

Extracellular vesicles from olive co-products: Isolation, characterization and antioxidant activity.

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Extracellular vesicles (EVs) are nanoscopic membranous structures which are involved in intercellular communication between cells and could have interesting antioxidant/anti-inflammatory properties. Recent works highlighted the existence of EVs secreted by plant cells.

The objective of our study, is to characterize the main physico-chemical properties of EVs isolated from two types of olive coproducts (wastewater and pomace) and compare the properties of EVs to the one of the initial co-products they were extracted from.

Isolation was carried out using successive centrifugations using as raw materials: one wastewater and two pomaces (one mono varietal, one plurivarietal). Pomaces were either used without pretreatment or after lyophilization. In total, EVs were isolated from four types of raw material: wastewaters (WW), fresh mono varietal pomace (MP), fresh plurivarietal pomace (PM), lyophilized plurivarietal pomace (LP). The main physical characterizations were size and charge using Dynamic Light Scattering (DLS) and Zeta Potential (ZP). The chemical characterization included phenolic compounds characterization using HPLC and lipid classes with TLC. Furthermore, test of their antioxidant activity was conducted using DPPH and CAT (Conjugated autoxidizable triene) assay. DPPH gave access to reducing power in homogeneous media and gave an insight into intrinsic reducing power of the constituent EVs molecules. CAT assay gave access to antioxidant activity in dispersed (emulsion based) media and preserve the bioassembly effect of EVs.

EVs isolated from the four co-products (WW, MP, PP, LP) presented distinct physico-chemical properties. EVs diameter ranged 20-300nm according to the type of co-product. Most EVs presented on a [2-7] pH range an evolution of charge from positive values at acidic pH to negative ones at basic pH, with an isoelectric point ranging between [2-3]. EVs isolated from wastewaters were the most concentrated with phenolic compounds (0.5mM eq HT) and contained more neutral lipids than EVs isolated from pomace. However similar polar lipids classes (PA>PC>MGDG>PE) were found in all fractions. Wastewaters EVs was the fraction with the highest antioxidant activity in both antioxidant activity tests: DPPH and CAT.

Physical characteristics (size, charge) of isolated fractions are in agreement with values reported for vesicles in literature. EVs isolated from wastewaters were more concentrated in polar lipids (membranous lipids) and phenolic compounds. Furthermore DPPH and CAT assay showed an antioxidant effect for putative EVs, indicating promising potential of these assemblies.