SUA FHLE

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IMPRESUM

METHODS FOR ESTIMATION OF SELECTED BIOLOGICALLY ACTIVE SUBSTANCES

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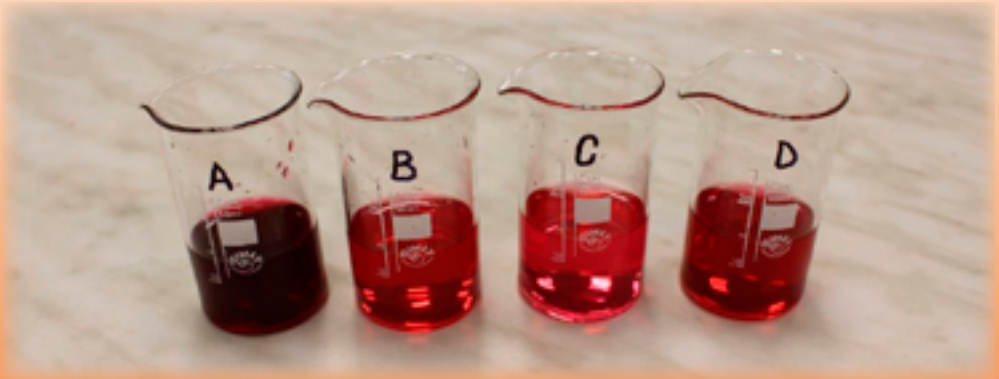

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Department of Vegetable Production

Slovak University of Agriculture in Nitra, Faculty of Horticulture and Landscape Engineering

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Methods for estimation of selected biologically active substances

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Introduction

The book *Methods for estimation of selected biologically active substances* are designed for students of engineer study stage at the studying programme Horticulture at Faculty of Horticulture and Landscape Engineering, Slovak University of Agriculture in Nitra. The book is designed for study of subject Bioactive substances in horticultural crops, compulsory for mentioned studying programme, as well as for other university students which can study this subject as optional.

The book is divided to five chapters which include spectrophotometric, non-spectrophotometric or other methods for estimation of selected biologically active substances which can be applicable within practical subject studying, as well as in the case of final thesis solved within studying programme Horticulture. The estimation methods are approached to students by innovative way with using of detail photos in an effort to modernize the practical side of studied subject. The book was prepared in cooperation with several ranking specialists from practice.

Authors

1 OPTICAL METHODS

Optical methods represent the group of methods which are characterized by common mechanism based on the interaction of mass and electromagnet radiation. These methods can be divided on:

1. **spectroscopic** – atomic spectral or emission absorption analysis, spectrophotometry, infrared spectrometry, luminescence analysis, nuclear magnetic resonance etc.,
2. **non-spectroscopic** – refractometry, polarimetry.

Typical character for mechanism of spectroscopic methods is that atoms, molecules, ions, radicals or their groups are excited or deactivated by interaction with electromagnetic radiation. Thus, spectroscopic methods are connected with absorption or emission of electromagnetic radiation.

In non-spectroscopic methods, some properties of radiation, e.g. speed or plane of polarisation, are only changed within its junction through sample.

From spectroscopic methods **UV-VIS spectrometry** and from non-spectroscopic methods **refractometry** are used to determine selected bioactive substances with antioxidant properties in agricultural products at the Department of Vegetable production, FHLE, SUA in Nitra.

1.1 UV-VIS spectrometry

The Ultra-violet visible (UV-VIS) spectrometry belongs to absorption spectroscopic methods. It is used for obtaining of substance absorption spectrum in solutions or solid matters.

Principle

UV-VIS spectrometry records information flowing from electron junction in atoms and molecules. The photon energy is absorbed by atoms or molecules of analyte. Electrons are changing from basic state to excited state, i.e. electron is getting from lower to higher energetic level and energetic difference of these two levels are corresponding to energy of absorbed photon. The intensity change of transmitted radiation is evaluated by absorption at selected wave lengths. Substances absorbing radiation in visible part of light spectrum, e.g. substances of transient elements or organic pigments, are coloured. Substances absorbing radiation in UV spectrum are colourless.

The UV-VIS energetic area of electromagnetic spectrum is ranged from 1.5 to 6.2 eV. It responds to the range of wave lengths from 800 and 200 nm. The evaluation of absorbed radiation and estimation of analyte concentration is based on Lambert-Beer law:

$$A = \varepsilon \times l \times c$$

where:

A – absorbance for one wave length (non-dimensional quantity, often marked as abs.)
ε – molar absorption coefficient of substance or molecule in solution (defined in $M^{-1}.cm^{-1}$)
l – cuvette diameter (usually 1 cm)
c – solution concentration ($mol.dm^{-3}$ or M)

There are known three types of UV-VIS spectrometers:

1. **single-beam,**
2. **double-beam,**
3. **simultaneous.**

All apparatuses content radiation source (usually deuterium or wolfram lamp), sample holder and detector. The single-beam spectrometer has filter or monochromator between source and sample which provide a transmitting of monochromatic radiation.

Evaluation

The method of calibration curve is used for evaluation of detector response change at different analyte concentration. Modern apparatuses are equipped by complex assessment unit – needed hardware and software which enable to evaluate analysis result, including calibration power.

1.1.1 Estimation of total carotenoid content

Extraction of solid material

One gram of fresh homogenised vegetable sample is weighted and quantitatively washed by acetone into the grinding mortar. The sample is grinded by sea sand with small acetone volume which is sequentially poured to the separatory funnel. This process is repeated until the extract becomes a colourless solution (needed about 50.0 cm^3 of acetone).

Extract preparation

Obtained acetone extract are two-times shaking in separatory funnel with 10 cm^3 petroleum ether (2 × 10 cm^3). Within this process, carotenes with other pigments get to the top, petroleum ether layer. Petroleum ether layers are drained to the next separatory funnel and shaken with 50 cm^3 of distilled water at which acetone remains are diluted. After separation of phases, water phase are drained and petroleum ether layer are dried by anhydrous sodium sulphate, quantitatively transferred to the volumetric flask and filled by petroleum ether to the volume of 25 cm^3. The absorbance is measured after 5 minutes at wave length of 450 nm, compared to the petroleum ether (Hegedűsová et al., 2007).

Calculation of total carotenoid content (Biehler et al., 2010):

$$C\ [\mathrm{mg.kg^{-1}}] = (A450 \times V1 \times M \times 1{,}000 \times 1{,}000 \times V2/V3/d\ [\mathrm{cm}]/\varepsilon\ [\mathrm{l/mol/cm}]/w\ [\mathrm{g}]/1{,}000$$

where:

$A450$	–	sample absorbance at 450 nm
$V1$	–	volume filled after extraction (cm^3)
$V2$	–	volume filled at dilution – if it is needed (cm^3)
$V3$	–	pipetted volume at dilution (cm^3)
d	–	cuvette width (1 cm)
M	–	average molecular weight of carotenoids (548 $g.mol^{-1}$)
ε	–	specific average absorbance of carotenoids (135,310 $l.mol^{-1}.cm^{-1}$)
w	–	sample weight (g)
1,000	–	recounting of flask volume to one litre

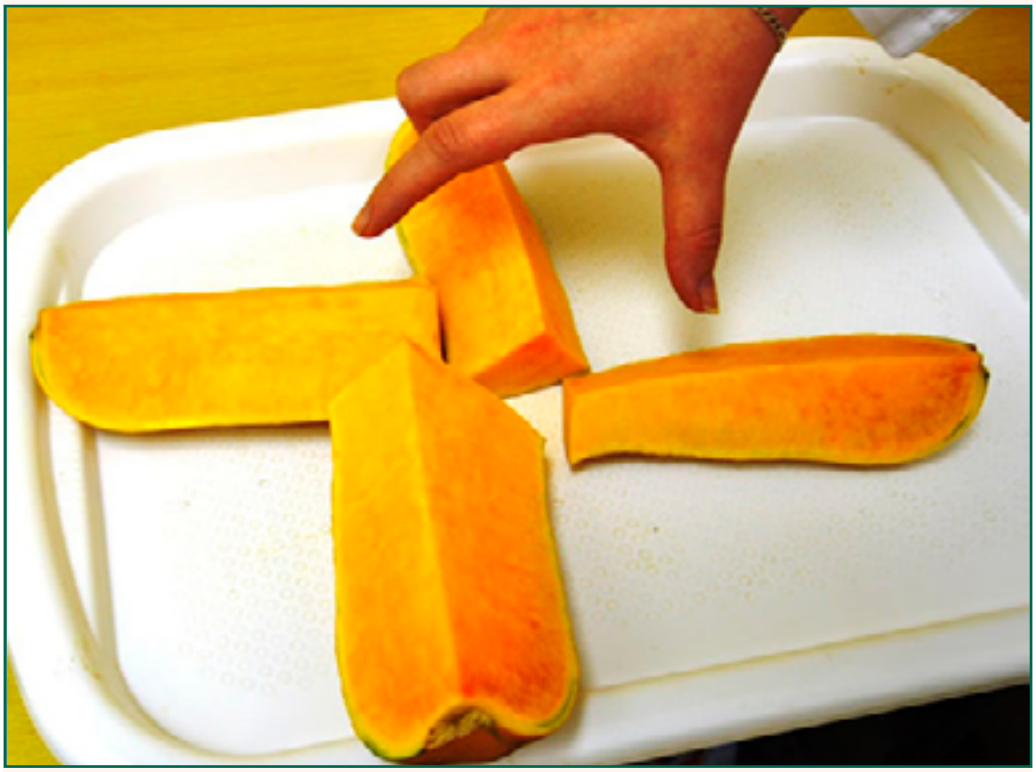
Practical demonstration of total carotenoid content estimation in butternut squash (*Cucurbita moschata* Duch.)

Material:

- separatory funnel, grinding mortar, laboratory spoon, beaker, volumetric flask, scales, spectrophotometer (Spektroquant PHARO 100).

Chemicals:

- acetone p.a., petroleum ether p.a., sea sand, anhydrous sodium sulphate (Na_2SO_4), distilled water.



The sample of butternut squash is washed, dried, cut and opposite quarters are used for average sample preparation. It must be prepared from several fruits within the same cultivar or tested treatment

Cut fruits are put into the homogenizer

The homogenised mass is formed by sample mixing

Approximately one gram of homogenised sample is weighted

The sea sand and acetone is added to the sample

contens

Pigments are flowed to the organic solvent after sample grinding with sea sand and acetone

The acetone extract are orange-coloured because of flowed pigments

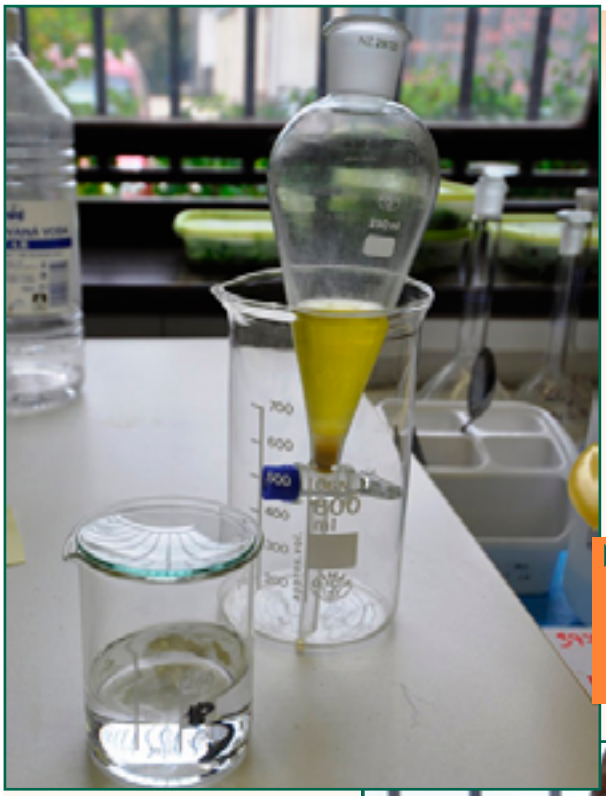
contens

The analytical funnel with cotton, moistened by acetone, is put into the separatory funnel. The coloured acetone extract is drained through prepared funnel with cotton

The grinding of plant material with acetone is realised until the extract is uncoloured in grinding mortar (maximally to the volume of 50 cm^3). Acetone extracts must be carefully and quantitatively drained to the separatory funnel after each grinding phase

In the end of plant pigment extraction, sea sand and plant remains become colourless in the grinding mortar

After draining of acetone extract to the separatory funnel, coloured cotton is washed by small volume of acetone



The extract is two-times shaken (circular motion) by 10 cm^3 of petroleum ether. Within this process, carotenes get to the top, petroleum ether layer

The small volume of distilled water (approximately 2 cm^3) is added for radical separation of acetone and petroleum ether layers

The bottom layer (water with acetone) is drained out of separatory funnel without shaking. The petroleum ether layer, coloured by abundant carotenoids, remains in separatory funnel

Connected petroleum ether phases are mixed with 50 cm^3 of distilled water by circular motion for removal of acetone remains from petroleum ether layer. Because of vacuum created within mixing, glass stopper of separatory funnel must be several times loosen

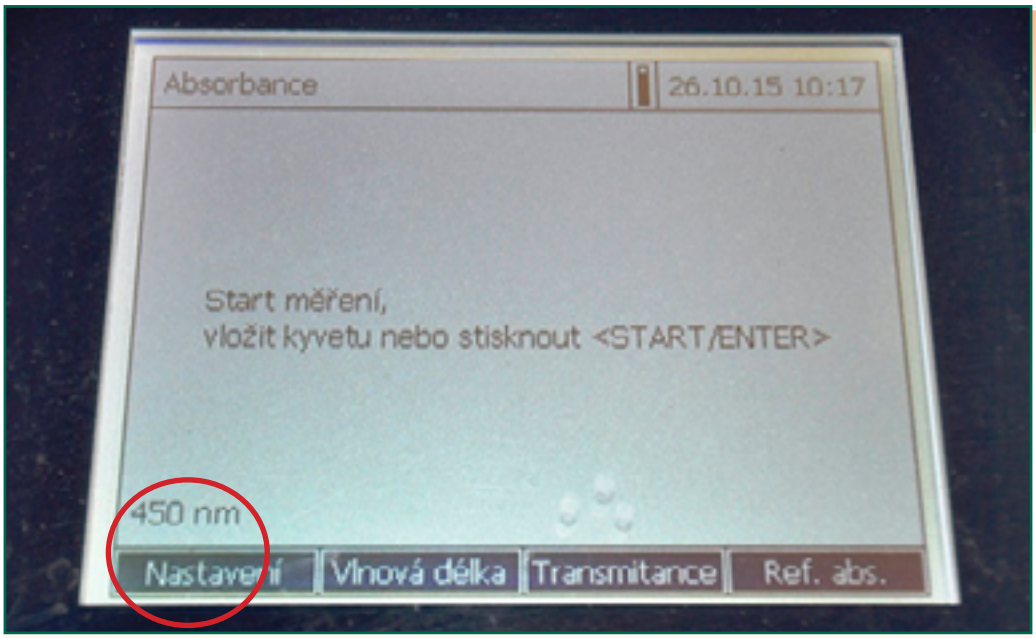
The bottom layer (water) is drained out after separation of petroleum ether and water phase (approximately after 5 minutes)

