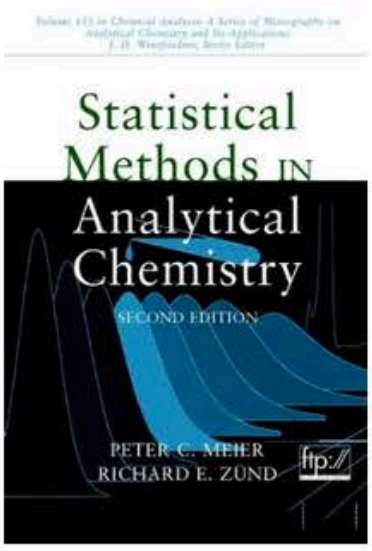
Scientifically validated sources

This new edition of a successful, bestselling book continues to provide you with practical information on the use of statistical methods for solving real-world problems in complex industrial environments. Complete with examples from the chemical and pharmaceutical laboratory and manufacturing areas, this thoroughly updated book clearly demonstrates how to obtain reliable results by choosing the most appropriate experimental design and data evaluation methods.



Title: Statistical Methods in Analytical Chemistry
Author(s):
Peter C. Meier, Richard E. Zünd
First published:30 March 2000
Print ISBN:9780471293637
Online ISBN:9780471728412
DOI:10.1002/0471728411

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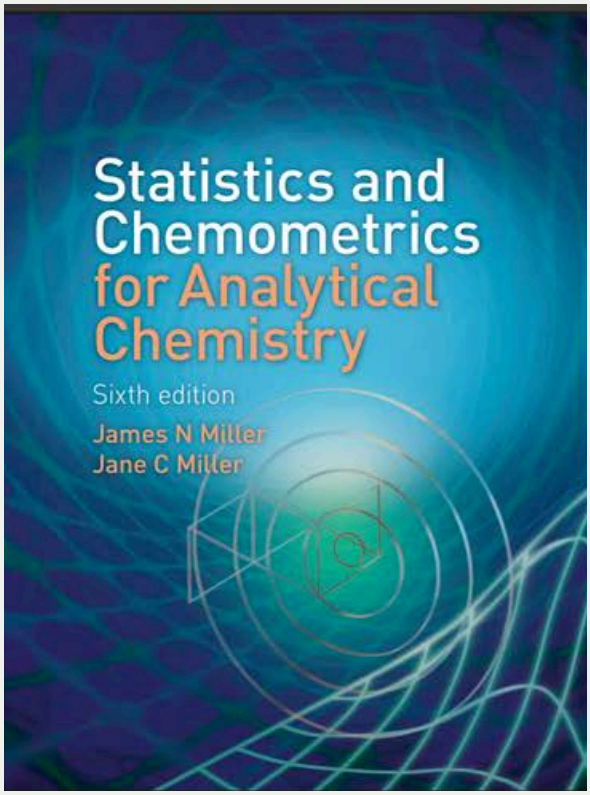
This popular textbook gives a clear account of the principles of the main statistical methods used in modern analytical laboratories. Such methods underpin high quality analyses in areas such as the safety of food, water and medicines, environmental monitoring, and chemical



Title: Statistics and Chemometrics for Analytical Chemistry
Authors: James N. Miller, Jane Charlotte Miller
Publisher: Pearson Education, 2005
ISBN: 0131291920, 9780131291928

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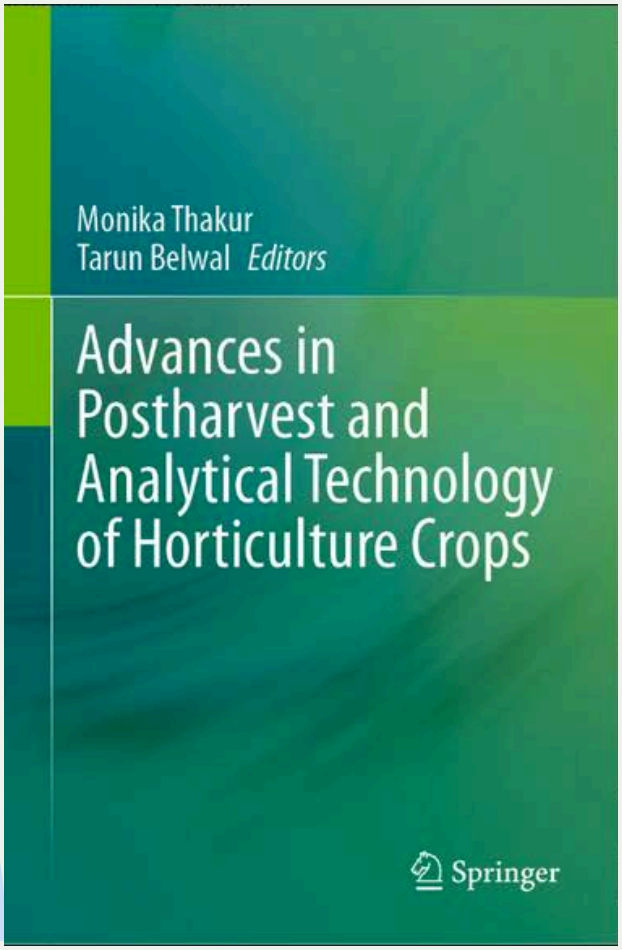
This book discusses advances in postharvest and analytical technology for horticulture crops and challenges to meet future needs. The horticulture crops (fruits and vegetables) need a systematic and scientific postharvest handling and management system for securing both physical and chemical attributes while prolonging their shelf life. Postharvest technologies include storage, drying, packaging, extraction of components, and preparation of juice and wine from the collected fruits and vegetables. Parallel development of analytical techniques has also evolved to monitor the quality of fruits and vegetables during postharvest processing and thus provide a rapid and efficient method for delivering safer food products.



