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A novel approach for analyzing gas chromatography-mass spectrometry/olfactometry data

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ABSTRACT

GC-MS/O (gas chromatography-mass spectrometry/olfactometry) is an indispensable technique to associate individual volatile odorants to odors perceived by human assessors. Interpretation of GC-MS/O data is, however, hampered in practice by different factors related to the instrumental set-up and by heterogeneity among odor descriptions given by the assessors (olfactometer). In this paper, a novel automated approach is presented, which deals with these GC-MS/O challenges and enables visualization and interpretation of GC-MS/O data. It includes signal warping via COW (correlation optimized warping), synchronizing MS and O data via detection of odor areas and construction of a TOC (total odor count) to visualize odor heterogeneity, respectively. Our approach is implemented in practice, and we successfully associated odors to compounds in data sets of two alcoholic beverages with different flavor compositions. It leads to a faster and less biased association of odors to compounds compared to current practice, reducing the time and effort needed for interpreting GC-MS/O data.

1. Introduction

GC-MS/O (gas chromatography coupled to mass spectrometry and olfactometry) is often used for identification of odor-active compounds ('odorants') in a complex mixture [1–4]. It is especially useful in food research, where identification of odorants is an important issue. Examples include the analysis of the molecules that make up the odor of fruit juice [5–7], wines [8,9], and cheeses [10–12]. In GC-MS/O analyses, a mixture is injected in a gas chromatograph to separate the constituents of the mixture, and detection takes place via both a mass spectrometer and an olfactometer simultaneously. The mass spectrometer provides chemical information about the constituent molecules via mass spectral data, while sensory information about these molecules is provided at the olfactometer, using the human nose as detector [13,14]. The detection of a compound by a human assessor is called an *odor event* in the remainder of this text.

For optimal interpretability of GC-MS/O data, chemical (GC-MS) and sensory information (olfactometry) need to be associated for each constituent. However, automated association is hampered in practice by three challenges: chromatographic elution time differences between different runs, detection time differences between mass spectrometer and olfactometer, and heterogeneity among odor descriptions by different assessors for identical odorants.

The problem of differences in chromatographic elution time is well-recognized within the field of chromatography. These differences often occur because experimental chromatographic conditions—like column temperature or pressure—slightly change over time. Numerous 'warping' (or 'alignment') methods are devised that correct these elution time differences [15–18]. They can be easily adapted for GC-MS/O data.

The detection time difference between mass spectrometer and olfactometer is specific for GC-MS/O experiments. This difference depends on the instrumental set-up (i.e. the arrival time of a compound at both detectors), as well as on variations in experimental conditions such as temperature changes and assessor response. Indeed, assessors need time to perceive an odorant and give a response, often by pressing buttons with predefined odor descriptions or by using a microphone [13]. This is the main source of the detection time difference. Continuous training of the assessor panel will minimize but not remove detection time differences occurring because of the assessor response.

Heterogeneity in odor descriptions is inherent to using the human nose as detector. This is due to the natural variation among humans with regard to their sensory perception and experience, even after extensive panel training. Moreover, some compounds cannot be detected at all by some assessors because of the same reason [13].

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These three challenges prevent the availability of simple tools to associate odors to compounds based on GC-MS/O data. Usually, a substantial amount of manual labor is involved [1,2,19]. The exact procedure to associate odors to compounds currently seems to rely much on human interpretation, which may be biased. Therefore, in this paper, a generally applicable, novel approach is proposed and validated. It deals with the non-informative differences discussed before in GC-MS/O data, aiding in fast and unbiased association of odors to compounds. In this paper, it has been applied to two GC-MS/O data sets of different complexity (i.e. different amounts of odorants).

2. Materials and methods

For validation and illustration of the proposed approach, two GC-MS/O data sets were recorded, to which will be referred as 'data set A' and 'data set B' in the remainder of this text. Both data sets relate to the analysis of a flavored malt-based beverage.

2.1. Chemicals

Pure standards of ethyl butanoate, ethyl 3-methylbutanoate, isopentyl acetate, camphene (and alpha-fenchene[1]), beta-myrcene, ethyl hexanoate, 1,4-cineole, 1,8-cineole, terpinolene, 3-methyl-1-butanol, 3-methyl butanoic acid, octanal, alpha-terpinene, limonene, terpinolene, and *cis*-beta-terpineol were purchased from Sigma-Aldrich Corporation (St. Louis, Missouri, USA).

2.2. Samples

The two malt-based beverages used in this work were provided by Heineken Supply Chain BV (Zoeterwoude, The Netherlands). The sample used for data set A was a malt-based beverage with a simple flavor, whereas for data set B, a malt-based beverage with a more complex flavor (i.e. more odorants) was used. The simple malt-based beverage—which had common odorants with the complex one—was spiked with 4 flavor volatile compounds suspected to be important for the flavor of the complex beverage. Those four flavor-active compounds were camphene,[2] 1,4-cineole, 1,8-cineole (eucalyptol), and terpinolene. All volatiles were added in the concentrations as known to be present in the complex beverage. The simple malt-based beverage with spiked compounds is used to test if spiked compounds can be properly found and identified in GC-MS-O data with the developed approach. The complex beverage is used to examine whether the approach is able to perform well in the case of GC-MS-O data with many closely eluting compounds.

