Selective phenolic-rich extracts from olive pomace by sequential supercritical CO_2 extraction and Pressurized Liquid Extraction with ethanol-water mixtures

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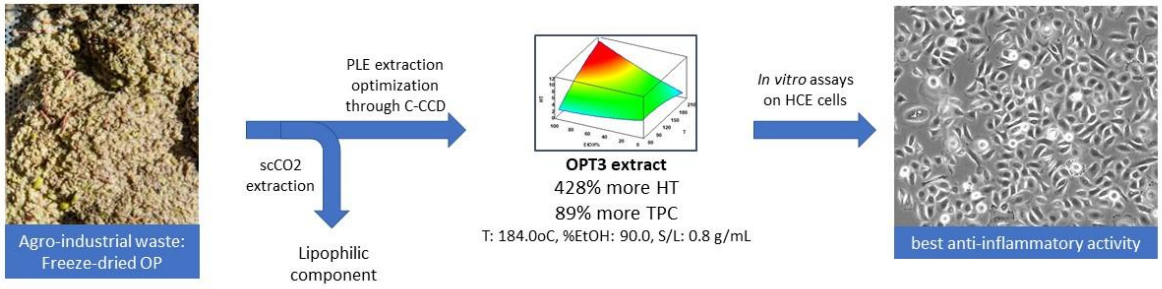
Olive Pomace (OP), olive oil´s industry main by-product, poses a great environmental threat due to its high organic load and phenolic content. In this work, the olive pomace was evaluated as a source of high value bioactive polyphenols with potential application as treatment for ocular surface diseases.

The material was freeze-dried (FDM; ca. 3% moisture) and, then, supercritical carbon dioxide (scCO2) extraction was applied as pretreatment to minimize its lipophilic content and enhance phenolic extraction (FDM-DO). The operation conditions were pressure of 30 MPa, temperature (T) of 62°C and 76.3 kg_{CO2}/g_{FDM}. The goal was to achieve the maximum extract richness in oleuropein (OL), hydroxytyrosol (HT), Total Phenolic Content (TPC), as well as the maximum extraction yield (EY), using an industrially applicable and environmentally friendly approach. A Pressurized Liquid Extraction system (PLE) was developed applying Design of Experiments (DoE) using the Circumscribed Central Composite Design (C-CCD) to optimize the extraction parameters for the responses mentioned. The factors set were the T (65-185°C), the percentage of ethanol (%EtOH) in water (8-92%) and the solid/liquid (S/L) ratio (0.2-0.8 $g_{OP}/mL_{SOLVENT}$), resulting in 24 experiences. Pressure and extraction time were kept at 10 MPa and 20 minutes, respectively. A conventional phenolic extraction (50% vol. EtOH; 70°C; 0.5 g/mL) with FDM-DO was used as reference. Decarboxymethyl oleuropein aglycone dialdehyde (3,4-DHPEA-DEDA) was identified by HPLC-DAD-MS/MS as the most abundant polyphenol in the reference extract and studied for the first time for OP. The Chemical Antioxidant Activity (CAA) of the extracts was evaluated (ORAC assay) and correlated with all the responses. The *in vitro* anti-inflammatory and antioxidant effects of the reference and 3 selected optimal extracts were evaluated in Tumor Necrosis Factor-α and ultraviolet-B radiation stimulated Human Corneal Epithelial (HCE) cells, by measuring interleukin (IL)-6, IL-8 and interferon γ-induced protein-10 secretion and Reactive Oxygen Species (ROS) production, respectively.

Different PLE conditions were found to optimize each response, achieving a clear enhancement of the dry extract richness. Thus, 3 extraction conditions were selected: low T and EtOH%, and high S/L, giving an extract with 373% more 3,4-DHPEA-DEDA and 89% more CAA (OPT1), compared to the reference; low T, and high S/L and EtOH%, producing an extract enriched in OL (475% increase) (OPT2); high T, EtOH% and S/L, obtaining an extract with 428% more HT and 89% more TPC (OPT3). In addition, CAA correlated with TPC and 3,4-DHPEA-DEDA. OPT3 had the best *in vitro* anti-inflammatory activity, significantly reducing the secretion of all IL measured, while all extracts inhibited ROS production equally.

Thus, a sustainable, selective, scalable, and effective extraction process has been established, producing a high-value phenolic extract with demonstrated potential as treatment for inflammatory and oxidative-related ocular surface diseases from an environmentally hazardous agro-industrial by-product. Results are patent pending.



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