Book Title: Advances in Postharvest and Analytical Technology of Horticulture Crops
Editors: Monika Thakur, Tarun Belwal
DOI: https://doi.org/10.1007/978-981-97-7247-6
Publisher: Springer Singapore
ISBN 978-981-97-7246-9
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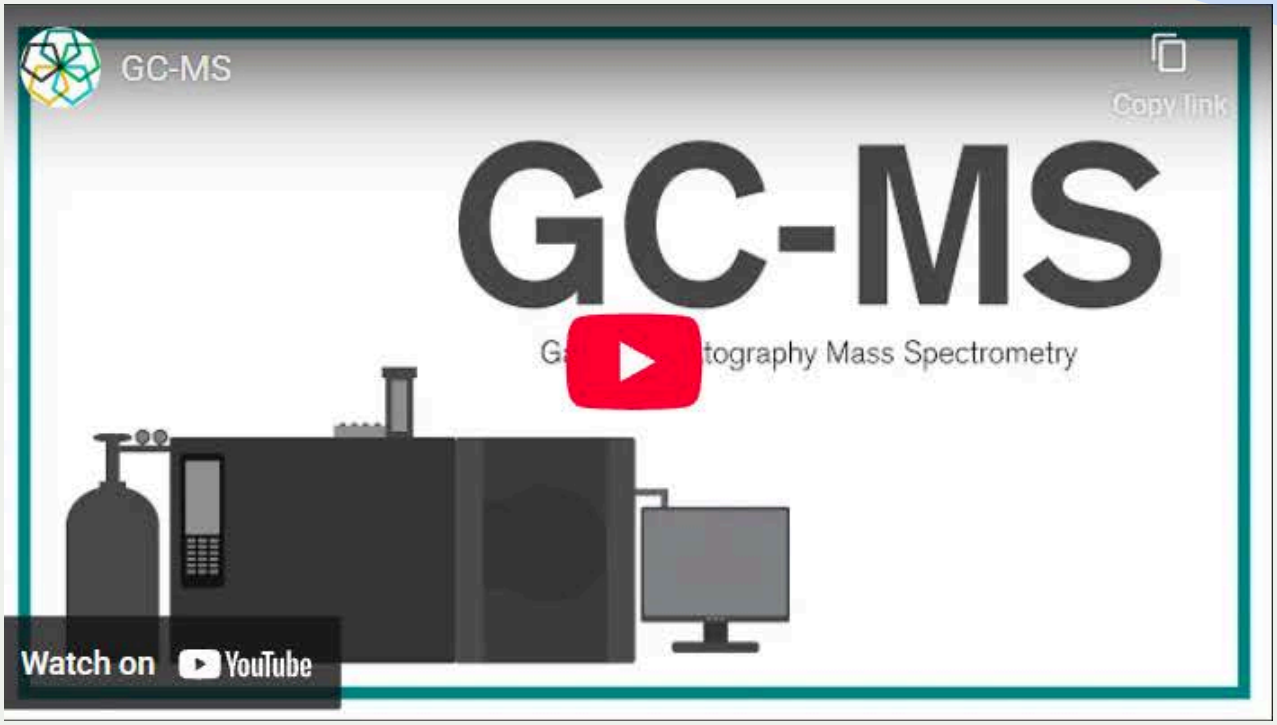

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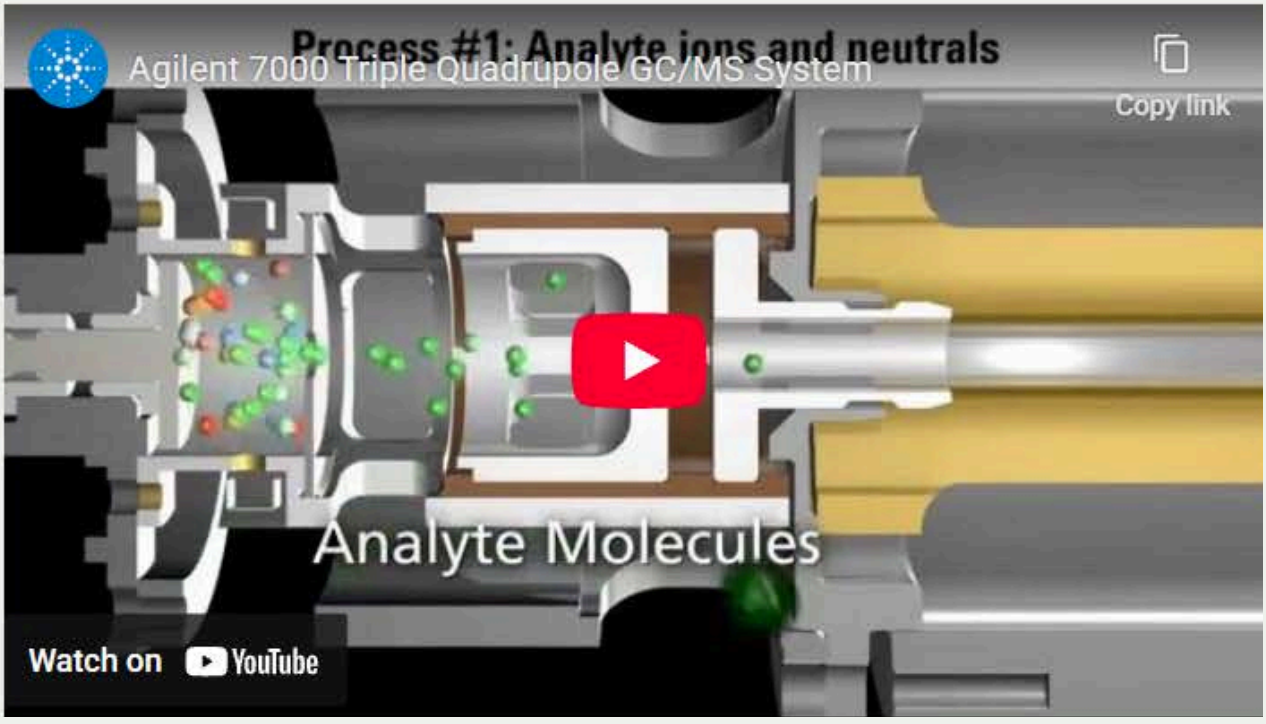

