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Sorting out the phytoprostane and phytofuran profile in vegetable oils

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ABSTRACT

Phytoprostanes (PhytoPs) and phytofurans (PhytoFs) are prostaglandin-like compounds, contributing to defense signaling and prevention of cellular damage. These plant oxylipins result from autoxidation of α-linolenic acid (ALA) and have been proposed as new bioactive compounds due to their structural analogies with isoprostanes (IsoPs) and prostanoids derived from arachidonic acid in mammals, which have demonstrated diverse biological activities. The present work assesses a wide range of vegetable oils - including extra virgin olive oils (n = 7) and flax, sesame, argan, safflower seed, grapeseed, and palm oils - for their content of PhytoPs and PhytoFs. Flax oil displayed the highest concentrations, being notable the presence of 9-*epi*-9-D_{1t}-PhytoP, 9-D_{1t}-PhytoP, 16-B_1-PhytoP, and 9-L_1-PhytoP (7.54, 28.09, 28.67, and 19.22 μg mL^{-1}, respectively), which contributed to a total PhytoPs concentration of 119.15 μg mL^{-1}, and of *ent*-16-(*RS*)-9-*epi*-ST-Δ^{14}-10-PhytoF (21.46 μg mL^{-1}). Palm and grapeseed oils appeared as the most appropriate negative controls, given the near absence of PhytoPs and PhytoFs (lower than 0.15 μg mL^{-1}). These data inform on the chance to develop nutritional trials using flax and grapeseed oils as food matrices that would provide practical information to design further assays intended to determine the actual bioavailability/bioactivity *in vivo*.

1. Introduction

Phytoprostanes (PhytoPs) are regio- and stereo-isomeric prostaglandin-like compounds found in plants, being products of α-linolenic acid (ALA, C18:3 n−3) autoxidation (Barbosa et al., 2015; Mueller, 2004). To date, two regioisomeric series (16-G_1-PhytoPs and 9-G_1-PhytoPs) have been proposed as precursors of the diverse classes of cyclic PhytoPs (Jahn, Galano, & Durand, 2010). In the last few years, novel non-enzymatic pathways have been described that lead to the synthesis of phytofurans (PhytoFs) - additional oxygenated metabolites of ALA synthesized under higher oxygen partial pressure (Cuyamendous et al., 2015; Fessel, Porter, Moore, & Sheller, 2002) - so named for their homology with the 3-hydroxy-2,5-disubstituted tetrahydrofuran structures occurring in mammals, derived from arachidonic acid (AA) (isofurans), docosahexaenoic acid (neurofurans), and adrenic acid (dihomo-isofurans) (de la Torre et al., 2015; Roberts & Fessel, 2004). Both PhytoPs and PhytoFs are constitutively synthesized in plant cells in response to increased levels of reactive oxygen species (ROS), being part of the molecular tools of higher plants involved in defense signaling and in the prevention of cellular damage caused by redox imbalance (Loeffler et al., 2005).

The demonstration of the biological activities of PhytoPs and PhytoFs has been based on *in vitro* mechanistic studies, whilst addressing the existing gap of information concerning their actual biological activity *in vivo* requires further experimental support. In this sense, *in vivo* assays (regarding not only bioactivity but also bioavailability) would provide valuable data on underexplored issues essential to the understanding of the biological relevance of plant oxylipins (PhytoPs and PhytoFs) in mammals after their dietary intake (Barden et al., 2009; Dupuy et al., 2016). Indeed, the lack of information on the actual concentration reached in the diverse cells and tissues at a systemic level constitutes a major constraint to the development of mechanistic studies into a realistic mode of action, due to the use of improper concentrations exceeding those occurring *in vivo* and chemical forms not matching those present *in vivo* in cells and tissues.

For the development of nutritional trials, vegetable oils merit

Abbreviations: ALA, α-linolenic acid; AA, arachidonic acid; BHT, butylated hydroxytoluene; MRM, multiple reaction monitoring; PCA, principal component analysis; PhytoFs, phytofurans; PhytoPs, phytoprostanes; PUFAs, polyunsaturated fatty acids; ROS, reactive oxygen species; SPE, solid-phase extraction; UHPLC-ESI-QqQ-MS/MS, ultra-high performance liquid chromatography coupled to electrospray and triple quadrupole mass spectrometry

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consideration as sources of PhytoPs and PhytoFs given their broad inclusion in the diet in their raw form. In this regard, though a number of scientific articles have addressed the profile and content of PhytoPs in vegetable oils (Collado-González et al., 2015; Collado-González et al., 2015; Karg et al., 2007), the diversity evaluated has not been enough to identify true controls, essential to retrieve consistent results.