The anhydrous sodium sulphate (Na_2SO_4) is prepared to the beaker for petroleum ether drying

The pure petroleum extract is quantitatively transferred to the volumetric flask with volume 25 or 50 cm^3 and filled by petroleum ether up to the scale line of flask. The volume of used flask is dependent on the extract colour intensity

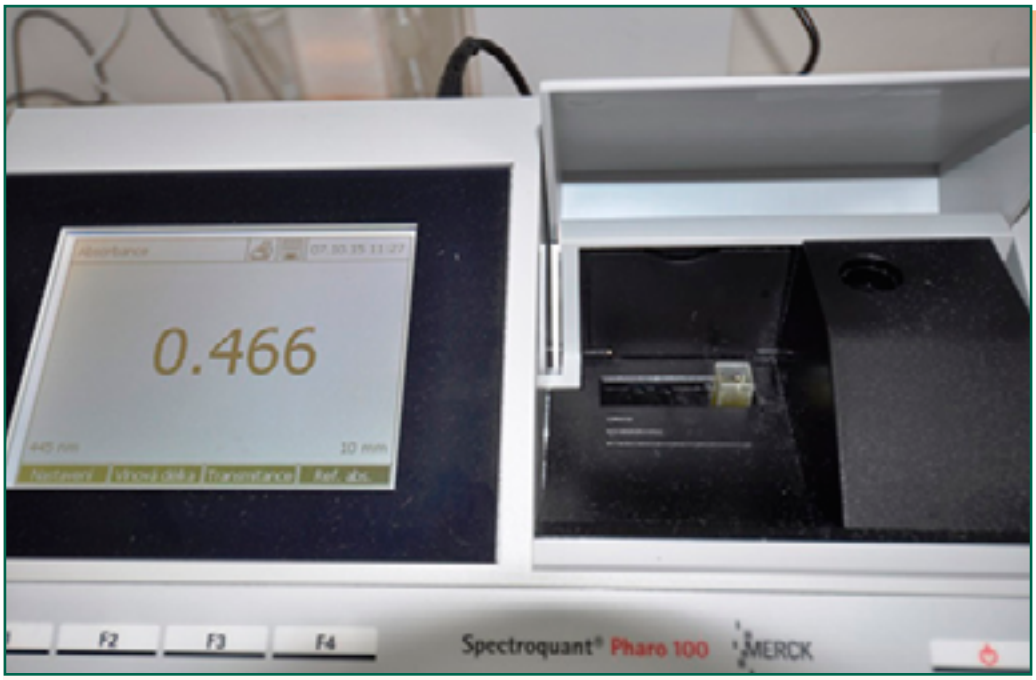
The petroleum extract is poured from volumetric flask to the dark bottle and colour intensity is measured on the spectrophotometer

contens



The wave length for total carotenoids (450 nm) is set on the spectrophotometer

The solution of pure petroleum ether is used for setting of calibration zero (BLANK ZERO)



The prepared petroleum ether extract is poured to the silica cuvette (1 cm) and sample absorbance is sequentially measured. This step is repeated for all samples and obtained values are marked into the prepared table. If absorbance values are ranged from 0.3 to 0.7, extracts are not diluted

1.1.2 Estimation of chlorophyll *a* and chlorophyll *b* content

Extract preparation

One or two grams of fresh vegetable sample (according to the expected chlorophyll content) is weighted and homogenised with sea sand and acetone (approximately 3–4 cm^3) in the grinding mortar. It is recommended to add 0.2–0.5 g of magnesium carbonate ($MgCO_3$) which fixes acids and protects to the destruction of chlorophyll to the pheophytin. After absolute homogenisation, acetone extract is carefully poured into the glass frit filter. This process is repeated until the extract becomes a colourless solution. The pure extract is quantitatively poured into the volumetric flask and filled by acetone up to flask scale line. The intensity of extract colour is measured at wave lengths of 649 nm (chlorophyll *a*) and 665 nm (chlorophyll *b*). The zero position is controlled by pure acetone at 750 nm. The possible dispersion value is deducted from individual absorbance values (Hegedűsová et al., 2007).

Warning:

- Acetone etches PVC cover of spectrophotometer cuvette!

Calculation of chlorophyll content:

- in $mg.dm^{-3}$:

$$Ch_a^* = 11.64 \times A_{665} - 2.39 \times A_{649}$$

$$Ch_b^* = 20.11 \times A_{649} - 5.18 \times A_{665}$$

- in $mg.kg^{-1}$:

$$Cha_a^* = \frac{Cha_a^* \times df \times 1{,}000}{20 \times w}$$

$$Cha_b^* = \frac{Cha_b^* \times df \times 1{,}000}{20 \times w}$$

where:

Ch_a^* – chlorophyll *a*
Ch_b^* – chlorophyll *b*
A_{665} – absorbance at 665 nm
A_{649} – absorbance at 649 nm
df – dilution factor (if it is needed)
w – sample weight

Practical demonstration of chlorophyll *a* and chlorophyll *b* content estimation in broccoli (*Brassica oleracea* sk. Italica)

Material:

- grinding mortar, laboratory spoon, glass frit filter, filter apparatus according to Morton, volumetric flask, spectrophotometer (Spektroquant PHARO 100).

Chemicals:

- acetone p.a., sea sand, magnesium carbonate ($MgCO_3$) p.a.

- Fresh broccoli florets are cut and opposite quarters are taken for average sample preparation which must be prepared from several florets of the same cultivar/treatment

Cut parts of broccoli are homogenised

The homogenised broccoli sample is marked and prepared for analysis

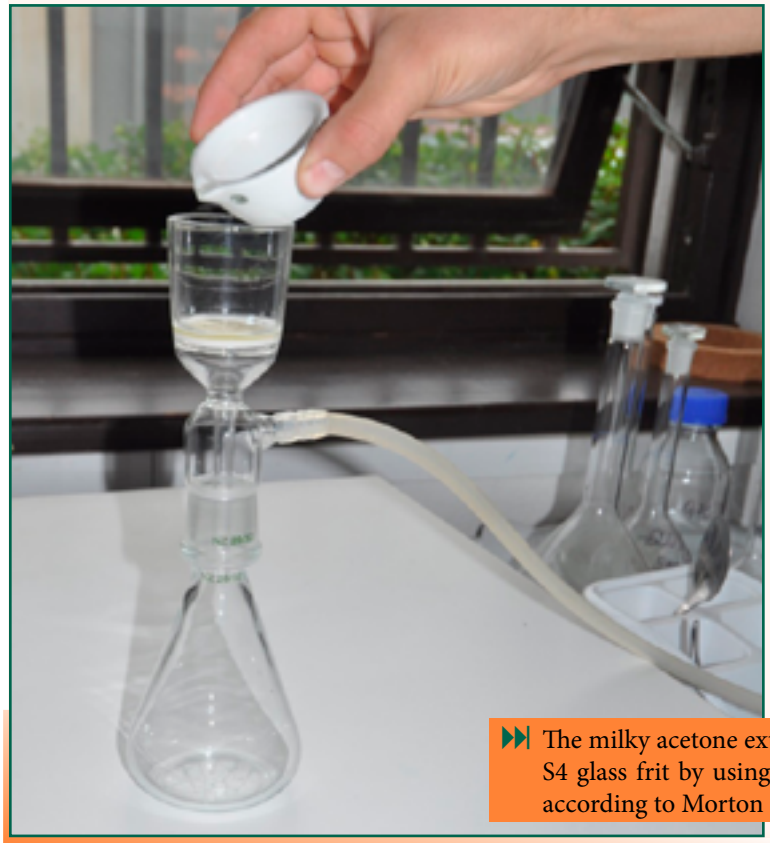
contens

One gram of prepared sample is weighted

The weighted sample is grinded with sea sand, magnesium carbonate (one laboratory spoon) and acetone (small volume) and pigments are sequentially flowed to the organic solvent

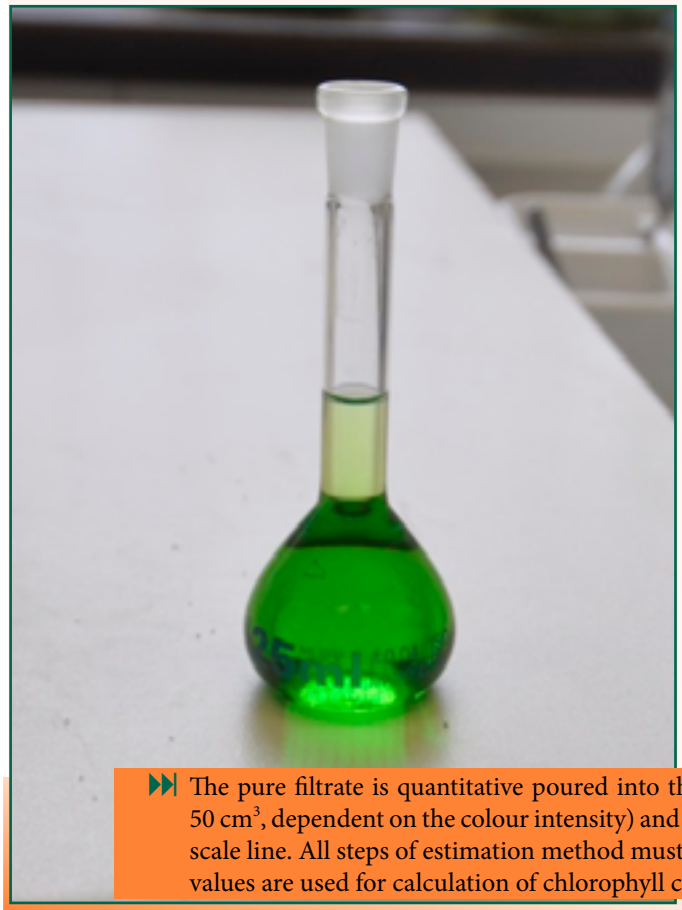
contens

The acetone extract is green-coloured because of abundant chlorophyll

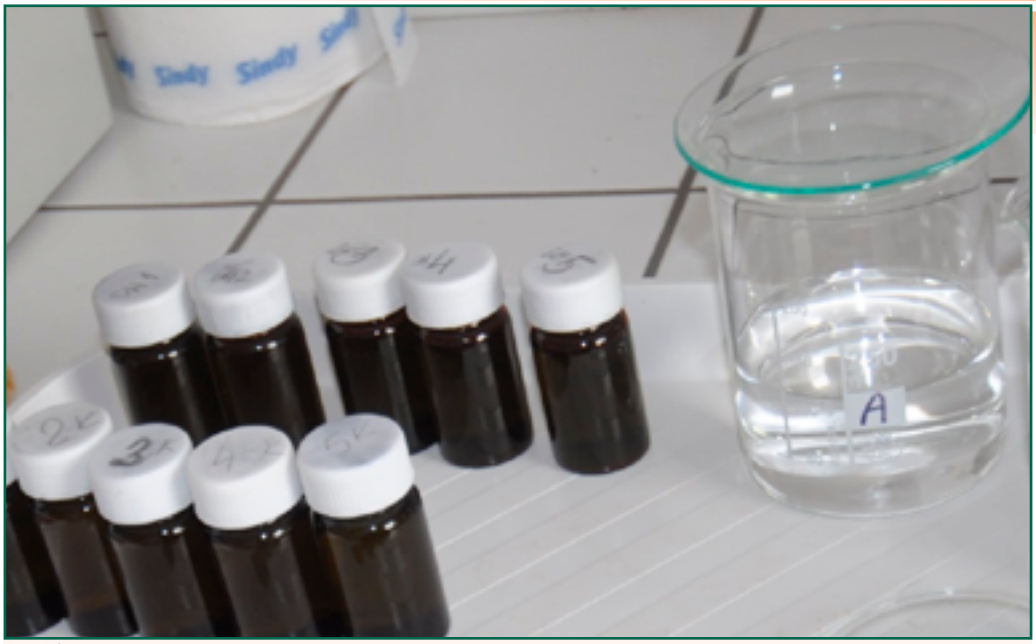


The milky acetone extract is filtered through S4 glass frit by using of filtration apparatus according to Morton

The process of sample grinding by acetone and filtration is repeated until the plant material remains become uncoloured



The pure filtrate is quantitative poured into the volumetric flask (25 cm^3 or 50 cm^3, dependent on the colour intensity) and filled by acetone up to the flask scale line. All steps of estimation method must be marked because numerous values are used for calculation of chlorophyll content



Acetone extracts are poured from volumetric flask to the marked dark bottle and colour intensity is measured at the spectrophotometer

The spectrophotometer must be turned on 10 minutes before measurement for lamp warming-up. The calibration zero is set for acetone at wave length of 750 nm. The silica cuvette is carefully put into the apparatus because acetone etches plastic (PVC) material

The prepared acetone extract is poured into the silica cuvette (1 cm) and sample absorbance is measured. This step is repeated for all samples and obtained values are marked into the prepared table

The chlorophyll absorbance is measured at wave lengths of 645 nm (Chl *a*) and 665 nm (Chl *b*)

1.1.3 Estimation of total anthocyanins content

Extract preparation

Fifty grams of fresh sample are weighted, homogenised and extracted with 80% ethanol within 12 hours. The total anthocyanins content is sequentially estimated in obtained extract by modified method according to Lapornik et al. (2005). The principle of this method is based on the decrease of pH extract value at 0.5–0.8 connected with transformation of all anthocyanins to the red-coloured flavilic cation.

One cm^3 of 0.01% solution of HCl in 80% ethanol is pippeted into the two tubes. Sequentially, ten cm^3 of 2% water solution of HCl is added to the first tube and ten cm^3 of buffer solution with pH = 3.5 (c = 0.2 $mol.dm^{-3}$ of Na_2HPO_4 and c = 0.1 $mol.dm^{-3}$ of citric acid) is added to the second tube. The absorbance of both samples is measured spectrophotometrically at wave length of 520 nm, compared to the blind experiment.

Table 1 Combinations of used chemicals and their volume at anthocyanins estimation

	Sample (extract)	0.01% HCl in 80% ethanol	2% HCl	Buffer solution
Tube 1	1 cm^3	1 cm^3	10 cm^3	–
Tube 2	1 cm^3	1 cm^3	–	10 cm^3
Tube – blind experiment 1	–	1 cm^3	10 cm^3	–
Tube – blind experiment 2	–	1 cm^3	–	10 cm^3

The total content of anthocyanins is expressed in $mg.kg^{-1}$ of fresh weight. The calculation of its value is following:

$$\text{Total anthocyanins } (TA)\ (mg.dm^{-3}) = (A1 - A2) \times f$$

where:

$A1$ – absorbance of first tube (2% solution of HCl)
$A2$ – absorbance of second tube (buffer solution)
f – 396.598

Practical demonstration of total anthocyanins content estimation in purple-coloured potatoes (*Solanum tuberosum* L.)

Material:

- laboratory shaker, automatic pipettes, pH meter, centrifuge, tubes for centrifuge, spectrophotometer (Spektroquant PHARO 100).

