INSTITUTE OF CHEMICAL TECHNOLOGY, PRAGUE
Faculty of Food and Biochemical Technology
Department of Food Analysis and Nutrition

ANALYSIS OF FOOD AND NATURAL PRODUCTS

LABORATORY EXERCISE

Determination of carbohydrates in foodstuff

(LC/RID method)

Exercise guarantor: Assoc. Prof. Karel Cejpek, Ph.D.

CONTENT

Required knowledge

1. Chemistry of saccharides (within lectures on Food Chemistry)
   - structure of saccharides (mainly monosaccharides and disaccharides)
   - profile and content of carbohydrates in food commodities
   - significant properties and reactions of carbohydrates.
2. Food adulteration, methods of detection.
3. Methods of determination
   - physical methods for the determination of saccharides
   - principle and application of refractometric determination of saccharides and dry matter
   - principles of liquid chromatography (types of stationary phase, mobile phase, detectors)
   - preparation of working solutions, basic calculations.

Test questions

- Define carbohydrates and classify them. Give examples of different types of carbohydrates.
- Which carbohydrates are found in following commodities and what is their approximate content:
  - ♣ sweets (various types)
  - ♣ honey
  - ♣ fruit (various types)
  - ♣ fruit juices
  - ♣ vegetable juices
  - ♣ soft drinks.
- How much of D-glucose (D-fructose, sucrose) should be weighed to prepare 25 ml of single component standard solution at a concentration of 1 g / 100 ml?
- What are the volumes of standard solutions of sugars D-glucose, D-fructose and sucrose at a concentration 1g/100ml that we need to prepare 10 ml of mixed solution at a concentration of 0.1 g/100ml (a) and 0.3 g/100ml (b) of individual sugars?
- What is the role of Carrez solutions and what are their components?
- Which calibration method would you employ for the determination of sugars using HPLC with refractometric detection?
- What are the standard conditions for the refractometric measurements? Which parameters can affect the refractive index?
- How to measure the refractometric dry matter, if the refractometer is not equipped with a linear automatic temperature compensation?
- What is the subject of Brix scale and what the scale means? Which other scale for the determination of the dissolved solids´ concentration by refractometry do you know?

Evaluative criteria

- proved theoretical knowledge
- quality of practical work execution
- compliance with laboratory work rules (safety, order)
- protocol – integrity of experimental data, results calculation and discussion

Laboratory exercise content

A. **Determination of mono-and disaccharides in sweets and beverages by HPLC with refractometric detection** (based on CSN 56 8543, Apple juice, 2001).

B. **Refractometric determination (estimation) of the content of dry matter (sugars) by the use of portable refractometer** (based on CSN 56 0414, Estimation of soluble dry matter - refractometric method, 1998)**.**

Specification to the exercises:

- each pair in the working group analyzes one sample
- the calibration curve is prepared by all members of working group, except for students who are preparing food sample with more complex matrix
- injections of the standards of saccharides (for calibration curve construction) have to be done as soon as possible in order to carry out the exercise in time.

Laboratory exercise schedule

<table>
<tr><th colspan="2">TASK</th><th>DURATION (min)</th></tr>
<tr><td colspan="2">Introduction and testing, preparation of the HPLC system</td><td>30 min</td></tr>
<tr><td colspan="2">Discussion on the experimental procedure, organizing and scheduling</td><td>30 min</td></tr>
<tr><td>Preparation of the solutions of analytical standards</td><td>Samples´ preparation</td><td>60 min</td></tr>
<tr><td rowspan="2">HPLC measurements for calibration curves</td><td>Sample preparation (filtration, complex matrices)</td><td>30 min</td></tr>
<tr><td>Calibration of portable refractometer</td><td>30 min</td></tr>
<tr><td>Sample analysis (HPLC)</td><td>Sample analysis (portable refractometer)</td><td>30 min</td></tr>
<tr><td colspan="2">Evaluation of data + data for protocols + cleaning</td><td>30 min</td></tr>
</table>

A. Determination of mono-and disaccharides in sweets and beverages by HPLC with refractometric detection

1. USEFULNESS OF THE METHOD

The method is used to determine the content of monosaccharides and disaccharides in foodstuffs, especially in sweets and beverages.

2. THE PRINCIPLE OF THE METHOD

Saccharides are extracted from sample by the mixture of acetonitril : water. They are determined in the extract after filtration eventually purification by the liquid chromatography method with refractometric detection (RID). Identification and quantification is done by the method of external standard calibration curve.

3. INSTRUMENTS AND EQUIPMENTS

- High pressure isocratic pump HPP 5001 (Laboratorní přístroje Praha)
- Injection valve with a loop (Rheodyne, USA)
- Analytical column - NH_2 phase, 4,6 mm x 250 mm (or 4,6 mm x 125 mm)
- Refractometric detector (RID) (Knauer, Deutschland)
- Analytical scales
- Ultrasonic bath
- Common laboratory glass and other equipment

4. STANDARD AND CALIBRATION SOLUTIONS

- glucose (p.a.)
- fructose (p.a.)
- sucrose (p.a.)

