Food Chemistry 212 (2016) 730–738

Contents lists available at ScienceDirect

Food Chemistry

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

Characterisation of minor components in vegetable oil by comprehensive gas chromatography with dual detection

Giorgia Purcaro [a,*], Laura Barp [a], Marco Beccaria [b], Lanfranco S. Conte [a]

[a] Dipartimento di Scienze degli Alimenti, University of Udine, via Sondrio 2A, I-33100 Udine, Italy
[b] Dipartimento di Scienze del Farmaco e dei Prodotti per la Salute (SCIFAR), University of Messina, Viale Annunziata, 98168 Messina, Italy

ARTICLE INFO

Article history:
Received 26 February 2016
Received in revised form 9 June 2016
Accepted 15 June 2016
Available online 16 June 2016

Keywords:
Authentication
Multidimensional comprehensive gas chromatography (GC × GC)
Vegetable oils minor compounds
Sterols
Waxes

ABSTRACT

The profile of minor compounds, such as alcohols, sterols, free and alkyl fatty acids, waxes, etc., was investigated in different vegetable oils by a comprehensive gas chromatographic system, coupled with a simultaneous dual detection (flame ionisation detector and mass spectrometer) for quantitative and qualitative purposes. Such a system generated a unique two-dimensional chromatogram to be used as a chemical fingerprint. Multi-level information, due not only to a more "comprehensive" preparation technique, but also thanks to the exploitation of a more powerful and sensitive analytical determination allowed the extrapolation of diagnostic information from the minor components profile of different vegetable oils, along with their characteristic profile. Furthermore, an admixture of an extra virgin olive oil with a low amount of sunflower and palm oils was evaluated, attesting to the powerful diagnostic information provided by the proposed approach.

1. Introduction

Two-dimensional comprehensive gas chromatography (GC × GC) is among the most powerful chromatographic techniques available (Liu & Phillips, 1991; Seeley & Seeley, 2013; Tranchida, Donato, et al., 2013). The well-known advantages of this technique (separation power enhancement, increased sensitivity, and rationalised chemical patterns of compounds over the chromatographic plane) can provide a great advancement of knowledge within the food field. In particular, the unique two-dimensional chromatograms obtained can be handled as a chemical fingerprint to catalogue sample features in a comparable way across samples to classify them with different criteria in order to verify product origin, quality and safety (Kiefl et al., 2012; Nicolotti et al., 2013; Purcaro, Cordero, Liberto, Bicchi, & Conte, 2014).

The term "fingerprint" in the edible oil field immediately remind of the minor components (which represent less than 5% of the entire oil composition), since the characteristic composition of this fraction provides specific information about the identity of an oil (Belitz, Grosch, & Schieberle, 2009). To investigate such a fraction is necessary to isolate it from triacylglycerols (TAGs), which represent over 95% of the sample. The most employed approach to remove TAGs is saponification, but along with TAGs, other informative classes of compounds are hydrolysed, such as waxes, fatty acid alkyl esters (FAAEs), and esterified minor compounds. A valid alternative is the use of a silica column separation step (off-line or on-line), which retains TAGs and more polar compounds (e.g. free sterols and alcohols) (Amelio, Rizzo, & Varazini, 1993; Biedermann, Bongartz, Mariani, & Grob, 2008; Grob & Läubli, 1986; International Olive Council, 2010; Mariani & Fedeli, 1989; Reiter, Lechner, Aichholz, & Lorbeer, 1999). In 1989 Grob et al. proposed a derivatisation step using pivalic anhydride to obtain alcohols, sterols and triterpenic alcohol moieties, which eluted in the same fraction as waxes, FAAEs and esterified sterols, using a silica column in an on-line liquid chromatographic (LC)–gas chromatographic (GC) system (Grob, Lanfranchi, & Mariani, 1989). Shortly after, the derivatisation agent was replaced with *N*,*O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA) to improve stability of the derivatives and their chromatographic separation from naturally occurring esterified compounds (Mariani, Fedeli, & Grob, 1991). Such an application was carried out off-line using a 15-g silica column. Using this approach, a more complex profile was obtained, mainly including FAAEs, free and esterified sterols, and waxes. Some of these classes are investigated to evaluate both quality and authenticity, in particular for high value oils, such as extra virgin olive oil (EVO) (Pérez-Camino, Cert, Romero-Segura, Cert-Trujillo, & Moreda, 2008; Reg. EU n. 1348/2013; Reg. EU n. 61/2011).