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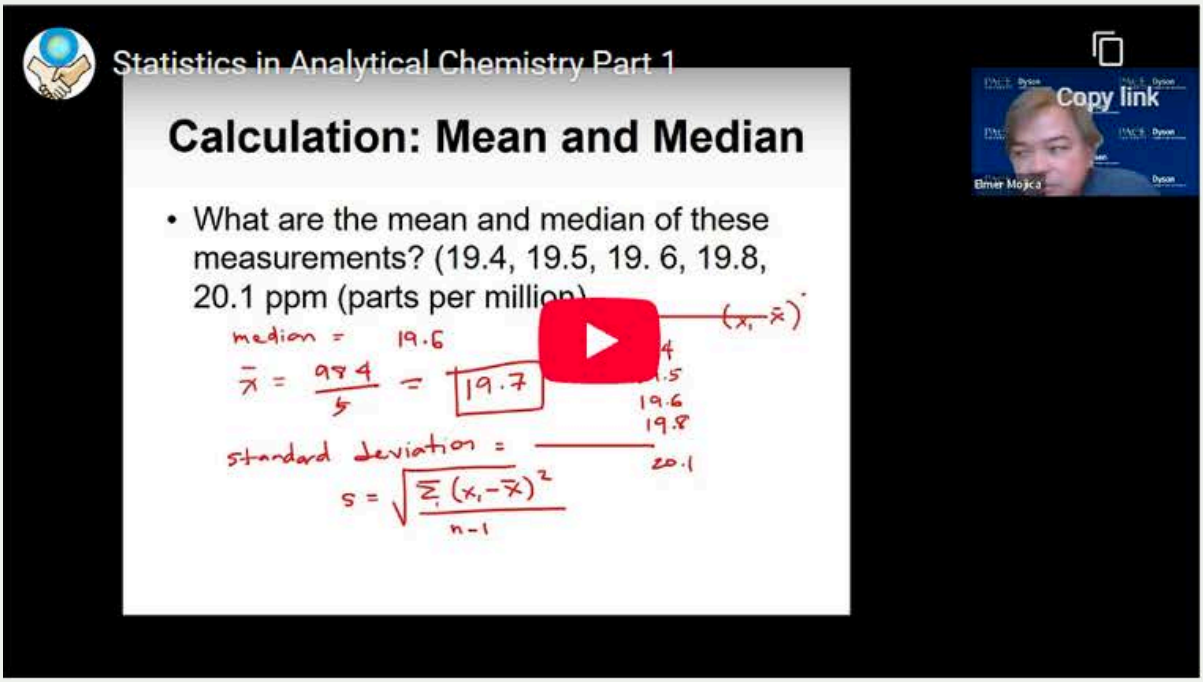
Statistics in Analytical Chemistry Part 1
Calculation: Mean and Median
• What are the mean and median of these measurements? (19.4, 19.5, 19. 6, 19.8, 20.1 ppm (parts per million)
median = 19.6
x̄ = 98.4/5 = 19.7
standard deviation =
s = √(Σ(xi − x̄)²/(n−1))
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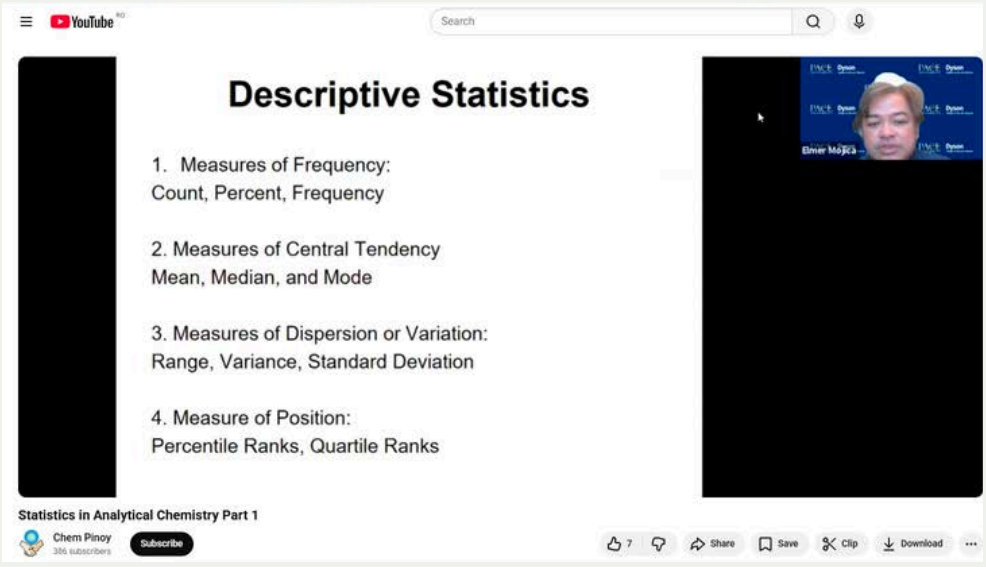
