



Università
degli Studi
della Campania
Luigi Vanvitelli

*Dipartimento di Scienze e Tecnologie
Ambientali Biologiche e
Farmaceutiche*



Valorisation of forest by-products: the chestnut case as renewable source of bioactives for sustainable livestock diets

Marialuisa Formato^{1*}, Simona Piccolella¹, Alessandro Vastolo², Serena Calabrò², Monica Isabella Cutrignelli², Christian Zidorn³, Severina Pacifico¹

¹Department of Environmental Biological and Pharmaceutical Sciences and Technologies, University of Campania “Luigi Vanvitelli”, via Vivaldi 43, Caserta, Italy.

²Department of Veterinary Medicine and Animal Production, University of Naples Federico II, Via F. Delpino 1 – 80137, Napoli.

³Pharmazeutisches Institut, Abteilung Pharmazeutische Biologie, Christian-Albrechts-Universität zu Kiel, Gutenbergstraße 76, 24118 Kiel, Germany

*email: marialuisa.formato@unicampania.it



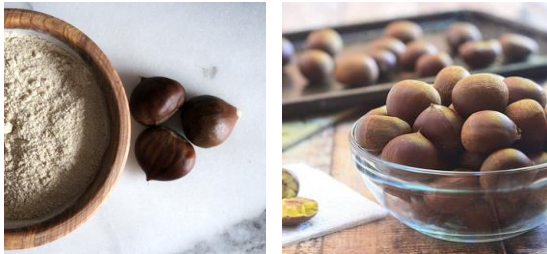
VIII CONVEGNO NAZIONALE
DEL CASTAGNO

14, 15 e 16 settembre 2022 - PORTICI - NAPOLI

Castanea sativa Mill.