The total content and the profile of sterols after saponification are considered in order to assess the botanical origin of the oil and to verify admixture of extraneous oils (CODEX-STAN

* Corresponding author.
E-mail address: giopurcaro@gmail.com (G. Purcaro).

http://dx.doi.org/10.1016/j.foodchem.2016.06.048
0308-8146/

210-1999), for instance, to olive oil (Reg. EEC n. 2568/91; Reg. EU n. 1348/2013). In fact, sterols naturally occur as a mixture of free and esterified sterols with fatty acids (FAs) (Jacini, Fedeli, & Lanzani, 1967), and their profile suggests a bio-guided formation rather than a random esterification (Johansson & Appelqvist, 1978; Johansson & Hoffman, 1979). Finally, waxes are present in the cuticle layer of plants and when solvent extraction is performed a higher amount is found in the final product (as in the case of pomace oil). In the case of mechanically extracted oil, such as olive oil, a high amount can be related to a bad conservation status of olives (*i.e. lampante oil*). A limit for the sum of wax esters from C40 up to C46 was established for high quality pressed olive oils in 2007 (Reg. EC n. 702/2007), but modified in 2013 excluding C40 waxes (Reg 1348/2013), due to the finding of anomalous values in oils from some certain geographical origins (e.g. Argentina) (Ceci & Carelli, 2007). Very recently, upon performing the analysis of one of these samples by a GC × GC system, it was suggested that the odd values found were rather related to the coelution of the C40 wax group with a phytol ester (Purcaro, Barp, Beccaria, & Conte, 2015).

Very few works have exploited the potential of GC × GC to investigate minor compounds in edible oil. Waxes were investigated in different plant oils, using normal-phase LC to isolate the fraction of interest before the GC × GC analysis (Biedermann, Haase-Aschoff, & Grob, 2008). The total sterols profile was studied by GC × GC after a traditional saponification step (Tranchida, Salivo, et al., 2013). Only one paper (Purcaro et al., 2015) extended the classes of minor compounds analysed in a single GC × GC run, by performing a preparation step combining the International Olive Council (IOC) method for FAAEs and wax esters determination (International Olive Council, 2012) with a derivatisation step (Mariani & Fedeli, 1991), followed by dual detection (FID and MS). This method allowed other informative classes of compounds to elute in the same fraction, such as alcohols, free sterols and FAs, present as trimethylsilyl (TMS) derivatives along with FAAEs and waxes (whose determination is required by the olive oil legislation) and esterified sterols. From the chromatographic viewpoint, several compromises were necessary to obtain more information in a separation time comparable to the official method (about 60 min) (International Olive Council, 2012). The use of a short column in the first dimension (1D) was necessary to assure elution of high boiling compounds (such as waxes) in a reasonable time. Therefore, compounds eluted faster and at a lower temperature, determining longer residence times in the 2D column, thus causing undesirable broadening of peaks and wrap-around. An oven offset (+25 °C) was applied to avoid a too extensive wrap-around of some classes of compounds (e.g., free sterols). The structure of the chromatogram was assured up to wax C46, while information related to esterified sterols was lost, since they eluted in the isothermal part of the chromatogram. A simultaneous dual detection, namely FID and MS, was carried out for quantitative and qualitative purposes. The reliability of the method for FAAEs and waxes analysis in olive oil has already been proven (Purcaro et al., 2015), and it was applied here to further characterise edible oils and to prove the potential to detect illegal admixture of olive oil with other extraneous oils.