Chemicals:

- ethanol p.a., Na_2HPO_4 . 12 H_2O, concentrated HCl, citric acid.

Potato tubers are washed and dried

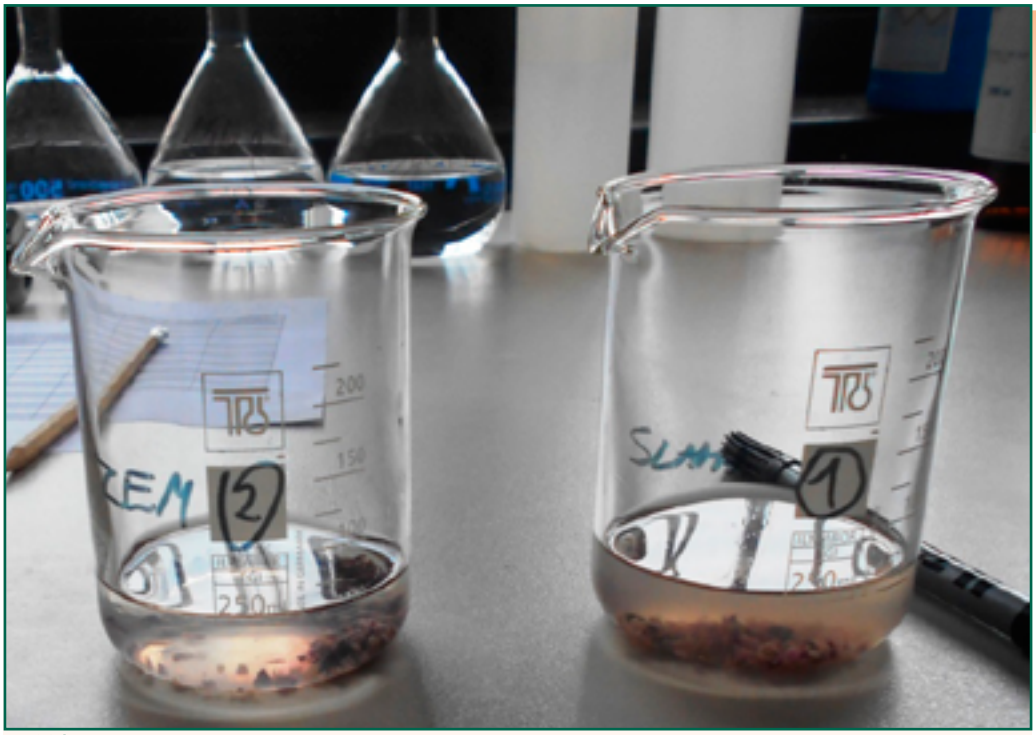
Potato tubers (5–10) are cut and two opposite quarters of each tuber are used for sample preparation

All parts of potato tubers are homogenised

contens

The sample weight (5–50 g) is chosen according to the colour intensity of potato flesh tubers. The potato sample is weighted directly into the beaker and accurate sample weight is marked

All samples must be clearly marked



Weighted samples are poured by 50 cm^3 of 80% ethanol

The beaker is covered by aluminium foil and anthocyanins extraction to the ethanol is realised by shaking at laboratory shaker (100 revolutions per minute) within 12 hours

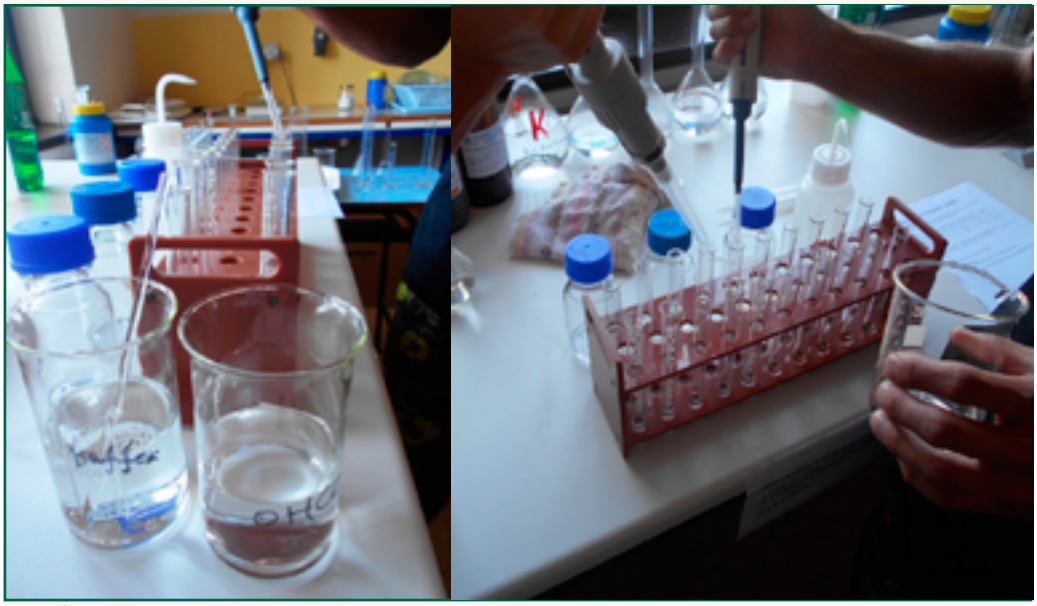
The ethanol extract of plant material is sequentially filtered

The pure filtrate is poured to the closable flask and all samples are marked

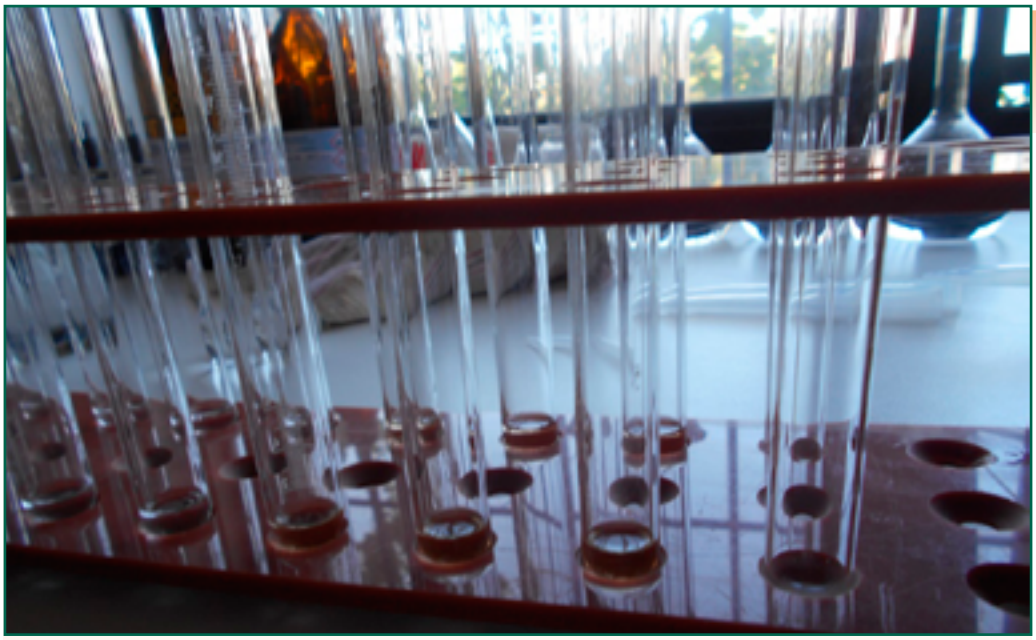
The buffer solution (pH = 3.5) is prepared by solution mixing of disodium phosphate (c = 0.2 mol.dm^{-3} Na_2HPO_4) and citric acid (c = 0.1 mol.dm^{-3} citric acid)

It is also needed to prepare other solutions for analysis: 0.01% solution of HCl in 80% ethanol and 2% water solution of HCl

One cm^3 of plant ethanol extract and one cm^3 of 0.01% solution of HCl in 80% ethanol is added to two tubes



Sequentially, $10cm^3$ of 2% HCl solution is added to the first tube. Into the second tube, 10 cm^3 of prepared buffer solution is added. All tubes must be carefully marked in case of many samples and measurements. Samples with HCl solutions are put into the first line and samples with buffer solution into the second line of tube clamp



The last step of estimation method is blind experiment. The blind experiment for samples with 2% HCl solution is following mixture: 1 cm^3 of 0.01% HCl solution in 80% ethanol + 10 cm^3 of 2% HCl solution. Within the blind experiment with buffer solution, 1 cm^3 of 0.01 HCl solution in 80% ethanol is mixed with 10 cm^3 of buffer solution (tab. 1)

The absorbance of both samples is measured spectrophotometrically at wave length of 520 nm, compared to the blind experiment. The total anthocyanins content is expressed in $mg.kg^{-1}$ of fresh weight according to mentioned calculation formulas

1.1.4 Estimation of antiradical activity by DPPH method

Principle

The elimination ability of free radicals is tested in ethanol solution of 2,2-Diphenyl-1-picrylhydrazyl – DPPH (Lee et al., 1998). The level of solution decolouration indicates the ability to eliminate radicals by the help of added samples (filtrates). The result is expressed as antiradical activity (ARA).

Material:

- spectrophotometer, horizontal laboratory shaker, tubes with volume 10 cm^3, micropipettes, solution batcher.

Chemicals:

- 2,2-Diphenyl-1-picrylhydrazyl (DPPH), ethanol or methanol (70% water solution).

Sample extraction

Analysed samples of crop products are cleaned from unwanted parts and washed from impurity (e.g. soil). If analysed sample (fruit) has higher weight or volume and it is not possible to analyse whole product, sample characterized the concentration composition of measured substances is taken and prepared for analysis. Similarly, several fruits are processed and average sample is sequentially prepared for extraction. The sample is homogenised during one minute and 10 grams of fine mass is mixed with 40 cm^3 of 70% ethanol/methanol water solution. In case that sample consistency does not allow to thorough homogenisation, the same amount of deionised water is added to the sample and mixture is homogenised. Twenty grams of mixture are weighted and 30 cm^3 of 96% ethanol/methanol is added to it. The prepared mixture is stored at room temperature during 20 hours. Sequentially, mixture is extracted in the horizontal shaker during 4 hours and it is filtered. If next methods steps are not done immediately, extracts can be stored in the fridge at 5–8 °C within needed time period. Extracts must be secured against solvent evaporation.

Estimation process

From each sample extract, 0.2 cm^3 is pipetted to the tube; though, the pipetted volume can be ranged from 0.1 cm^3 to 0.8 cm^3 in dependency on the plant sample character. Sequentially, the 70% ethanol/methanol is added up to the volume of 2 cm^3 (1.9–0.2 cm^3 of ethanol/methanol). After that, 4 cm^3 of DPPH solution (25 mg DPPH.dm^{-3}) is added to the mixture. Within reference sample (blind experiment), simultaneously realised to plant sample, 2 cm^3 of 70% ethanol/methanol is mixed with 4 cm^3 of DPPH solution. The absorbance is measured at wave length of 517 nm after 30 minutes.

Note:

1. **in case of extracts with high antioxidant activity** (exocarp of royal walnut, elderberry, sallow-thorn berry, black chokeberry etc.): necessary to dissolve it before analysis by 70% ethanol minimally in ratio 1 : 9 (extract : ethanol);
2. **in case of red-coloured extracts** (abundance of anthocyans and betanin): recommended to measure colour change kinetics. Method: after adding of all reagents, solution is quickly mixed and absorbance is immediately measured (time T_0). The measurement of the same solution is sequentially repeated after 10, 20 and 30 minutes. The initial absorbance T_0 is estimated and considered as reference absorbance.

contens

Calculation

The antiradical activity (*ARA*) is calculated as % of DPPH decolouration by using of following formula:

$$ARA = (1 - A_s \times n/n_{10}/(V1/V2)/A_{ref}) \times 100$$

where:

A_s – sample absorbance
w – sample weight (g)
w_{10} – recalculated factor to 10 g of sample
$V1$ – pipetted value of extract (0.2–1.8 cm^3)
$V2$ – standardly pipetted volume (always 0.2 cm^3)
A_{ref} – absorbance value of reference (blind) sample

Practical demonstration of antiradical activity (*ARA*) by DPPH method in redbeet (*Beta vulgaris* sk. Conditiva)

⏭ Analysed samples must be cleaned and washed from soils or other impurities

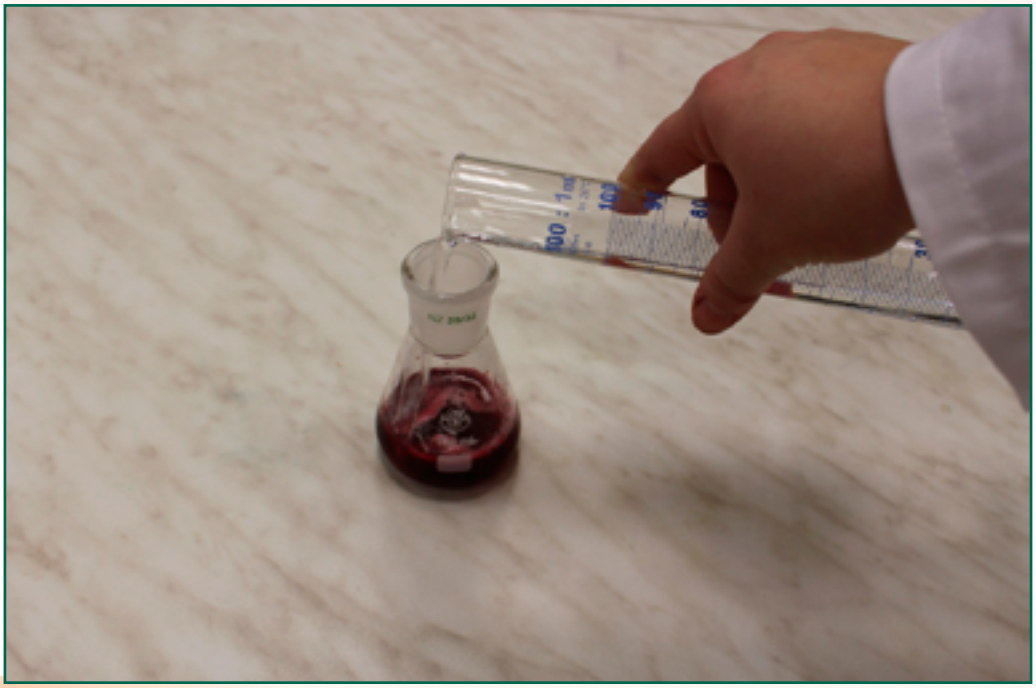
If the analysed fruit has higher weight/volume and it is not possible to analyse whole fruit, sample, characterized concentration composition of measured substance, is taken and used for analysis

