Analysis and Quantification of Short Chain Fatty Acids with Airsense Analyzer

A. Aranyos[1], E. Pinteaux[1], S. Morel[1], C. Leidlmair[2], A. Genuin, S. Praun[2]

[1]Alpha MOS France, [2]V&F Analyse – und Messetechnik GmbH, Austria

Amongst other volatiles that are formed during industrial fermentation processes, the short chain fatty acids (SCFA) are important products to investigate as they significantly impact the quality of the ferment and the organoleptic properties. This study demonstrates how we can use on-line, ion molecule reaction mass spectrometry for detection and quantification of these compounds in ferment medium

Materials & Methods

Samples

Known concentrations of various SCFA were used to develop a detection and quantification model. For validation, mixtures were prepared out of the calibration solutions.

Media Composition

Component	Concentration
$K_2HPO_4 \cdot 3H_2O$	2.6 g/l
$NaHCO_3$	0.2 g/l
NaCl	4.5 g/l
$MgSO_4 \cdot 7H_2O$	0.5 g/l
$CaCl_2 \cdot 2H_2O$	0.3 g/l
$FeSO_4 \cdot 7H_2O$	0.005 g/l

Acids Ranges

SFCA	Concentrations
Acetic Acid	0.1g/l - 0.5 g/l - 2.0 g/l
Propionic Acid	0.1g/l - 0.5 g/l - 2.0 g/l
Valeric Acid	0.1g/l - 0.5 g/l - 2.0 g/l
Iso-Valeric Acid	0.1g/l - 0.5 g/l - 2.0 g/l
Butyric Acid	0.1g/l - 0.5 g/l - 2.0 g/l
Iso-Butyric Acid	0.1g/l - 0.5 g/l - 2.0 g/l

Table 1 : Samples

Equipment

The analysis was conducted using an Airsense.net multi component analyzer.

IMR-MS system
1. Primary ion source
2. Octopole separation device
3. Charge exchange cell
4. Quadrupole – Mass filter
5. Particle detector
6. Vacuum system
7. Gas inlet system
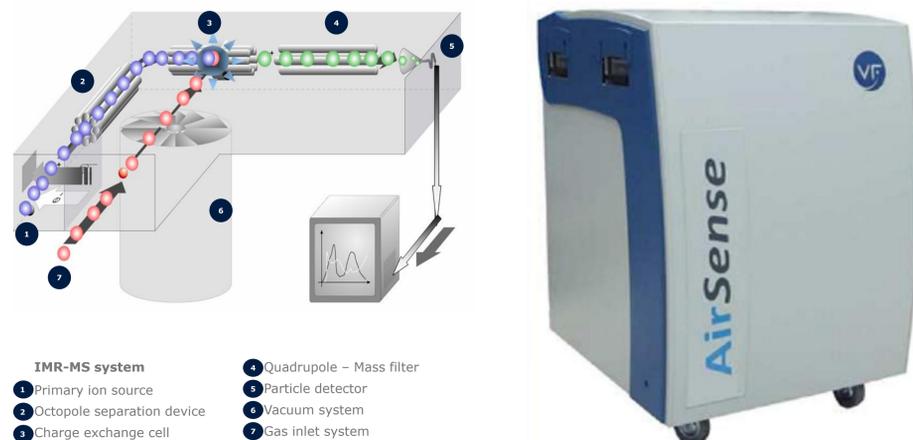
Fig. 1: Airsense analyzer and schematics of working principle

Low energy (10 - 14 eV) and highly efficient ionization allows for low level detection of volatiles with minimal fragmentation. After the ionization the analyte ions are separated using a quadrupole mass filter and an electron multiplier.
The software interface allows for real-time readouts of the signals. In this study the data for multi-variate regression were exported, however, such results can also be programmed directly into the operating software.

Analytical Conditions

Samples were prepared in 20 ml headspace vials. Headspace was generated in an auto sampler under typical fermentation temperatures (39°C) with a short (20min) equilibration time. The headspace was pumped to the analyzer and the sample pressure was set to 25 mbars. Both Hg (low energy) and Xe (medium energy) ionization modes were used, as acetic acid detection requires higher energy than the other acids. The pH of the solution was kept constant using a buffer solution.

