

Scientific Report
**Short term scientific mission, COST 863:
From genomic to sustainable production, quality and health**

Determination of Ellagitannin-Diversity in Strawberry

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1 Objective

Ellagitannins are a main class of polyphenols with health-promoting effects. The aim of the short term scientific mission was to characterize the occurrence of ellagitannins in strawberry, furthermore to evaluate differences in cultivars, location and cultivation techniques. Therefore 27 samples were analyzed at the host institute with two different methods.

2 Material and Methods

2.1 Plant Material

Strawberry samples of four different cultivars were collected 2009 from an experimental field in Geisenheim, Germany. One variety was also cultivated under plastic tunnels. The samples were directly snap frozen with liquid nitrogen and milled to a fine powder. Additionally, fruit material of two different cultivars from Agroscope, Changins -Wädenswil, Switzerland and the University of Copenhagen, Denmark were analyzed. Strawberries were sampled and prepared comparably.

2.2 Extraction Method

The frozen powder was weighed and an equal volume of acetonitrile containing 0.2% formic acid was added (1g fruit powder + 1 mL extracting agent). The acetonitrile was evaporated and the remaining water was dried by lyophilization. The dried extracts were brought to the SCRI, Invergowrie, Scotland.

At the host institute, the extracts were dissolved in aqueous ethanol containing a total phenol content of 4000 µg/mL. One fraction of this extracts was measured to determine all containing phenols. The main part was purified with Sephadex LH-20.

2.3 Sephadex LH-20 clean up

Sephadex LH-20 was swollen with aqueous ethanol overnight. The redissolved extracts were mixed with an equal volume of Sephadex LH-20 slurry, equilibrated and centrifuged. The supernatant was collected and the LH-20 was washed with 50 % ethanol. Bound components removed with 70 % acetone. The bound fractions were dried with speed-vac and resuspended in 50% acetonitrile/water containing 0.1 % formic acid with a total phenol content of 4000 µg/mL.

2.4 Liquid Chromatography-Mass Spectrometry (LC-MS) analysis

Samples of redissolved extracts (section 2.2) and the bound fraction (section 2.3) were analysed on a LCQ-DECA system, comprising Surveyor autosampler, pump and photo diode array detector (PDAD) and a ThermoFinnigan mass spectrometer iontrap controlled by the XCALIBUR software as described previously (McDougall et al., 2008) with minor modifications. The PDA detector scanned three discrete channels at 280, 365, 520 nm. Solvent A was 0.1% formic acid in ultra pure water and solvent B 0.1% formic acid in acetonitrile. The bound fraction was prior analyzed on a short column.

Method 1 (partial separation with short column):

Column: Synergi, (20 x 2 mm, 2.5µm) MAX-RP 100A Mercury (Phenomenex).

Gradient: 98:2 solvent A:B at time=0min, 50:50 at time=3min, 35:65 at time =5min, 5:95 at time=6.5min The duration of one run was 10 minutes.

Flow: 200µL/min

Method 2 (tall length column)

Column: Synergi (150 x 4.6 mm, 4 μ m) Hydro-RP C18 80A (Phenomenex).

Gradient: 95:5 solvent A:B at time=0min to 55:45 A:B at time=30min

The duration of one run was 45 minutes.

Flow: 400 μ L/min

2.5 Direct infusion mass spectroscopy (DIMS)

The direct infusion MS (DIMS) technique was also carried out on the LCQ–DECA system of one purified sample to prove the occurrence of ellagitannins in the bound fraction resulting from 2.3. The sample was injected directly into the ESI (electrospray ionization) source. ESI-MS spectra (negative mode) were acquired from m/z 80–2000 for 2 min.