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Descriptive Statistics
1. Measures of Frequency:
Count, Percent, Frequency
2. Measures of Central Tendency
Mean, Median, and Mode
3. Measures of Dispersion or Variation:
Range, Variance, Standard Deviation
4. Measure of Position:
Percentile Ranks, Quartile Ranks
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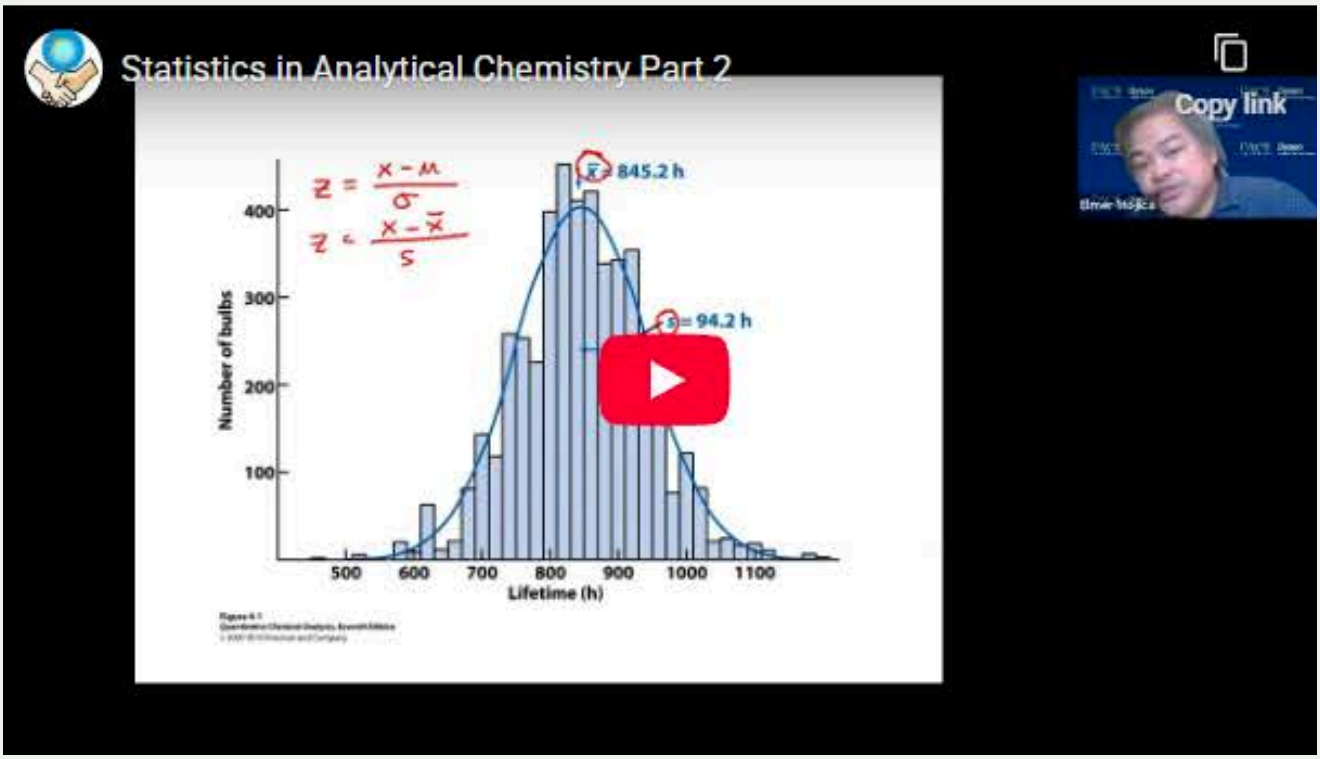