According to the previous step, sample is taken from several fruits

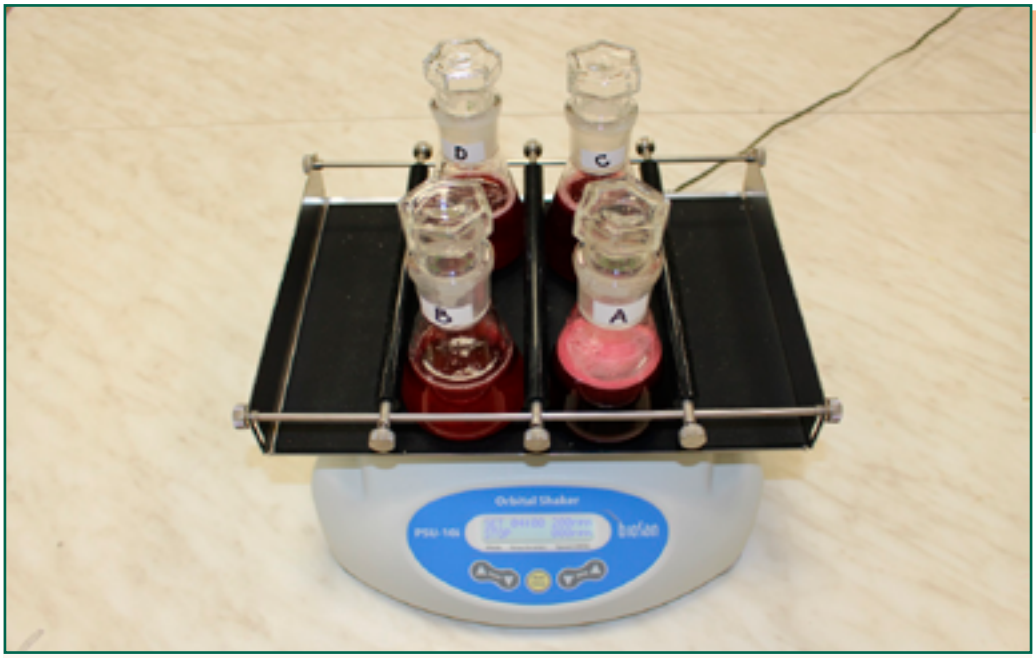
The average sample is prepared from taken samples which are cut into the smaller pieces

The sample is homogenised during one minute

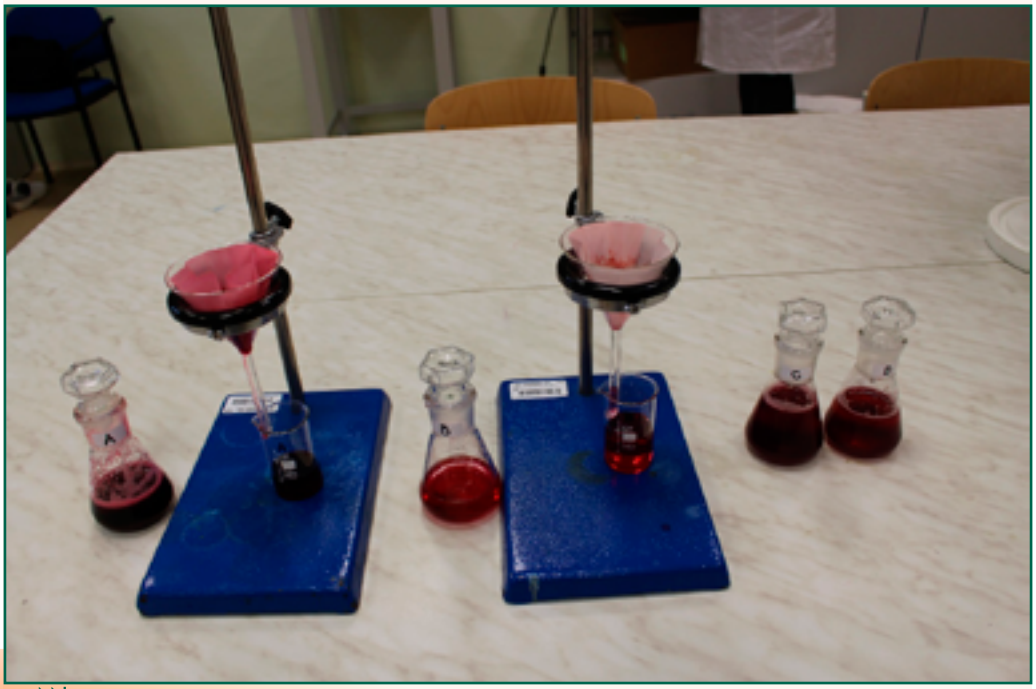
Ten grams of homogenised sample is weighted and used for analysis



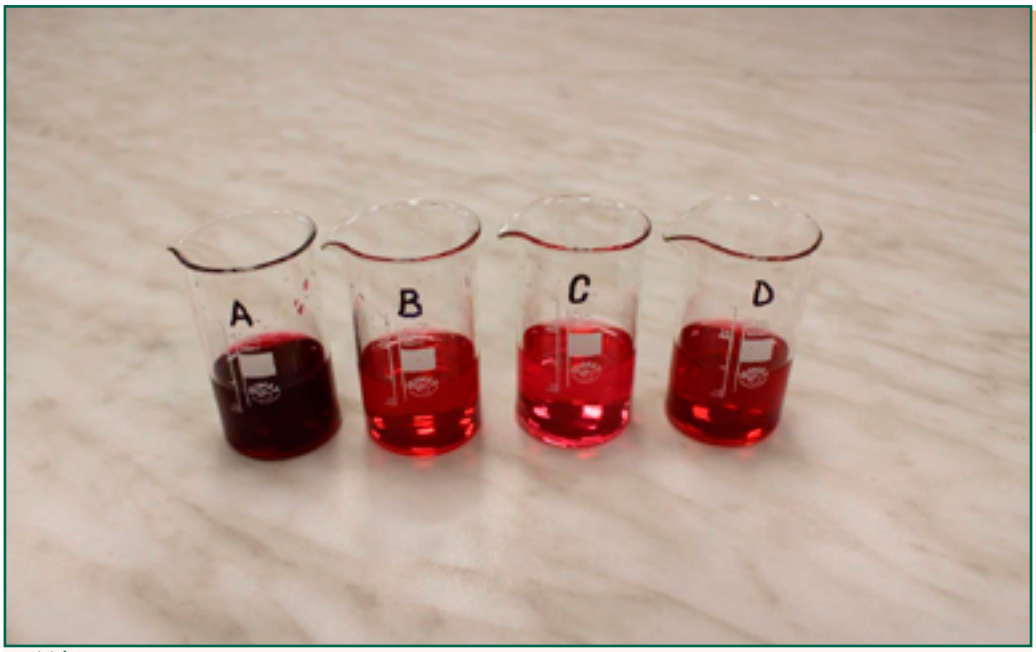
The sample is mixed with 40 cm^3 of 70% water solution of ethanol or methanol



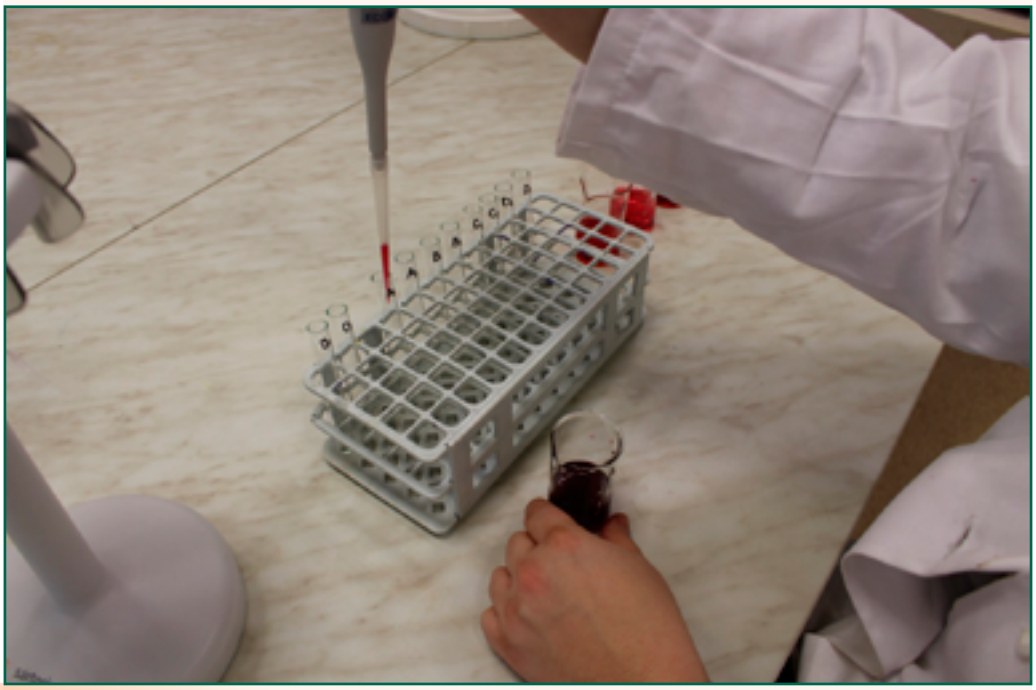
The sample with ethanol/methanol is stored at room temperature within 20 hours and it is sequentially extracted in the horizontal shaker within 4 hours



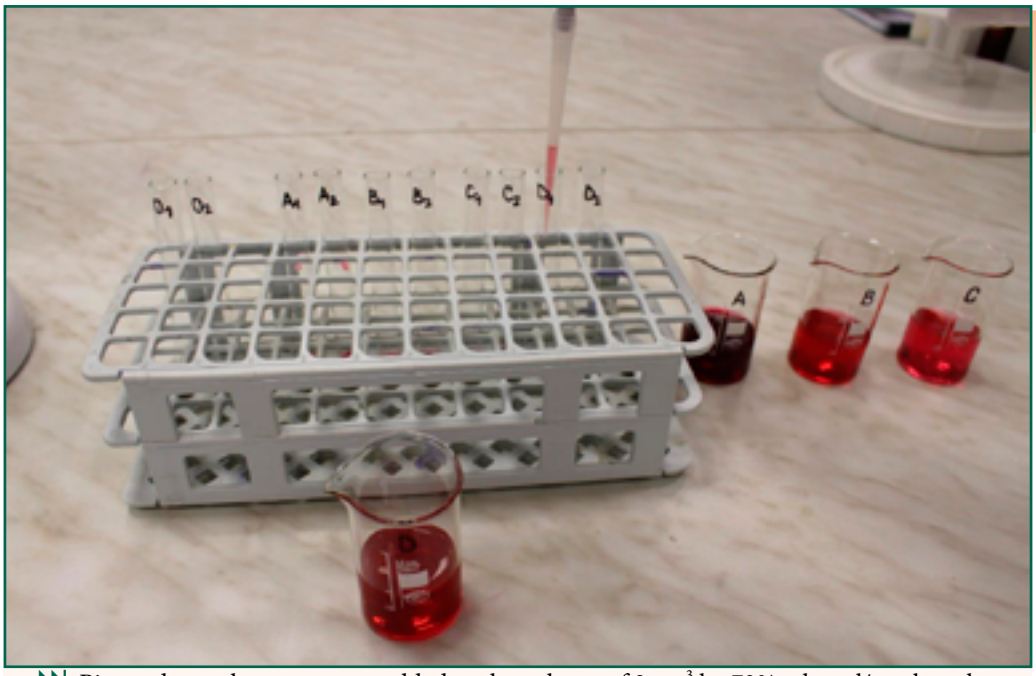
After extraction, sample is filtered through fanfold filtration paper



Filtered alcoholic extracts are prepared for analysis

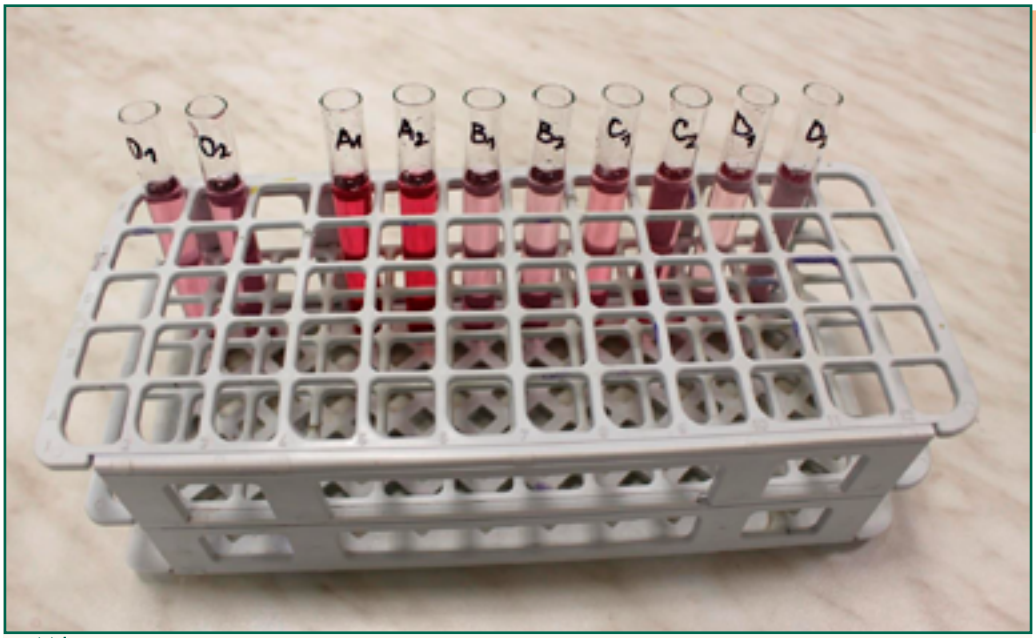


From each sample extract, 0.2 cm^3 (volume can be ranged from 0.1 to 0.8 cm^3, according to the antioxidant content) is pipetted to two tubes



Pipetted sample extracts are added to the volume of 2 cm^3 by 70% ethanol/methanol

Into the each tube, 4 cm^3 of DPPH solution (25 mg DPPH.dm^{-3}) is added



Simultaneously to the sample, reference sample (O1 and O2 in the figure) are prepared by following way: 2 cm^3 of 70% ethanol/methanol and 4 cm^3 of DPPH solution is mixed in the tube

The zero absorbance for 70% ethanol/methanol is set on the spectrophotometer at wave length of 517 nm

Samples are pipetted to the spectrophotometer cuvette after 30 minutes

The filled cuvette is put into the spectrophotometer

The absorbance of all samples is measured at wave length of 517 nm

1.2 Refractometry

Refractometry is a method based on the refractive index measurement. Based on the refractive index, the concentration of compounds in solution, their purity or their structure can be determined.

It is the ratio of the ray rate in two optical environments or the ratio of sinus of incidence angle α and sinus of refraction angle β.

The refractive index is not an absolute quantity, it depends on:

- temperatures (mainly in liquids and gases),
- pressure (gases),
- the wavelength of the used light.

When measuring the refractive index with respect to the used radiation, it can be used:

1. monochromatic radiation source,
2. polychromatic radiation source (the refractometer is equipped with a compensator).

For qualitative analysis any inaccuracies are compensated by the use of a calibration curve (errors are the same for all determinations).

The refractive index is of additive character. This means that the refractive index value for multicomponent mixtures is given by the sum of the contributions of individual components according to their relative proportion in the mixture.