Distribution

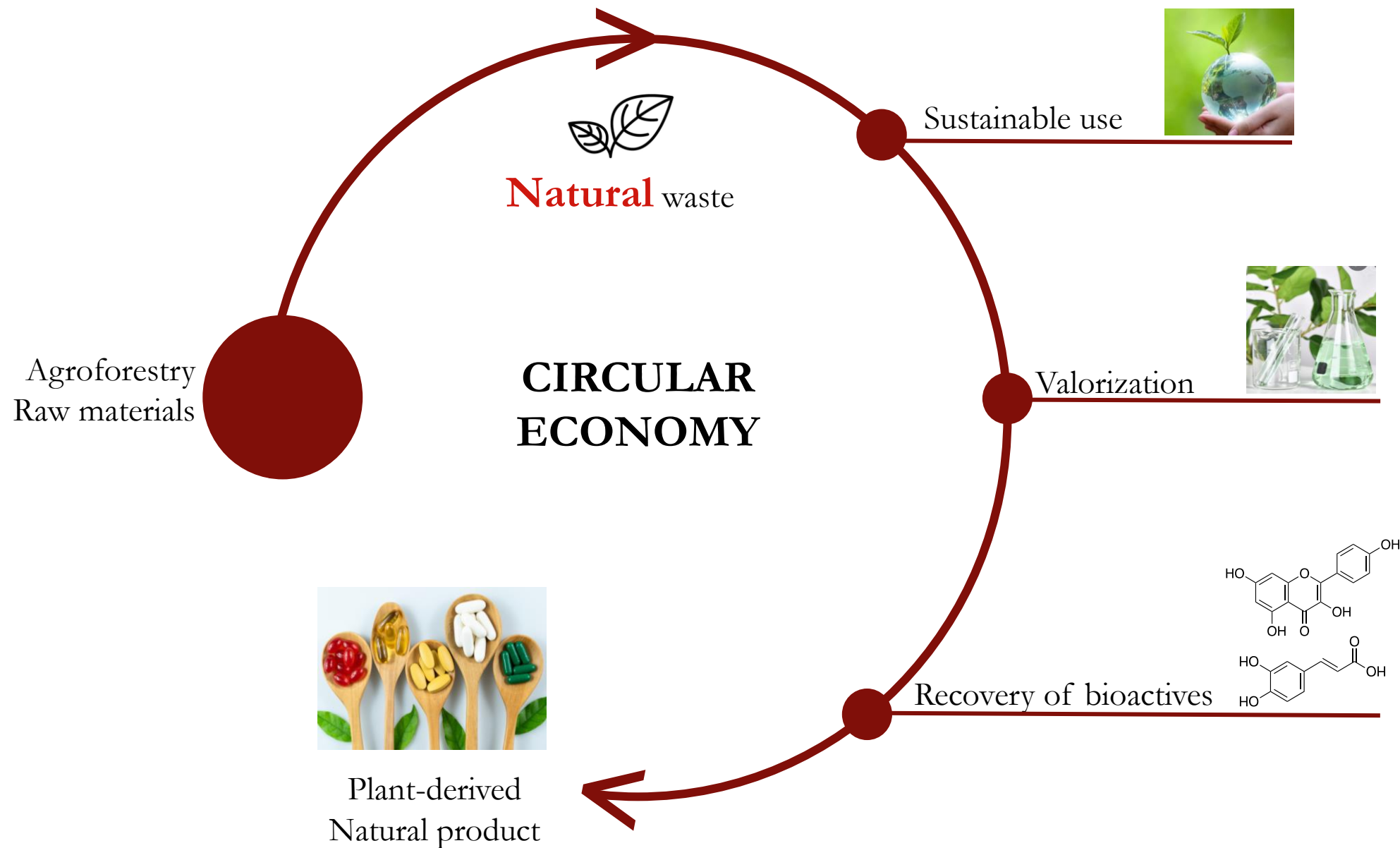


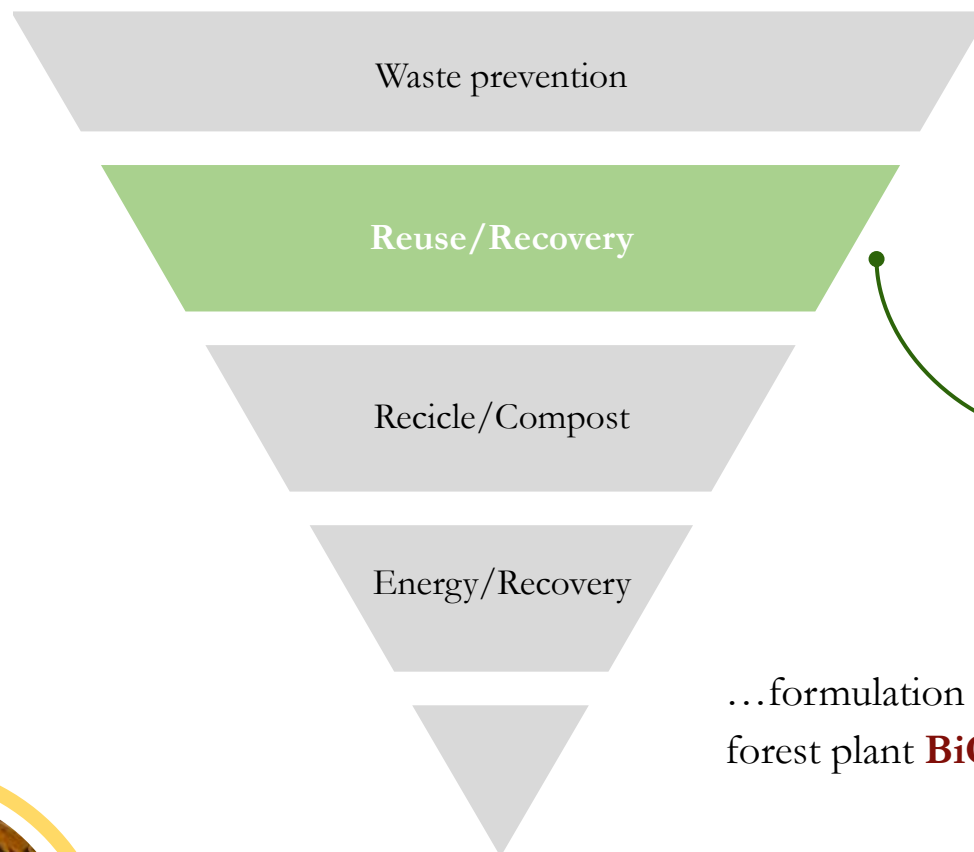
chestnut fruits can be used as good source of energy due to its **starch**, **carbohydrates** and **fatty acids**

Chestnuts are finding a new application in nutrition; an example is a significant importance of chestnut fruits in **coeliac disease** as regard the production of marron glacé, purée and **chestnut flour**, which find application as an ingredient in gluten-free diets



The **waste** of *Castanea sativa* Mill., namely **leaf**, **shell**, and **bur**, could contain bioactive with marked biological activity





molecules



Article

UHPLC-ESI-QqTOF Analysis and In Vitro Rumen Fermentation for Exploiting *Fagus sylvatica* Leaf in Ruminant Diet

Marialuisa Formato ¹, Simona Piccolella ¹, Christian Zidorn ², Alessandro Vastolo ³, Serena Calabrò ³, Monica Isabella Cutrignelli ³ and Severina Pacifico ^{1,*}

...not waste but **RESOURCE**

...formulation of **animal feed** and/or supplements using forest plant **BiOaCtIvE** compounds

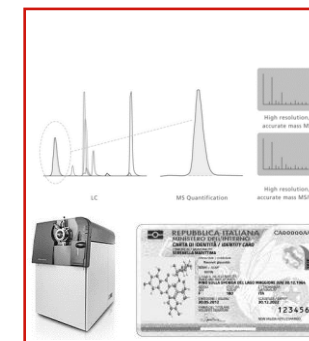
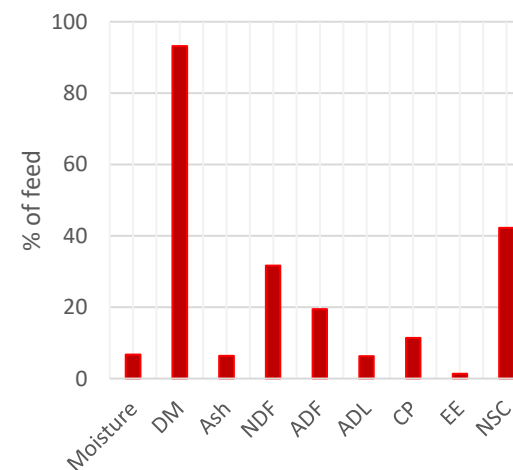
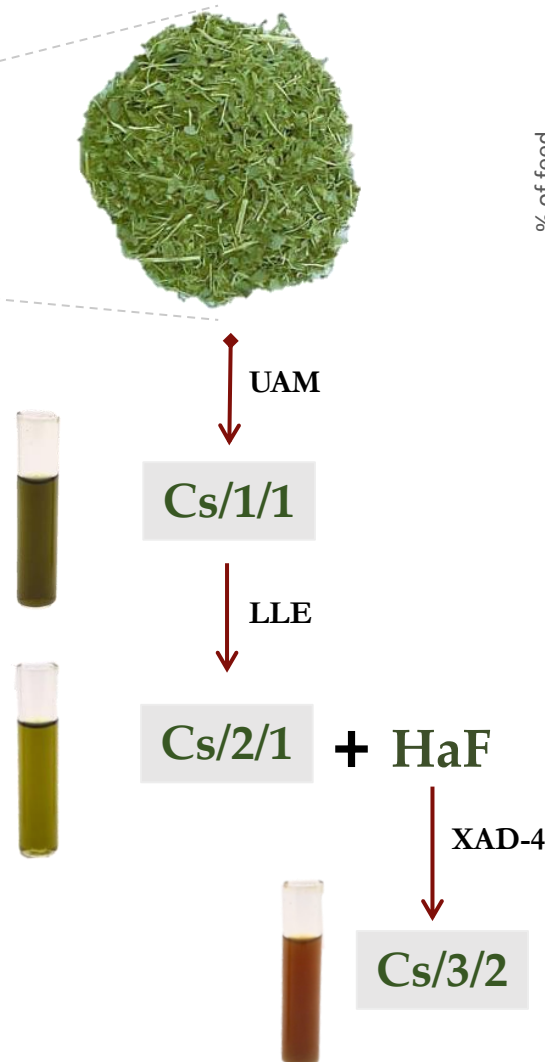
- ↓ greenhouses gasses (i.e, feed production, digestion process)
- ↑ animal health condition (i.e., SARA, mastitis)
- ↑ quality of products (i.e., milk, meat)