Results & Discussion

Molecule	Formula	MW (g/mol)	Ion energy (eV)
Acetic acid	C2H4O2	60.05	10.65
propionic acid	C3H6O2	74.08	10.44
butyric acid	C4H8O2	88.10	10.17
Isobutyric acid	C4H8O2	88.10	10.24
isovaleric acid	C5H10O2	102.13	10.51
valeric acid	C5H10O2	102.13	10.53

Gas	Symbol	Ion energy (eV)
Mercury	Hg	10.44
Xenon	Xe	12.13
Kr	Kr	14.00

Table 2: Ionisation energies

Results and Discussion

A detailed energy analysis of the various fragments indicated that interfering fragments from the higher acids should be expected on the molecule ions corresponding to the shorter acids (for example: valeric acid will also give a signal on the mass 88). This was confirmed by the analysis of pure compounds, and for each acid the relative mass ratios were recorded for concentration calculations.

Valeric acid mass ratios
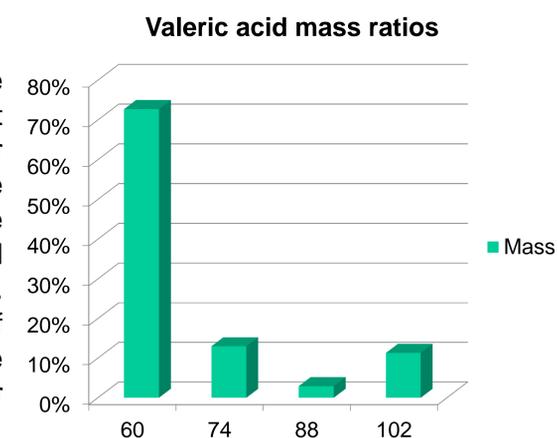
Fig. 2: Example: Signal Coefficients for valeric acid

Apart from the overlapping fragments another major challenge was to differentiate acids from their isomers. Since the resolution of our measurement is 1 amu, we had to find the significant difference in the signal pattern. For all the acids, the iso form gave relatively lower intensity on the protonated molecule ion.
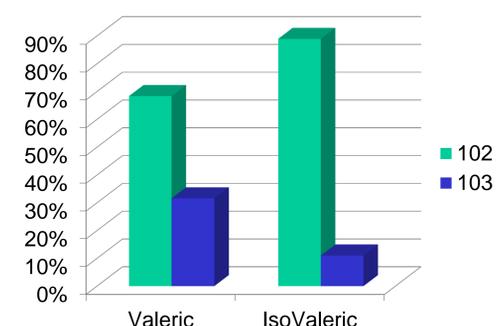
Fig. 3: Example: Differentiation patter between isobaric acids

With this data at hand it was possbile to calculate:
- First, the valeric and isovaleric concentrations form M102 / M103
- The butyric and isobutyric concentrations from M88 / M 89 and the now known valeric and isovaleric contributions
- The propionic from M74 and the contributions from the four higher acids
- Finally, the acetic acid from Xe based M60 adjusted for the five other acids

	Acetic		Propionic		Isobutyric	
	Real	Model	Real	Model	Real	Model
Test Mix 1*	0,200	0,200	0,125	0,125	0,188	0,190
Test Mix 2*	0,000	0,000	0,000	0,000	0,000	0,000
Test Mix 3*	0,000	0,000	0,000	0,000	1,330	1,328

	Butyric		IsoValeric		Valeric	
	Real	Model	Real	Model	Real	Model
Test Mix 1*	0,125	0,126	0,188	0,188	0,125	0,125
Test Mix 2*	0,000	0,000	1,330	1,330	0,670	0,670
Test Mix 3*	0,670	0,669	0,000	0,000	0,000	0,000

CONCLUSION

In this study the feasibility of detecting and quantifying short chain fatty acids in a fermentation medium could be demonstrated . The application is very promising for on-line analysis and control of industrial fermentation processes.