2. Experimental

2.1. Chemicals

n-Hexane, diethyl ether, and pyridine of Pestanal grade were purchased from Sigma-Aldrich (St Louis, MO). Dodecyl arachidate (C12:0-C20:0, C32) (99% purity) was from Larodan (Malmo, Sweden). Alkanes mixture (from *n*-C7 to *n*-C40) and *N*,*O*-bis(trimethyl silyl)trifluoroacetamide (BSTFA) with 1% of trimethylchlorosilane (TMCS) were purchased from Supelco (Bellefonte, PA). A working internal standard (IS) solution was obtained by mixing methyl heptadecanoate (Me-C17:0, 99%), dodecyl arachidate (C32, 99%), α-cholestanol (Cho, ⩾95%), and 1-eicosanol (OH-C20, ⩾98.5%) at a concentration of about 0.05 mg mL^{-1} each. All the standards were purchased from Sigma Aldrich. Quantification was performed using the internal standard approach, as reported in the IOC method (International Olive Council, 2012).

2.2. Sample and sample preparation

Edible oils considered were: 2 EVO, 2 high oleic sunflower oils (HOSO), 1 palm oil (PO), 1 corn oil (CO), 1 high oleic rapeseed oil (HORSO), 1 sacha inci (*Plukenetia volubilis* L.) oil (SIO). A mixture of 90% of EVO-2, with 5% of palm oil and 5% of sunflower oil was made for method evaluation in the assessment of authenticity.

Sample preparation was carried out using a combination of two different methods, according to the method proposed by Purcaro et al. (2015). Briefly, 100 mg of sample were treated adding 150 μL of BSTFA + 1% TMCS and 150 μL pyridine; it was left to react for 30 min at room temperature (Mariani & Fedeli, 1991), before performing a silica column purification (International Olive Council, 2012). Briefly, silica gel (60–200 μm mesh, Supelco) was placed in a muffle oven for at least 4 h at 500 °C. After cooling, 2% water was added and the silica was stirred for at least 1 h and then left to equilibrate for 12 h. The deactivated silica (3 g) was used to pack a glass column. The prepared column was washed with 10 mL of *n*-hexane and the derivatised oil sample (in 1 mL of *n*-hexane) was loaded. The *n*-alkanes naturally present were first eluted with 12 mL of *n*-hexane, then the fraction of interest (containing FAAEs, free alcohols and sterols as TMS derivatives, waxes, and esterified sterols) was eluted with 45 mL of an *n*-hexane/diethyl ether (99:1, *v:v*) mixture. The exact volume of elution used in this purification step had to be adjusted and/or verified for each different batch of silica and solvents used. A work-flow of the sample preparation procedure is reported in Supplementary Fig. 1.

2.3. GC × GC-FID/MS analysis

GC × GC analyses were performed on a system consisting of two GC2010 gas chromatographs linked through a heated (350 °C) transfer line (Shimadzu, Kyoto, Japan). The GC was equipped with an AOC-20i auto-injector and a split-splitless injector, and a QP2010 Ultra quadrupole mass spectrometer (Shimadzu). A second GC was located between the primary GC and the MS.

A lab-made prototype of a loop-type dual-stage thermal modulator was employed in the GC × GC experiments (Purcaro et al., 2014). Liquid CO_2 was used as cooling agent and a modulation time of 5 s (4.1 s CO_2 flow on and 0.9 s off) was applied.

Columns configuration and conditions were as reported in a previous study by Purcaro and co-workers (Purcaro et al., 2015). Briefly, an 8 m × 0.25 mm i.d., 0.25 μm d_f Rxi-5Sil MS column (Crossbond® 1,4-bis(dimethylsiloxy)phenylene dimethyl polysiloxane located in the first oven) was connected to an uncoated capillary segment (1.5 m × 0.25 mm i.d.) and then to the second analytical column, which was an Rxi-17Sil MS (midpolarity Crossbond®) segment (1.5 m × 0.15 mm i.d., 0.15 μm d_f) located in the second oven. The Rxi-17Sil MS fragment was connected, *via* a capillary column splitter, to two uncoated capillaries linked to the FID (0.3 m × 0.1 mm i.d.) and to the MS (0.5 m × 0.1 mm i.d.) detectors. The eluent was diverted 59% to the MS and 41% to the FID at the beginning of the analysis, changing to 46% and 54% at 350 °C, respectively. All columns were from Restek (Bellefonte, PA).

GC oven temperature program: from 50 °C (hold 1 min) to 140 °C at 40 °C min^{-1}, then to 350 °C (hold 5 min) at 4 °C min^{-1}