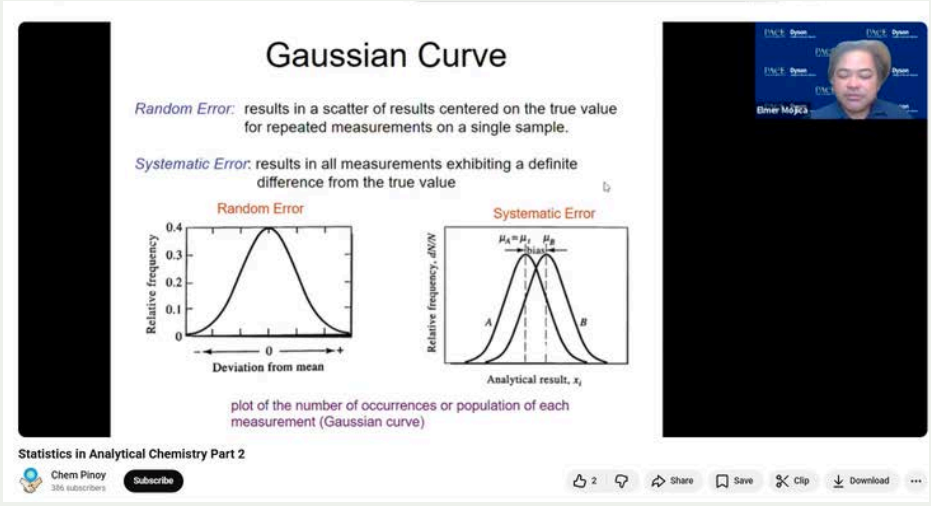

Statistics in Analytical Chemistry Part 2
Copy link
$z = \frac{x - \mu}{\sigma}$
$z = \frac{x - \bar{x}}{s}$
$\bar{x}$ = 845.2 h
s = 94.2 h
Number of bulbs
400
300
200
100
500
600
700
800
900
1000
1100
Lifetime (h)


Gaussian Curve
Random Error: results in a scatter of results centered on the true value for repeated measurements on a single sample.