Preparation of standard solutions:

Prepare a stock solution of glucose, fructose, and sucrose of the exact concentration of approx. 1.0 g /100 ml (0.25 g/25ml + 10 ml acetonitrile:water 1:1, v/v, extraction solvent acetonitrile:water 3:1, v/v is added to the mark). For the completion of calibration curve, prepare mixed standard solutions at concentrations of 0.1, 0.3, and 1.0 g/100 ml for each sugar (solutions of concentrations 0.1 and 0.3 are mixture of glucose, fructose, and sucrose of this concentration). Use mixture acetonitrile:water 3:1, v/v, for the dilution of standards.

5. CHEMICALS, SOLUTIONS, AND MATERIALS

- Acetonitrile, grade for HPLC
- demineralised water
- Carrez I solution: 30% $ZnSO_4$
- Carrez II solution: 15% $K_4Fe(CN)_6$
- Folded paper filter at medium porosity
- membrane filter of 0.2-0.45 µm porosity
- unless specified otherwise, use chemical of cleanliness class p.a.

6. WORKING PROCEDURE

6.1. Preparation of the laboratory sample

6.1.1. *Solid Samples*

Pulverize the solid samples (e.g. honey, dia sweets, dry milk) in mortar with pestle and mix it perfectly. Weigh the amount of 5-10 g of homogenous sample into 250 ml Erlenmayer flask, add 100 ml of hot water, and ultrasonicate the sample for 15 min. To clear up the sample mixture, add 3 ml of Carrez I solution and, after mixing, also 3 ml of Carrez II solution. After additional mixing of the sample, filter it and transfer the filtrate quantitatively to 200 ml volumetric flask. After chilling of the solution to the laboratory temperature, add the extraction solvent to the 200 ml mark. Before introduction the sample into liquid chromatograph, filter all sample solutions using a membrane filter.

6.1.2. *Liquid Samples*

Stir properly the samples of fruit beverages, lemonades, and alcoholic beverages and dilute them by the mixture of acetonitrile:water (in relation according to assistant instruction). Before introduction the sample into liquid chromatograph, filter all sample solutions using a membrane filter.

6.1.3 *Other Samples*

Tang – approx. 0.06 g to 10 ml (acetonitrile:water 3:1)
Syrup – approx. 0.1 g to 10 ml (acetonitrile:water 3:1)
Drops – 1 drop (pulverize, weigh) + 40 ml hot water + 3 ml Carrez I and 3 ml Carrez II to 100 ml (top up by acetonitril)
Before introduction the sample into liquid chromatograph, filter all sample solutions using a membrane filter.

6.2. Analysis by liquid chromatography

Column: analytical column filled with NH_2, mobile phase (4.6 x 250 mm, or 4.6 mm x 125 mm, Lichrospher, Spherisorb) with a precolumn
Mobile phase: acetonitrile:redistilled water (80:20, v/v; alternatively 75:25, v/v)

(the mobile phase prepared is degassed in ultrasonic bath for 10 min)

Flow of mobile phase: 1 ml/min (alternatively 1.2 ml/min)

Detection: refractometric detector KNAUER
Volume to be introduced to chromatograph: 20 µl
Temperature: laboratory temperature

Chromatograms are evaluated by software CSW32

6.3. Identification and quantification

Identification is done by the comparison of retention time of an analyte in analyzed sample with the retention time of the calibration standard.

Quantitative analysis is done by the external standard method by reading the concentration in analytical sample from the calibration curve (the dependence of the area or height of the peak on the

concentration of standard in g/l; concentration in the analytical sample must be in interval between the lowest and highest point of calibration curve) and by recalculation on the original sample by the following formula:

$$c_a = x \cdot V_a / n$$

c_a - analyte concentration [g/kg, or g/l]
x - analyte concentration read from calibration curve [g/l]
V_a - the volume of sample solution after completion of the volume of volumetric flask [l]
n - sample weight [kg, or l]

Linearity of the method

The response is linear in the range 0.05-1.00 g/100ml

Limit of detection, limit of quantification, and repeatability of the method

Limit of detection (LOD) is the lowest amount of analysed compound in the sample detectable by this method. Limit of detection is calculated as 3x of the noise in real sample.

Limit of quantification (LOQ) is the lowest amount of analysed compound in the sample detectable by this method. Repeatability was calculated from repeated analyses of real sample and expressed as relative standard deviation (RSD %).

	Limit of detection (g/100ml)	Limit of quantification (g/100ml)	RSD (%)
sucrose	0.01	0.05	4.3
glucose	0.01	0.06	2.5
fructose	0.01	0.06	1.8

B. Refractometric determination of dry matter (sugars) by the use of portable refractometer

1. USEFULNESS OF THE METHOD

The method is used to determine the sugar content of syrups, fruit juices, ferments, vegetable juices and milk-based drinks, and to estimate the total concentration of monosaccharides and disaccharides in any solution.

