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New isobaric lignans from Refined Olive Oils as quality markers for Virgin Olive Oils

Lorenzo Cecchi [a,b], Marzia Innocenti [a,b], Fabrizio Melani [a,b], Marzia Migliorini [c], Lanfranco Conte [d], Nadia Mulinacci [a,b,*]

[a] Dipartimento di NEUROFARBA, Università degli Studi di Firenze, Via Ugo Schiff 6, 50019 Sesto Fiorentino, Firenze, Italy
[b] Multidisciplinary Center of Research On Food Sciences (M.C.R.F.S.-Ce.R.A), Università degli Studi di Firenze, Italy
[c] PromoFirenze, Azienda Speciale della CCIAA di Firenze, Divisione Laboratorio Chimico, via Orcagna 70, 50121 Firenze, Italy
[d] Dipartimento di Scienze degli Alimenti, Università degli Studi di Udine, via Sondrio 2/a, 33100 Udine, Italy

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ABSTRACT

Herein we describe the influence of olive oil refining processes on the lignan profile. The detection of new isobaric lignans is suggested to reveal frauds in commercial extra-Virgin Olive Oils. We analyzed five commercial olive oils by HPLC-DAD-TOF/MS to evaluate their lignan content and detected, for the first time, some isobaric forms of natural (+)-pinoresinol and (+)-1-acetoxypinoresinol. Then we analyzed partially and fully-refined oils from Italy, Tunisia and Spain. The isobaric forms occur only during the bleaching step of the refining process and remain unaltered after the final deodorizing step. Molecular dynamic simulation helped to identify the most probable chemical structures corresponding to these new isobars with data in agreement with the chromatographic findings. The total lignan amounts in commercial olive oils was close to 2 mg/L. Detection of these new lignans can be used as marker of undeclared refining procedures in commercial extra-virgin and/or Virgin Olive Oils.

1. Introduction

All over the world, the Mediterranean diet is recognized for its health benefits. Olive oil is one of the most important components of this diet (Buckland & Gonzalez, 2015) with special combination of fatty acids and bioactive minor constituents, which are particularly abundant in the highest quality Extra-Virgin Olive Oils (Beauchamp et al., 2005; Cecchi, Migliorini, Cherubini, Innocenti, & Mulinacci, 2015; Cecchi et al., 2013; Frankel, 2011; Grossi et al., 2013; Migliorini, Cherubini, Cecchi, & Zanoni, 2013; Salvini et al., 2006).

According to the International Olive oil Council (IOC), Virgin Olive Oils are obtained from olive fruit solely by mechanical or physical means under conditions that do not lead to alterations in the oil. Depending on their chemical and organoleptic properties, Virgin Olive Oils are classified as Extra Virgin Olive Oil (EVOO), Virgin Olive Oil (VOO), Ordinary Virgin Olive Oil (OVOO) or Lampante Virgin Olive Oil (LVOO). The oils belonging to this latter category need to be refined to make it edible.

VOOs and, above all EVOOs, are widely appreciated for their health benefits and sensorial properties. The health benefits are mainly related to a high presence of monounsaturated fatty acids, mainly oleic acid (Cohen, Epstein, Pittman, & Rivenson, 2000), and phenolic compounds (Coccia et al., 2014; Covas et al., 2006). The presence of these latter compounds has allowed the EFSA to approve the health claim, "the olive oil polyphenols contribute to the protection of blood lipids from oxidative stress" (EFSA Panel on Dietetic Products, 2011). The sensory properties are also influenced by this fraction together with the volatile compounds mainly derived from the lipoxygenase pathway (Andrewes, Busch, De Joode, Groenewegen, & Alexandre, 2003; Bendini et al., 2007; Gutièrrez-Rosales, Rios, & Gomèz-Rey, 2003). All these properties justify the higher price of the EVOOs and VOOs when compared with other edible oils (Tena, Wang, Aparicio-Ruiz, Garcìa-Gonzalez, & Aparico, 2015).

Oils of lower commercial value include Olive Oil (OO), which, according to the IOC, consists of a blend of Refined Olive Oil (ROO) and Virgin Olive Oils (IOC). The country of retail sale may

Abbreviations: EVOO, Extra Virgin Olive Oil; VOO, Virgin Olive Oil, as sub category of virgin olive oil; OVOO, Ordinary Virgin Olive Oil; LVOO, Lampante Virgin Olive Oil; OO, Olive Oil; ROO, Refined Olive Oil; cv, cultivar; EI, Extract ion; IOC, International Olive oil Council; EFSA, European Food Safety Authority.

* Corresponding author at: Dipartimento di NEUROFARBA, Università degli Studi di Firenze, Via Ugo Schiff 6, 50019 Sesto Fiorentino, Firenze, Italy.
E-mail address: nadia.mulinacci@unifi.it (N. Mulinacci).

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require a more specific designation; regarding Italy, Virgin Olive Oil used to prepare OOs has to be different from LVOO, according to European Regulation 1308/13 (2013).

Oil refining is a physical-chemical multi-step process applied to LVOOs and other common seed oils to make them edible. This process requires, at least, a deacidification treatment and a deodorization as last step. It has been reported that phenolic compounds disappear in olive oil after the refining process, with the exception of lignans, (+)-pinoresinol and (+)-1-acetoxypinoresinol (Garcìa, Ruiz-Mendez, Romero, & Brenes, 2006). Nevertheless, the amount of these lignans in VOOs or crude LVOOs is consistently higher than in the refined oils (Garcìa et al., 2006; Owen et al., 2004).

