Evaluation of the methane mitigating potential of Hemp using Solid Phase Extraction, Untargeted metabolomics and *in vitro* rumen fermenation

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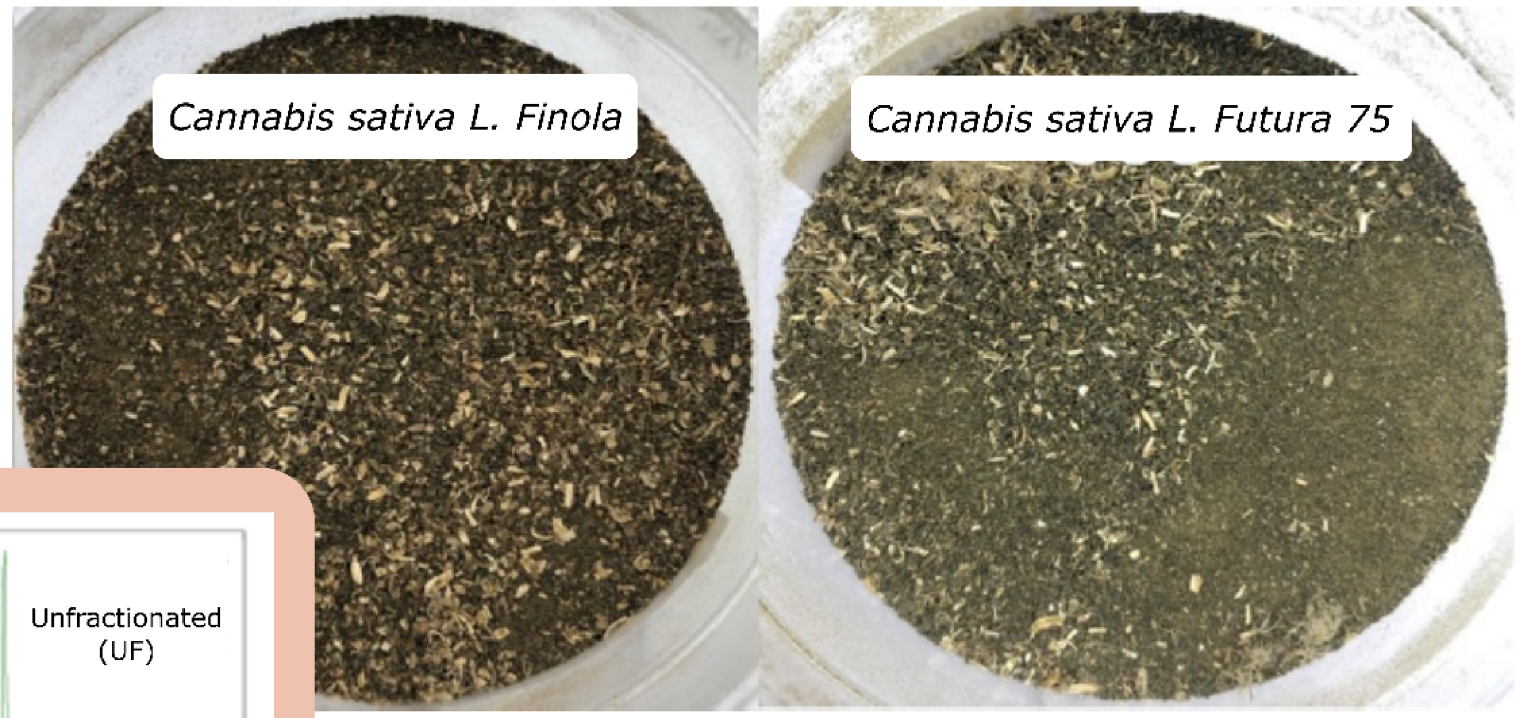
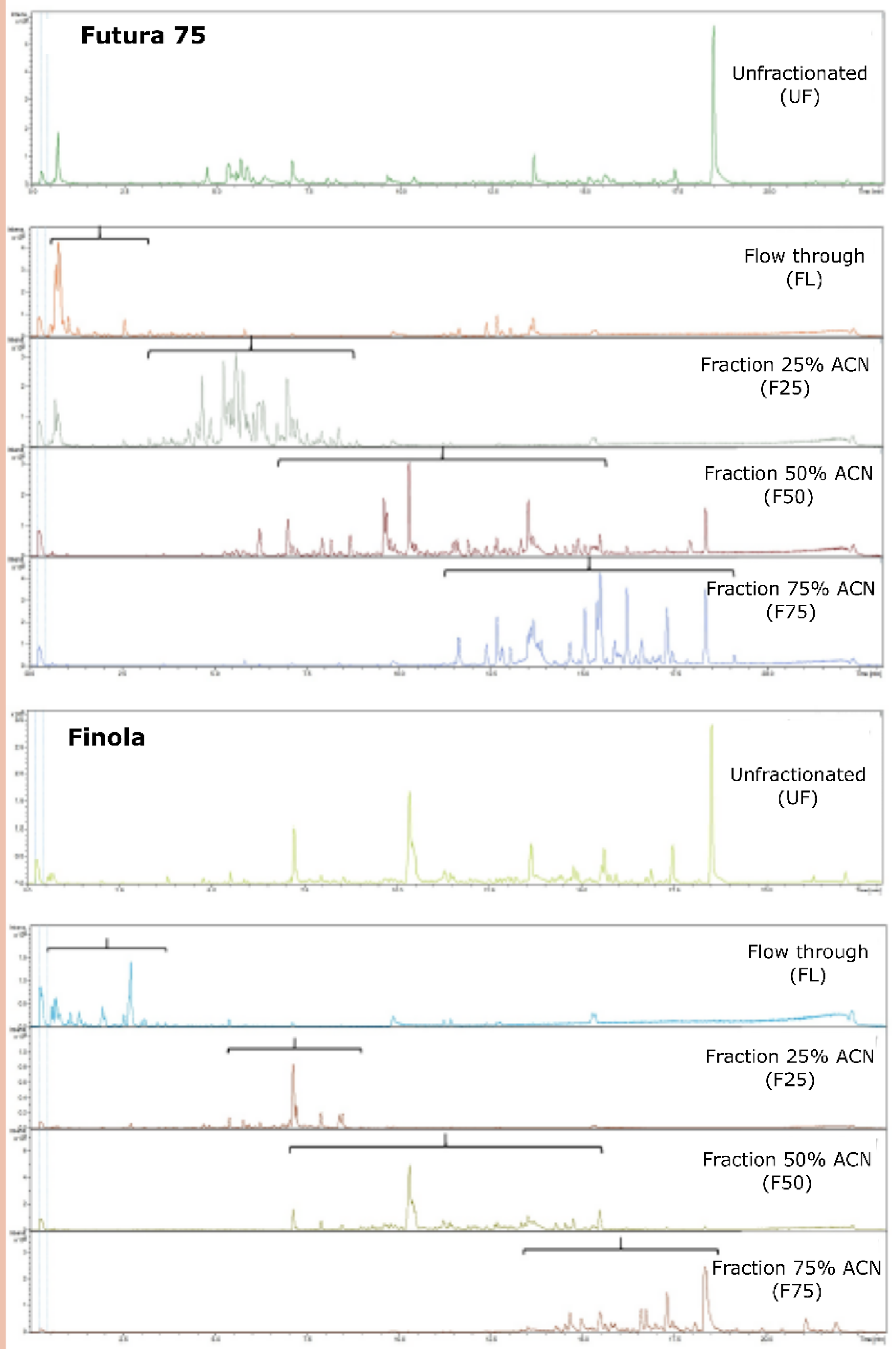
INTRODUCTION