2.6 Work carried out during the visit

Week 1:

- LC/MS analysis of extracts
- Testing abbreviated method for Sephadex LH-20
- direct infusion mass spectroscopy
- sample purification of 27 strawberry samples

Week 2:

- LC/MS analysis: short column (Method 1)
- LC/MS analysis: tall length column (Method 2)
- comparison of “short column” results

Week 3:

- analyzing of data
- statistical analysis

3 Main obtained results

3.1 LC/MS characterization of strawberry extracts prior to purification

The measurement of the whole strawberry extract including all phenols showed that all samples had similar phenolic profiles. However, variation among cultivars and identical cultivars from different origins were observed in their phenolic profiles.

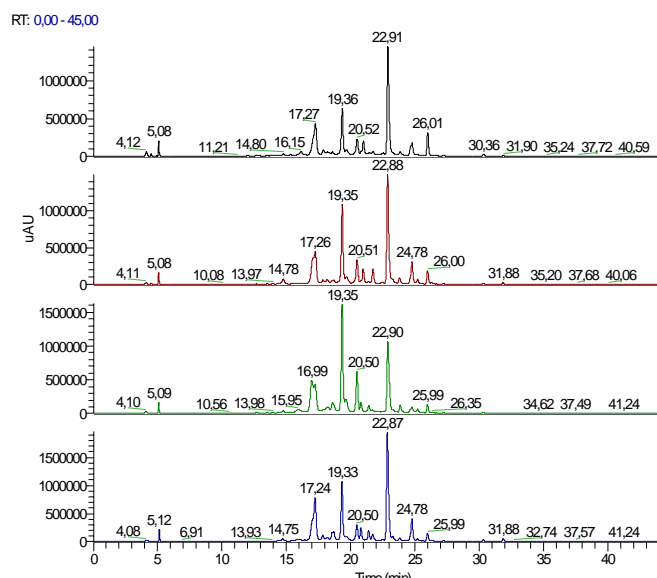


Figure 1: HPLC-DAD chromatograms of polyphenols in strawberry fruits of four different cultivars recorded at 280 nm,

3.2 Direct infusion mass spectroscopy (DIMS) of tannin-rich fraction

The DIMS method was used to verify the existence of ellagitannins in the bound fraction of one sample. The characteristic masses of ellagitannins and also proanthocyanidins were identified. This was the motivation to use the abridged Sephadex LH-20 clean-up for all samples.

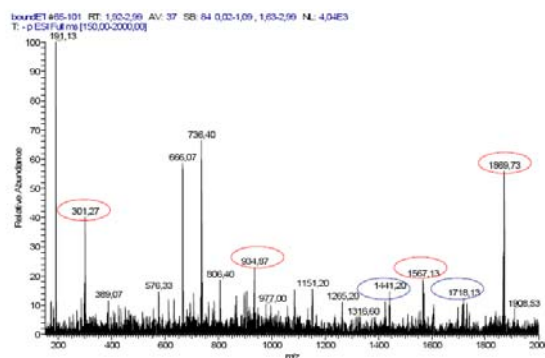


Figure 2: DIMS-mass spectra of the tannin-rich fraction. The red circles show typical masses of ellagitannins, the blue circles of proanthocyanidins.

3.3 Partial separation of tannin-rich fraction (short column method)

The short column method is with 10 minutes a very fast and effective method for a screening of many samples. Differences in cultivar and origin but not in cultivation technique could be observed with this method. The main differences between cultivars were due to the composition of proanthocyanidins and ellagitannins.

