Comparison and Differentiation of Flavor Profiles for Vegan and Non-Vegan Cheese

Introduction

An increased awareness to health, environment and animal welfare as well as a growing acceptance of vegan products has led to a rise in non-dairy product sales [1]. Consequently, the market for meat- and dairy-free alternatives is growing rapidly. Two main selling strategies exist for meat- and dairyfree alternatives. One of them is the imitation of popular non-vegan products. This strategy has the advantage that customers are already familiar with a product and may want to switch to a vegan alternative with similar taste and affection. Hence, characterization of flavor and aroma profiles is of particular interest during the development of these types of products.

A large part of flavor and aroma perception arises from volatile and semi volatile compounds exhibited from the food product [2]. Gas chromatography coupled to mass spectrometry (GC-MS) is particularly useful at investigating these types of compounds. Typically, 70 eV electron ionization (EI) is used as ionization method of choice in GC-MS. Unfortunately, this ionization method often misses molecular ion information, which results in largely reduced compound identification certainties, especially when appropriate reference standards for compounds are not available and library match information is insufficient [3]. Softer ionization techniques such as chemical ionization (CI) can overcome this issue and provides molecular information of compounds when using accurate mass high-resolution mass spectrometers. Furthermore, under controlled conditions CI can yield additional information about compounds of interest. The ecTOF is simultaneously operating EI and CI [3] and detects both types of ions in one TOF analyzer in parallel (Figure 1). With this, data alignment is inherently provided, and tentative identification of unknown compounds, especially when combined with GC analysis, made simpler and faster. In this application note, we will show the potential of the ecTOF in comparing flavor profiles of both vegan cheese (New Roots AG, CH) and its non-vegan flavor equivalent.

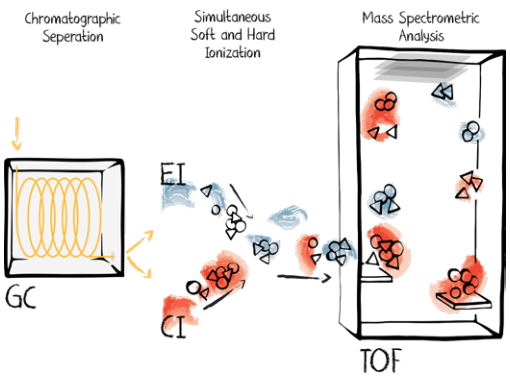

Figure 1. Principle of operation of the GC-ecTOF.

Keywords:
flavor, vegan, food analysis, ecTOF, non-target, compound identification, EI&CI, GC-HRMS

Marleen Vetter, Steffen Bräkling and Sonja Klee
TOFWERK, Thun, Switzerland

Identification of compounds of interest by the ecTOF

In a headspace vial, 10 g of a vegan cheese from New Roots AG as well as its supposed non-vegan equivalent (Appenzeller Classic, Appenzeller Käse GmbH, Appenzell Switzerland) were incubated for 10 mins.

Afterwards, each cheese was sampled in triplicate at 60 °C for 30 mins using a Restek Polyacrylate SPME fiber (BGB Analytik, Switzerland). Full details on the method are found in Table 1.

Table 1: Method Parameters.

SPME (Polyacrylate, Restek) Method	10 g sample into 20 mL screw top vial, incubated for 15 mins at 60 °C, 2 cm SPME fiber exposed to headspace for 30 mins at 60 °C
Desorption and Injection	SPME held in injector port (235 °C) for 3 mins, splitless injection into Agilent GC 7890A with SPME injection sleeve (0.75 mm ID)
Carrier Gas Flow	1.0 mL/min sccm He
Purge Flow	10.0 mL/min
Column	Restek Stabilwax-MS (30 m x 0.25 mm x 0.25 µm)
Septum Purge	3.0 mL/min
Temperature Program	*35 °C for 4 mins, 10 °C/min 250 °C held for 5 mins*
Flow Split	1:1 CI/EI
Heated Transfer Line Temperature	250 °C
Ionization Sources	StarBeam 70 eV EI source HRP CI source reagent: H_3O^+ [4]
Ionization Source Temperatures	EI 280 °C CI 300 °C
Mass Range	1 - 450 *m/z* (Th)
Mass Accuracy	< 1 ppm (lock mass)

Statistical analysis of ecTOF data

Initially, only the main flavor compounds of interest were investigated (Figure 2).