The present study profiles and quantifies PhytoPs and PhytoFs in vegetable oils - including extra virgin olive oil (EVOO) from seven distinct varieties of drupes, as well as argan, flax, sesame, grapeseed, safflower, and palm oils. The qualitative and quantitative data were obtained by UHPLC-ESI-QqQ-MS/MS and inform on the best vegetable oil to be included in further bioavailability and clinical trials. The application of biostatistical techniques allowed the determination of both the correlations between individual compounds and the potential of the information obtained for the categorization of the vegetable oils considered.

2. Materials and methods

2.1. Chemical and reagents

The PhytoPs 9-F_{1t}-PhytoP, 9-*epi*-9-F_{1t}-PhytoP, *ent*-16-F_{1t}-PhytoP, *ent*-16-*epi*-16-F_{1t}-PhytoP, 9-D_{1t}-PhytoP, 9-*epi*-9-D_{1t}-PhytoP, 16-B_1-PhytoP, and 9-L_1-PhytoP and the PhytoFs *ent*-16-(*RS*)-9-*epi*-ST-Δ^{14}-10-PhytoF, *ent*-9-(*RS*)-12-*epi*-ST-Δ^{10}-13-PhytoF, and *ent*-16-(*RS*)-13-*epi*-ST-Δ^{14}-9-PhytoF (Fig. 1) were synthesized according to procedures described in the literature (Cuyamendous et al., 2015; Cuyamendous et al., 2017; El Fangour et al., 2004; El Fangour, Guy, Vidal, Rossi, & Durand, 2005; Guy, Flanagan, Durand, Oger, & Galano, 2015; Oger, Brinkmann, Bouazzaoui, Durand, & Galano, 2008; Pinot et al., 2008). They were provided by the *Institut des Biomolécules Max Mousseron* (IBMM) (Montpellier, France). Hexane and chloroform were obtained from Panreac (Castellar del Vallès, Barcelona, Spain), Bis–Tris (bis(2-hydroxyethyl)amino-tris (hydroxymethyl)methane) was purchased from Sigma–Aldrich (St. Louis, MO, USA), and all LC–MS grade solvents, methanol, and acetonitrile were from J.T. Baker (Phillipsburg, NJ, USA). The Strata solid-phase extraction (SPE) cartridges used (Strata x-AW, 100 mg 3 mL^{-1}) were acquired from Phenomenex (Torrance, CA, USA).

2.2. Vegetable oil samples: preparation and extraction

The samples consisted of the commercial oils: 0.1° extra virgin olive (*Olea europaea* L.) oils made using the drupe varieties 'Arbequina', 'Coupage', 'Koroneiki', 'Cuquillo', 'Hojiblanca', 'Cornicabra', and 'Picual' (Oilmedros S.L., Jumilla, Spain), and bio-organic virgin flax (*Linum usitatissimum* L.), virgin sesame (*Sesamum indicum* L.), and virgin argan (*Argania spinosa* L.) oils (Laboratorios Almond, NaturGreen, Librilla, Spain). In addition, safflower (*Carthamus tinctorious* L.) seed (Nutrition & Santé Ibérica SL, Sant Cugat del Vallès, Spain), grapeseed (*Vitis vinifera* L.) (La compagnie des saveurs, Novers-Sur-Cher, France), and palm (*Elaeis guineensis*) (Gracomsa alimentaria S.A., Valencia, Spain) oils were also assessed for their content of PhytoPs and PhytoFs. The oils were stored at room temperature (RT), protected from light, until extraction and analysis.

The PhytoPs and PhytoFs present in the oils were extracted following the methodology described by Leung, Chen, Zhong, Yu, and Lee (2014) and Yonny et al. (2015), with minor modifications for its

