

Evaluating the effects of tannins on the extent and rate of *in vitro* measured gas and methane production using the Automated Pressure Evaluation System (APES)

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MESSAGE

1. Measuring methane production kinetics using an automated gas production system allows to study shifts in methanogenic activity
2. Valonea and Myrabolan tannins seem very successful in reducing methane production with only minor effects on over all fermentation
3. Chebulic-type ellagitannins appear to be of particular interest in their ability to reduce methanogenesis

Objective

- Assess the efficacy of some hydrolysable and condensed tannin isolates to change the extent and rate of *in vitro* methane production using an Automated Pressure Evaluation System (APES)
- Identify the tannin assignments involved in reducing methane production by use of MALDI-TOF MS technique

Materials & Methods

MALDI-TOF MS analyses of tannin isolates

Hydrolysable

- Chestnut (91.6%)
- Myrabolan (14.5%)
- Tara (95.1%)
- Valonea (27.0%)

Condensed

- Grape seed (33.3%)
- Green tea (84.9%)
- Quebracho (33.6%)

Experimental design

Donor Animals

- 4 lactation HF cows
- FPCM: 35.5 ± 8.6



Inocula



Substrate

- Lucerne

Treatments

- Tannins added (10%)
- PEG6000 added (+) or not (-)
- level PEG:substrate = 1:1

Automated Pressure Evaluation System

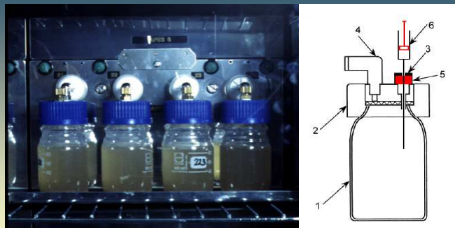


Figure 1. Modified APES. (1) 100 mL culture bottle; (2) lid; (3) screw cap insert with a small aperture; (4) elbow connection; (5) gastight septum in screw cap; (6) gas tight syringe for headspace sampling.

Data collection, Sampling & Analyses

Gas & methane production

- Cumulative gas production was measured for 72h (Davies et al., 2000)
- 25 µL gas samples taken at 0, 1, 4, 7, 11, 15, 23, 30, 46, 52 and 68 h and injected onto a gas chromatograph for methane analyses
- Gas & CH₄ production curves fitted with a monophasic model (Groot et al., 1996)

Tannin analyses

- Tannin isolation as described by (Frazier et al., 2010)
- Structural characterisation of tannins by use of MALDI-TOF MS

RESULTS

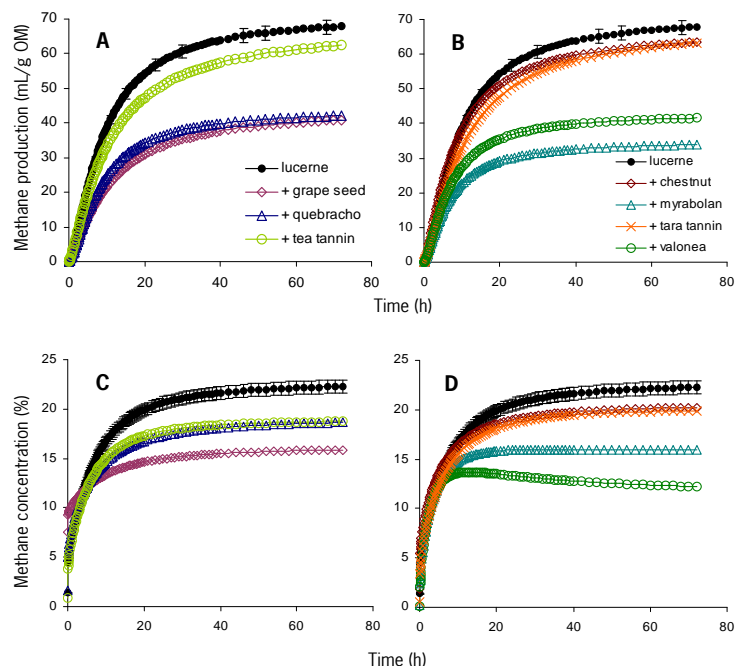


Figure 2. Cumulative methane production (A & B) and concentration of methane in total fermentation gas (C & D) for condensed and hydrolysable tannins.

Conclusions

- Measuring methane production in an automated gas production system gives opportunities to study shifts in fermentation activity in more detail.
- The ability of PEG to bind and alter biological activity of tannins depends on the type of tannin.
- The MALDI-TOF MS analyses proved to be a very powerful tool, revealing many new candidate tannin structures in the case of valonea.
- Methane was strongly reduced by valonea and myrabolan tannin, whilst over all fermentation was only affected to some extent.
- Chebulic-type ellagitannins appear to be of particular interest in their ability to reduce methanogenesis as they are present in both valonea and myrabolan extracts.