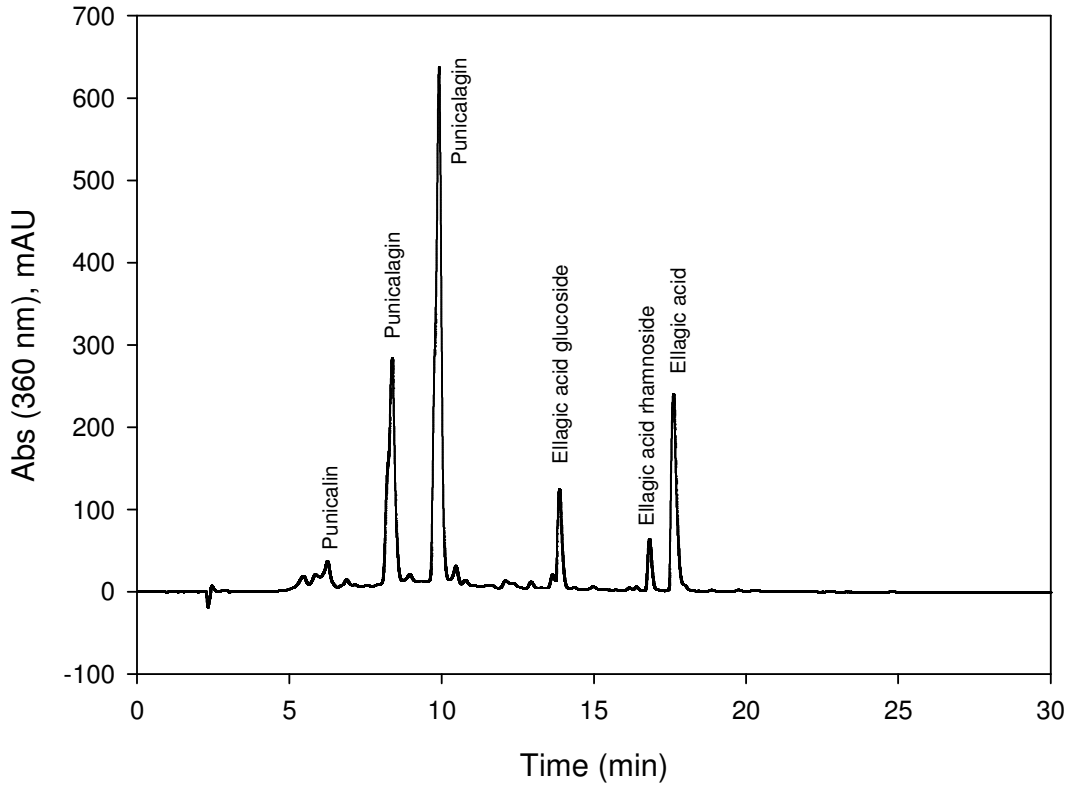
Six months report of activity CSIC-CEBAS WP2

Task 2.2.2 Development and ring-testing of an accurate quantitative method for the determination of ellagitannins, ellagic acid derivatives and other polyphenols in pomegranate extracts and other foods.