1.2.1 Determination of refractometric soluble solids (RSS)

A refractometric soluble solid (RSS) is an additive quantity and it expresses the content of dissolved compounds (sugars, acids, etc.) in vegetable extracts. To measure refractometric soluble solids in juice of horticultural crops a manual refractometer is used and its value is expressed in °B (Brix degrees). The tested material is dropped onto the dry prism of the refractometer, and on the scale it is read directly so called refractometric sugar solids (i.e. percentage by weight of soluble sugar and other compounds). Measurement is performed at room temperature.

Practical demonstration of refractometric soluble solids (RSS) determination in fresh red pepper (*Capsicum annum*)

Material:

- refractometer (type DR201-95), hand press.

Chemicals:

- distilled water.

Calibration of the refractometer is checked by distilled water that has refractometric soluble solids (RSS) equal to zero

Average homogenized sample from selected pepper fruits is placed in a manual press

contens

The juice from the homogenized sample is printed on a clean and dry prism of the refractometer

After pressing the READ button the value on the scale is read. The result is an average of three measurements

2 SEPARATION METHODS

Separation methods are used for isolation (separation) of determined compound in the analysed mixture and for removing of disruptive components from the analysed sample. Separation (segregation) includes operations in which the sample is divided into at least two parts with different composition.

Separation methods can be divided according to two criteria:

1. **Separation methods based on phase balances.** This includes traditional partitioning methods such as distillation, sublimation, crystallization, but also modern instrumental techniques such as liquid and gas chromatography.
2. **Separation methods based on different rates of compounds movement.** From traditional separation methods are here included for example diffusion and dialysis, and from modern instrumental techniques at the present there is increasing the use of mass spectrometry or electrophoretic techniques, such as for example capillary and gel electrophoresis, or isotachophoresis.

At the Department of Vegetable Production the **distillation** for the determination of **essential oils content** in spices and aromatic plants is used from separation methods based on phase balances, and liquid **chromatography** for the determination of ascorbic acid in vegetable and fruit species.

2.1 Determination of essential oils by using SFK 1 method, 1997 (Slovak Pharmacopoeia)

Determination of essential oils content in plant drugs is carried out by distillation with water vapour in a special apparatus under the conditions listed below. The distillate is captured in a calibrated tube, the aqueous phase is automatically returned back to the distillation flask.

Before the distillation, the dry drug of the plants is grinded into smaller fractions. Subsequently, the necessary quantity in grams is weighted, suitable for distillation in the distillation apparatus. The distillation apparatus consists from a distillation flask which has a round bottom and a high neck with a draft tube. Upon reaching the boiling point, boiling fluid vapour passes into the cooler while condensing. The steam and condensate pass in one direction of the cooler and the water flows through the cooler jacket in the opposite direction. The obtained distillate flows through the allonge in to the holding tank. The entire distillation apparatus must be fixed by the help of brackets and clamps on iron stands. An electrically heated nest is used to heat the bank.

The prescribed amount of drug is transferred to the flask followed by the distillation as described above for prescribed time and in prescribed rate. After 10 minutes when the heating is completed the essential oils content in splitting funnel is counted. The essential oil content is calculated according to the formula:

$$x\,(\%) = 100 \times V \times \gamma / w$$

where:

V – volume of the essential oils (cm^3)
γ – specific weight of essential oils of plant drug
w – weight of the sample (g)

Practical demonstration of determination of essential oil content in basil (*Ocimum basilicum* L.)

Material:

- distillation apparatus, boiling flask with round bottom, heating nest

A suitably dried average basal drug sample taken from several plants from the given variant is homogenized on the mill for approximately 10–15 seconds as required until the desired fineness and homogeneity of the material is achieved

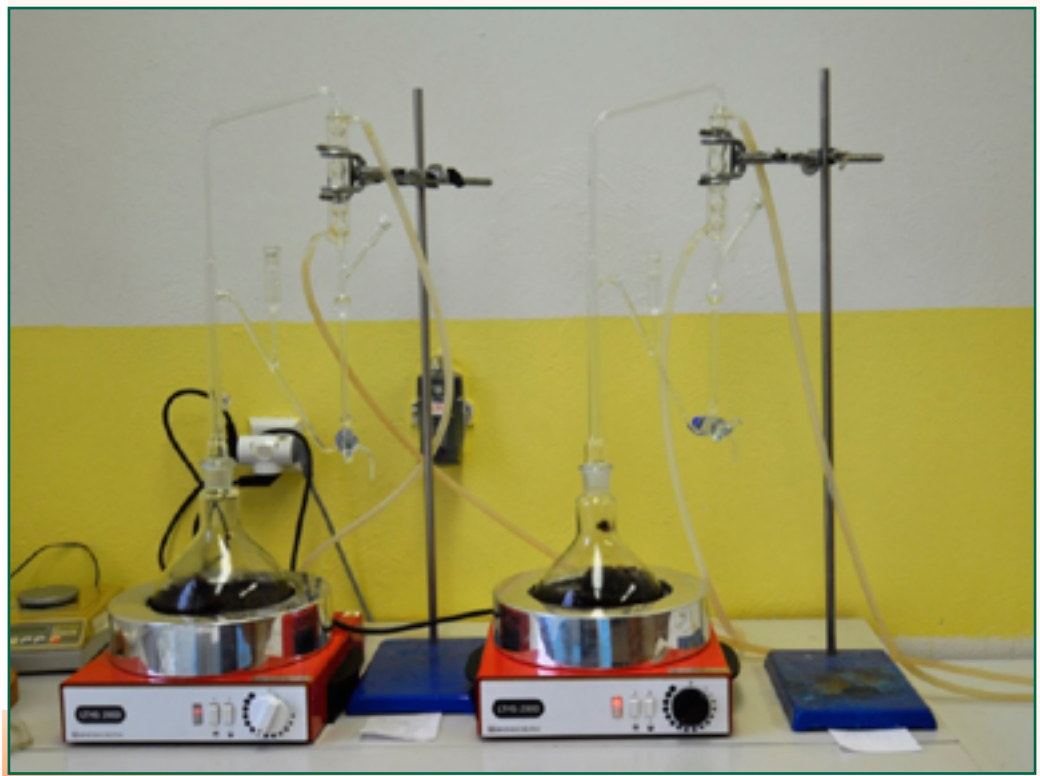
The homogenized sample (20 g) is weighted of and marked appropriately. The same homogenization time is chosen for each variant, since the size of the milled particles is important for the amount of distilled essential oils

Clean dry boiling flask and heating nests are prepared

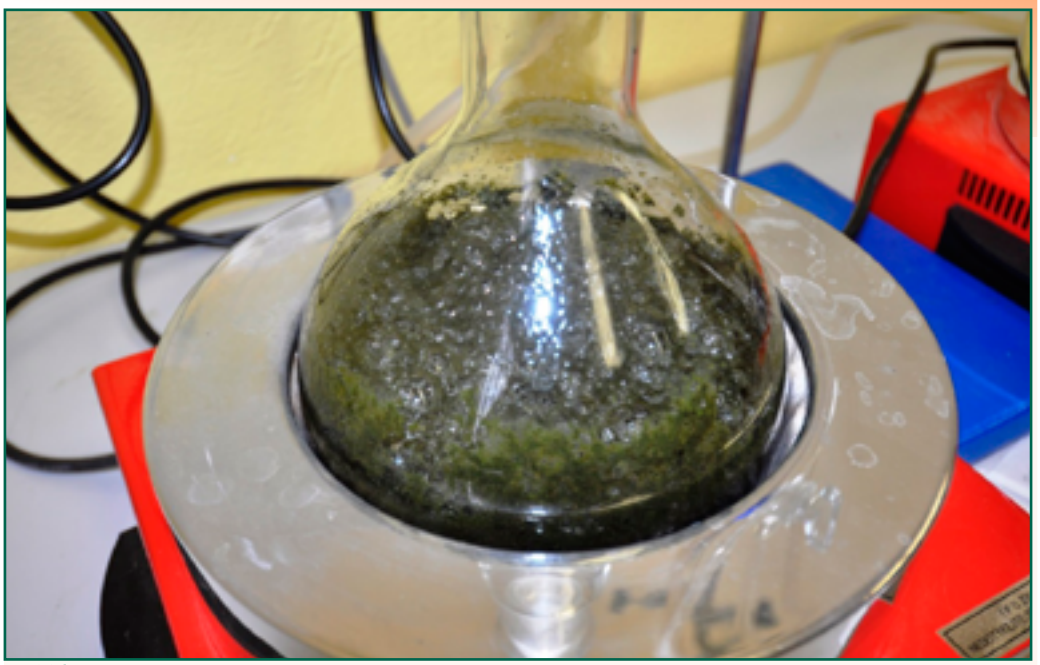
- Weighted homogeneous sample (20 g) is put to round bottom flask through a glass funnel with a wide neck
- The volume of water is added so that it does not exceed 2/3 of boiling flask volume. In case of 2 l boiling flask the water content is 1,200 ml

- The distillation apparatus is assembled; the condensation part adheres to the boiling flask with the finger notch the way that they form a single unit together
- A waste hose and a water supply hose is attached to close the circulating water circuit

The water supply with a slight flow is set and the amount of water in the circuit is regulated according to the drainage flow rate

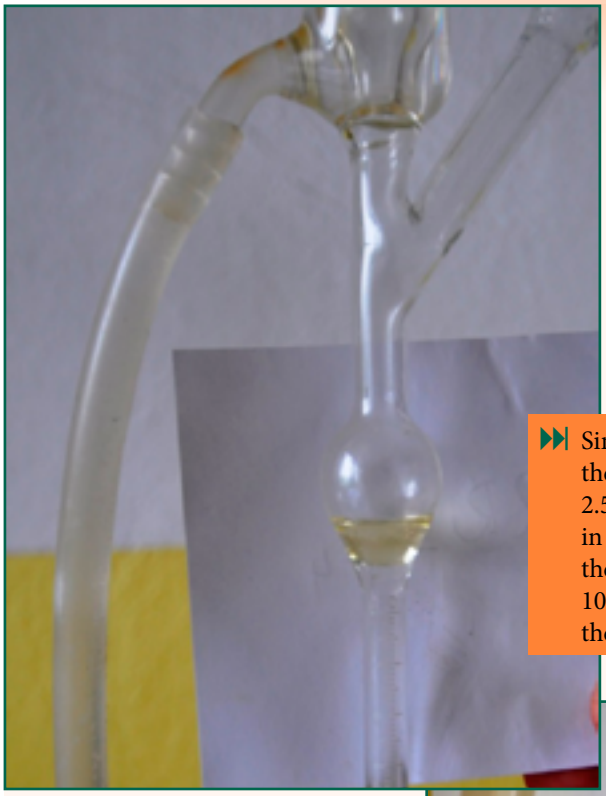


The heating nest is turned on for maximum heating and the content of the flask is brought to boiling point



The dynamics of boiling is monitored, as soon as the boiling point is occurred; the temperature of the heating nest is decreased to keep the mixture simmer for all time. These way solid particles of the sample with water will not enter to the condenser (cooler). In the condensation section the water vapour is circulated with the essential oils

In the cooler the water vapour is condensed to water, and together with the essential oils is captured in the splitting funnel of the condensation apparatus. Whole distillation of the basil takes 2.5 hours



Since essential oils are lighter than water, they accumulate in the upper part. After 2.5 hours, when the volume of basal drug in the reservoir is no longer changed, the heating nests are switched off for 10 minutes to allow the stabilization of the essential oils volume

Using a triggering valve the essential oil is moved into the dividing tube. There is deducted the volume in ml (cm^3). The essential oil content is calculated according to the formula given in the methodology (the specific weight of basil is 0.910 $g.m^3$)

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2.2 Determination of ascorbic acid by HPLC

The principle

The method is used to determine ascorbic acid in non-alcoholic beverages, syrups, wines, lunch and ready-made foods as well as in other foods and nutritional supplements. In the sample, after thorough homogenization and further adjustment by defecation, dilution and filtration the ascorbic acid is determined by HPLC.

Solutions:

- **2% – solution of oxalic acid:** 20 g of oxalic acid is added to 1000 ml volumetric flask and replenished with demineralized water followed by degassing in an ultrasonic bath for 10 minutes.
- **Carrez solution I:** 15.0 g $K_4Fe(CN)_6 \times 3\ H_2O$ is dissolved in 100 ml of demineralized water.
- **Carrez solution II:** 30.0 g $ZnSO_4 \times 7\ H_2O$ is dissolved in 100 ml of demineralized water.
- **Phosphate buffer:** 1.9 g K_2HPO_4 + 2.5 g KH_2PO_4 is dissolved in 1,000 ml of demineralized water, pH = 3.5. If necessary, the pH is adjusted with orthophosphoric acid.
- **Standard solution of ascorbic acid:** on an analytical balance 0.1000 g of ascorbic acid p.a. is weight, transfered to a 100 ml volumetric flask and filled up with 2% solution of oxalic acid (c = 1 mg.ml^{-1}) till mark. The solution is prepared always fresh.

Increase of the prepared solutions stability:

1. To prevent access of oxygen to minimize the risk of oxidation in the preparation of solutions – gently transfer liquids and salts.
2. Prepared solutions of standards and samples after degassing in an ultrasonic bath (approximately 10 minutes) to process within 45 minutes.

Preparing the calibration graph:

To 100 ml volumetric flasks the base solution is pipetted according to following table and it is filled up with a 2% solution of oxalic acid. The prepared calibration solutions (Table 2) are used to prepare the calibration graph. The solutions are prepared always fresh.

Table 2 Preparation of calibration standard solutions

Pipetted volume of the basic solution (ml)	5.0	10.0	15.0	20.0	25.0
Ascorbic acid content (mg.100 ml^{-1})	5.0	10.0	15.0	20.0	25.0

Operating procedure

10 g of homogenized sample is weight into 90 g of oxalic acid (in 2% concertation) followed by mixture homogenization and then it is placed in an ultrasonic bath for 10 minutes. After that 1 ml of Carrez I solution is added to clarify the solution, it is mixed and then 1 ml of Carrez II solution is added. After mixing the extract is filtered and from the filtrate it is dosed for analysis. If necessary the sample is appropriately diluted so that the analyte content is within the range of the calibration graph.

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Conditions on HPLC:

- WATERS HPLC system: Waters 2489 UV/VIS Detector, Waters 1525 Binary HPLC Pump,
- chromatografic column: RP C-18, 5 μ, ID 4,6, length 150 mm,
- mobile phase: Acetonitrile – Phosphate Buffer Solution (pH = 3.5) 5 : 95,
- flow rate: 0.5 ml.min^{-1},
- UV Detection 264 nm.

Calculation

As a calibration method, the calibration curve method is used at 5 concentration levels within the linear response of the test substance. Quantitative evaluation is performed from the measured areas of chromatographic peak standards and samples. For the calibration curve in the linear field the relationship is effective:

$$A_x = k \times c_x + A_0$$

from which the calculation of the sample concentration shall be applied:

$$c_x = (A_x - A_0) \times V_1/V_2 \times 1{,}000/k/n$$

where:

c_x – ascorbic acid concentration (mg.kg^{-1})
A_x – peak area of sample
A_0 – shift of calibration curve
k – directive of calibration curve
V_1 – volume of completed sample
V_2 – volume of pipetted sample for dilution
n – weight (g)

Determination of ascorbic acid by the method of DPPH in Sea-buckthorn (*Hippophae rhamnoides* L.)

