2.21.4 Short Chain Fatty Acids (Distillation Method)

Medium chain fatty acids arise from the metabolic activity of the yeast. Their concentration depends upon the yeast strain, fermentation temperature, intensity of wort aeration and duration of lagering the beer.

Principle

Volatile substances in beer are concentrated through distillation and the distillate is extracted using dichloromethane. The solvent phase is concentrated in the rotary vacuum evaporator and subsequently analyzed using a gas chromatograph. The linearity of the detector and the determination of the concentrations of analytes in the sample is achieved by using multiple concentration levels within the relevant range and through evaluation of the relative area under the peaks.

Apparatus

Capillary gas chromatograph with flame ionization detector (FID)
Capillary column:
Phase: FFAP 25 m × 0.2 mm × 0.3 µm (DB-FFAP, J & W Scientific no. 122 3232, HP-FFAP, Hewlett Packard no. 19091F-102 or comparable column, packed columns may also be used)
Liquid autosampler
Electronic integrator or PC workstation
Analytical balance, 0.1 mg readability
Laboratory scale, accurate to 0.1 g
Rotary vacuum evaporator
Water bath
Test tube shaker
Steam distillation apparatus (Büchi, Gerhardt or comparable)
Graduated cylinder with ground glass joint, 100 ml
GC syringe, 10 µl
Volumetric flask, 50 ml
Graduated cylinder, 100 ml
Graduated cylinder for shaking, 100 ml
GC Operating Conditions:

GC:	capillary gas chromatograph with FID
Capillary column:	see Apparatus section

Carrier gas:	hydrogen (4.8), flow rate: programmed, 1.3 ml/min
Injector temperature:	200 °C
Oven temperature:	60 °C, 1 min; 20 °C/min → 220 °C; 220 °C, 7 min
Detector temperature (FID):	250 °C
Injection volume:	1 µl; split ratio: 50 : 1

Reagents

Ethanol, p. a.
Sodium chloride, p. a.
Dichloromethane, p. a.
Sulfuric acid, 0.5 mol/l
Silicone antifoam agent or glycerin monostearate
Note: antifoam reagents should be tested for purity.
Internal standard:
4-Methylpentanoic acid (concentration: approx. 300 mg/l in ethanol), stable for three months if refrigerated
Reference substances (Aldrich, Fluka, Merck, Roth or comparable):
Butyric acid
3-Methylbutyric acid
4-Methylpentanoic acid
Hexanoic acid
Octanoic acid
Decanoic acid

Procedure

- measure 100 ml beer (0 °C) in a graduated cylinder, or alternatively weigh out 100 g of beer
- add 2 ml of sulfuric acid (0.5 mol/l) to a pH of 2.5–3.0
- pipette 1 ml internal standard into the beer, add antifoam agent
- distill until 50 ml has been collected in an ice-cooled receiver
- bring the distillate to 20 °C and fill to 50.0 ml
- pour the distillate into a 100 ml graduated cylinder suitable for shaking, add 10 g sodium chloride and 15 ml dichloromethane
- shake for 30 min
- cool the contents in a freezer for phase separation

- pipette 10 ml of the organic phase into a 50 ml flat bottom flask and concentrate using the rotary vacuum evaporator under a vacuum at 40 °C until dry
- dissolve the residue in 0.5 ml dichloromethane by shaking briefly
- perform an analysis of the dichloromethane phase using the gas chromatograph

Comparable procedures may also be used for this analysis.

Calibration

Calibration is performed by using different concentration ranges. Different amounts of reference substance of a known concentration is added to beer and the relative area measured under the peak is determined. Depending on the linearity of the detector, the calibration factors are determined by means of regression analysis.

Concentration levels used for calibration:

Butyric acid	0.10, 0.15, 0.30, 1.5, 2.0 and 3.0	mg/l
3-Methylbutyric acid	0.05, 0.10, 0.20, 0.30, 0.80, 1.0 and 1.6	mg/l
2-Ethylhexanoic acid	0.05, 0.10, 0.20, 0.30, 0.80, 1.0 and 1.6	mg/l
Hexanoic acid	0.10, 0.15, 0.30, 1.5, 2.0 and 3.0	mg/l
Octanoic acid	0.15, 0.30, 1.20, 2.5, 3.5 and 10.0	mg/l
Decanoic acid	0.15, 0.30, 1.20, 2.5, 3.5 and 5.0	mg/l

Comparable procedures may also be used for this analysis.

Calculation

Calculation of the concentrations is performed through comparison of the area under the peaks. The concentration of the substances analyzed in the sample can be calculated as follows:

$$C\ [\text{mg/l}] = \frac{A_{SUB}}{A_{ISTD}} \times CF$$

C = concentration of the substance in the sample
A_{SUB} = area under the peak for the substance in the sample
A_{ISTD} = area under the peak for the internal standard in the sample
CF = calibration factor of the substance

Comparable procedures may also be used for this analysis.

Results

In mg/l; over 1 mg/l to one decimal place, under 1 mg/l to two decimal places

Reference Values (bottom-fermented beer, original gravity 11–13 %)

Butyric acid:	0.2–1.0 mg/l
3-Methylbutyric acid:	0.2–1.0 mg/l
2-Ethylhexanoic acid:	0.5–2.0 mg/l
Hexanoic acid:	0.5–2.0 mg/l
Octanoic acid:	2.0–5.0 mg/l
Decanoic acid:	0.1–2.0 mg/l

References

1. L. Narziß, H. Miedaner, H. Schöndorfer, BWiss *35*, 109 (1982)

Sample Chromatogram