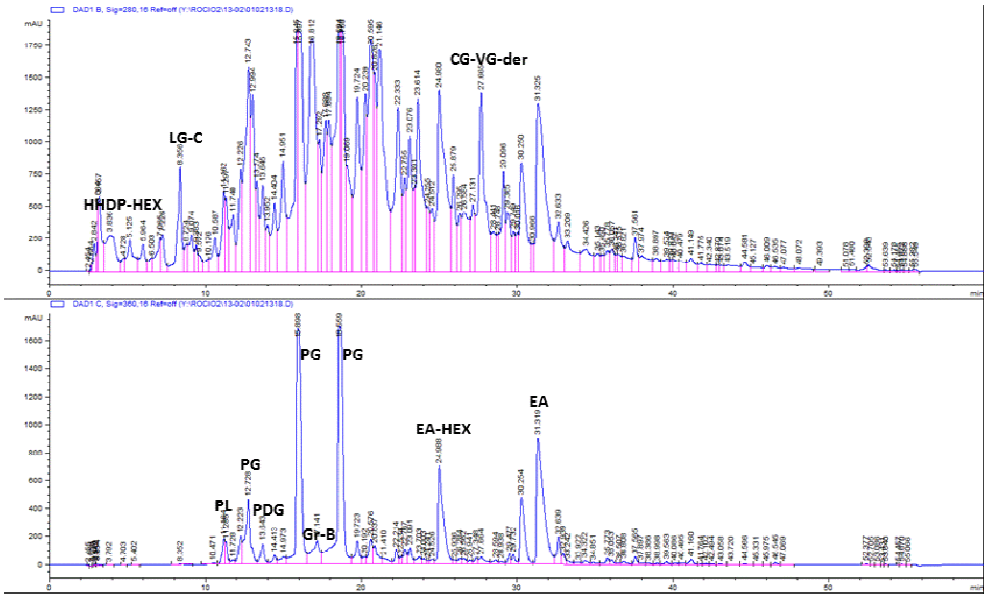
ADMIRA has prepared a number of pomegranate extracts, and polyphenols fractions from pomegranate that have been analysed at CSIC-CEBAS using HPLC-DAD-MS methods. Extracts enriched either in punicalagins, in punicalin or in ellagic acid conjugates were prepared. These extracts and fractions will be used by CSIC-CEBAS to purify individual compounds using preparative chromatography methods. The isolated compounds will be tested for purity (probably using NMR), and will be circulated for the ring-test analytical assay.

An optimized analytical method for the main pomegranate ellagitannins has been developed. This uses reversed phase columns and detection with UV detectors at 360nm. This method works excellent for the quantification of punicalagins, punicalin and ellagic acid and its conjugates with rhamnose, glucose and arabinose.



HPLC chromatogram of a characteristic pomegranate extract (detector set at 360 nm).

A hydrolytical method is actually being assayed to hydrolyze ellagitannins using HCl in methanol/water extracts, and to quantify the hydrolysis products that include gallagic acid, ellagic acid and valoneic acid bilactone in the case of pomegranate extracts. Small amounts of gallate and methyl gallate were also observed. These metabolites differ from those found after the hydrolysis of other ellagitannin-containing foods. The metabolites from other food products can be much more complex including conjugates with glucose in ellagitannin-C-glycosides and conjugates with proanthocyanidins, in other food products. This is actually under development. The analytical methods will be checked at CSIC-CEBAS within the next few months and the method will be submitted to a ring-test within the BACHUS project starting in October (just after the harvest of pomegranates). The methodology and the standards for quantification (punicalagin and ellagic acid) will be circulated among the partners that participate in the ring-test.



HPLC chromatograms of pomegranate juices. Top chromatogram detected at 280 nm (ellagitannins). Bottom chromatogram detected at 360 nm; punicalagins (PG), punicalin (PL), pedunculagin (PDG), granatin B (Gr-B), ellagic acid hexoside (EA-HEX) and ellagic acid (EA).

Task 2.2.3 Pomegranates and other ellagitannins-containing foods.

The extracts to be used in the intervention studies for WP6 are prepared by ADMIRA in the form of capsules. The extract selected is being fully characterized and is very rich in punicalagins and ellagic acid conjugates . The bioavailability/pharmacokinetics study that will be carried out in WP4 will help making the decision of the best particle size to be used to improve the bioavailability of the ellagic acid or the urolithins.

Deliverables.

D.2.2 Month15 SOPs ellagitannins method of analysis. (Will be delivered in due course)

D.2.3 Month 18 Report on bioactive and nutritional composition of the fruit polyphenol-rich food and beverage products to be used in human studies. (Will be delivered in due course)

M.2.5 Month 18 Development of ellagitannin method and standards completed. Will be reached on time.