Plant sampling, extraction and analysis

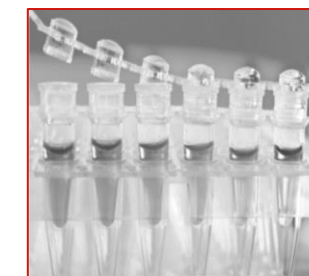


Castanea sativa Mill.

UAM: Ultrasound assisted maceration
LLE: Liquid-liquid extraction
XAD-4: Amberlite absorbent resin
HaF: Hydroalcoholic fraction



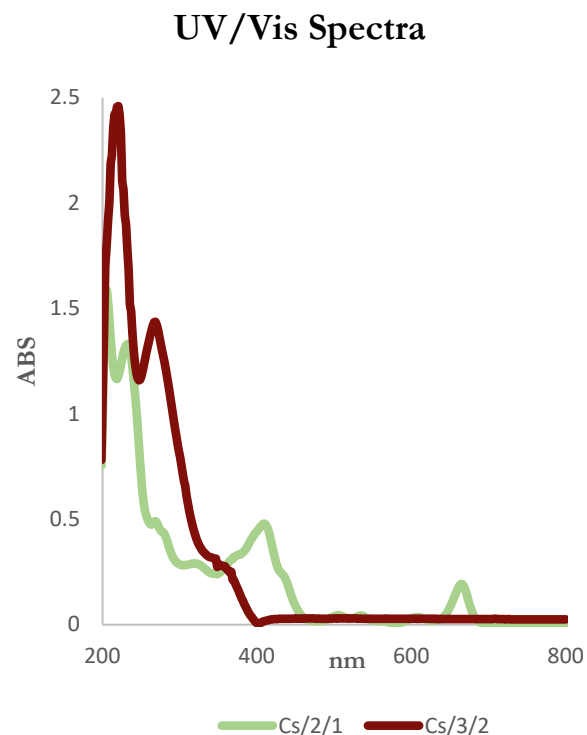
UHPLC-HRMS/MS



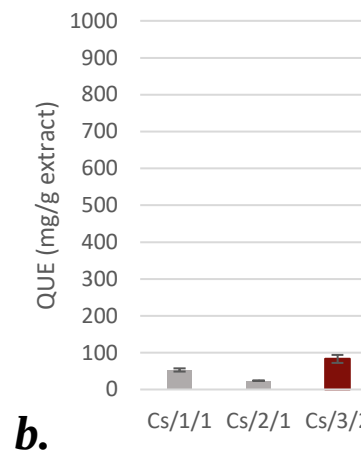
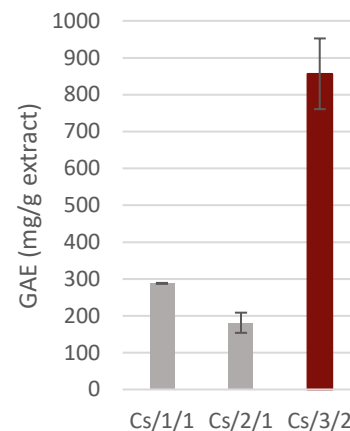
Colorimetric assays



