Journal of Chromatography A, 1334 (2014) 101–111

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Toward a definition of blueprint of virgin olive oil by comprehensive two-dimensional gas chromatography☆

Giorgia Purcaro[a,*], Chiara Cordero[b,**], Erica Liberto[b], Carlo Bicchi[b], Lanfranco S. Conte[a]

[a] *Dipartimento di Scienze degli Alimenti, Università di Udine, via Sondrio 2A, I-33100 Udine, Italy*
[b] *Dipartimento di Scienza e Tecnologia del Farmaco, Università di Torino, via Pietro Giuria 9, I-10125 Torino, Italy*

ARTICLE INFO

Article history:
Received 26 July 2013
Received in revised form 17 January 2014
Accepted 24 January 2014
Available online 31 January 2014

Keywords:
Olive oil
Sensomics
Volatile compounds
Comprehensive multidimensional gas chromatography (GC × GC)
Fingerprinting
Pattern recognition
Aroma defects

ABSTRACT

This study investigates the applicability of an iterative approach aimed at defining a chemical blueprint of virgin olive oil volatiles to be correlated to the product sensory quality. The investigation strategy proposed allows to fully exploit the informative content of a comprehensive multidimensional gas chromatography (GC × GC) coupled to a mass spectrometry (MS) data set. Olive oil samples (19), including 5 reference standards, obtained from the International Olive Oil Council, and commercial samples, were submitted to a sensory evaluation by a Panel test, before being analyzed in two laboratories using different instrumentation, column set, and software elaboration packages in view of a cross-validation of the entire methodology. A first classification of samples based on untargeted *peak features* information, was obtained on raw data from two different column combinations (apolar × polar and polar × apolar) by applying unsupervised multivariate analysis (i.e., principal component analysis—PCA). However, to improve effectiveness and specificity of this classification, *peak features* were reliably identified (261 compounds), on the basis of the MS spectrum and linear retention index matching, and subjected to successive pair-wise comparisons based on 2D patterns, which revealed peculiar distribution of chemicals correlated with samples sensory classification. The most informative compounds were thus identified and collected in a "blueprint" of specific defects (or combination of defects) successively adopted to discriminate Extra Virgin from defected oils (i.e., *lampante* oil) with the aid of a supervised approach, i.e., partial least squares-discriminant analysis (PLS-DA). In this last step, the principles of sensomics, which assigns higher information potential to analytes with lower odor threshold proved to be successful, and a much more powerful discrimination of samples was obtained in view of a sensory quality assessment.

1. Introduction

Modern-omics disciplines investigate sample constituents considered collectively (primary and secondary metabolites, compounds generated by thermal treatments and/or enzymatic activity) [1,2] and represent the route of choice for a comprehensive evaluation of food attributes, in particular if sensory properties (sensomics and flavoromics) are unequivocally correlated to a specific distribution of chemicals [3–5].

Sensomics focuses analytical efforts on revealing sensorially-active compounds (odorants and tastants) in order to link the chemical composition of a food with its sensory quality (flavor) [6]. From this perspective, the analytical process through which the peculiar product profile (i.e., the blueprint) is revealed closely fits the aims of the above mentioned—omics disciplines, in addition to comprehensive information on chemical composition, quality and authenticity.

However, the number of volatiles effectively contributing to food aroma is relatively small, and the detection, identification, and quantitation of odor-active components, sometimes occurring at trace (ng/kg) levels, require complex procedures that combine effective extraction, powerful separation and highly informative (identity confirmation and mass quantitation) detection.

In this perspective, two-dimensional comprehensive gas chromatography (GC × GC) coupled to mass spectrometry (MS) can be successful in revealing the sensory-related chemical blueprint [7] of high quality foods since it fully exploits the informative potential of each analytical dimension (separation and detection) by (a) increasing the separation power, (b) ordering analytes through logic structures over the chromatographic plane and (c) by enhancing detection sensitivity. In particular, the possibility of extending

---

☆ Presented at the 37th international symposium on capillary chromatography and 10th GC × GC symposium, Palm Springs, CA, 12–16 May 2013.

* Corresponding author at: Dipartimento di Scienze degli Alimenti, Università di Udine, via Sondrio 2A, I-33100 Udine, UD, Italy. Tel.: +39 432558170.

** Corresponding author at: Dipartimento di Scienza e Tecnologia del Farmaco, Università di Torino, via Pietro Giuria 9, I-10125 Torino, Italy. Tel.: +39 0116707662.

E-mail addresses: giopurcaro@gmail.com, giorgia.purcaro@uniud.it (G. Purcaro), chiara.cordero@unito.it (C. Cordero).

0021-9673/$ – see front matter © 2014 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.chroma.2014.01.067

the analytical investigation to a broader range of chemicals, if compared to mono-dimensional approaches, improves fingerprinting sensitivity and effectiveness of sample classification [8,9].

Methods for reliable selections of 2D peak information and alignment across set of chromatograms were recently reviewed by Reichenbach et al. [10]. In particular, the most performing approaches are those referred to as *peak feature* methods, i.e., methods that enable not only, untargeted analytes alignment (based on peak features, i.e., retention times and MS fragmentation pattern similarity) but also pattern recognition and pair-wise comparisons based on 2D peaks distribution over the chromatographic plane.

In this study, the informative potentials of GC × GC–MS in revealing the chemical blueprint of (Extra) Virgin Olive Oil (EVO and VO) have been investigated by adopting advanced fingerprinting methods for reliable peak selection and focusing on peculiar distributions of sensory-active volatiles (aroma compounds) related to quality attributes currently regulated by international organisms (European Union (EU), International Olive Council (IOC), and the Codex Alimentarius) [11–14].

