Characterisation of sainfoin (*Onobrychis viciifolia*) condensed tannins by MALDI-TOF mass spectrometry

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Introduction.
- Sainfoin (*Onobrychis viciifolia*) was grown in Europe before the widespread use of commercial fertilisers in the 1950’s.
- It is an excellent fodder legume and it contains tannins.
- It can lead to more efficient utilisation of proteins.
- It is an excellent fodder legume and it contains tannins.
- Its tannins may also reduce pollution in terms of emission of nitrogen and methane, one of the most damaging greenhouse gases.
- It possesses anthelmintic properties.
- It could contribute to more sustainable farming systems.

Hypothesis.
Sainfoin condensed tannins are responsible for its beneficial nutritional and anthelmintic effects.

Materials and Methods.
MALDI-TOF spectra were collected on an Ultraflex Tof/Tof mass spectrometer (Bruker Daltonics) equipped with delay extraction and a N₂ laser set at 337 nm. In the positive reflectron mode an accelerating voltage of 25.0 KV and a reflectron voltage of 26.3 KV were used. All spectra were the sum of 200 shots. Spectra were calibrated with ClinProt Standards (Bruker). 2,5-dihydroxybenzoic acid (2,5-DHB); sinapinic acid (SA); α-cyano-4-hydroxycinnamic acid (HCCA) and α-cyano-4-chlorocinnamic acid (CCCA) were used as matrices. The matrix and the sample solutions were then mixed at 1/1 (v/v) ratio, spotted on the target and left to dry according to the dried droplet method.

Results.
- Polymers consisting of up to 12 flavan-3-ol units were detected in the MS spectra (Fig.2). A further series of peaks differing by 16 m/z units was observed within each of these oligomers. They correspond to etero- and homopolymers containing catechin and epicatechin units (C) and gallocatechin and epigallocatechin units (G) in all possible combinations (Fig.2 inset). Peaks in the range 3800-5040 m/z may correspond to polymers containing 13-16 units.
- Trimers galloylated and dimers glycosylated and galloylated were observed for the first time.
- Further research is needed to improve the MS detection and resolution of polymers with mDP>12.
- Sainfoin contains a highly complex mixture of condensed tannins in terms of PC:PD ratios and molecular weights. Comparisons between the tannins from sainfoin, *Lotus pedunculatus* and *Lotus corniculatus* are ongoing in order to determine if this complexity is the key to their beneficial effects of sainfoin.

References.

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