STANDARD OPERATION PROCEDURE
Faculty of Biosciences, NMBU

Method name: Sugar
BIOVIT-nr.: Arb1018

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1. Introduction:

Sucrose, glucose, and fructose are separated by liquid chromatography (HPLC) and detected with a refraction index (RI) detector. Column, flow rate and temperature are taken from the application note "sugar screening using Eurokat column". (Application No.: VFD0190). www.knauer.net/en/sugar-screening-using-eurokat-column/a41993

The method detects and quantifies the individual levels of sucrose, glucose, and fructose, contrary to the WSC (water soluble carbohydrates) method, and the ESC (ethanol soluble carbohydrates) method which normally gives the total amount of sugar in one result which also includes fructans (fructose polymers, which usually have a glucose moiety).

WSC = sucrose+glucose+fructose+fructans
- the samples are extracted with water and most of the fructans are extracted.

ESC = sucrose+glucose+fructose+short-chain fructans (degree of polymerization up to about 20 fructose units in length)
- the samples are extracted with 40% ethanol and only the short-chained fructans are extracted.

In the Sugar method the samples are extracted with water, meaning that most of the fructans are extracted in the same way as for the WSC method. But since the samples are analyzed with chromatography the fructans are separated from the sucrose, glucose, and fructose peaks in the chromatogram. The method can be used for a rough estimation of the levels of fructans, but not quantification, since fructans are a very inhomogeneous class of compounds.

2. Reagents:

- Mobile phase: MilliQ water
- Extraction solvent: MilliQ water

- Glucose, fructose and sucrose standard (Art.nr: CAR10-1KT - Merck).

Preparation of Stock solution of glucose, fructose, and sucrose:
Weight in 150 mg of each sugar and dissolve in 10 mL of milliQ water = 15 mg/mL

Mixing equal parts of these three solutions will give a stock solution containing 5 mg/mL of each sugar. This stock solution is diluted to give standards containing 0,25, 0,5 and 1 mg/mL

3. Risk assessment

No risks

4. Equipment

- 300 mL Erlenmeyer flask
- HPLC-RI: (Azura, Knauer).
- Software: Chromeleon (Thermo Scientific).
- Column: Eurokat Pb; 300 mm x 4 mm ID, 10 µm (Knauer, Art No.: 30DX350EKN).
- Thermostatic water bath 60 °C

5. Special remarks

6. Sample material:

Freeze dried silage and forages.

7. Work procedure

1. Weight in approximately 1g sample in a 300 mL Erlenmeyer flask

   (the sample weight can be adjusted based on sample type and expected levels of sugar)
2. Add 50 mL of preheated milliQ water (60 °C)
3. Extract the samples in a water bath at 60 °C for 40 minutes.
   a. Cover the flasks with counting glasses to avoid evaporation.
   b. Use metal rings to stabilize the flasks in the water bath.
4. Cool down the samples quickly in cold water.
5. Filtrate the samples with folding filter.
6. Transfer approximately 1 mL sample to a 2mL vial with lid
7. The samples are ready for analysis on the HPLC system.

HPLC parameters:

Column: Eurokat Pb; 300 mm x 4 mm ID, 10 µm (Knauer)

Column temperature: 75 °C

Injection volume: 40 µL (can be increased/decreased, if necessary, max 100µL)

Mobile phase: MilliQ

Flow rate: 0,7 mL/min (increased from 0,5 due to low back pressure)

Detector temperature: 35 °C

Time per analysis: 25 minutes

8. Calculations

Three standard samples containing sucrose, glucose, and fructose in three different concentrations are used to construct standard curves, one for each sugar.
The concentration of the individual sugars in the samples are then calculated using the regression equation from the standard curves. $Y = ax + b$

Where:
Y = the concentration of the sugar (e.g., sucrose)
X = the area of the peak relating to the sugar (e.g., sucrose)
a = the slope of the standard curve
b = the intercept with the Y-axis

The concentration can then be calculated:

$X = (Y - b)/a$

Example: (regression equation for sucrose)
$Y = 5{,}66x + 0{,}078$

If the area of the sucrose peak in a sample = 14,64, and 200 mg sample was weight out:

$X = (14{,}64 - 0{,}078)/5{,}66 = 2{,}57$ mg/mL

$\frac{2{,}57 \text{ mg/mL} * 8 \text{ mL}}{0{,}2 \text{ g}} = 102{,}8$ mg/g (same as g/kg)

Excel sheet for calculating is to be found under:

Labmal -> diverse analyser -> sukker
«standardkurve sukker»