Systematic Error: results in all measurements exhibiting a definite difference from the true value
Random Error
Systematic Error
Relative frequency
0.4
0.3
0.2
0.1
0
Deviation from mean
Relative frequency, dN/N
Analytical result, x_i
A
B
plot of the number of occurrences or population of each measurement (Gaussian curve)
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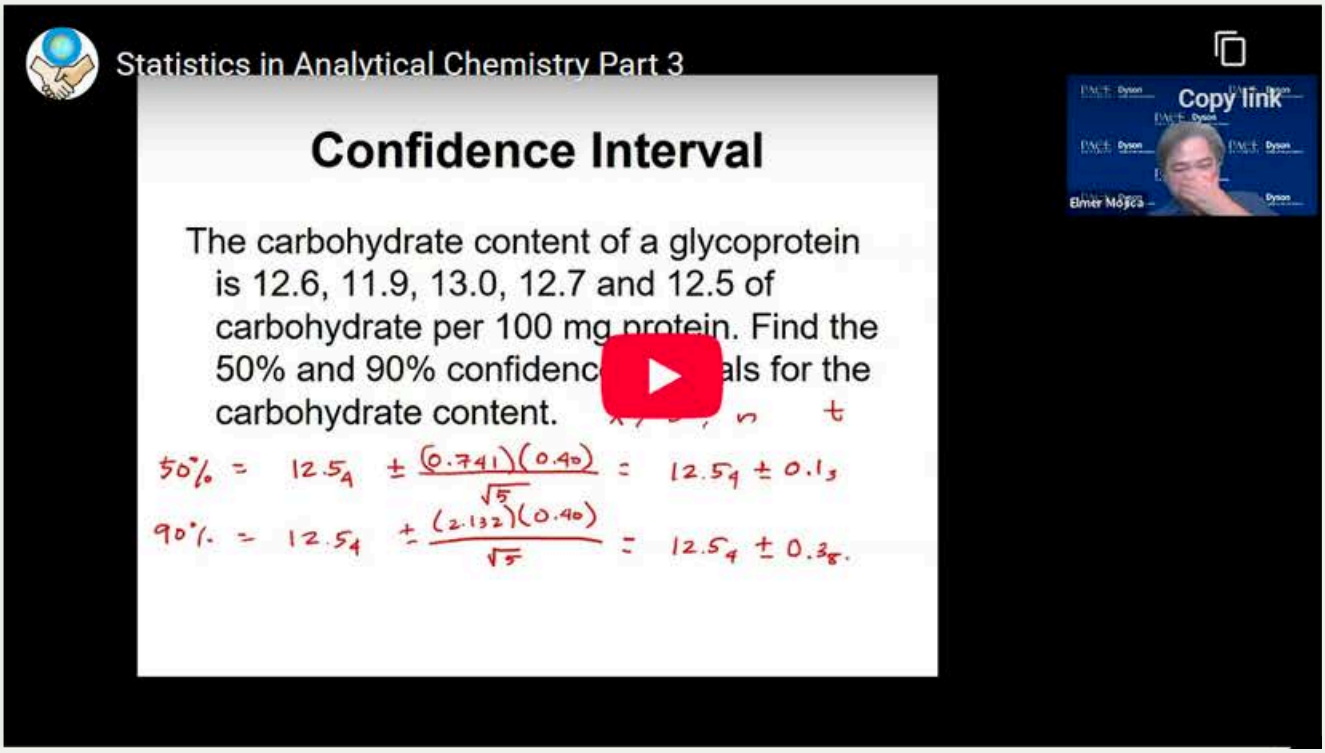
Statistics in Analytical Chemistry Part 3
Confidence Interval
The carbohydrate content of a glycoprotein is 12.6, 11.9, 13.0, 12.7 and 12.5 of carbohydrate per 100 mg protein. Find the 50% and 90% confidenc als for the carbohydrate content.
50% = 12.54 ± (0.741)(0.40)/√5 = 12.54 ± 0.13
90% = 12.54 ± (2.132)(0.40)/√5 = 12.54 ± 0.38
