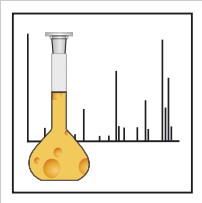
doi:10.2533/chimia.2023.622 Chimia 77 (2023) 622



Highlights of Analytical Sciences in Switzerland

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A Simple, Rapid and Validated Method for the Determination of Free Volatile Carboxylic Acids in Cheese by GC-FID

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Keywords: Bacterial cultures · Cheese · Flavour · Free volatile carboxylic acids · Free volatile fatty acids · GC-FID · Headspace

Free volatile fatty acids, or more precisely free volatile carboxylic acids (FVCA), are formed during cheese ripening by microorganisms *via* heterofermentative lactic acid fermentation or amino acid deamination. FVCAs content varies greatly from a cheese to another, depending on the type of bacteria or yeasts present, and contributes significantly to the final taste and flavor of each cheese. While the presence of some FVCAs is associated with desirable typical flavors (*e.g.* propionic acid in Emmental cheese), other FVCAs, resulting from fermentations by undesirable microorganisms, can lead to unpleasant flavors or eye formation. Therefore, the quantification of FVCAs is of great interest in cheese manufacturing as it can reveal ripening defects.

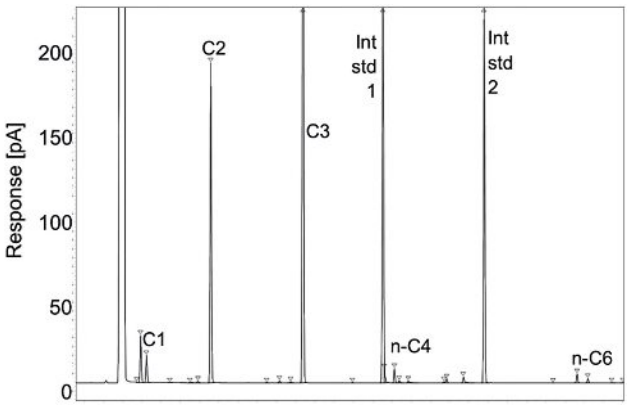
Many different approaches for FVCAs quantification have been proposed over the years, improving sensitivity, but remaining complex to implement. Such methods combine extraction (with polar or apolar solvents, dynamic headspace, solid phase (micro)extraction), pre-purification with various adsorbents, conversion into sodium salts, drying, esterification and analysis using gas-chromatography coupled with flame ionization (GC-FID), thermal conductivity or mass spectrometry detectors.

Our new method allows the simultaneous quantitative determination of FVCAs, and has been validated for eight target analytes. It uses a weakly basic aqueous extraction, followed by an esterification with ethanol directly from the aqueous phase in a headspace vial. The ethyl esters thus formed are then analyzed by GC-FID. The conditions for the extraction, esterification, and headspace (amount of ethanol, sodium hydroxide concentration, time and temperature) were optimized over the years. The limits of detection (LOD) in cheese were less than 0.3 µmol kg^{-1}. The lower limits of quantitation (LOQ) were better than 0.001 mmol kg^{-1}. The upper LOQ varied from 39 to 136 mmol kg^{-1} depending on the analyte. The Horwitz ratio showed good precision for all analytes (less than 0.77).

Our new method is simple and rapid to implement. The validation parameters of trueness, specificity, precision, LOD, and LOQ demonstrate that it is sensitive, robust and suitable for accurate quantification of FVCAs over a wide range of measurements in cheese, in bacterial culture, and potentially in other type of matrices.

Reference
R. Badertscher, C. Blaser, P. Noth, *Food Chemistry* **2023**, *398*, 133932, https://doi.org/10.1016/j.foodchem.2022.133932.

Agroscope's experimental cheese factory (A), Agilent 8890 gas chromatograph coupled with a flame ionization detector and a PAL3 autosampler in headspace mode (B).



Chromatogram of the free volatile carboxylic acids in their ethyl ester form in a hard cheese sample. Formic acid (C1), acetic acid (C2), propionic acid (C3), isobutyric acid (i-C4), n-butyric acid (n-C4), isovaleric acid (i-C5), isocaproic acid (i-C6) and n-caproic acid (n-C6). Samples' headspace were analyzed with an Agilent 8890 GC-FID equipped with an Agilent HP-5 cross-linked phenyl methyl silicone fused silica capillary column, using helium as the carrier gas. The GC temperature program was 40 °C (4 min), 8 °C.min^{-1} to 144 °C, then 30 °C.min^{-1} to 279 °C (0.5 min).

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