2.3. Sample preparation

All samples were kept cool until they were analyzed. For the spiked samples (data set A), 30 g of the beverage was mixed with 30 μl of a mixture of the 4 volatiles, diluted in ethanol. In this mixture, the concentrations of the 4 spiked compounds were as follows: camphene 0.91 μg/L, 1,4-cineole 357 μg/L, 1,8-cineole 291 μg/L, and terpinolene 39.8 μg/L. The malt-based beverage with complex flavor was analyzed by using 30 g of sample (data set B).

All samples were put in a 40 mL glass vial, and a 10 mm length, 3.2 mm o.d., and 0.5 mm thick polydimethylsiloxane-coated stir bar ('twister') from Gerstel was added to the solution (Mülheim an der Ruhr, Germany). Vials were closed with a screw cap and stirred at room temperature for 45 minutes at 500 rpm. After extraction, the twister was removed, rinsed briefly in distilled water, and placed in a glass thermal desorption tube.

[1] Camphene in its pure form always contains some alpha-fenchene.
[2] Including alpha-fenchene.

2.4. GC-MS/O conditions

The GC analysis was performed using a TDU (thermal desorption unit) combined with a MPS (multipurpose sampler), a CIS 4 cooled injection system PTV (programmed temperature vaporization) type inlet, and an Olfactory Detector Port 2, all from Gerstel. An Agilent 6890A gas chromatograph with a 5973 MSD (mass selective detector) was used (Palo Alto, CA, USA). The GC analysis system was operated under MAESTRO software control (version 1.4.16.9, Gerstel) integrated with ChemStation software (version E.02.02.1431, Agilent) using one integrated method and one integrated sequence table. Compounds were separated on a 30 m × 0.25 mm i.d. fused silica capillary column from Agilent (CP8944 Vf-5 ms) with a film thickness of 0.25 μm. The GC column was maintained at 50 °C for 2 minutes and subsequently ramped at a rate of 10 °C/min to 280 °C. Carrier gas was helium at 2.0 mL/min with a split ratio of 20 for data set A and 10 for data set B. After the column, the effluent was split 1:1 into the MS and the sniffing port. EI mass spectra were generated scanning from mass 33 to 300. The length of the transfer line to the sniffing port was 148 cm, with a constant temperature of 220 °C. This length was optimized as much as possible to ensure that components are detected at the same time by both MS and sniffing port. Humidified air was added at the sniffing port.

2.5. Olfactometric procedure

Each panelist assessed each sample by smelling directly from the sniffing port, continuously from time 3 to 20 minutes of the chromatographic run, recording their impressions on a touchscreen. A predefined vocabulary of odor descriptors was used for recording the perceived odors. Individual descriptors were laid out on the touchscreen, for each assessor to choose one or more descriptors that fit the perceived odor best. Additionally, the option of a 'START' button was given for the occasions in which assessors perceived an odor but were not immediately sure of its exact odor description. Olfactory data were collected with the use of AromaTrax software from Microanalytics (version 9.10, Round Rock, TX, USA).

The spiked sample (data set A) was assessed by a panel of 15 trained assessors in duplicate. The complex beverage (data set B) was assessed by a panel of 10 trained assessors—of which five were also in the panel for the spiked sample—in triplicate. Each sample was evaluated using a vocabulary of 53 descriptors organized in a flavor wheel of ten main odor categories ('Fruity', 'Vegetative', 'Floral', 'Chemical', 'Sulphury', 'Earthy/Musty', 'Microbiologica', 'Vinous/Acidic', 'Roasted', 'Spicy'), split in subcategories and precise flavor examples per main category/subcategory. All main odor categories and certain subcategories of the flavor wheel were embedded on the touchscreen data collection system of AromaTrax for the assessors to choose from.

2.6. Data

Data set A contains data from 30 runs (15 assessors in duplicate). For each run, 7113 mass spectra were recorded in a time frame from 0 to 25 minutes. Within this time frame, 407 odor events were recorded in total for all runs. Data set B contains data from 29 runs: 9 assessors in triplicate and 1 assessor in duplicate. 7118 mass spectra were recorded for each run, again from 0 to 25 minutes, and all runs together led to a total of 744 odor events.

2.7. Data analysis

All methods employed in this approach were programmed in MATLAB (version 7.12, The MathWorks Inc., Natick, MA, USA). The approach consists of a single workflow (Fig. 1) that associates the qualitative olfactory perception—the odor—to small time regions called 'odor areas' (OAs). After identification of the compound within the time