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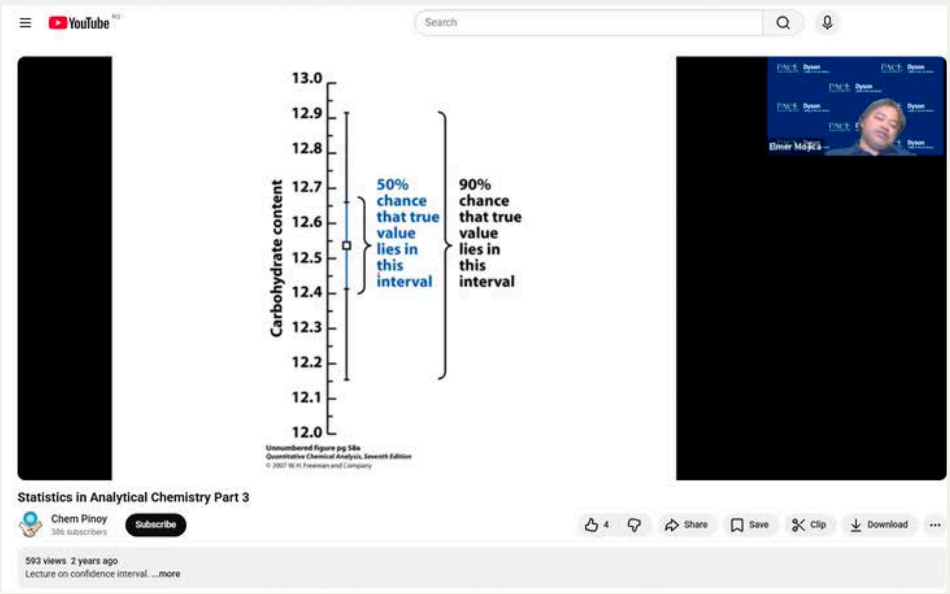
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13.0
12.9
12.8
12.7
12.6
12.5
12.4
12.3
12.2
12.1
12.0
Carbohydrate content
50% chance that true value lies in this interval
90% chance that true value lies in this interval
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Lecture on confidence interval. ...more

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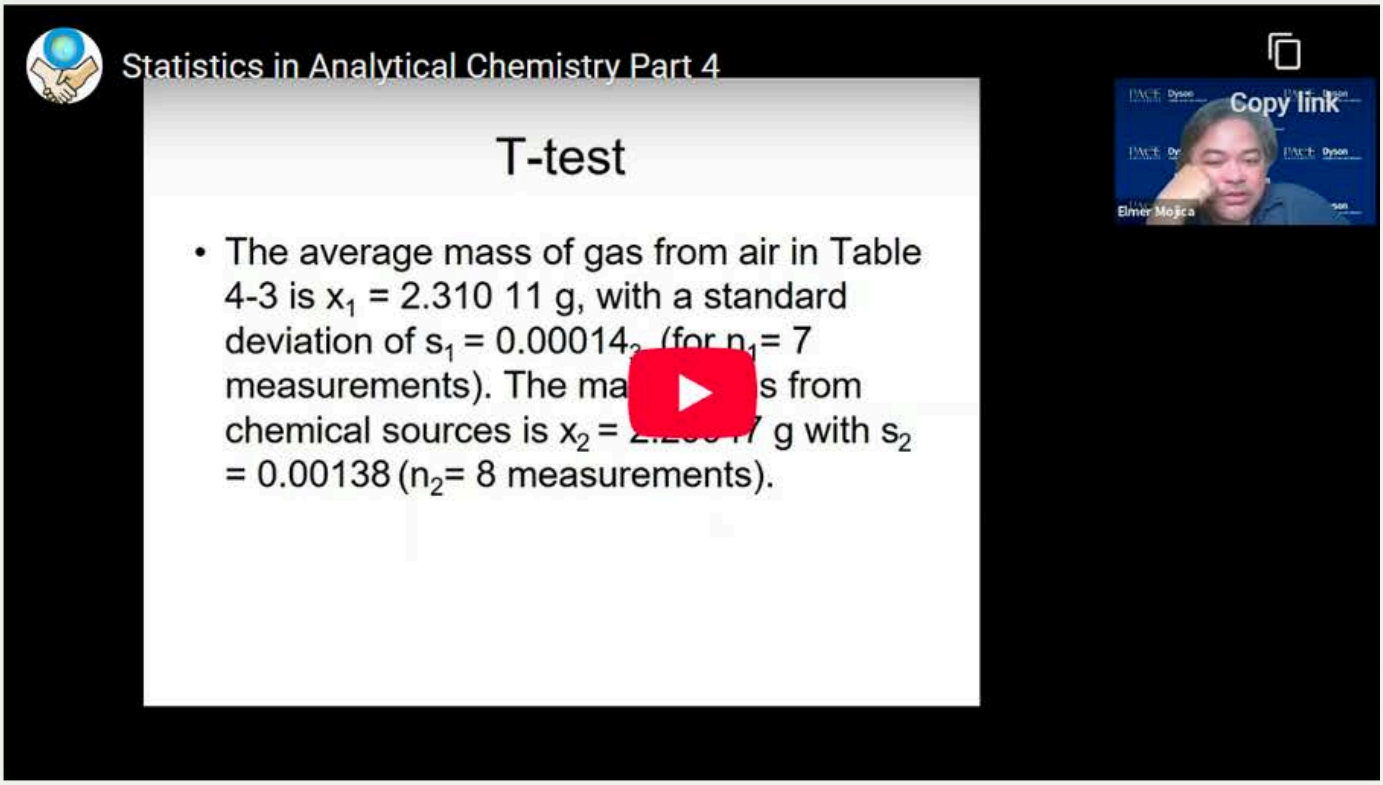
Statistics in Analytical Chemistry Part 4
T-test
• The average mass of gas from air in Table 4-3 is x_1 = 2.310 11 g, with a standard deviation of s_1 = 0.00014 (for n_1 = 7 measurements). The ma s from chemical sources is x_2 = g with s_2 = 0.00138 (n_2= 8 measurements).