Interestingly, olive oil is presently the only food product whose sensory attributes are officially regulated, and sensory assessment carried out through well-standardized protocols [11–14] by highly and continuously (permanently) trained panelists. On the basis of the presence (or absence) and the intensities of specific defects and the intensity of the "fruity" perception, evaluated by smelling and tasting, Virgin Olive Oil can be classified into three categories (determining its economic value) namely EVO, VO, and *lampante* oil [15].

Undoubtedly, volatile compounds play a crucial role in defining olive oil sensory quality and strong efforts have been dedicated to unravel the composition of this informative fraction, to understand correlations with quality attributes [16–18]. The relative distribution of volatiles depends on several parameters (i.e., cultivar, geographical origin, fruit ripeness, processing practices, and storage [18–27]) and the identification of an unequivocal fingerprint correlated to quality may be a difficult task. Most of these variables concur to determine the intensity and quality of the green and fruity perception, while the presence of defects is mainly due to inappropriate manufacturing practices. Olive storage has to last no longer than 24–48 h under appropriate conditions to avoid the formation of three main defects, namely mustiness or mold, winey or vinegary, and fusty. Such defects are mainly due to yeast and fungi metabolic activity and aerobic or anaerobic fermentations, respectively [13]. While inappropriate oil storage conditions after production may lead to muddy and rancid defects, the former may be originated by an excessive contact of the oil with sediment in tanks, and the latter by an auto-oxidation process. The descriptor of the fusty and muddy defects was recently unified due to several misinterpretations [12], although the origin of such defects is very different both from the technological and chemical viewpoint.

In the panorama of existing studies, just few of them focused on the identification of markers of the specific defects by adopting reference standard samples provided by the IOC [27,28]. Morales and co-workers [26], developed a dynamic headspace (D-HS) sampling procedure to extract and concentrate informative volatiles from oil samples, to establish correspondences between specific odorants and related sensory attributes, with known metabolic pathways from endogenous or exogenous (from microorganism) enzymes. The results, normalized and interpreted on the basis of target analytes odor thresholds (OT), placed in a more realistic perspective the sensory significance of specific volatiles suggesting an effective strategy of investigation on this subject.

However, despite an extensive knowledge on the volatile composition of olive oil, sensory evaluation has not yet been replaced nor supported by any recognized analytical procedure. This is probably due to several aspects, recently discussed by Aparicio et al. [29], among which the complexity of sensory perception, the fuzziness of the semantics of some descriptors, and at the same time, the necessity to improve analytical selectivity and resolution along with the pre-concentration procedure, represent objective limits of current methodologies adopted for volatile profiling of olive oil.

In this perspective, the present work aims at evaluating whether GC × GC–MS, in combination with both headspace sampling carried out with solid phase microextraction (HS–SPME) and implemented with advanced data elaboration tools, can be a valuable and more informative analytical approach for a high throughput detailed profiling and an effective fingerprinting of the volatile fraction of olive oil in view of its quality assessment.

The chemical blueprint of samples corresponding to regulated defects and authentic EVO samples, provided by IOC, has been adopted as a probe to classify commercial products and to guide an iterative process including sensory evaluation (performed by both an official panel and an internal one), volatiles distribution and their sensory relevance (OT).

2. Materials and methods

2.1. Reference compounds and solvents

Pure reference compounds for analyte identity confirmation and *n*-alkanes (*n*-C7 to *n*-C30) for linear retention index ($I^T{}_S$) determination were from Sigma-Aldrich and Supelco (Milan, Italy). Solvents for *n*-alkanes dilution (toluene and cyclohexane) were HPLC-grade from Riedel-de Haen (Seelze, Germany).

2.2. Samples

The IOC supplied five samples of standard defected oils (*muddy*, *vinegary*, *mold*, *fusty*, and *rancid*); five samples (EVO-1–EVO-5) were provided by an Official Panel (Stazione Sperimentale per le Industrie degli Oli e dei Grassi, Milano, Italy) after sensory evaluation; 9 samples (EVO-6–EVO-14) were purchased from the local market and evaluated by a trained internal panel (five people), according to the standardized official method approved by the IOC [14] and reported in Annex XII of Ref. [12]. The list of samples analyzed by both research units, along with label descriptions and sensory evaluation is reported in Table 1.

2.3. SPME devices and validation

The SPME devices and fibers were from Supelco (Bellefonte, PA, USA). A divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) d_f 50/30 μm, 2 cm length fiber was chosen and conditioned before use as recommended by the manufacturer.

The internal standard (ISTD) loading procedure onto the SPME fiber [30,31] preceded each sampling and was as follows: fiber was inserted into a 20 mL sealed vial containing 500 μL of ISTD (menthol standard solution at 50 ng/mL in dibuthyl phthalate) and exposed to the headspace at 50 °C for 40 min. After ISTD loading, the fiber was exposed to the oil sample headspace (2.000 g) at 50 °C for 40 min and subsequently introduced into the GC injector for thermal desorption for 10 min at 250 °C. Samples were analyzed in duplicate from the two research units. Precision data referred to intra-laboratory relative standard deviations (RSD %) for retention times of 5% maximum and of 18% on normalized 2D peak volumes (i.e., cumulative 2D peak area normalized over ISTD) for a selection of target analytes (Table 2). Inter-laboratory precision was evaluated on normalized 2D peak volumes and was always within a 25% (RSD) range.