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PC-B28: A bio-refinery approach for the recovery of compounds of interest and valorisation of winery waste

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Currently, a great part of the grapes cultivated worldwide are being directed to wine production, which involves the deposition of huge amount of vegetal biomass from the wine production process causing environmental pollution. Stems, seeds, dried pulp and skins are currently considered low-value by-products from the grape that could be valorized[1] and transformed into value-added products using environmentally friendly processes.[2] Grape stems are known to present an elevated content of minerals, being also a cheap source of phenolic compounds with antioxidant properties[3] while grape seeds can be used to obtain vegetable oil and are also rich sources of polyphenols.[4] These compounds are currently very appreciated in the food and pharmaceutical industries due to their nutraceutical and bioactive properties. Therefore, the present study aimed at the evaluation of the extraction process of natural value-added compounds, using green technologies such as ultrasound assisted extraction (UAE), microwave assisted extraction (MAE) and autohydrolysis. The selected approach followed the philosophy of biorefineries to valorise different bioactive fractions from grape seeds and stems obtained as a residue after wine production. Grape wastes from Touriga Nacional variety were kindly supplied by Adega Cooperativa de Silgueiros, Portugal. After manually separating the seeds and stems, the seeds were ground and submitted to oil extraction using a Soxhlet apparatus for 6 hours and to UAE using a frequency of 20 KHz and a potency of 150 W, at 30 ºC for 15, 30, 60, and 90 min. In both cases hexane was used as the extraction solvent with a ratio 1:40 (w/v) in Soxhlet and 1:8 (w/v) in UAE. The best results (4.3% extracted oil) were obtained with 90 min UAE extraction with no significant differences being observed compared with Soxhlet extraction. Subsequently, the obtained seeds' residue was dried overnight at ambient temperature and protected from light, to eliminate hexane residues. After that, MAE was used to extract bioactive compounds from the obtained residues. To this aim, different conditions were assayed, namely different solvents (water, ethanol:water (40:60, v/v) and ethanol:water (80:20, v/v)), extraction time (2 min and 5 min) and temperatures (80 ºC, 100 ºC, and 120 ºC). All experiments were performed using a solid to liquid ratio of 1:10. The obtained extracts were filtered, diluted, and submitted to total phenolic compounds determination using the Folin-Ciocalteau reagent and antioxidant activity evaluation using the DPPH method. Moreover, the extracts composition in phenolic compounds and anthocyanins was determined by LC-DAD-ESI-MS/MS allowing the identification of (+)-catechin, (-) epicatechin, type B (epi)catechin dimer, malvidin-*O*-hexoside, and malvidin-*O*-deoxyhexoside-hexoside. In general, water extracts showed the lowest values of bioactive compounds and the worst antioxidant activity while best results were obtained using MAE extraction with 40% hydroethanolic solution at 120 ºC for 5 min. In addition, the grape stems were submitted to autohydrolysis (AH) and MAE extraction, with both methods being carried out at 120 ºC, 150 ºC, 180 ºC, and 210 ºC. For AH treatment, distilled water and ground sample were processed in a 0.6 L stirred pressure reactor (Parr instruments series 4842) equipped with temperature controller and heater. MAE extraction was carried out in an Anton Parr, Monowave 450 reactor, with the equipment being set to reach the selected temperature in 2 min, after which the temperature was maintained for 5 min and then decreased to 55 ºC. Both obtained liquors were analysed by HPLC-RI for oligomeric carbohydrates and other derived groups allowing to detect the presence of glucose, xylose, arabinose and acetic acid. The highest content of sugars was obtained with MAE at 210 ºC. The obtained soluble extracts were used to formulate gelatin-based hydrogels and characterized by rheology. To conclude, the obtained results indicate that the use of MAE technology is a fast and efficient approach to recover natural and bioactive

compounds from wine industry wastes. Moreover, the obtained extracts showed adequate potential for the preparation of gelling matrices with potential food or cosmetic applications.

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