Several branches of sea buckthorn with mature fruits are prepared for analysis

The fruits are harvested from the branches and the average sample is prepared

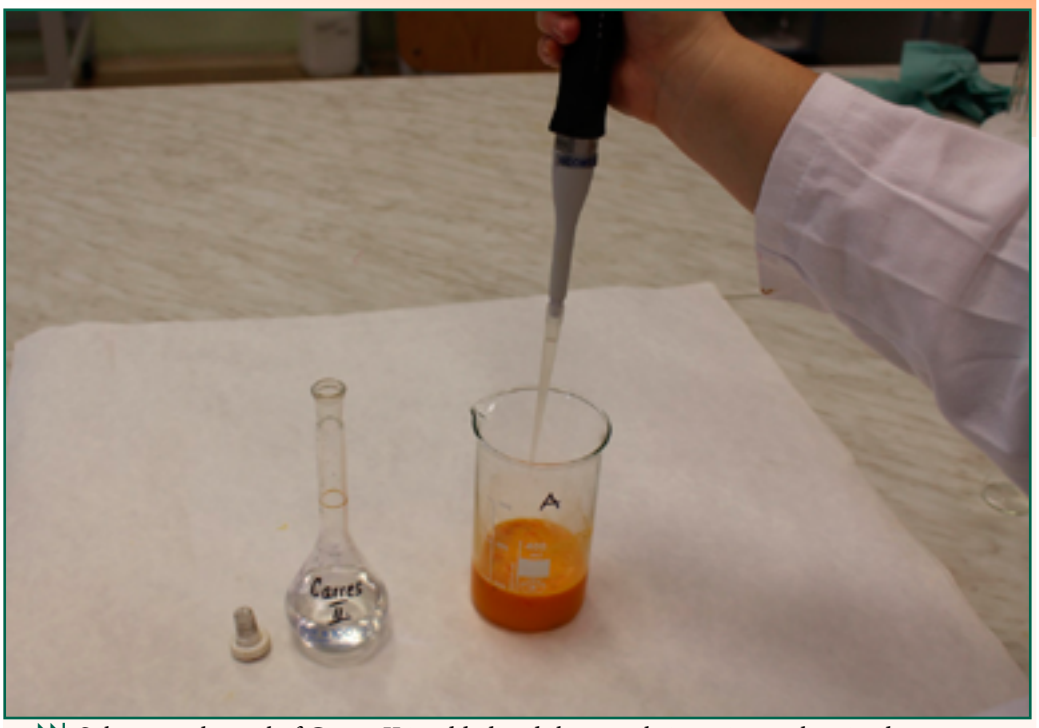
In the beaker 90 g of a 2% solution of oxalic acid is weighted out

⏭ To the prepared oxalic acid solution 10 g of the sample is weighted out

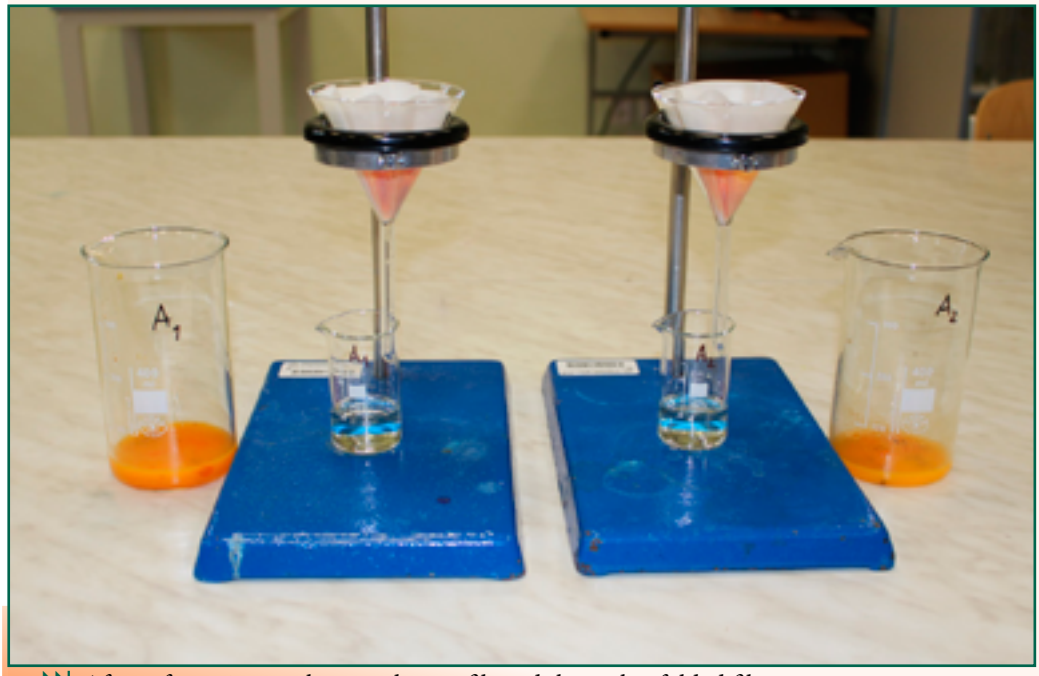
⏭ The weighted sample is homogenized for 1 min

The homogenate is placed in an ultrasonic bath for 10 minutes

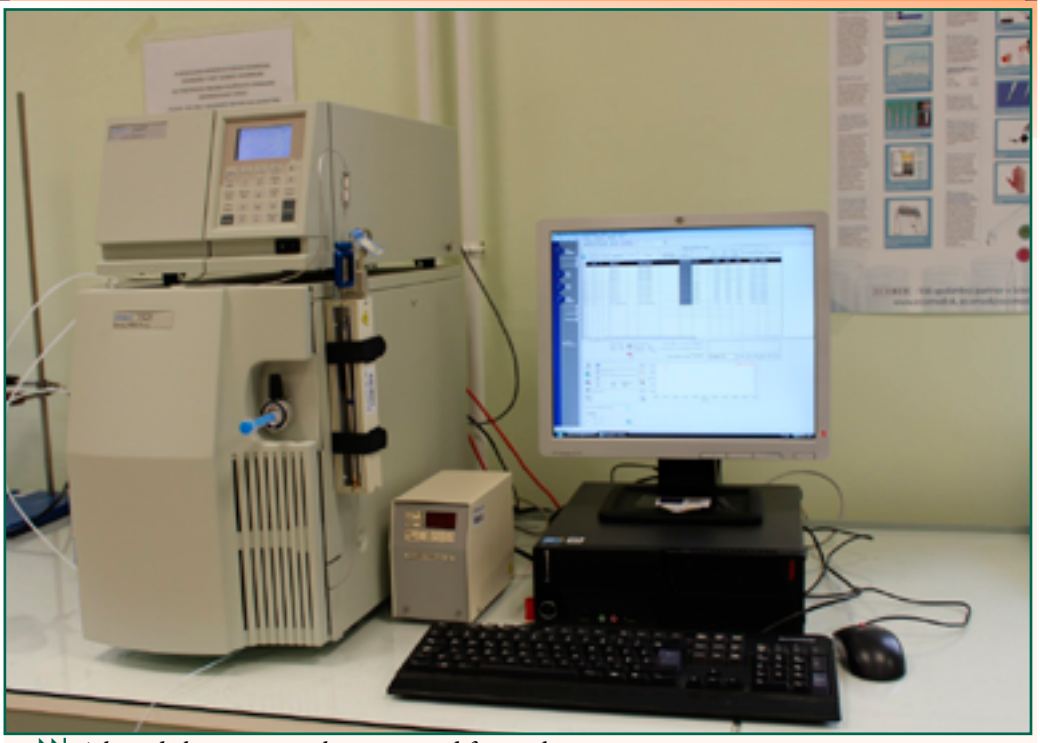
Carres I solution (1 ml) is added to the homogenized sample for clarification and the sample is mixed gently



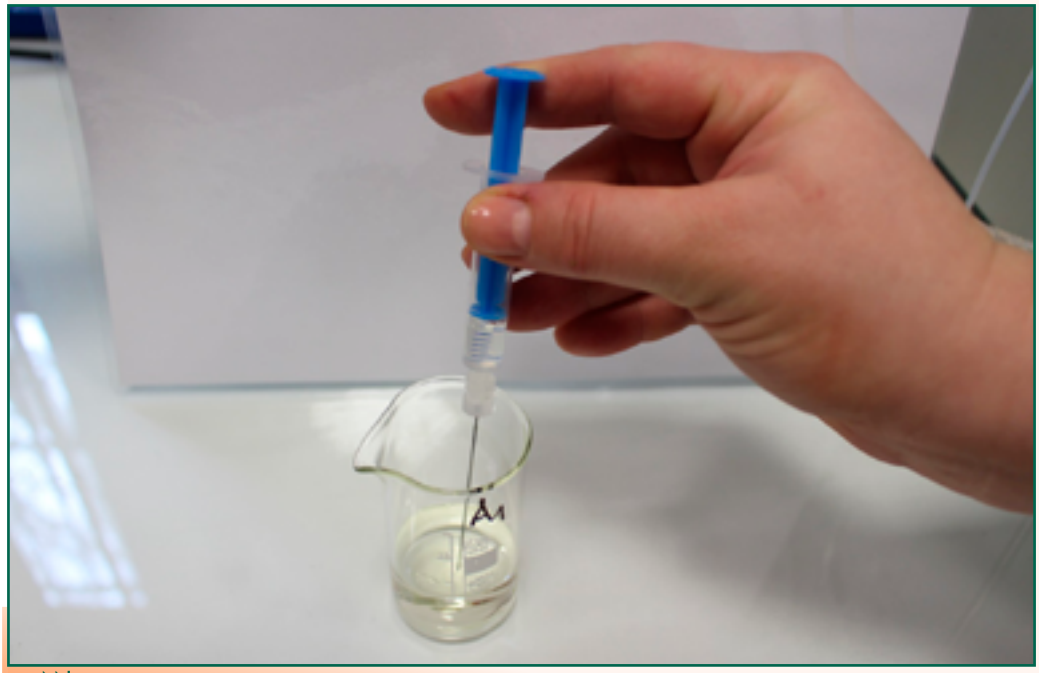
⏭ Subsequently 1 ml of Carres II is added and the sample is again gently mixed



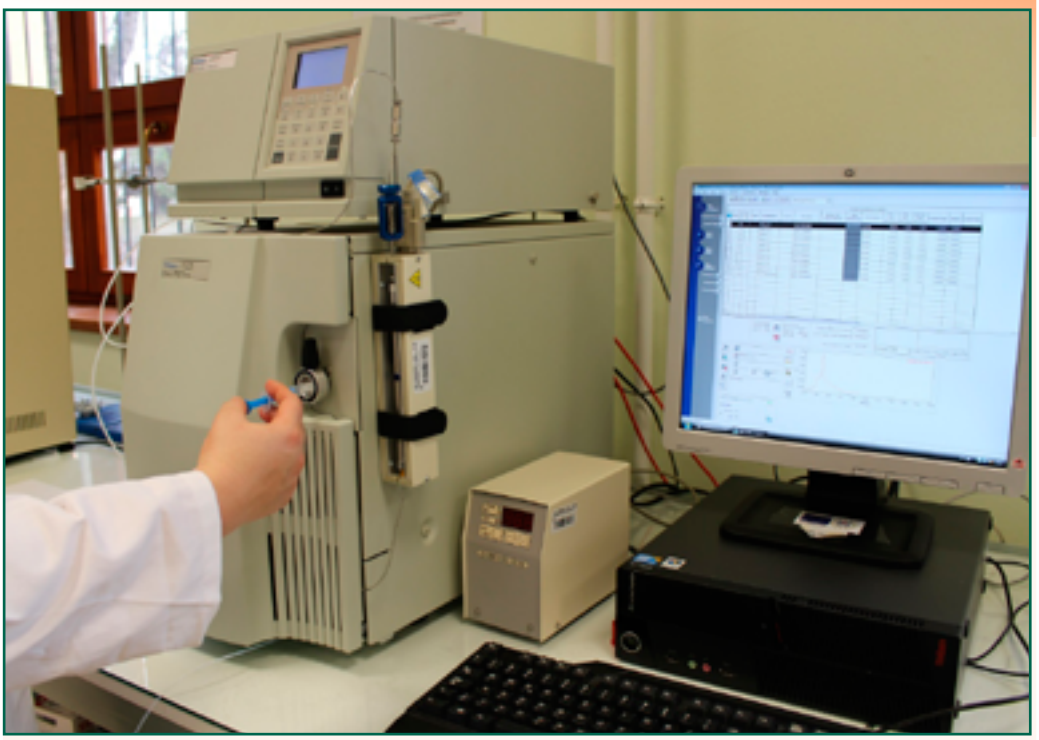
⏭ After a few minutes the samples are filtered through a folded filter



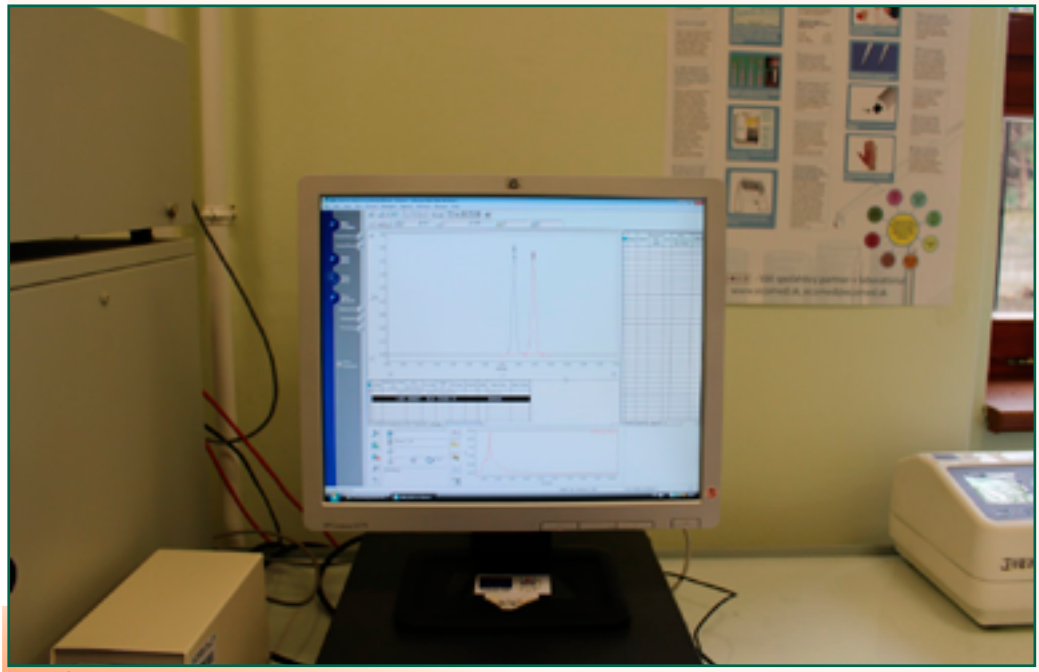
A liquid chromatograph is prepared for analysis



