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Differentiation of lemon essential oil based on volatile and non-volatile fractions with various analytical techniques: a metabolomic approach

Florence Mehl [a], Guillaume Marti [a], Julien Boccard [a], Benjamin Debrus [a], Philippe Merle [b], Estelle Delort [b], Lucie Baroux [b], Vilfredo Raymo [c], Maria Inés Velazco [b], Horst Sommer [b], Jean-Luc Wolfender [a], Serge Rudaz [a,*]

[a] School of Pharmaceutical Sciences, University of Geneva, University of Lausanne, 30, Quai Ernest-Ansermet, 1211 Geneva 4, Switzerland
[b] Firmenich, Corporate Research, Geneva, Switzerland
[c] Simone Gatto Company, San Pier Niceto, Italy

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ABSTRACT

Due to the importance of citrus lemon oil for the industry, fast and reliable analytical methods that allow the authentication and/or classification of such oil, using the origin of production or extraction process, are necessary. To evaluate the potential of volatile and non-volatile fractions for classification purposes, volatile compounds of cold-pressed lemon oils were analyzed, using GC-FID/MS and FT-MIR, while the non-volatile residues were studied, using FT-MIR, ^{1}H-NMR and UHPLC-TOF-MS. 64 Lemon oil samples from Argentina, Spain and Italy were considered. Unsupervised and supervised multivariate analyses were sequentially performed on various data blocks obtained by the above techniques. Successful data treatments led to statistically significant models that discriminated and classified cold-pressed lemon oils according to their geographic origin, as well as their production processes. Studying the loadings allowed highlighting of important classes of discriminant variables that corresponded to putative or identified chemical functions and compounds.

1. Introduction

Citrus is the most important fruit tree crop in the world, with an annual production of approximately 102 million tons. *Citrus limon* (L.) Burm. f. is considered the third most important citrus species after orange and mandarin. Its production quantity is estimated at 4.2 million tons. Argentina (1,400,000 t/year), Spain (750,000 t/year) and Italy (576,000 t/year) are among the major producers of lemon (González-Molina, Domínguez-Perles, Moreno, & García-Viguera, 2010). Citrus essential oils are characterized by volatile and non-volatile fractions that are composed of more than 200 compounds. Volatiles, which represent 85% to 99% of the entire oil, are well characterized in the literature (Dugo & Mondello, 2010). This fraction is mainly composed of monoterpene and sesquiterpene hydrocarbons, their oxygenated derivatives and aliphatic aldehydes, alcohols and esters. The non-volatile residue of cold-pressed lemon oils (CPLOR), which forms 1% to 15% of the oil, contains hydrocarbons, sterols, fatty acids, waxes, carotenoids, coumarins, psoralens, and flavonoids (Dugo & Mondello, 2010).

Cold-pressed lemon oils (CPLOs) are widely used in the perfume, cosmetic, food and beverage and pharmaceutical industries (González-Molina et al., 2010). CPLOs can be extracted by different extraction processes: oil recovery from peel after juice extraction (Sfumatrice), simultaneous extraction of juice and oil emulsion from whole fruit (Food Machinery Corporation (FMC) Inline Extractor) or recovery of oil from the peel flavedo after removal from the whole fruit by abrasion or shaving (Brown Oil Extractor, BOE and Pelatrice). Several techniques can be employed to analyze volatile and non-volatile fractions of CPLOs. Although chromatographic methods, such as gas chromatography (GC) (e.g., GC-MS (gas chromatography - mass spectrometry), GC-FID (gas chromatography coupled with flame - ionization detection), GC-O (gas chromatography - olfactometry)) and liquid chromatography (LC) (e.g., HPLC-UV (high performance liquid chromatography coupled with ultra-violet detection), HPLC-MS (high performance liquid chromatography - mass spectrometry)) are widely used, only a few publications describe the Fourier transform-middle infrared spectrometry (FT-MIR) and ^{1}H nuclear magnetic resonance

* Corresponding author. Tel.: +41 22 3796336; fax: +41 22 3796808.
E-mail address: serge.rudaz@unige.ch (S. Rudaz).

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(^{1}H-NMR) for the analyses of citrus cold-pressed oils (Schulz & Baranska, 2007; Sommer et al., 2003; Tomi, Barzalona, Casanova, & Luro, 2008).

GC-FID and GC-MS are the golden standard techniques for analyzing the volatile fractions of essential oils. They are sometimes associated with other spectroscopic techniques, including FT-MIR, which is a versatile technique with powerful qualitative and quantitative aspects. Moreover, it is a cheap, fast and reproducible analytical method. Attenuated total reflectance (ATR) sampling allows well-resolved FT-MIR spectra of the oil to be obtained without any specific sample treatment or preparation. FT-MIR spectra, recorded in the wave number range between 650 and 4000 cm^{-1}, provide a high level of specific molecular information and allow highlighting of characteristic vibrational bands that correspond to various chemical functional groups and families (Schulz & Baranska, 2007). FT-MIR associated with chemometrics was previously used to analyze natural products such as vegetable oils and essential oils (Charve, Chen, Hegeman, & Reineccius, 2011; Dupuy, Molinet, Mehl, Nanlohy, Le Dréau, & Kister, 2013) to follow aging or for authentication (e.g., determination of geographic origin, chemical composition). To the best of our knowledge, the only attempt to combine FT-MIR and NIR for characterizing the chemical composition of citrus oils was reported by Schulz and Baranska (2007).