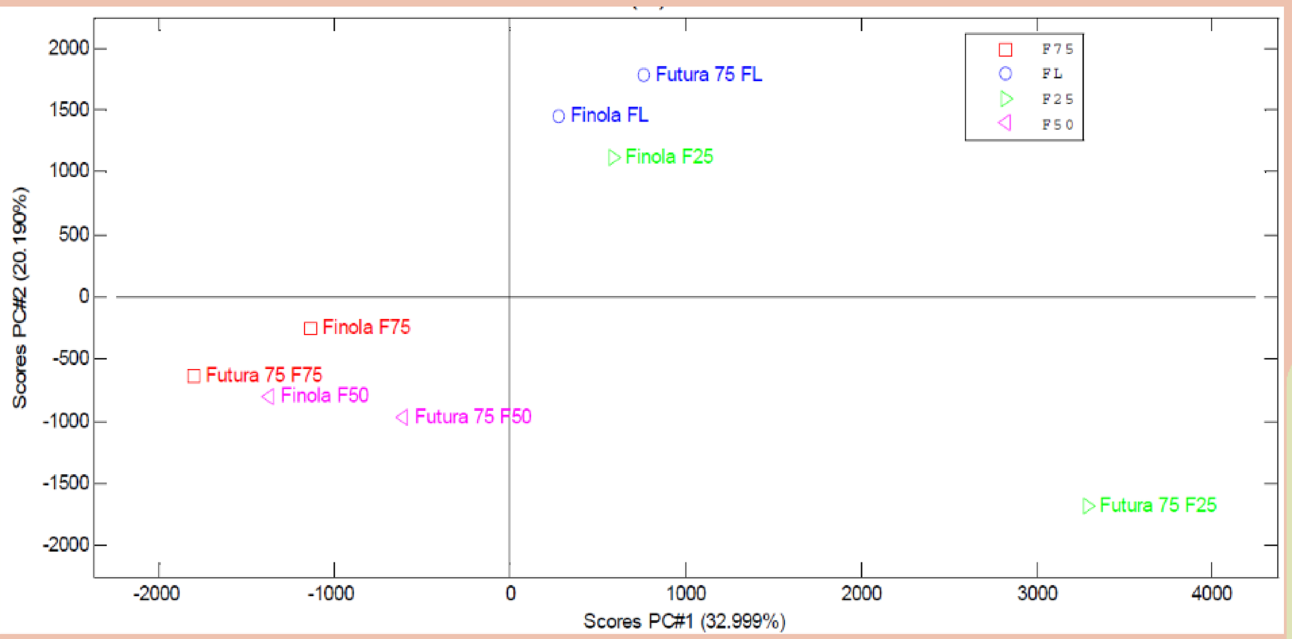
Methane is a highly potent greenhouse gas, which contributes to climate change. Therefore, there is an increasing need to develop additives to cow diets with methane mitigating potential.