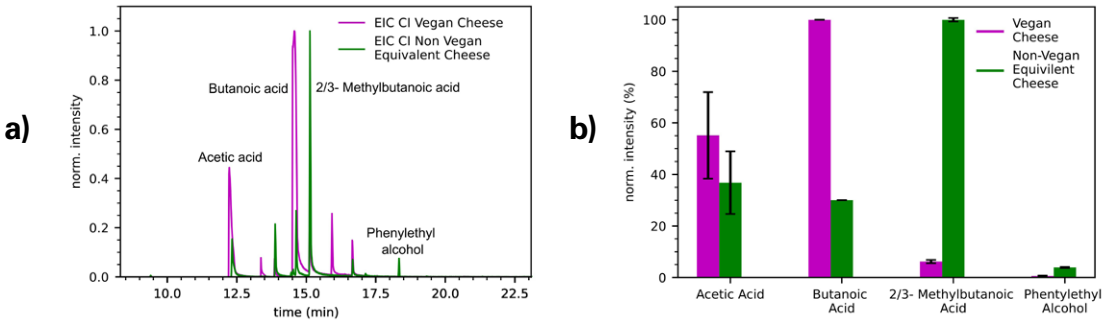

Figure 2. Comparison of key flavor compounds for the employed fermentation process. Ideally, both profiles should be very similar. However, a clear difference in peak intensities can be seen, especially between butanoic acid and 2/3-methyl butanoic acid. a) shows a comparison of the combined EICs of *m/z* 61, 89 and 103 and b) shows the proportional distribution of the peak ratios to one another.

Between the two types of cheese a clear difference between common flavor compounds such as butanoic acid and 2/3- methyl butanoic acid can be observed. While the first has a more pungent odor and acrid flavor, the second is known for its pleasantly sweet, fruity, and cheesy flavor profile. These compounds are classical compounds of interest for flavor profile determinations of cheese [5]. Next to a conventional target flavor compounds analysis, the ecTOF provides the possibility of investigating non-target compounds recorded in the same analysis run. Figure 3 shows the comparison of chromatograms and different mass spectra between the vegan cheese sample and the non-vegan equivalent cheese.