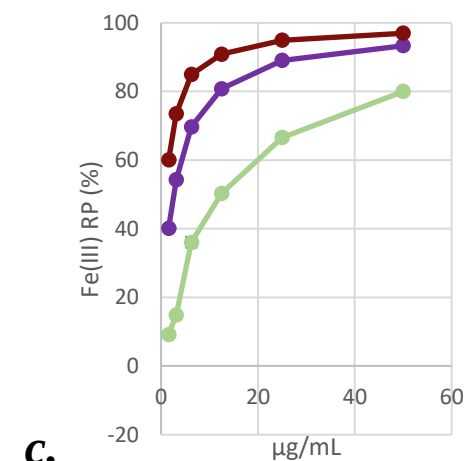
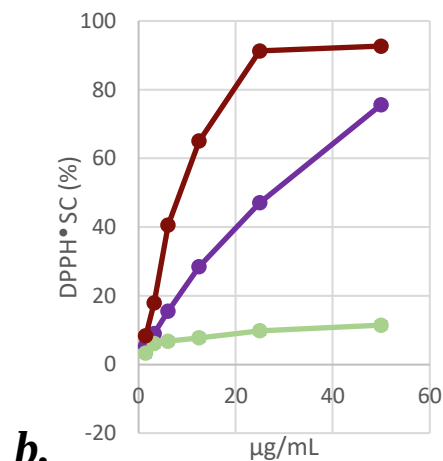
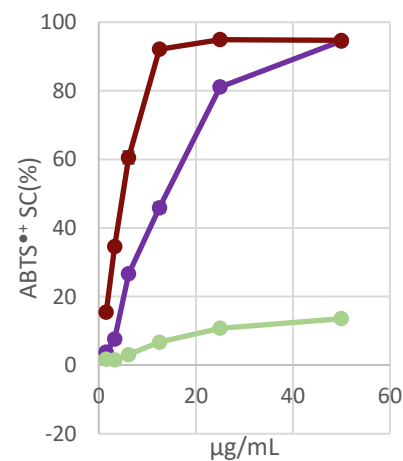
In vitro fermentation parameters



A)



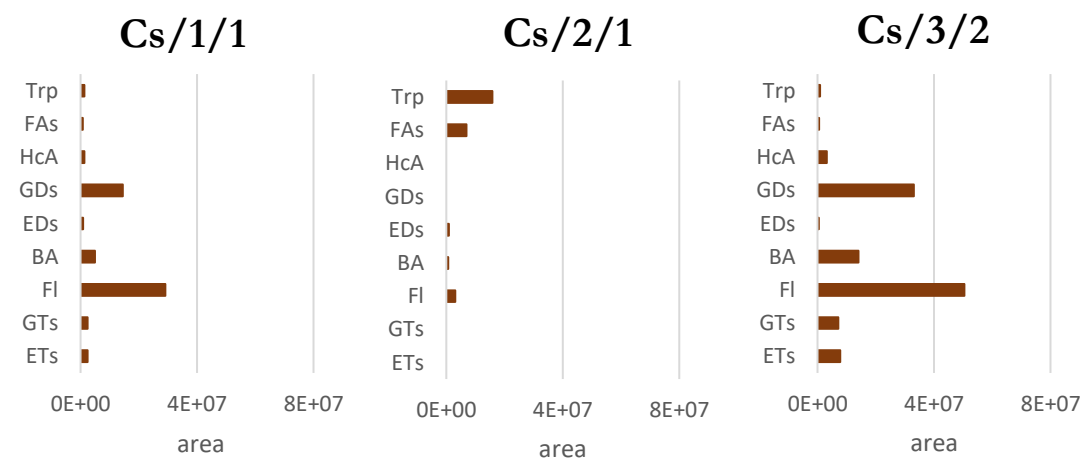
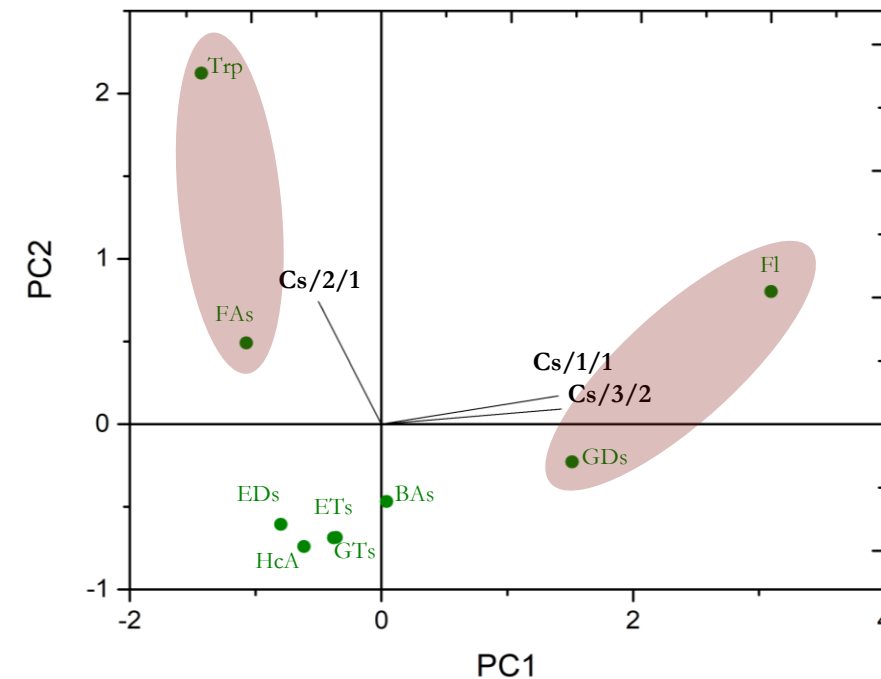
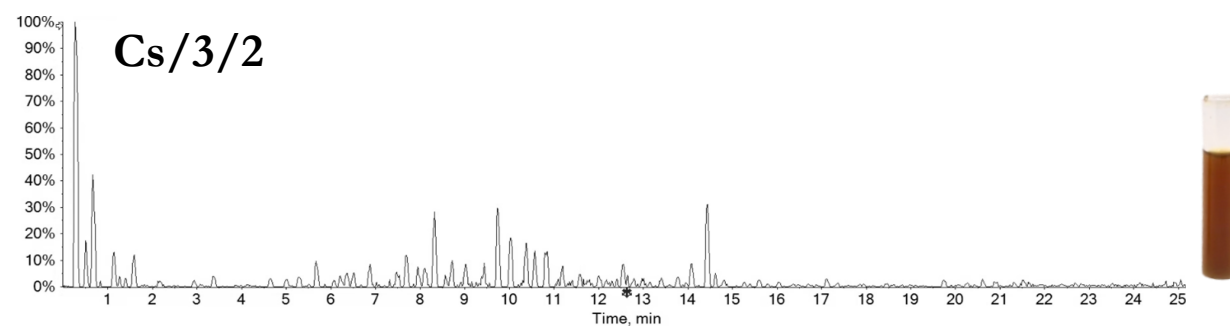
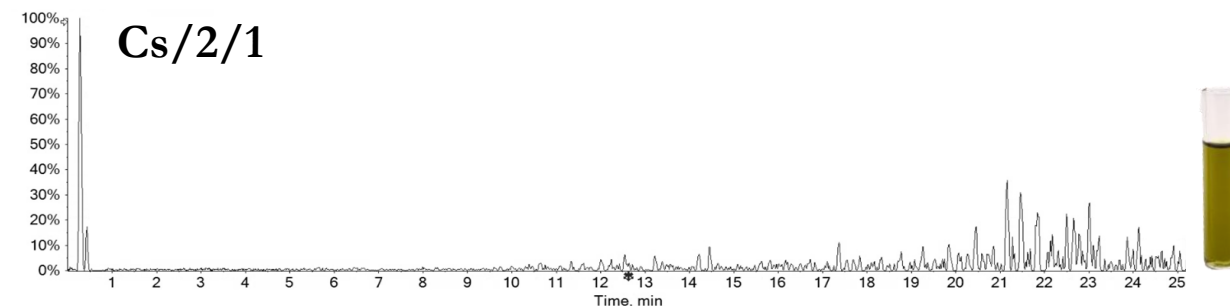
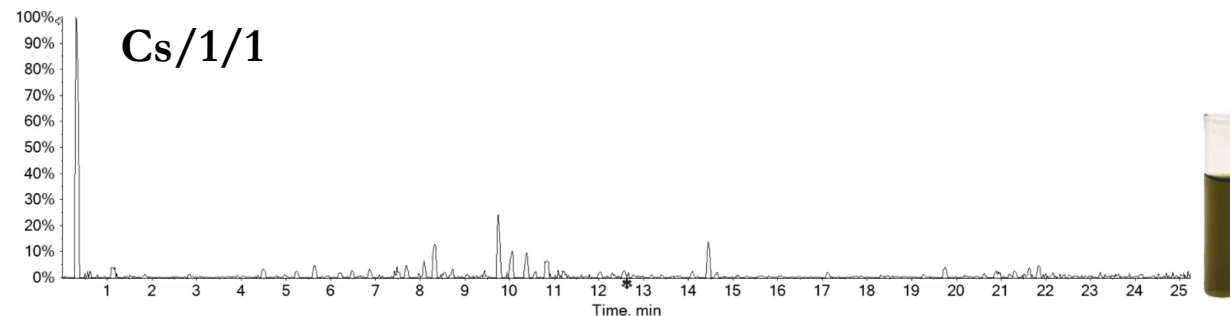
B)