2. THE PRINCIPLE OF THE METHOD

A liquid sample is pipetted to a measuring cell or the measuring cell is immersed in the sample. The instrument measures the sugar content in selected unit of measurement (concentration within the calibration curve, percentage of Brix scale, the sugar content of the grape juice, etc.) after previous adjustment and setting of temperature compensation coefficients.

3. INSTRUMENTS AND EQUIPMENTS

- Portable Refractometer Refracto 30PX (Mettler Toledo, Schweiz) with assessories
- Analytical scales
- Common laboratory glass and other equipment

4. STANDARD AND CALIBRATION SOLUTIONS

- glucose (p.a.)
- fructose (p.a.)
- sucrose (p.a.)

Preparation of standard solutions:
Prepare a stock solution of glucose, fructose, and sucrose of the exact concentration of approx. 20 g/100 ml after the instruction of the assistant. For the completion of calibration curve, prepare standard solutions of glucose, fructose, and sucrose at concentrations of **1**; 2; **3**; 4; 5; **10** and **20** g/100 ml. Prepare a calibration curve from 3-4 points, choose the concentrations in consultation with the assistant. Use water for the dilution of standards.

5. CHEMICALS, SOLUTIONS, AND MATERIALS

- demineralised water
- folded paper filter at medium porosity
- membrane filter of 0.2-0.45 μm porosity

6. WORKING PROCEDURE

6.1. Preparation of the laboratory sample

6.1.1. *Liquid Samples*
Stir properly the samples tested and filter them eventually - after instructions of the assistant - dilute them through a folded filter or membrane filter, respectively. The determination of insoluble solids is carried out without any dilution.

6.1.2 *Other samples*
Tang – approx. 1 g to 10 ml water
Syrup – approx. 1 g to 10 ml water

6.2. Setup and calibration of the portable refractometer

According to the instruction assistant, switch the Refracto device on and adjust it by distilled water: pipette carefully a few drops of water in the measuring cell. If the deviation is > 0.0005, calibration on the water shall be performed.
To completely remove the remaining sample, get the measuring cell dry using pulp.
Follow the instructions assistant and set a measurement unit after the application used:
a) "Sugar" (determination of sugars) - display of results (the content of soluble solids) in ° Brix (as g sucrose / 100 g sample). For citrus juices and concentrates correct acidity after the instructions of the assistant (see Appendix chapter 8 and Annex A).
b) "Conc." (concentration) - concentration measurement by entering the desired concentration conversion formula y = a + bx at the reference temperature, where
y = concentration in % or without units
a, b = coefficients depending on the sample
x = measured refractive index
Select an appropriate temperature compensation coefficient from the instrument memory according to the instructions of the assistant.
c) "Wine" (sugar content in grape juice)
The results are displayed in one of the selected units (° Oechsle, ° KMW, ° Baumé)

Calculation of the temperature compensation coefficient
- Measure the refractive index (n_D) of the sample:
• temperature (T_1) above the normal temperature measurements (n_D^{T1})
• temperature (T_2) under normal temperature measurements (n_D^{T2})
- Calculate α according to the formula:

$$\alpha = (n_D^{T1} - n_D^{T2}) / (T_2 - T_2)$$

- Insert the value of $\alpha * 1000$ into the device

6.3. Measurement

According to the instructions of the assistant, measure the prepared standard solutions and samples. Make sure that prism and measuring cell are totally clean before each measurement. Ensure that the measured samples are homogeneous and close to the laboratory temperature. Pipette a sample in the measuring cell. The measuring cell has to be filled to the mark. After temperature equalizing (about

20 s) carry out a measurement according to the instructions for use of the device. During the measurement, it is necessary to maintain a constant temperature. When measuring with the use of temperature compensation coefficient α, select desired coefficient and then measure the sample. Do not store the results in the device memory, but write it in your lab diary. The content of soluble solids (° Brix) is read directly in % of sucrose with 0.1% precision. Carry out two parallel measurements for each sample. The result obtained is the content of sugar in% determined by refractometry.

6.4. Repeatability of the method

Absolute difference between two single test results performed on the same test material by one operator, using the same equipment, in the shortest possible time interval does not exceed repeatability value r in more than 5% of cases.
The repeatability values are:

juices	r = 0,15 °Brix
concentrates	r = 0,2 °Brix

Appendix

ČSN 56 0414, 1998: Estimation of soluble solids content - refractometric method.

The text of the technical standard cannot be freely disseminated, and, therefore, this Annex cannot be included in electronic form of the laboratory manual. Students can study this standard during laboratory exercises, or can access the full texts of the Czech technical standard *via* the Central Library, ICT.