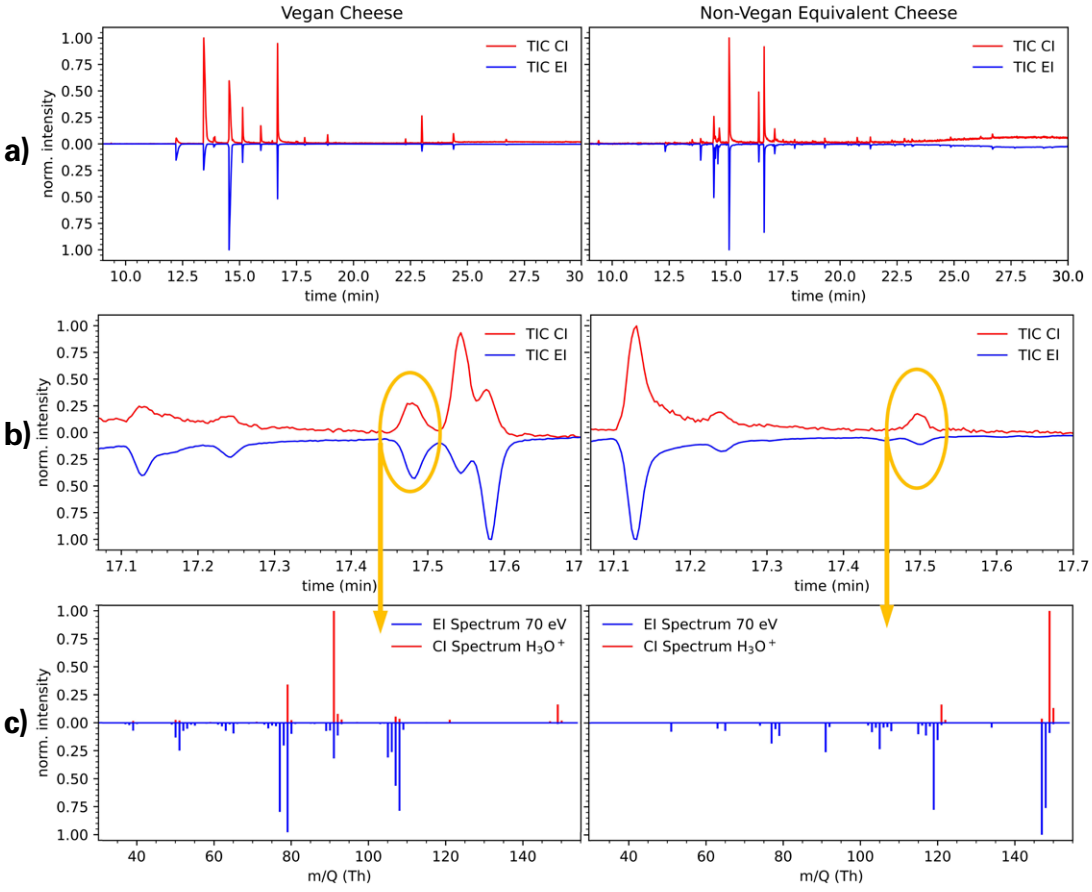

Figure 3. Comparison of vegan and non-vegan equivalent cheese flavor chromatograms. a) Comparison of the total ion chromatograms (TIC) for both EI and CI data of the two different samples. b) Zoom-in of the chromatographic peak of interest at around 17.5 mins. c) EI and CI mass spectra detected for the peak of interest for both samples are shown.

Significant differences between the vegan cheese and the non-vegan alternative can be observed using a volcano plot (Figure 4).

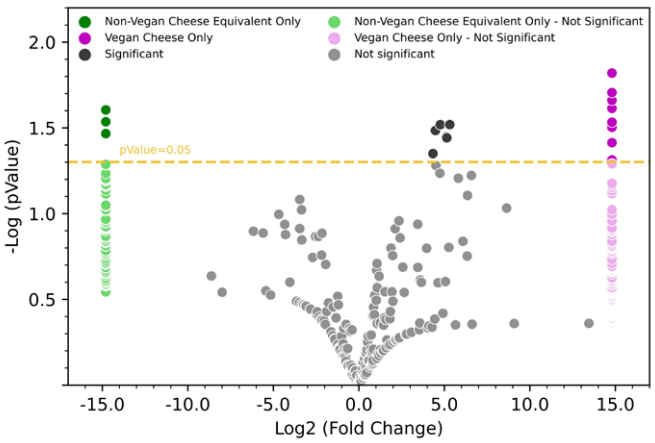

Figure 4. Volcano plot of the vegan cheese and non-vegan cheese ecTOF data.

A peak of interest for both the vegan and the non-vegan equivalent was selected at around 17.5 min (Figure 3b) and the EI and CI mass spectra for both runs are shown (Figure 3c). For the non-vegan cheese, both EI and CI information are in good agreement, pointing to 2,4,6-trimethyl-benzaldehyde (sum formula $C_{10}H_{12}O$) (isotopic pattern error of 5.2 % (Figure 5)) and NIST library match factor 899, reverse match factor 928 and probability of 51.6 %.