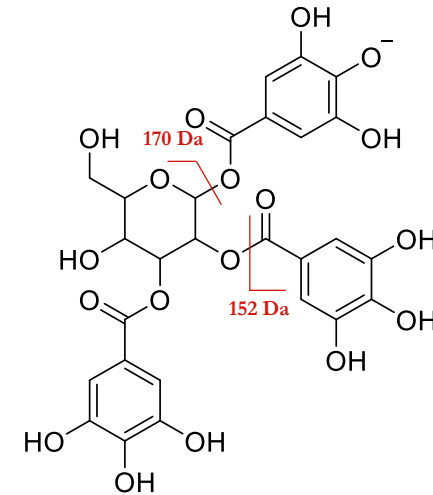
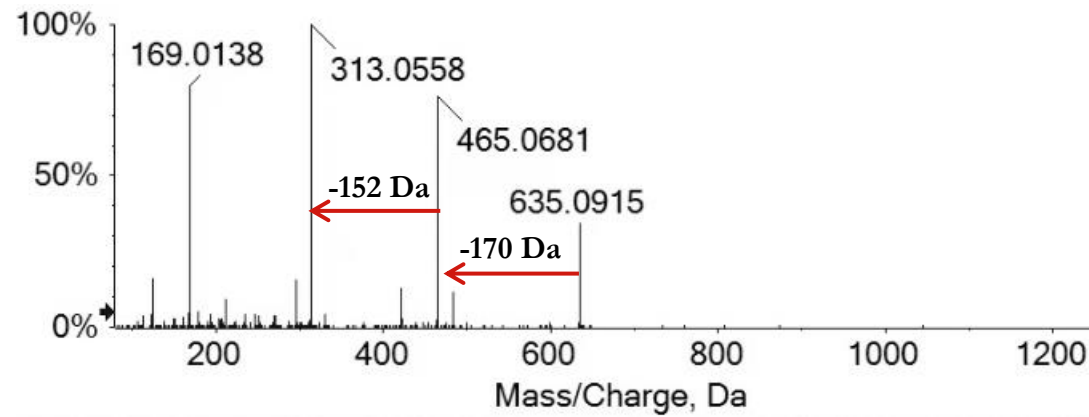
● Cs/1/1 ● Cs/2/1 ● Cs/3/2

A) a. Total Phenol Content, expressed as mg of gallic acid equivalent (GAE) per g of extract; **b.** Total Flavonoid Content, expressed as mg of quercetin equivalent (QUE) per g of extract; **B) a.** Scavenging activity (SC%) of *C. sativa* extract and organic fraction therefrom vs. 2,2'-azino-bis(3-ethylbenzothiazoline)-6-sulfonic acid (ABTS) radical cation; **b.** SC% vs. 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical; **c.** Fe (III) reducing power (RP).