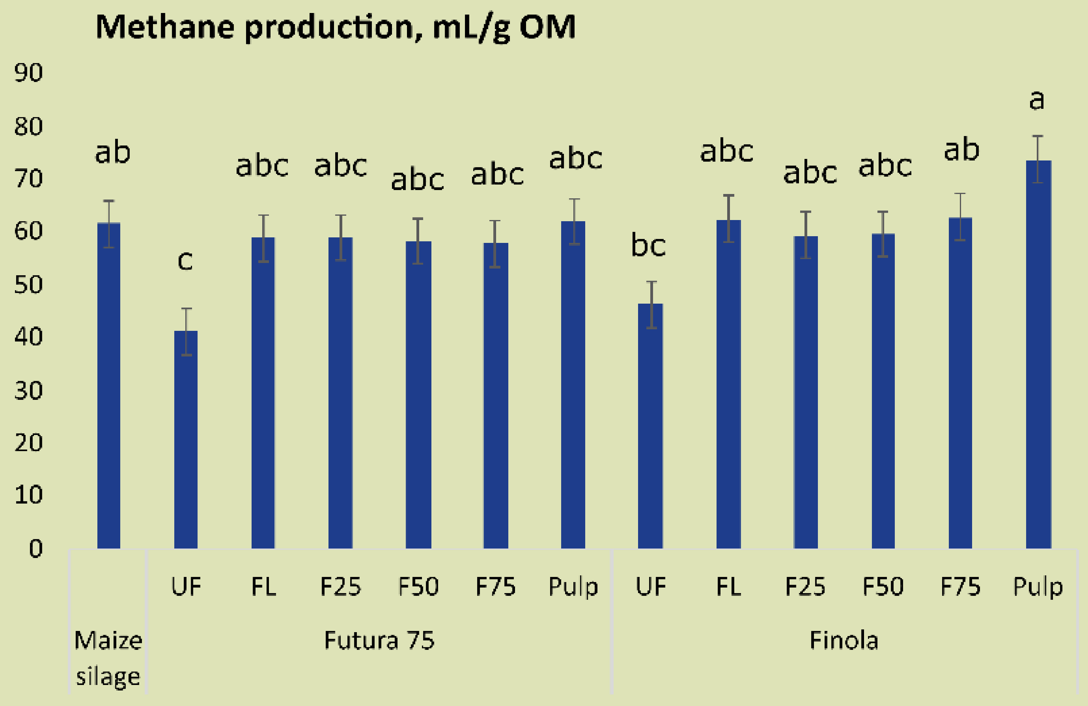
Hemp contains many bioactive compounds, which could potentially have anti-methanogenic effects. Two hemp (*Cannabis sativa L.*) varieties, Futura 75 and Finola were evaluated for their methane mitigating potential by extracting bioactive compunds with methanol and fractionation using Solid Phase Extraction (SPE) to screen for methane mitigating potential in an *in vitro* rumen fermentation system.