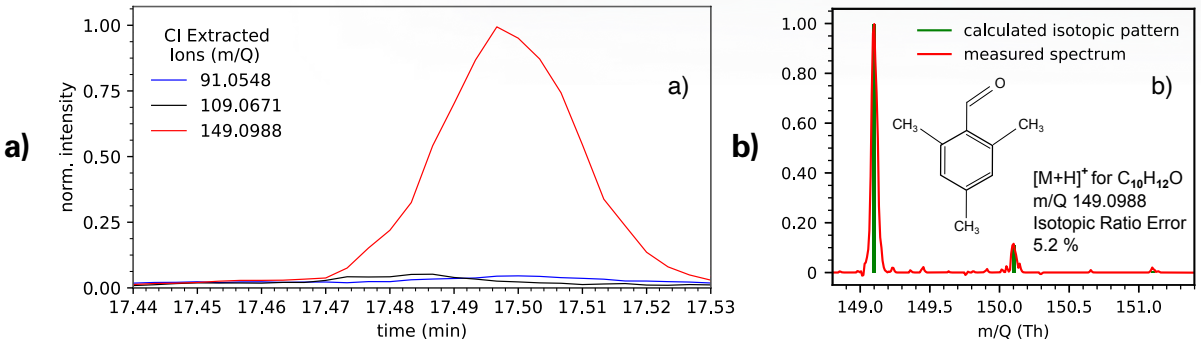

Figure 5. a) CI extracted ion chromatograms of the non-vegan cheese peak at 17.5 min b) mass spectrum zoom-in to the first three isotopic signals of $[M+H]^+$.

In the vegan cheese however, the EI mass spectrum clearly suggests that 2,3,4-trimetyl-benzaldehyde is not present, even though a chromatographic peak appears at the same retention time. The first suggested NIST hit for the peak at around 17.5 min (match factor 857, reverse match factor 878 and probability of 27.2 %) was benzyl alcohol (C_7H_8O). Not only the low probability in the NIST library search, but also a contradiction of the accurate mass of the possible molecular ion found in the CI mass spectrum (*m/z* 149.0968) alludes to an inconsistency of the recorded data with the NIST library match. For many compounds, the retention index (RI) or equivalent measure for polar columns is unfortunately not reported, which was the case in this investigation. Hence, the RI information was of limited added value.

While a more detailed examination of the peak specific mass traces in the EI chromatogram does not indicate a distinct coelution of substances, a coelution of two peaks is clearly visible the CI chromatogram (Figure 6a).