The non-volatile fraction of CPLOs (Cold Pressed Lemon Oil Residue, CPLOR) is also of great interest for the cosmetic industry and human health. Furocoumarins possess phototoxic properties and they are unfortunately believed to cause mutagenesis, carcinogenesis, and photodermatitis (Desmortreux, Rothaupt, West, & Lesellier, 2009). Conversely, polymethoxylated flavones display antioxidative, anti-mutagenic, and antitumor properties, and may therefore possess chemopreventive potential (Weber et al., 2005). In this study, CPLOR were analyzed by FT-MIR, ^{1}H-NMR and UHPLC-TOF-MS. ^{1}H-NMR spectra provide information on a wide range of compounds that are present in a complex matrix in a single experiment. This method is simple, comprehensive, reproducible for both long- and short-term studies and requires very limited sample preparation. It has been applied for many applications related to plant, natural product quality control and metabolomics (Eugster, Guillarme, Rudaz, Veuthey, Carrupt, & Wolfender, 2011), while high-resolution ^{1}H-NMR has been used, in combination with chemometrics, to address various issues of food authenticity and origin. This technique is commonly used to authenticate natural products, such as virgin olive oil, honey or citrus juice, i.e., to classify samples according to geographic origin and detect adulteration. ^{1}H-NMR spectroscopy has been used to qualitatively and quantitatively characterize essential oils (Rivero-Cruz, Rivero-Cruz, Rodríguez, Cerda-García-Rojas, & Mata, 2006) but has been infrequently used to analyze citrus oils (Tomi et al., 2008). Sommer et al. (2003) analyzed CPLORs, using HPLC-NMR and MS to identify coumarins and furocoumarins. Because of its high sensitivity, UHPLC-TOF-MS is a well-suited technique for the analysis of the non-volatile fraction of lemon oils. The coupling of UHPLC with MS is particularly attractive in terms of detection, potential quantification and identification of a wide range of natural products (Eugster et al., 2011; Guillarme & Veuthey, 2012).

As CPLOs are employed in a wide variety of products, their composition must be carefully characterized to ensure quality, genuineness and absence of contaminations or to determine their geographic origin. In particular, present-day adulterations of citrus oils are becoming increasingly difficult to reveal and highly selective and sensitive analytical methodologies are therefore required (Tranchida, Bonaccorsi, Dugo, Mondello, & Dugo, 2012). Due to differences of quality between essential oils from different geographic origins, varieties of fruit or extraction processes, and the importance of lemon oil in the industrial context, it is essential to develop methodologies that are able to reliably differentiate and classify CPLO samples.

The aim of this study was the development and evaluation of analytical methods, including sample preparation, for the discrimination and/or classification of lemon essential oils according to their geographic origin and extraction process. A multi-approach method, similar to that developed for metabolomics, was considered to differentiate the samples (Fig. 1). The volatile and oxygen heterocyclic fractions of the lemon oils were independently analyzed using spectral (i.e., FT-MIR, NMR) and separation (i.e., GC-FID, UHPLC-TOF-MS) techniques. The data obtained using these techniques were investigated with various chemometric tools. Data sets were first explored with non-supervised multivariate analysis (Principal Component Analysis (PCA) and Hierachical Cluster Analysis (HCA)) to gain an overview and detect possible outliers. Then, orthogonal partial least square discriminant analysis (OPLS-DA) was carried out to differentiate classes of samples and highlight biomarkers.

2. Materials and methods

2.1. Sample set

The sample collection consisted of 64 samples of cold-pressed lemon oil obtained by industrial extraction from 3 different

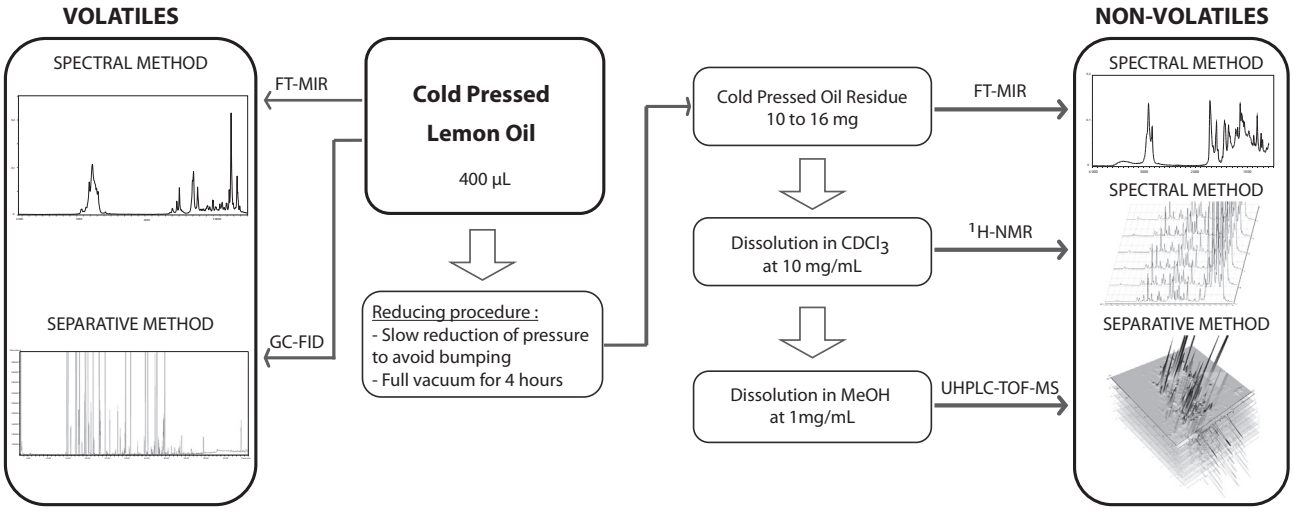

Fig. 1. Methodology used for the comprehensive characterization of volatile and non-volatile fractions of CPLOs.