STANDARD OPERATION PROCEDURE
Faculty of Biosciences, NMBU

Method name: Fermentation quality
BIOVIT-nr.: Arb1018

---

1. Introduction:

Lactic acid, acetic acid, butyric acid, propionic acid, formic acid, and ethanol is separated by liquid chromatography (HPLC) and detected with a refraction index (RI) detector. For a total fermentation quality Ammonuim-N (Arb1133) and pH is also analyzed. Column, flow rate and temperature are taken from the application note "separation of organic acids and alcohols with Eurokat H". (Application No.: VFD0054J).
https://www.knauer.net/en/separation-of-organic-acids-and-alcohols-with-eurokat-h/a628

2. Reagents:

- Mobile phase: 0,01 N H_2SO_4
- Extraction solvent: RO water (cold)

- Standards of lactic acid, acetic acid, butyric acid, propionic acid, formic acid

- Ethanol

Preparation of 2 mg/mL stock solution: dissolve 100mg of each in 50 mL milliQ water.

Make three standard samples of approximately 0,5 – 1 – 2 mg/mL

3. Risk assessment

No risks

4. Equipment

- 200 mL beaker
- Folding filter
- Plastic funnel
- HPLC-RI: (Azura, Knauer).
- Software: Chromeleon (Thermo Scientific).
- Column: Eurokat H; 300 mm x 8 mm ID, 10 µm (Knauer, Art No.: 30GX340EKN).

5. Special remarks

6. Sample material:

Fresh silage, about 20 g.

7. Work procedure

1. Weight in 20 gram of fresh sample in a 200 mL beaker and add 180 mL cold RO water
2. Cover the beaker with parafilm
3. Let the samples extract in a refrigerator for at least nine hours.
4. Filter the samples with folding filter and discharge the first 10 mL of the extract.
5. Collect the rest of the extract
6. Transfer approximately 1 mL sample to a 2mL vial with lid
7. The samples are ready for analysis on the HPLC system (see below)
8. For ammonium-N 4 mL of the extract is analyzed with method 1133
9. For pH, measure pH in the extract

HPLC parameters:

Column:	Eurokat H; 300 mm x 8 mm ID, 10 µm (Knauer)
Column temperature:	60 °C
Injection volume:	40 µL (can be increased/decreased, if necessary, max 100µL)
Mobile phase:	0,01 N H_2SO_4
Flow rate:	0,8 mL/min
Detector temperature:	35 °C
Time per analysis:	20 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 acid (e.g. lactic acid)
X = the area of the peak relating to the acid (e.g. lactid acid)

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 lactic acid)
$Y = 1{,}89x + (-0{,}0234)$

If the area of the lactic acid peak in a sample = 7,07 and 20 g sample was weight out:

$X = (7{,}07 + 0{,}0234) / 1{,}89 = 3{,}75$ mg/mL

$\frac{3{,}75 \text{ mg/mL} * 180 \text{ mL}}{20 \text{ g}} = 33{,}77$ mg/g (same as g/kg)

Excel sheet for calculating is to be found under:

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