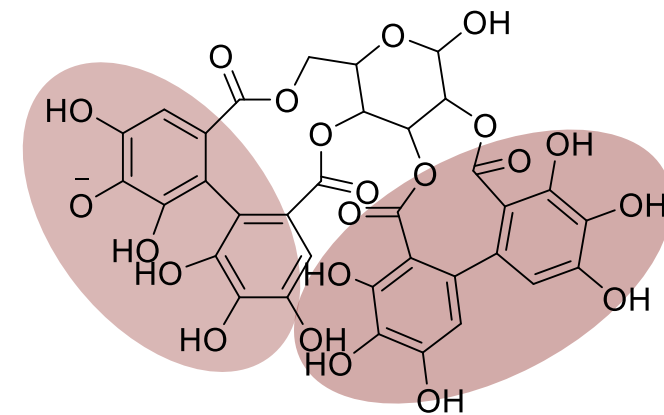
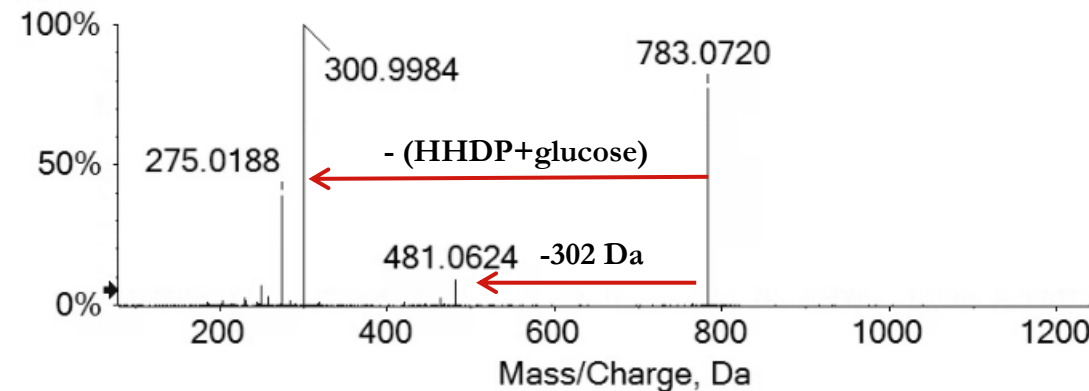
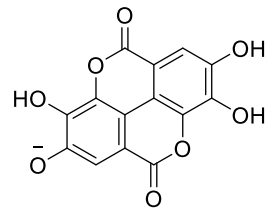
Chemical investigation



Ellagitannins and gallotannins in *C. sativa* leaves

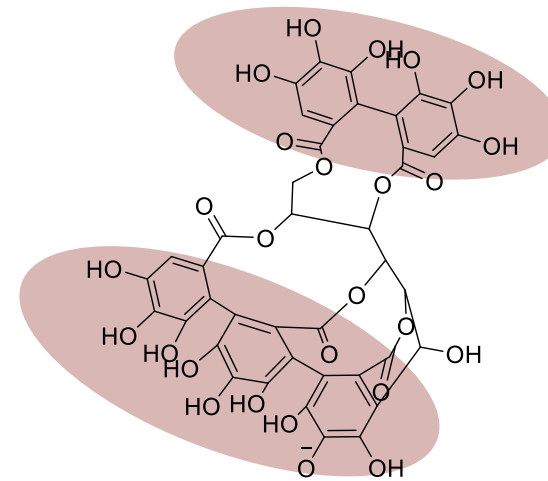
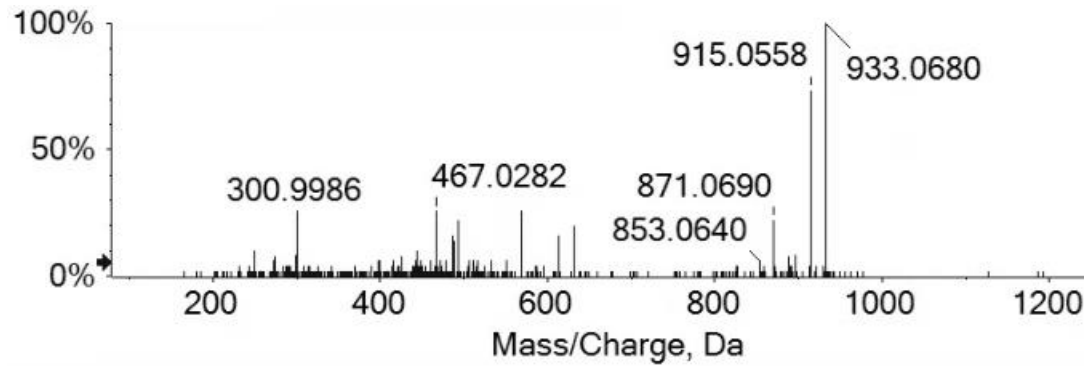