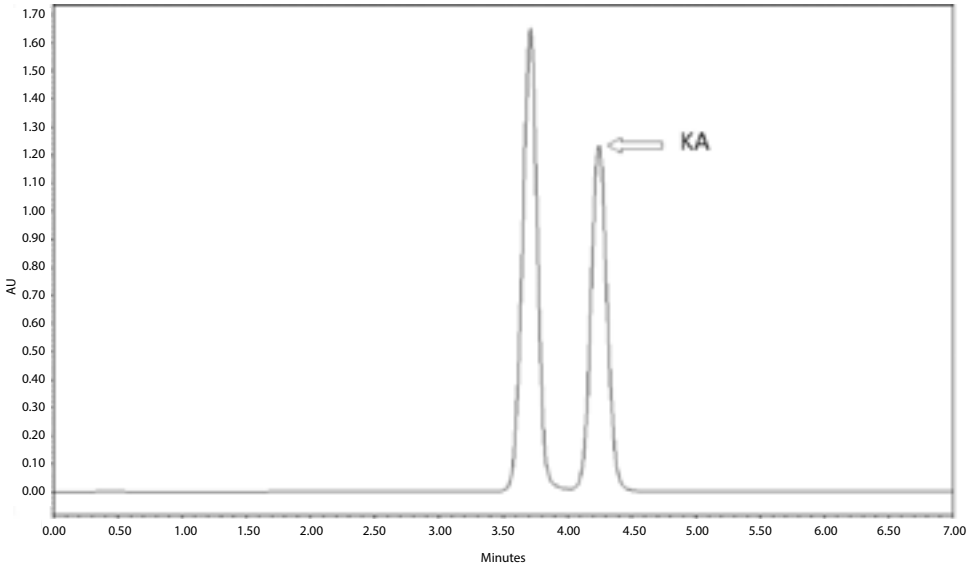
From the clear extract a sample is taken for analysis by the help of dosage syringe



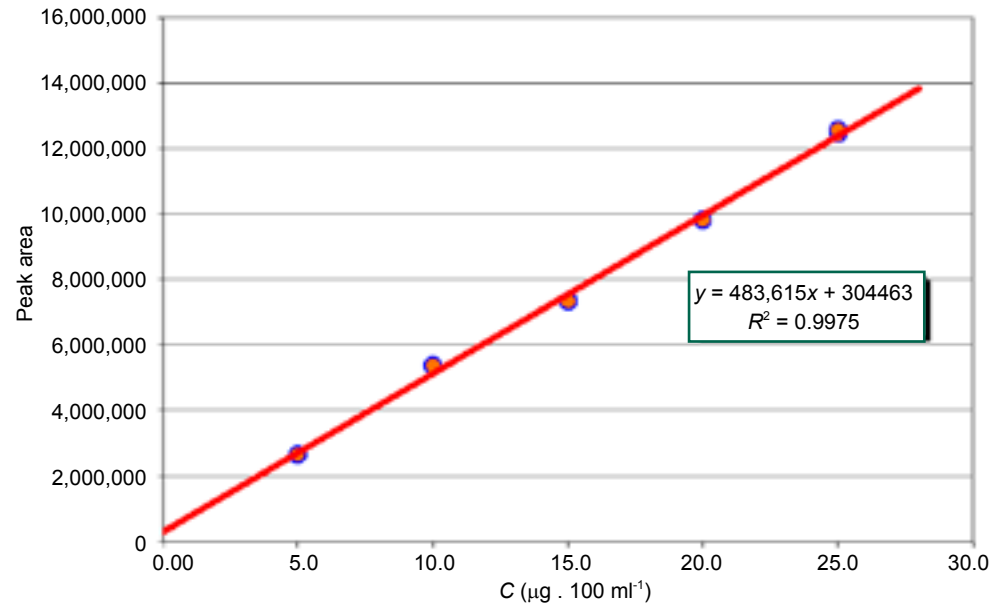
The clear filtrate obtained by filtration is dosed into a liquid chromatograph dispenser



When the analysis is completed, the ascorbic acid peak is evaluated from the obtained chromatogram



The peak of ascorbic acid (KA) of analysed sample



From the regression equation of the prepared calibration curve the ascorbic acid content of the sample is calculated

3 GRAVIMETRIC METHODS

The basis of this method is to **separate** and **weight** of enquired compound.

The separation may occur by:

- **solvent leaching** (most often with water);
- **ejection of determined component by raising the temperature** (when determining moisture: free water, crystal water (bound water) or annealing loss,
- **electrolytic exclusion of metals on platinum cathode** (Ag, Cu, Cd and others) or on platinum anode (PbO_2, MnO_2)
- excluding of a poorly soluble compound having a defined composition (annealing at a higher temperature).

At the Department of Vegetable Production the **gravimetry** is used from gravimetric method to determine dry matter.

3.1 Gravimetric determination of dry matter

The dry matter is non-evaporable residue of substance remaining after evaporation and heating at a maximum temperature of 105 °C to constant weight; in a state where all evaporable compounds are evaporated without residue and no further evaporates.

Determination of dry matter in percentages:

$$dry\ matter\ (\%) = \frac{dried\ sample}{weight\ (fresh\ sample)} \times 100$$

where:

dried sample = (*weight of the desiccator with lid + dried plant material*) - *weight of the desiccant with the lid*

weight = (*weight of the desiccator with lid + fresh plant material*) - *weight of the desiccant with the lid*

In practice it is determination of the exact percentage of matter from the original mass that does not evaporate at mentioned temperature. A complementary supplement to dry matter is moisture, which is the percentage of compounds that evaporate at temperatures below 105 °C. Dry matter is the percentage quantified residual of the mass after evaporation, and the moisture percentage expressed the weight loss after evaporation.

The sum of the two complementary percentages aliquot parts (dry matter + moisture) must always give 100% of the original weight. An example of 100% moisture and 0% dry

matter is distilled water – all matters evaporate to the atmosphere by evaporating of the distilled water without any residue – 100% of the weight of the distilled water is converted to water vapour and there is not any non – evaporable residue at all, it has 0% of dry matter.

Practical demonstration of dry matter and water content determination in pea (*Pisum sativum* L.)

Material:

- aluminium desiccator (evaporating bowl) with lid, laboratory dryer, desiccator.

Empty the aluminium desiccator with the lid is marked and weight. The weight of the empty desiccator is noted

An average pea sample of approximately ±10 g is placed in the desiccator

The aluminium desiccator with the lid and the fresh pea seeds is weighted and the numerical value is marked

The aluminium desiccator with lid and fresh pea seeds is dried at 105 °C to constant weight (approximately 24 hours)

The constant weight of the sample is checked after cooling in the desiccator and after re-drying by further weighing

If the weight of the sample does not change, the sample is considered to be dry

The weight of the aluminium desiccator with the lid and dry pea seeds is written down. The dry matter in percent is calculated the by the formula given in the methodology

ALŽBETA HEGEDŰSOVÁ, IVANA MEZEYOVÁ, ALENA ANDREJIOVÁ

4 MECHANICAL PROPERTIES OF MATERIAL

4.1 Material hardness

Hardness is the mechanical property of the material expressed by resistance to deformation of the surface caused by the geometrically defined body. It is a rheological property, not defined as a physical quantity; its value depends on the complex properties of the surface of the tested material and on the test conditions under which hardness is detected. The hardness of the crops depends on the temperature (e.g. cherries have at 30 °C three times lower hardness than at 0 °C).

4.1.1 Measurement of hardness by using a penetrometer

The method of hardness measuring the by the help of penetrometer is using the measurement of the pressure required to damage of the fruit. It is determined by measuring of the force required to push the tip or spheres of standard size into the crop. It is expressed in Pascal (Pa) or Newton (N). From the measured values of hardness the technological data are processed for transport, harvesting operations and crop storage.

Practical demonstration of hardness (*N*) determination of tomato fruits (*Lycopersicon esculentum* Mill.) by using a penetrometer

Material:

- penetrometer (type PCE-FM200) and accessories for hardness measurement

⏭ Five fruits of different sizes from the variant are selected

Digital penetrometer PCE-PTR 200

- ∅ of senzor 6 mm → grapes, cherries
- ∅ of senzor 6/8 mm → medium hard fruits (plums, lemons)
- ∅ of senzor 8/11.3 mm → hard fruits (apples, pears)
- ∅ of senzor 11.3 mm → very hard fruits

⏭ Appropriate tip according to the classification of plants from hard to soft fruits is selected

⏭ For the tomato a tip with a diameter of 6–8 mm is chosen

The pressure unit (N) is set and the device reset

The fruit is grasped firmly, supported on the pad and pressed in its centre till the area of the tip. The spent force of the pressure is read on device. We reset the device and repeat the procedure from the other side of the fruit. We will penetrate the next 4 fruits of tomatoes in the same way to get 10 values

5 TITRATION ANALYSES

The accurately measured and suitably adjusted sample of solution under the specified conditions is titrated drop by drop under the persistent mixing of the volumetric solution to the point of equivalence.

Equivalent point – is a condition where the ratio between the determined substance and the titrating agent is stoechiometric and exactly corresponds to the defined reaction product.

Achieving of the equivalence point can be indicated:

1. **visually,**
2. **instrumental.**

The visual indication is allowed by so-called chemical color indicator that is deliberately added to the reaction system.

The indicators serve to indication of achieving of the conditions of the equivalence point by changing of the coloration and thus allow finishing of addition of further shares (volumes) of titration agent.

Titration is a method of quantitative chemical analysis based on the accurate measurement of the volume of added measuring agent.

The result of the titration is expressed as:

- the amount of the determined substance *n*,
- the weight of the constituent substance *m*,
- the relative proportion of the constituent substance, e.g. in %.

To calculate the titration result itself is needed to know:

- the stoechiometric equation of determination,
- the volume of the volumetric reagent with known concentration,
- weight of the weighted sample if the result should be expressed by proportional
- representation.

Distribution of titration methods in analytical chemistry according to chemical nature:

1.
   - ⏭ acidimetry (titrated with acid),
   - ⏭ alkalimetry (titrated with a base).
2. Oxidation-reduction titrations:
   - ⏭ oxidometry,
   - ⏭ reductometry.
3. Precipitation titrations.
4. Complex-formation titrations.

contents

The methods are relatively fast, available financially as well as from equipment point of view, accurate and easy to implement.

The device for volumetric analysis consists of:

- measuring glass (pipettes, burettes, measuring banks),
- titration flasks, which are most commonly the wide-angle titration banks of conical shape.

In manual titration the titration bank is moved manually in circular motion under the persistent mixing during adding of the volumetric solution from burette to mix the agent with titrated solution properly. Magnetic mixers may also be used to mix the solution. The volumetric solution is added first in the stream and then dropwise, which may be separated if necessary by a clean stirrer. In case of the last drops there is always need to wait for the stabilization of chemical balance.

In visual titration colour changes are best observed against to filter paper.

Sludge is well identified against black glossy paper.

Basic compounds

In volumetric analyses the volumetric solutions are most often prepared at an approximate concentration.

The exact concentration of the volumetric solutions is determined by the basic compounds which must be:

- a precisely defined composition,
- a purity above 99.99%,
- high molar weight, related to the reduction of errors in weighting,
- fastness to air (humidity, CO_2, ox.-red.),
- good solubility in water,
- fast, stoechiometrically complete reaction with the volumetric agent without side reactions,
- easy determination of the equivalence point,
- harmlessness from the safety at work point of view.

Determination of exact concentration

By titration with volumetric solution of basic compounds:

1. the base substance is weighted directly (not differentially);
2. the concentration of a properly prepared volumetric solution of the basic compound is considered to be the exact concentration.

Calculation:

$$c_t = \frac{c_z \times V_z}{V_t}$$

where:

c_t – calculated concentration of the volumetric solution (mol.dm^{-3})
c_z – the exact concentration of the base compound solution (mol.dm^{-3})
V_z – volume of the base compound solution (dm^3)
V_t – volume of the volumetric solution (dm^3)

At the Department of Vegetable Production **acid-basic titration** are used to determine **the acid value** and the **oxidation-reduction titration** for the determination of **ascorbic acid.**

5.1 Acid-basic titration

The basis of the neutralizing analysis is the mutual reactions of acids and bases. It's protolytic process because there is an exchange of a proton between acid and basic.

The reactions take place in the pH range depending on the environment:

Aquatic environment:

- is limited by the ionic composition of water Kv (autoprotolytic constant) 1.10–14, which means – pH from 0 till 14.

Solutions with value:

- **pH <7** are acid solutions – high concentration of H_3O^+,
- **pH = 7** are neutral solutions – equal concentration H_3O^+ and OH^-,
- **pH >7** are basic solutions – high concentration OH^-.

Acidimetry – titrated with acid – HCl, HNO_3, H_2SO_4...

Alcalimetry – titrated with base – NaOH, KOH, $NaHCO_3$...

5.1.1 Determination of titration acidity by STN560240-5

The basis of the determination consists in neutralizing of the acids by using a suitable indicator, which is in case of determination in fruit and vegetable products the phenolphthalein. The result is converted to citric or malic acid.

25 g of the homogenized sample is quantitatively rinsed with distilled water to volume of 250 cm^3, than it is mixed and filtered. The filtrate (50 cm^3) is pipetted into an Erlenmeyer flask and heated to 60–70 °C, 3–5 drops of phenolphthalein are added and titrated hot with a solution of NaOH in a concentration of $c_{(NaOH)}$ = 0,1 $mol.dm^{-3}$ until pink coloration which lasts for at least 30 seconds.

Calculation:

$$x = \frac{a \times 0.64}{n}$$

where:

a – consumption of NaOH (cm^3)
0.64 – factor for conversion to citric acid
n – weight (proportion of used filtrate)

Practical demonstration of total acids titration in tomato (*Lycopersicon esculentum*)

Chemicals:

- NaOH (c_{NaOH} = 0.1 mol.dm^{-3}), 1% phenolphthalein.

Material:

- cooker, Erlenmayer bank, cooking glass, titration apparatus, distilled water.

From 8 to 10 fruits from each tomato variants is chosen, washed and dried, cut into quarters. Opposite parts of each fruit are used for homogenization

The homogenized samples (by the help of laboratory homogenizer) are appropriately stored and marked. After homogenization they are immediately analysed

25 g of the homogenized sample is weighted and quantitatively rinsed with distilled water into a 250 cm^3 graduated flask

The mixture in the volumetric flask is mixed followed by filtering

50 cm^3 of clear filtrate is pipetted into an Erlenmeyer flask and carefully marked

The filtrate is heated to 60–70 °C (indicator is the dewing of the walls of the Erlenmeyer flask) and then 3–5 drops of phenolphthalein are added

The contents of the flask is titrated under the heat conditions with a NaOH solution (c = 0.1 mol.dm^{-3}) until a pink colour lasts at min. 30 seconds

The acid content is calculated according to the methodology to citric acid and expressed in % or in g.100 g^{-1} of fresh matter

5.2 Oxidation-reduction titrations

The methods are based on the oxidation and reduction reactions of the determined ions and compounds.