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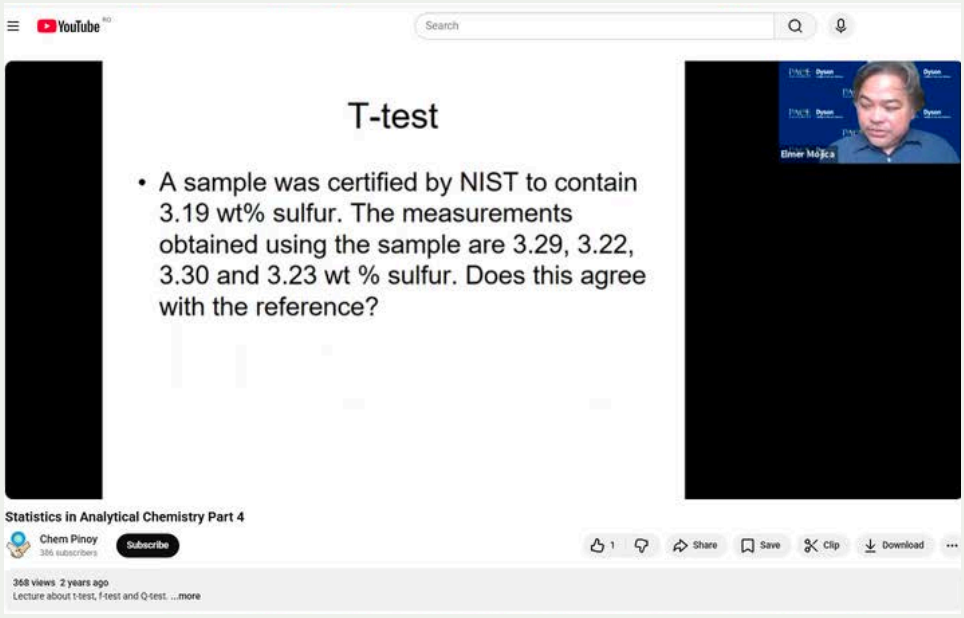
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T-test
• A sample was certified by NIST to contain 3.19 wt% sulfur. The measurements obtained using the sample are 3.29, 3.22, 3.30 and 3.23 wt % sulfur. Does this agree with the reference?
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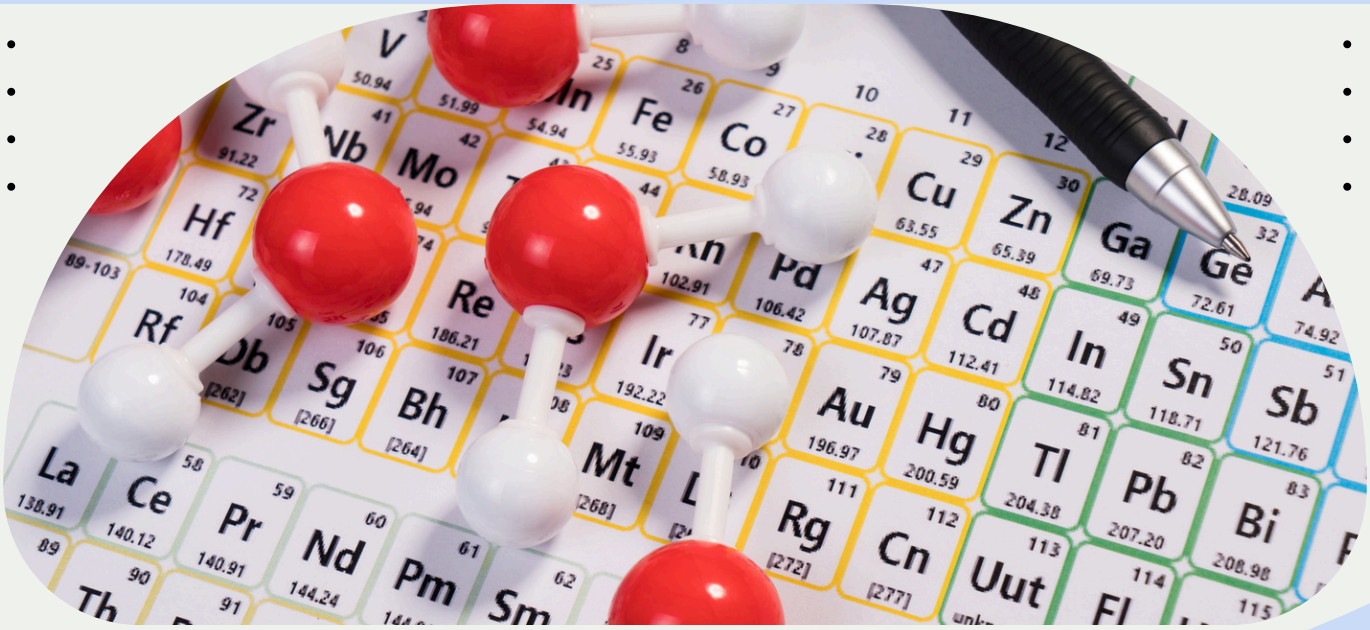

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