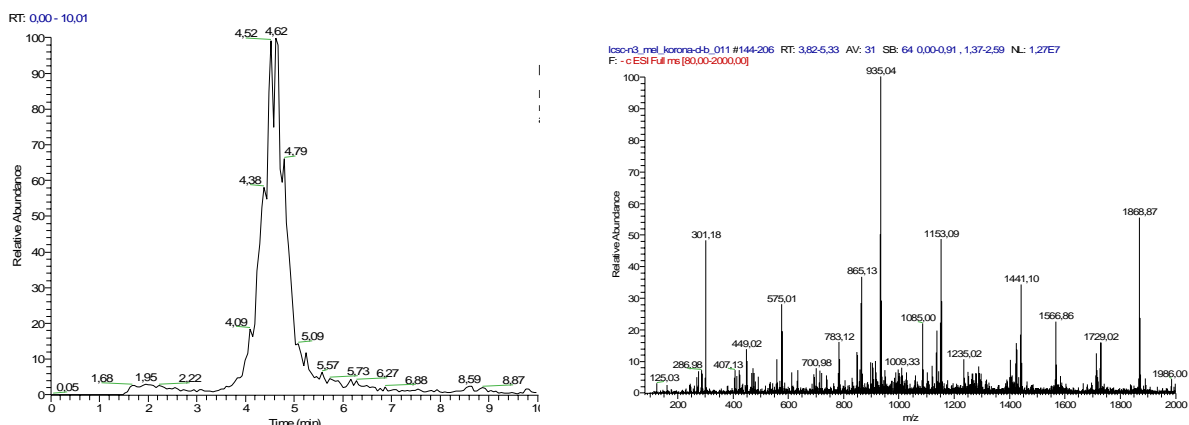


Figure 3: Example of MS-chromatogram (left) and MS-spectrum (right) resulting from the partial separation of the short column method.

3.4 Characterization of tannin-rich strawberry extracts

The tannin-rich fraction was analyzed with the same method as the redissolved strawberry extract. The major peaks belonged to ellagitannin related compounds. The signals of proanthocyanidins were weak and separation declined with the polymerization degree. In any case, the analyzed samples showed considerable variation.

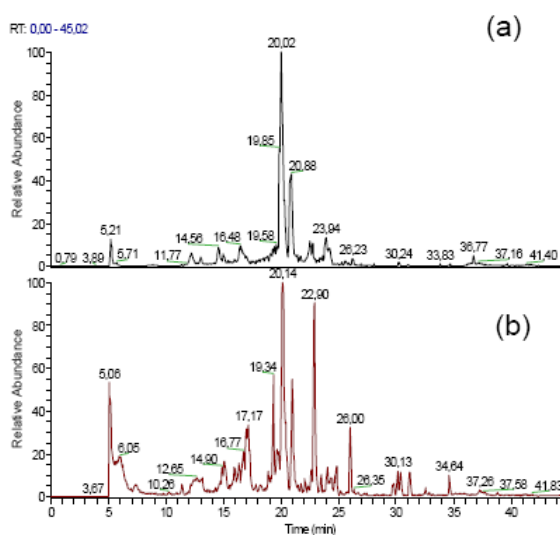


Figure 3: Example of MS-chromatogram of the purified and concentrated tannin-fraction (a) and the strawberry extract containing all phenols (b).

4 Conclusion

The partial separation with the short column method was an effective tool screening differences of samples and qualifying the main ellagitannin structures. Further this screening method supported the tall length LC/MS method which also identified and quantified tannins. Until now, less is known about variability of tannins among strawberry cultivars or variability of the growing location. This short term scientific mission was a successful study to extend the knowledge to this topic. We need to complete analysis of the results and plan future collaborations.

5 Acknowledgement

I like to thank Gordon McDougall for hosting me in his laboratory and his scientific assistance. Many thanks to all the staff of the SCRI (section of plant products and food quality) for being always helpful and welcoming. The COST scientific programme thanks for the financial support.

6 References

- Hager TJ, Howard LR, Liyanage R, Lay JO, Prior RL: Ellagitannin Composition of Blackberry As Determined by HPLC-ESI-MS and MALDI-TOF-MS. *J Agric Food Chem* 2008;56:661-669.
- McDougall GJ, Martinussen I, Stewart D: Towards fruitful metabolomics: High throughput analyses of polyphenol composition in berries using direct infusion mass spectrometry. *Journal of Chromatography B* 2008;871:362-369.