Depending on the nature of the process, they are divided into oximetric and reductometric titrations.

Oxidation is increasing of the oxidation degree of the ion or element – handing over of the electron:

$$Fe^{II} - e \Leftrightarrow Fe^{III}$$

In case of organic complex compounds: the intake of oxygen or the breakaway of hydrogen.

Reduction is the decreasing of the oxidation degree of the ion or element – receiving of the electron.

$$Cr^{VI} + 3e \Leftrightarrow Cr^{III}$$

In case of organic complex compounds: the breakaway of oxygen or the intake of hydrogen.

Oxidation and reduction always takes place at the same time!

Oxidation – reducing pairs:

- Half-reaction: $Ag^{+} + e \Leftrightarrow Ag^{0}/.2$
- Half-reaction: $Zn^{0} - 2e \Leftrightarrow Zn^{2+}$

$$2Ag^{+} + Zn^{0} \Leftrightarrow 2Ag^{0} + Zn^{2+}$$
$$Ox_1 + Red_2 \Leftrightarrow Red_1 + Ox_2$$

Oxidimetric titrations are:

1. **manganometry** – volumetric solution $KMnO_4$,
2. **bichromatometry** – volumetric solution ($K_2Cr_2O_7$),
3. **iodometry** – volumetric solution $Na_2S_2O_3(I_2)$.

$$Me^{2+} - e \rightarrow Me^{3+}$$

The reductometric titrations are:

1. titanometry – volumetric solution $TiCl_3$.

$$Me^{3+} + e \rightarrow Me^{2+}$$

Indicators in oxidation-reduction titrations

The equivalent point does not demonstrate itself spontaneously by colour change except of manganometry. In such cases **indicators** are used.

Oxidation-reduction indicators are agents whose colour is changing with the change of the oxidative-reduction potential of the environment.

5.2.1 Determination of vitamin C (ascorbic acid) content by titration method

The content of vitamin C (ascorbic acid) is determined by a conventional titration method, where the titrating agent is a solution of 2.6-dichlorophenolindophenol.

The sample is homogenized in a 5% solution of trichloroacetic acid and in the extract is estimated by titration with 2.6-dichlorophenolindophenol. In first excess of the titration solution is stained in pink.

Preparation of a solution of 2.6-dichlorophenolindophenol – titration reagent

0.2 g of 2.6-dichlorophenolindophenol is weight out and in hot is dissolved in a small amount of water. It is filtered through a blue filter and transfer quantitatively to a 500 ml volumetric flask. After cooling it is added to the mark. A solution of 2.6-dichlorophenolindopenolus is stored in the cold for max. 10 days.

Preparation of solution of ascorbic acid – standard solution (*Va*)

The solution of ascorbic acid is prepared explicitly on the day of determination of vitamin C! It is weighted 0.05 g of ascorbic acid, dissolved in a small amount of water, transferred quantitatively to a 50 ml volumetric flask and filled to the mark with distilled water.

Preparation of plant extract

10 g of homogenized plant material is weighted and immediately is overflowed with 30 ml of 5% trichloroacetic acid solution. The mixture is macerated for two hours in the dark and then filtered. The colourless clear filtrate is used for titration.

Factor (*f*) determination

The factor indicates the number of mg of ascorbic acid corresponding to 1 ml of the titrating agent:

1. **titration of blank experiment** – 10 ml of trichloroacetic acid is titrated as a blank (V_c),
2. **titration of the standard solution** – 10 ml of trichloroacetic acid + 1 ml of ascorbic acid solution is titrated (V_b).

The factor which gives the number of mg of ascorbic acid corresponding to 1 ml of the titrating agent is calculated:

$$f = \frac{V_a}{V_b - V_c}$$

where:

V_a – the volume of standard solution (ml)
V_b – the consumption of the titrating agent for the standard solution with trichloroacetic acid (ml)
V_c – consumption of titration reagent for blank experiment (ml)

Titration of plant material extract

From the filtrate it is pipetted 2 × 10 ml and it is titrated with 2.6-dichlorophenolindophenol to a light pink colouring.

Calculation of vitamin C content:

$$\text{mg of vitamin C} \,.\, 100\ \text{g}^{-1} = \frac{(V_{sample} - V_c) \times f \times 100 \times b}{d \times m}$$

where:

V_{sample} – consumption of 2.6-dichlorophenolindophenol at the titration of the sample (ml)
V_c – consumption of titration reagent for blank experiment (ml)
F – factor
b – the volume of trichloroacetic acid (ml) in what the sample is digested
d – pipetting (ml) (certain fraction of filtrate)
m – weight of sample (g)

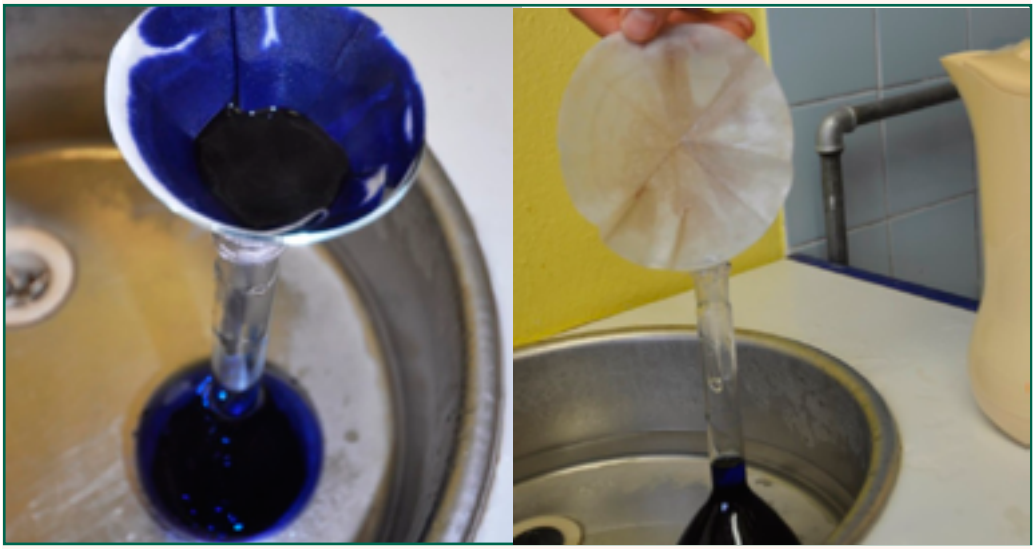
Practical demonstration of vitamin C determination in *Capsicum annuum* L. by titration method

Material:

- stands, titration burette, funnels, volumetric flasks, beakers, pipettes, filter paper.

Chemicals:

- 2.6-dichlorophenolindophenol, trichloroacetic acid p.a., ascorbic acid p.a., distilled water.



Preparation of a solution of 2.6-dichlorophenolindophenol: into the beaker with a weighted of 2.6-dichlorophenolindophenol (0.2 g) is added the hot distilled water in which it is dissolved. After each addition of hot water, the contents of the beaker are quantitatively passed through the filter paper into a measuring flask (500 ml). By successively adding of hot distilled water and pouring through the filter, the blue dye is completely dissolved, washed in solution till the filter paper is again white

The hot 2.6-dichlorophenolindophenol solution in a volumetric flask is cooled in a cold water bath, and after complete cooling distilled water is added to the mark. The solution can be stored in the cold for max. 10 days. Volumetric flask is marked with the date of preparation

A 5% solution of trichloroacetic acid is obtained by dissolving of 50 g of the acid in crystalline form in distilled water and over-spilled it into a volumetric flask (1 l)

An average sample of plant material is washed and dried

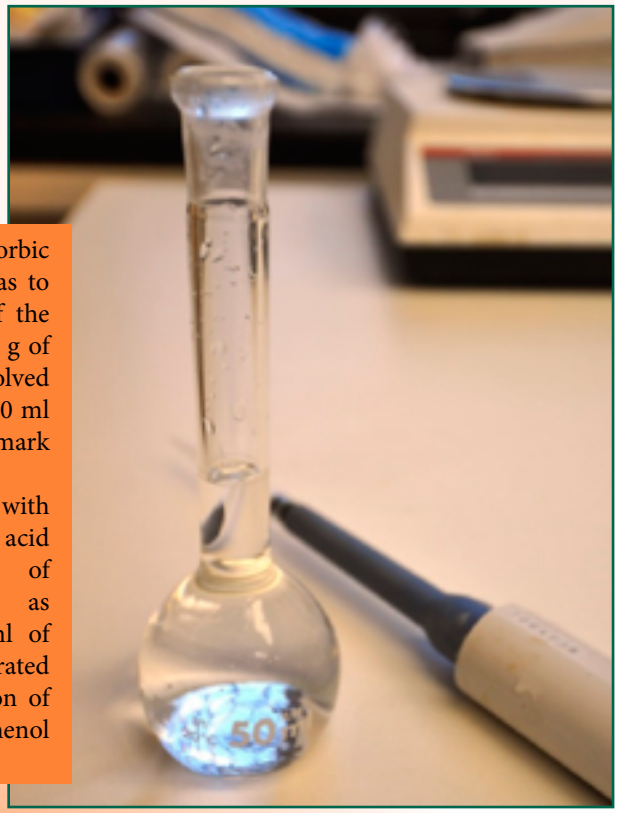
The selected washed fruits are cut, following by the opposite parts selection which are homogenized by a laboratory homogenizer

Each sample is homogenized in the same way

Weighted sample of fresh pepper (10 g) is watered immediately with a 5% solution of trichloroacetic acid in a volume of 30 ml

contents

The samples stored in the dark are for two hours macerated

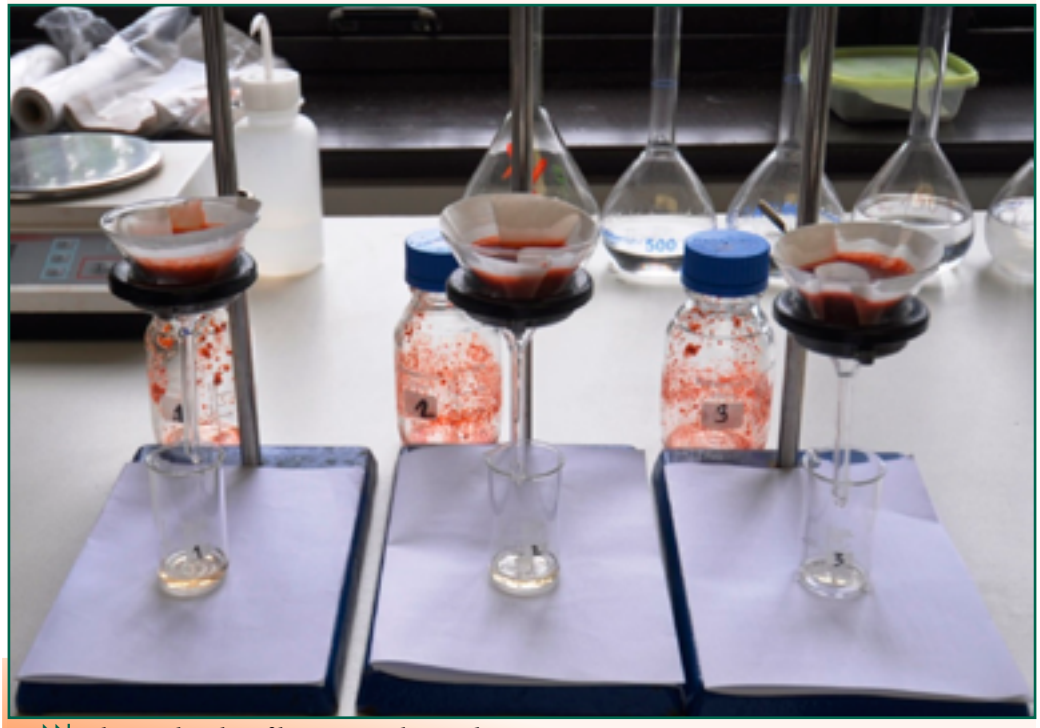


The standard solution of ascorbic acid is prepared. The solution has to be prepared only on the day of the determination of vitamin C! 0.05 g of ascorbic acid is weighted, dissolved in distilled water, poured into a 50 ml volumetric flask and filled to the mark with distilled water

1 ml of ascorbic acid with 10 ml of trichloroacetic acid is titrated with a solution of 2.6-dichlorophenolindophenol as a standard solution (V_b). 10 ml of trichloroacetic acid solution is titrated as a blank test (V_c). Consumption of the 2.6-dichlorophenolindophenol solution is recorded continuously

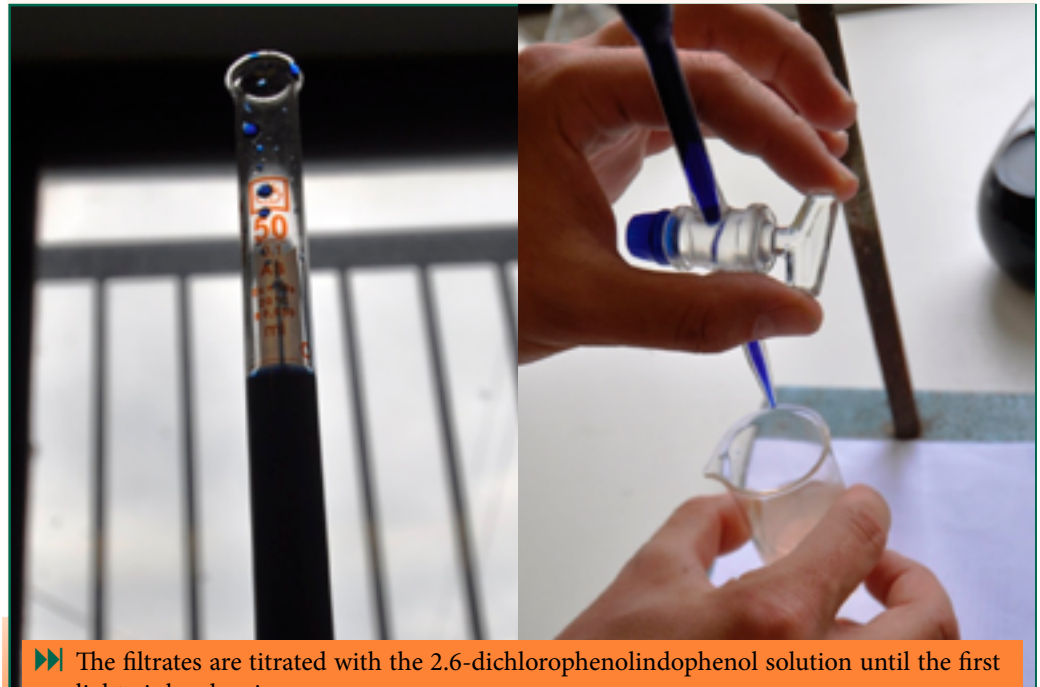
contents

The mixture of red pepper and trichloroacetic acid is filtered after two hours of maceration



Clear, colourless filtrates are obtained

From the clear filtrate 10 ml for titration is pipetted



- The filtrates are titrated with the 2.6-dichlorophenolindophenol solution until the first light pink colouring
- The vitamin C content of fresh pepper is calculated according to the formulas given in the methodology

contents

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