

DMAC-Analysis

The photometric analysis was done according to Prior et al. (2010). In short: the samples were dissolved or diluted in acetone+water+acetic acid (74.5+25+0.5, v+v+v) and incubated with the coloring agent (4-dimethylaminocinnamaldehyde (DMAC)) in acidified ethanol. The absorbance was read at 545 nm. For quantifying a calibration curve of procyanidin A2 was used.

HPLC-Analysis

For analysis of the procyanidins by UHPLC, the juice was filtered and analyzed without further treatment. The granulate was dissolved in acetone+water (70+30, v+v). For analysis of the procyanidins an Acquity UPLC system by Waters (Milford, MA, USA) consisting of a binary pump (BSM), an autosampler (SM; cooled to 10 °C), a column oven (CM) set at 40 °C, a diode array detector (PDA), and a triple-quadrupole mass spectrometer (Acquity TQD) with electrospray interface operating in negative mode was used. An Acquity BEH Shield RP18 column (150 mm × 2.1 mm, 1.7 µm; Waters) was used for separation. The whole system was controlled by MassLynx 4.1 software. The solvents were LC-MS grade water with 0.1% (v/v) formic acid (mobile phase A) and acetonitrile with 0.1% (v/v) formic acid (mobile phase B). The UHPLC gradient was as follows:

0–28 min, 98–76% A; 28–29 min, 76–0% A; 29–31 min, 0% A; 31–33 min, 0–98% A; 33–35 min, 98% A; flow rate = 0.4 mL/min.

Two microliters of each sample extract was injected. For quantification of A-type procyanidins, the mass spectrometer was tuned using a standard solution of procyanidin A2. The resulting parameters were as follows: capillary voltage, –2.0 kV; cone voltage, 46 V; extractor voltage, 2.0 V; RF voltage, 0.20 V; source temperature, 150 °C; desolvation temperature, 450 °C; cone gas (nitrogen) flow, 50 L/h; desolvation gas (nitrogen) flow, 800 L/h.

For quantification purposes mass traces of procyanidins m/z were measured by using selected reaction monitoring (SRM) with the following compound-specific transitions of parent and product ions: A-type dimers m/z 575 → 449, A-type trimers m/z 863 → 575 and 863 → 573. The dimers and trimers were quantified as procyanidin A2 dimer equivalents (A2 equiv) with an external calibration curve of procyanidin A2 in the range of 10–100 µg/mL.

All UHPLC-MS² analyses of each sample were done in duplicate.

Mass traces of A-type dimers (m/z 575 → 449) and A-type trimers (m/z 863 → 575 and 863 → 573) in lingonberry granulate and lingonberry juice. Peak labels according to Jungfer et al. 2012.