

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

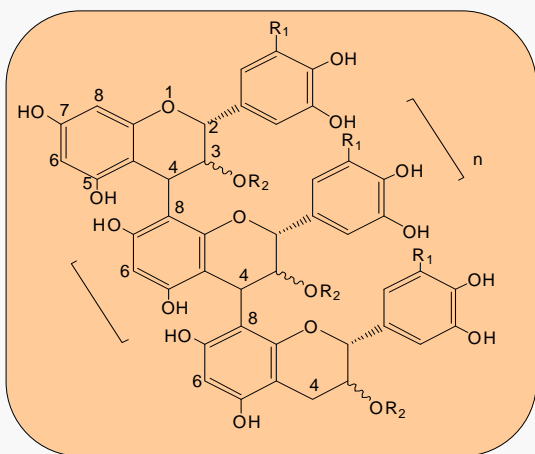
Elisabetta Stringano,<sup>§</sup> An Gea,<sup>§</sup> Rainer K. Cramer,<sup>†</sup> Wayne Hayes<sup>‡</sup> & Irene Mueller-Harvey<sup>§</sup>

<sup>§</sup> Chemistry & Biochemistry Laboratory, Agriculture; <sup>†</sup> BioCentre Facility; <sup>‡</sup> School of Chemistry, Food and Pharmacy; University of Reading, UK;

\* corresponding author: [e.stringano@reading.ac.uk](mailto:e.stringano@reading.ac.uk)

## 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<sup>2</sup>.
- 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.

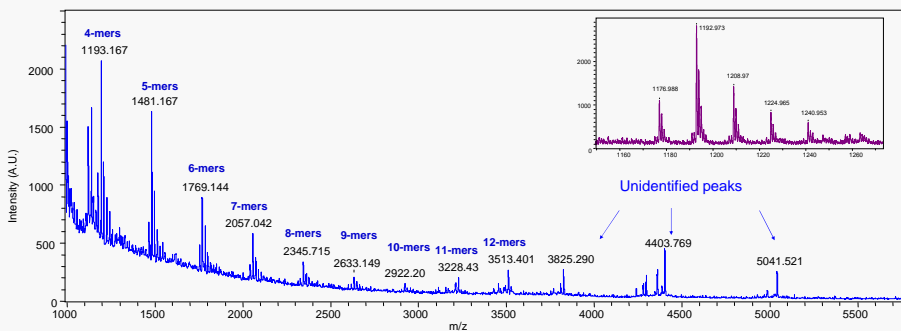


**Figure 1:** Structure of condensed tannins. R<sub>1</sub> = H (procyanidins) or OH (prodelphinidins); R<sub>2</sub> = gallic acid

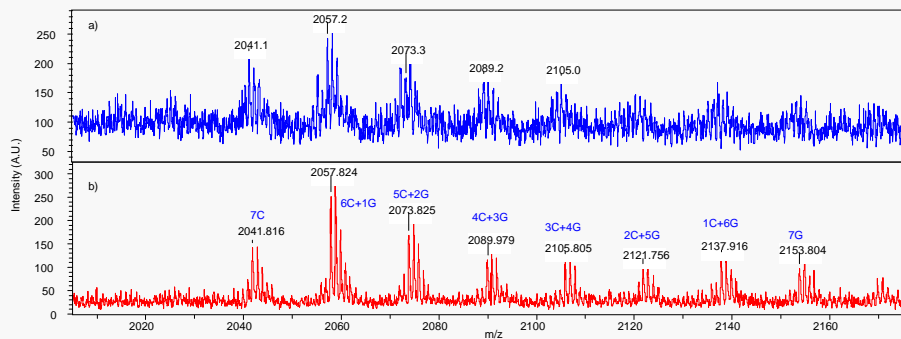
## Hypothesis.

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 N<sub>2</sub> 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); super-DHB; sinapinic acid (SA); a-cyano-4-hydroxycinnamic acid (HCCA) and a-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<sup>3</sup>.



**Figure 2:** MALDI-TOF MS spectrum of condensed tannins in sainfoin. Inset shows a series of tetramers.



**Figure 3:** MALDI-TOF spectrum of 7-mers obtained using CCCA (a) and sDHB (b) as matrices. The peaks correspond to homo- and heteropolymeric 7-mers containing C and G units. (C = (epi)catechin; G=(epi)gallocatechin).

## 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.

## Acknowledgements.

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- MALDI-TOF MS analyses were carried out at the BioCentre Facility (Reading RG6 6AS).

## References.

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