



SEMI-PREPARATIVE FRACTIONATION OF THE MAIN HYDROSOLUBLE ELLAGITANNINS FROM POMEGRANATE HUSK.

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Introduction. The ellagitannins are water-soluble polyphenolic compounds with molecular weight between 300 and 20,000 Da (1). The monomeric unit of ellagitannins possesses a hexahydroxydiphenic (HHDP) acid esterified generally to a glucose core. Ellagitannins can be defined in a narrow sense as HHDP esters of carbohydrates, while in a wider sense includes compounds derived from oxidative transformations. It is well known that ellagitannins possess important biological benefits. The common described are antioxidant, antiviral and anticancer (2). Ellagitannins purification involves many steps and techniques. One of the most reported technique employed in the separation of ellagitannins is liquid chromatography (3).

The objective of the present work was to fractionate and to characterize the hydrosoluble ellagitannins from pomegranate-husk polyphenols

Methodology. 200 mg of polyphenols was used. The preparative purification of ellagitannins was carried out using an MPLC (Medium Pressure Liquid Chromatography) equipment (PDA detector, preparative reversed phase C18 column and three solvents delivery units). Mobile phase was methanol 5% in acetic 3%. Isocratic method was used (80 min, flow rate 8 mL/min, detection wavelength 378 nm). The analysis of fractions was developed using an HPLC equipment (analytical reversed phase C18 column). The mobile phase was methanol, acetic 3% and acetonitrile. A gradient method was employed (60 min, flow rate 1.2 mL/min, detection wavelength 280 nm). The LC-MS analysis was in negative mode using an electrospray ionization. The mass scan range was between 100-2000 m/z.

Results. Three fractions were obtained from semi-preparative separation of ellagitannins (Fig. 1). The HPLC-MS analysis for fraction 1 allowed to identify three compounds with m/z of 1083.23, 781.16 and 481.09 that corresponds to punicalagin, punicalin and HHDP-hexose. The chromatogram for fraction 2 showed three peaks with retention times of 5.9, 7.1 and 9.5 minutes. The peaks 1 and 3 corresponds to the punicalagin anomers α/β according to the standard and the MS analysis, and the peak 3 corresponds to punicalin with m/z 781.16. In

fraction 3 was isolated only punicalagin (Fig. 2). The mass fingerprint for punicalagin was 1083.23, 781.16, 601.01 and 299.01.

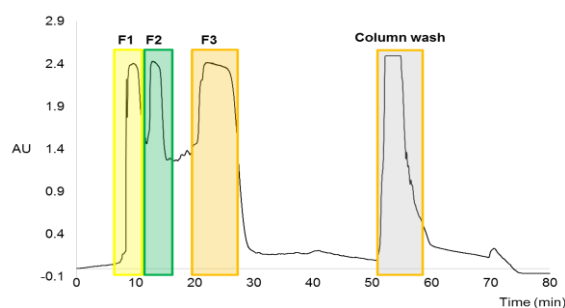


Fig. 1 Fractions from pomegranate polyphenols using HPLC-prep and detection of 378 nm

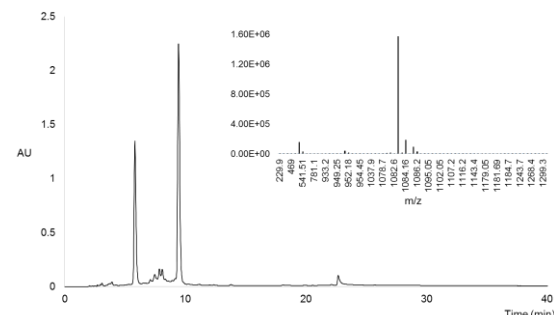


Fig. 2 Analytical chromatogram and mass spectrum for F3

Conclusions. The methodology developed allowed to obtain ellagitannins with potential uses in food industry.

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