Tri-galloyl hexose

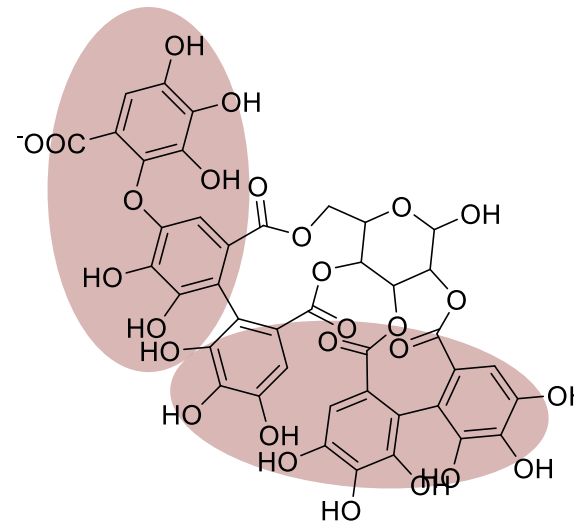
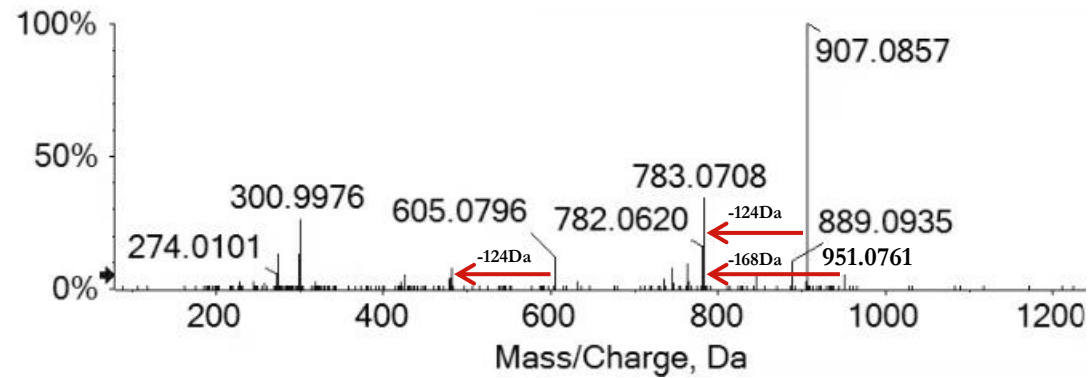


Bis-HHDP-glucose

Ellagitannins and gallotannins in *C. sativa* leaves

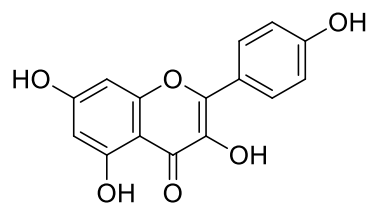


NHTP-HHDP-glucose

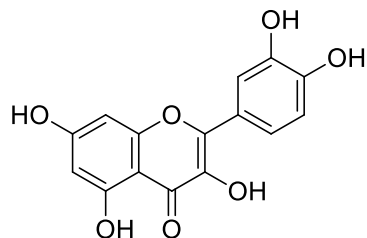


HHDP-valoneoyl-glucose

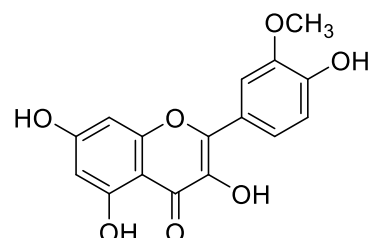
Flavonols in *C. sativa* leaves



Kaempferol

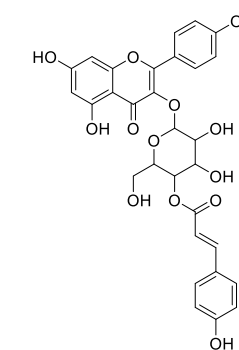
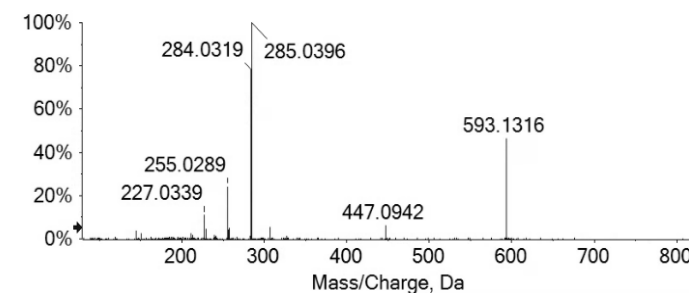
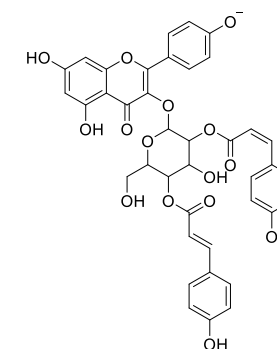
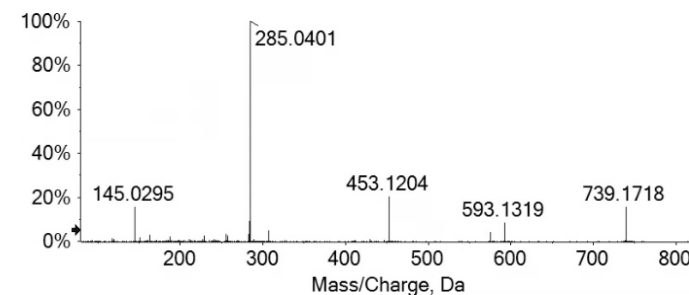
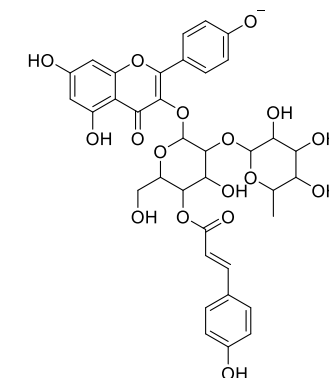
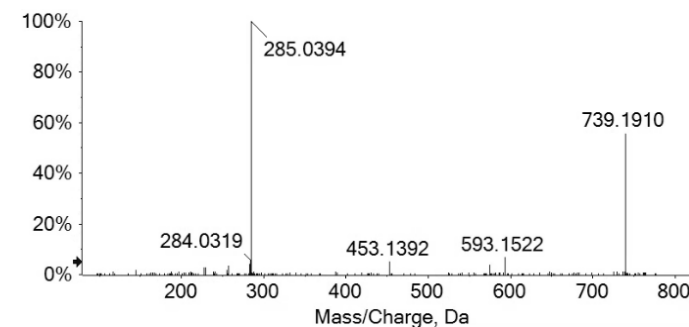