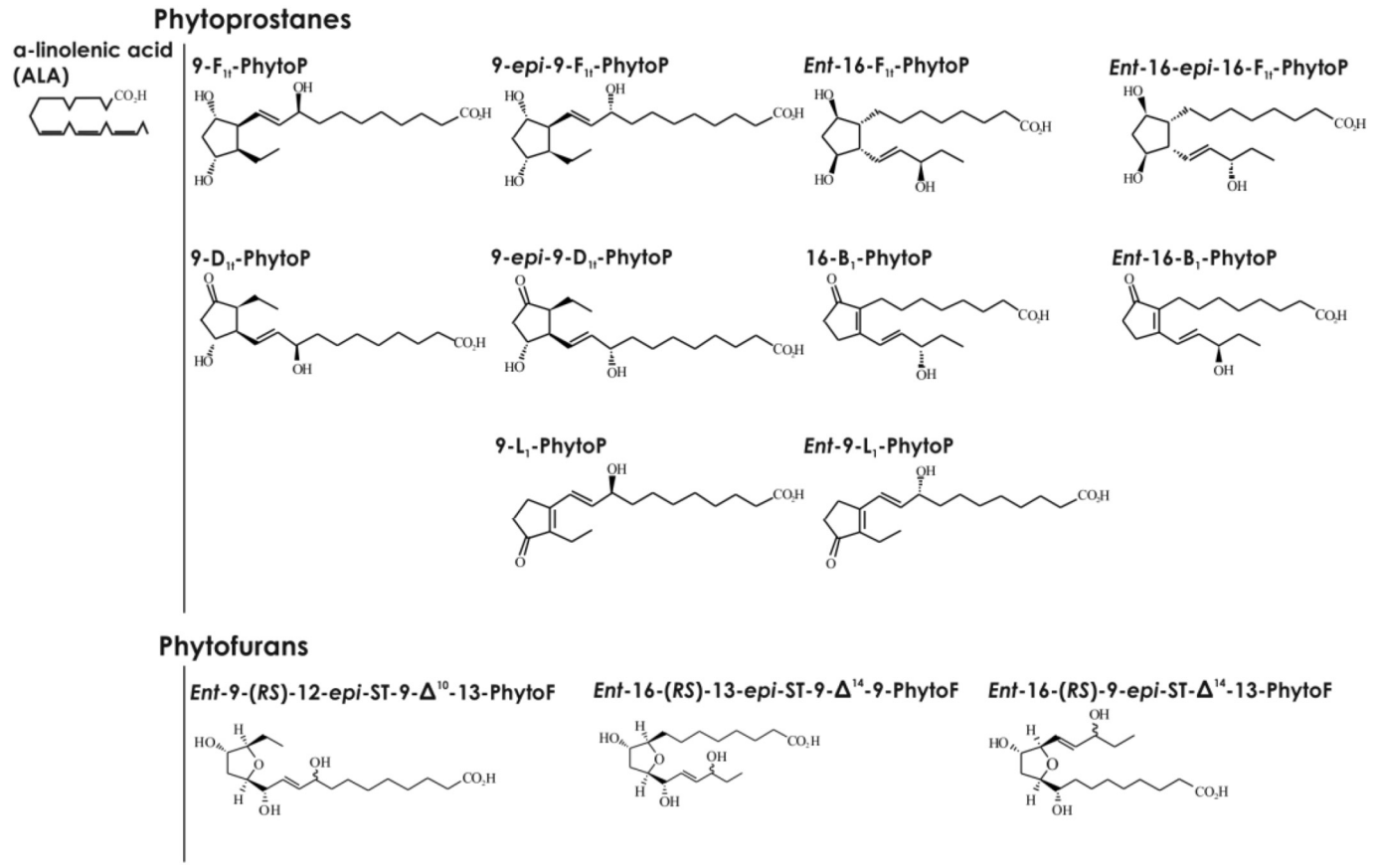

Fig. 1. Chemical structures of the phytoprostanes (PhytoPs) 9-F_{1t}-PhytoP, 9-*epi*-9-F_{1t}-PhytoP, *ent*-16-F_{1t}-PhytoP, *ent*-16-*epi*-16-F_{1t}-PhytoP, 9-D_{1t}-PhytoP, 9-*epi*-9-D_{1t}-PhytoP, *ent*-16-B_1-PhytoP, 16-B_1-PhytoP, *ent*-9-L_1-PhytoP, and 9-L_1-PhytoP; and the phytofurans (PhytoFs) *ent*-16-(*RS*)-9-*epi*-ST-Δ^{14}-10-PhytoF, *ent*-9-(*RS*)-12-*epi*-ST-Δ^{10}-13-PhytoF, *ent*-9-(*S*)-12-*epi*-ST-Δ^{10}-13-PhytoF, *ent*-9-(*R*)-12-*epi*-ST-Δ^{10}-13-PhytoF, and *ent*-16-(*RS*)-13-*epi*-ST-Δ^{14}-9-PhytoF, assessed in vegetable oils. Names are according to Taber, Morrow, and Roberts 2nd (1997) and Cuyamendous et al. (2016).