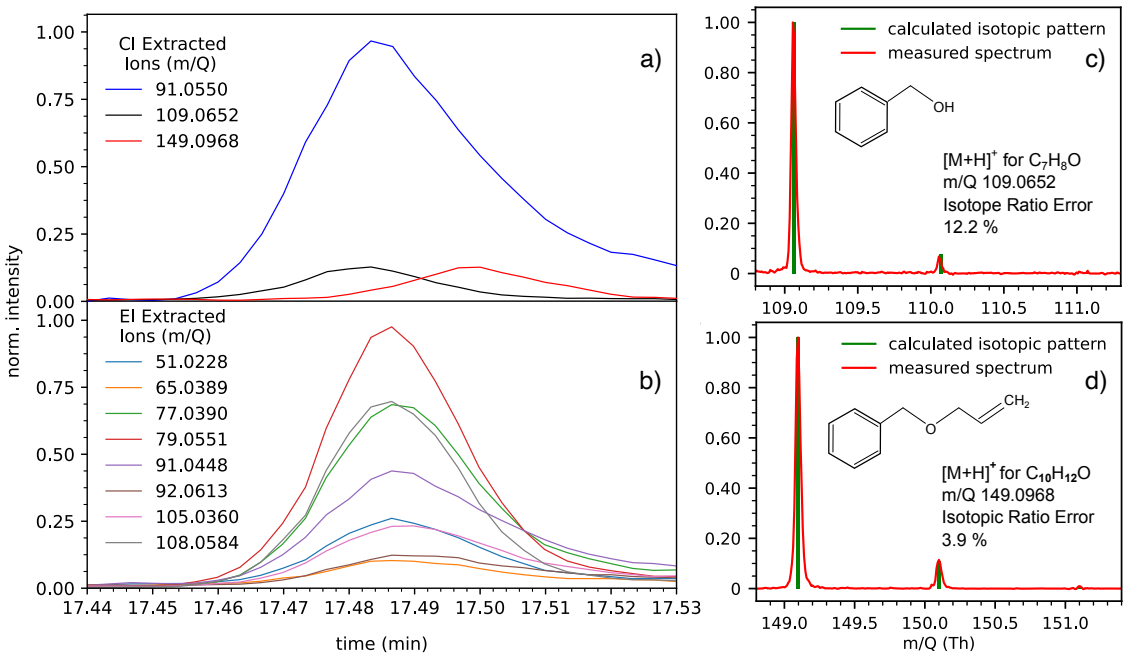

Figure 6. a) CI and b) EI extracted ion chromatograms of the vegan cheese peak at around 17.5 min. c) and d) mass spectrum zoom-in to the first three isotopic signals of $[M+H]^+$ of benzyl alcohol and allyl benzyl ether.

While the extracted ion chromatogram (EIC) of the CI chromatogram of *m/z* 91 shows the presence of the tropylium ion ($[C_7H_7]^+$) for both compounds, the EIC of *m/z* 109.0652 (predicted sum formula $[C_7H_8O]^+$) and *m/z* of 149.0968 (predicted sum formula $[C_{10}H_{12}O]^+$) show the presence of two different compounds. Using this and the NIST library search information, the presence of benzyl alcohol and allyl benzyl ether (NIST hit 7, match factor 778, reverse match factor 791, probability 1.28 %) can be predicted. Since these two substances produce almost identical fragments in the 70 eV EI spectra, a separation of the two peaks using EI only is not possible (Figure 6b). Due to shifted fragment ion ratios between the two compounds that mix within the EI mass spectrum, a NIST library search alone is not reliable.

Summary

This AppNote illustrates the advantages of incorporating CI alongside traditional EI in a single GC-MS analysis to enhance reliability, particularly when dealing with complex samples. Whereas the ecTOF can easily identify commonly investigated flavor compounds of interest, it is also able to identify and distinguish other volatile and semi-volatile compounds which may play a crucial role in flavor development or in later processing steps. With this, the strength of the ecTOF in identifying flavor compounds in cheese samples is demonstrated.

References

[1] Fortune Business Insights. The global vegan food market is projected to grow from $26.16 billion in 2021 to $61.35 billion in 2028 at a CAGR of 12.95% in forecast period, 2021-2028. Vegan Food Market Report, 2022, March Issue: 1-160.

[2] McSweeney, P.L.H. and Sousa, M.J.: Biochemical pathways for the production of flavour compounds in cheeses during ripening: A review. Lait, 2000, 80 (3): 293-324.

[3] Bräkling, S. et al: Parallel Operation of Electron Ionization and Chemical Ionization for GC-MS Using a Single TOF Mass Analyzer; Anal Chem., 2022, 94 (15): 6057-6064.

[4] Bräkling, S. et al: Hydrogen Plasma-Based Medium Pressure Chemical Ionization Source for GC-TOFMS; J. Am. Soc. Mass Spectrom., 2021, 33 (3): 499–509.

[5] Schlegel, K.; Ortner, E.; Buettner, A.; Schweiggert-Weisz, U. Contribution of S. Xylosus and L. Sakei ssp. carnosus Fermentation to the Aroma of Lupin Protein Isolates. Foods, 2021,10:1257.

Bruker Applied Mass Spectrometry is continually improving its products and reserves the right to change specifications without notice. © 2024, Bruker Applied Mass Spectrometry. AMS-014 1910126

For Research Use Only. Not for use in clinical diagnostic procedures.

Bruker Switzerland AG

Fällanden · Switzerland
Phone +41 44 825 91 11

Bruker Scientific LLC

Billerica, MA · USA
Phone +1 (978) 663-3660

marketing.bams.emea@bruker.com - www.bruker.com