Due to their high price, EVOOs, VOOs and OOs are very attractive targets for fraudsters. The most common frauds are: i) adulteration consisting of mixture of different categories of olive oils; ii) adulteration consisting of mixture with other vegetable oils. Consequently, there is a continuous search for new markers to detect adulterations and to guarantee the quality and safety of EVOO (Tena et al., 2015).

The lignans are a group of phytochemicals widespread in plants; they belong to the class of phytoestrogens and are beneficial for human health (Fini et al., 2008). Some of them are typical of *Olea europaea* L. and, although their quantity in olive oils is comparable to other classes of phenolic compounds, they were discovered 10 years after the first studies appeared on the oleuropein derivatives (Brenes et al., 2000). Lignans are the most abundant phenolic compounds after secoiridoids in the Virgin Olive Oils (Bonoli, Bendini, Cerretani, & Lercker, 2004). Their concentration mainly depends on the cultivar while the milling process does not affect their amount in a significant manner (Servili et al., 2014). To date, it is not yet clear how they are transferred from olives into oils (Oliveras-Lopéz et al., 2008) and how their amount changes after the chemical/physical treatment of oil.

The principal lignan in almost all Virgin Olive Oils from different cultivars is (+)-1-acetoxypinoresinol with minor amounts of (+)-pinoresinol (Owen et al., 2004), hydroxypinoresinol and syringaresinol (Ballus et al., 2015). On the other hand, (+)-1-acetoxypinoresinol is the minor lignan compared to (+)-pinoresinol in the Picual cultivar oils, which represent approximately 25% of the world's production of olive oil.

(+)-Pinoresinol has also been proposed as a marker to authenticate from Picual (Brenes, Garcia, Rios, Garcia, & Garrido, 2002).

The main goals of this study were to investigate the influence of the olive oil refining process on the lignan profile and to propose the detection of new isobaric lignans as chemical markers of undeclared refining procedures in commercial EVOOs. We analyzed five Italian commercial OOs and three series of partially and fully-refined oils from Italian, Tunisian and Spanish industrial production. A mechanism for the formation of new isobaric lignans during the bleaching step is proposed by a comparison between the chromatographic findings and data from a dynamic molecular modeling study.

2. Materials and methods

2.1. Chemicals

All chemicals for the analyses were of analytical reagent grade: deionized water was produced by the Milli-Q-system (Millipore SA, Molsheim, France). Ethanol and *n*-hexane of analytical reagent grade and formic acid and acetonitrile of LC-MS grade were from J. T. Baker (Phillipsburg, New Jersey, USA). (+)-Pinoresinol from Sigma-Aldrich (Steinheim, Germany) was used as a standard compound. All stock solutions containing the standard (+)-pinoresinol were prepared in ethanol.

2.2. Samples

We sampled five Italian commercial OOs and three series of samples including oils collected at different stages of the refining process, as summarized in Table 1. The oils codified as "Italy", "Spain" and "Tunisia" are samples purchased from Italy, Spain and Tunisia respectively and kindly obtained by an Italian factory of commercial olive oils. Each sample was representative of an industrial batch and was kept in the dark and stored at room temperature until the time of analysis. All the analyzed samples were purchased in 2014–2015 and identified as follow: L for Crude Lampante Olive Oil; N for Neutral Oil; D for Bleached Oil and R for Refined Oil (Table 1).

2.3. Refining process

The Italian Lampante Olive Oil (L_{ITA}) was subjected to a first chemical deacidification at room temperature, to obtain an oil with an acidity of approximately 7.8% and to a second step of deacidification at 90 °C, giving an oil with an acidity of approximately 1.0% (N_{ITA}). In the following step the oil was bleached by using 1.5% of active earth at 90–100 °C and 40–50 mmHg (D_{ITA}). Finally, the oil was deodorized for 2.5 h at 230 °C and 1.5 mmHg (R_{ITA}).

The Spanish Lampante Olive Oil (L_{SPA}) was subjected to a physical treatment consisting of a degumming process (N_{SPA}). The obtained oil was bleached with 0.8% of active earth (D_{SPA}) and then deodorized for 2.5 h at 230 °C and 1.5 mmHg (R_{SPA}).

The Tunisian Lampante Olive Oil (L_{TUN}) was subjected to a chemical deacidification at 90 °C, to obtain an oil with an acidity

Table 1
List of the analyzed samples: in the different columns are indicated the provenience, the sample derived by a specific refining step, the codifying and a short description of the treatment applied to each refining step.

Provenience	Sample type	Code	Treatment
Italy	Crude Lampante Olive Oil	L_{ITA}	Crude Lampante Olive Oil; acidity ≈ 8.8%
	Neutral Oil	N_{ITA}	Chemical deacidification at room temperature and then at 90 °C; acidity ≈ 1.0%
	Bleached Oil	D_{ITA}	Bleaching with 1.5% of active earth at 90–100 °C and 40–50 mmHg
	Refined Oil	R_{ITA}	Deodorization for 2.5 h at 230 °C and 1.5 mmHg
Spain	Crude Lampante Olive Oil	L_{SPA}	Crude Lampante Olive Oil; acidity ≈ 9.0%
	Neutral Oil	N_{SPA}	Physical treatment: degumming
	Bleached Oil	D_{SPA}	Bleaching with 0.8% of active earth
	Refined Oil	R_{SPA}	Deodorization for 2.5 h at 230 °C and 1.5 mmHg
Tunisia	Crude Lampante Olive Oil	L_{TUN}	Crude Lampante Olive Oil; acidity ≈ 9.0%
	Neutral Oil	N_{TUN}	Chemical deacidification at 90 °C; acidity ≈ 1.0%
	Bleached Oil	D_{TUN}	Bleaching with 0.8% of active earth
	Refined Oil	R_{TUN}	Deodorization for 2.5 h at 230 °C and 1.5 mmHg