Experimental

Total Phenolics:

Total phenolics were analyzed according to the Folin-Ciocalteu method using a ready-to-use reagent (Sigma-Aldrich, Steinheim, Germany). 200 µL of liquid samples were diluted with 1020 µL of ethanol containing 0.5% concentrated hydrochloric acid. Solid samples were extracted with ethanol + 1 N HCl (85+15, v+v). Reaction was started by addition of 7,5 % sodium carbonate (incubation 2 hours at 25 °C). Absorption was measured by a plate reader (FluoStar Optima, BMG Labtech, Ortenberg, Germany) at 720 nm. Dilutions of gallic acid in the extraction solvent at concentrations from 10 to 125 mg/L served for quantification.

UHPLC Analysis of Phenolics:

Sample Preparation:

3 mL of the juices or concentrates were filled up to 10 mL with acidified (acetic acid, 1%) methanol. Solid samples were extracted with water+acetic acid+methanol (29+70+1, v+v+v) according to Wu et al. (2004).

Chromatographic Conditions:

For analysis of phenolic compounds, an Acquity UHPLC system (Waters, Milford, MA, USA) consisting of a binary pump (BSM), an autosampler (SM) cooled at 10 °C, a column oven (CM) set at 40 °C, a diode array detector (PDA) scanning from 190 to 500 nm, and an Acquity TQD triple quadrupole mass spectrometer with an electrospray interface operating in positive (for anthocyanins) and negative (for all other polyphenols) ionization mode were used. All analyses were done in duplicate.

Anthocyanins were separated using an Acquity HSS-T3 RP18 column (150 mm x 2.1 mm; 1.8 µm particle size) from Waters with a guard column (5 mm x 2.1 mm). Eluent A was

water/1% formic acid, eluent B was acetonitrile/1% formic acid. The gradient program was as follows: 0 min: 0% B; 0.2 min: 5% B; 23 min: 28% B; 23.5 min: 98% B; 25 min: 98% B; 25.5 min: 0% B; 27.5 min: 0% B. The flow rate was 0.4 mL/min. The mass spectrometer was tuned using a solution of cyanidin 3-*O*-glucoside. The following parameters were obtained: capillary voltage 1.6 kV; cone voltage 32 V; extractor voltage 3.0 V; RF voltage 1.3 V; source temperature 150 °C; desolvation temperature 450 °C, cone gas (nitrogen) flow 50 L/h; desolvation gas (nitrogen) flow 800 L/h. Anthocyanins were quantified at 500 nm using cyanidin 3-*O*-glucoside as reference. Peak identity was confirmed by MS.

For the separation of punicalagins and ellagic acid, an Acquity BEH Shield RP 18 column (150 mm x 2.1 mm, particle size 1.7 µm) from Waters with a guard column (5 mm x 2.1 mm) was used. Eluent A was water/0.1% formic acid, eluent B was acetonitrile/0.1% formic acid. The gradient program at a flow rate of 0.4 mL/min was as follows: 0 min, 2% B; 20 min, 24% B; 20.5 min, 100% B; 22.5 min, 100% B; 23 min, 2% B; 25 min, 2% B. The mass spectrometer was tuned using a solution of punicalagin, resulting in the following parameters: Capillary voltage -2.0 kV; cone voltage 34 V; extractor voltage 2.0 V; RF voltage 0.2 V; source temperature 150 °C; desolvation temperature 450 °C; cone gas (nitrogen) flow 50 L/h; desolvation gas (nitrogen) flow 900 L/h. The MS system was controlled by MassLynx 4.1 software. Punicalagin was quantified as the sum of punicalgin A and B at 380 nm using punicalagin as reference, ellagic acid was quantified at 367 nm using ellagic acid as reference. Peak identity was confirmed by MS.