RT	M-H- (m/z) (Δppm)	Tentatively identified
5.35	609.1458 (0)	Kaempferol-7-sophoroside
5.46	593.1506 (1)	Kaempferol-3-O-neohesperidoside
5.55	447.0930 (1)	Orientin
5.55	895.1917	Orientin + sugar moiety
5.66	593.1506 (1)	Kaempferol diglycoside
5.66	875.2248	Kaempferol diglycoside + sugar moiety
5.77	577.1559 (1)	Daidzein diglycoside
6.01	431.0989 (1)	Vitexin
6.01	863.2014	Vitexin + sugar moiety
6.30	461.0719 (2)	Kaempferol glucuronide
6.30	923.1496	Kaempferol glucuronide + sugar moiety
7.05	445.0774 (1)	Genistein glucuronide
7.05	668.1190	Genistein glucuronide + sugar moiety
7.05	891.1607	Genistein glucuronide + sugar moiety
7.32	475.0876 (2)	Diosmetin glucuronide
7.32	951.1803	Diosmetin glucuronide + sugar moiety







METHOD

Cannabis sativa L. Futura 75 and Finola were harvested in Denmark, June 2018 and August 2019, resp. Upper third part of stem containing stalk and leaves were harvested, dried (seeds were threshed), and milled through 1-mm screen.

Metabolites were extracted from hemp samples with 75% MeOH, supernatant collected and pulp dried. Fractionation was performed on C18 SPE columns with solvents of ACN with different polarities giving four fractions; FL, F25, F50, F75. Fractions were concentrated by evaporation and reconstituted in water before they were added in the *in vitro* fermentation system.

The metabolic profiling of extracts and fractions and identification of bioactive metabolites were performed with Liquid Chromatography-Mass spectrometry (LC-MS).

Rumen fluid were collected from rumen cannulated maintenance Holstein cows and mixed with anaerobic buffer solution in the ratio 1:2. Maize silage (0.5g) served as a standard feed where 1 mL of fraction or 0.1g of pulp was added to bottles with 90 mL rumen inoculum. Total gas was automaticly measured with ANKOM pressure sensor. Methane, degradability, volatile fatty acid composition, pH were measured after 48h of fermentation.

CONCLUSIONS

- Extract of Futura 75 reduced methane without negative effect on degradability or content of volatile fatty acids.
- Same mitigating effect not possible to obtain from fractions of Futura 75 or pulp.
- Finola only numerically tendencies towards reduced methane production.
- Metabolic profiles differed in the content of flavonoid glycosides, whereby the difference in mitigating potential between Finola and Futura 75 could be related to differences in the content of these bioactive flavonoids.

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Nordic Metabolomics Conference 2022