Quercetin

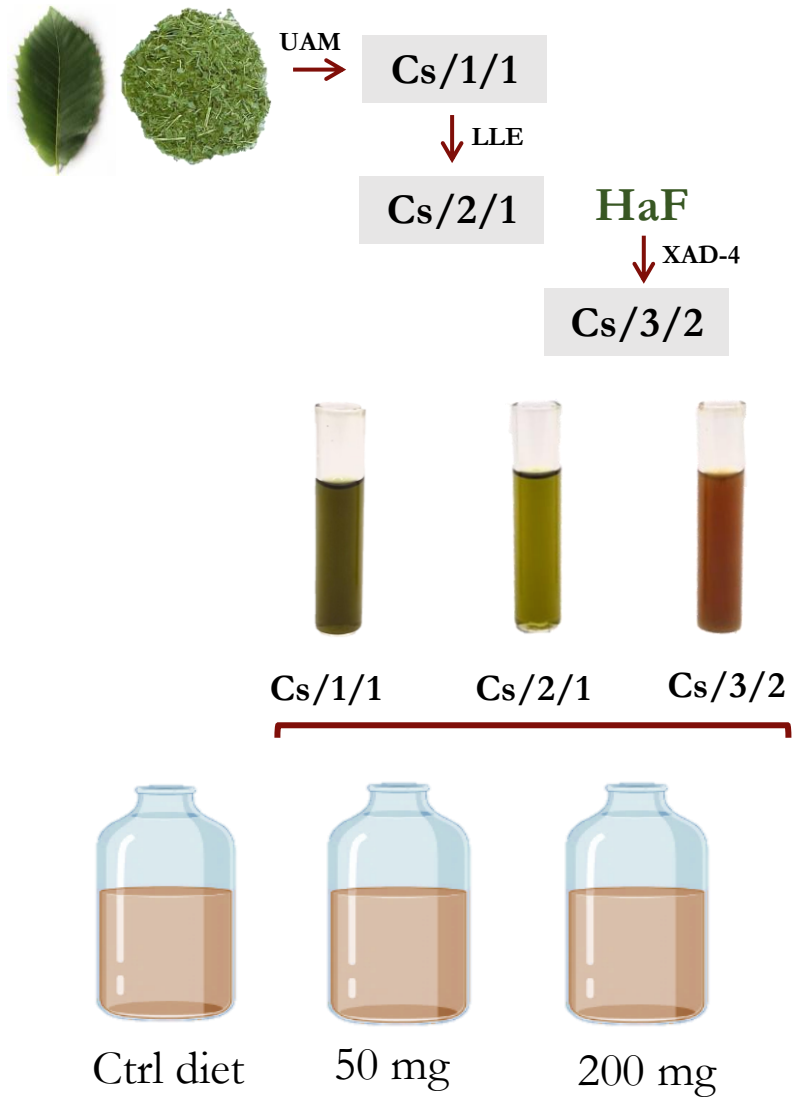


Isorhamnetin

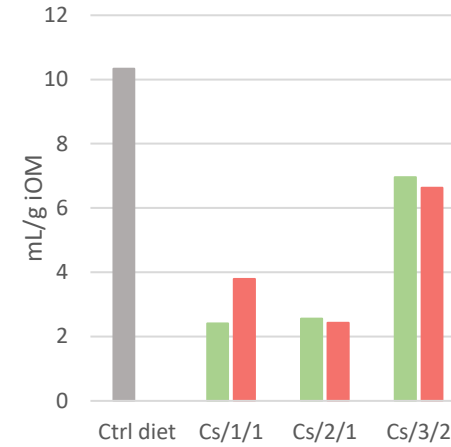
	Moiety	Neutral loss
<u>Monoglycosylated flavonols</u>	pentose	132.04 Da (C ₅ H ₈ O ₄)
	hexose	162.05 Da (C ₆ H ₁₀ O ₅)
	hexuronic acid	176.03 Da (C ₆ H ₈ O ₆)
	deoxyhexose	146.05 Da (C ₆ H ₁₀ O ₄)
<u>Diglycosylated flavanols</u>	pentosyl-hexose	294.09 Da (C ₁₁ H ₁₈ O ₉)
	hexosyl-deoxyhexose	308.11 Da (C ₁₂ H ₂₀ O ₉)
<u>Acylglycosylated flavonols</u>	acyl <i>p</i> -coumaroyl-hexose	350.10 Da (C ₁₇ H ₁₈ O ₈)
	acyl-hexose	204.06 Da (C ₈ H ₁₂ O ₆)
	<i>p</i> -coumaroyl hexose	308.08 Da (C ₁₅ H ₁₆ O ₇)
	<i>p</i> -coumaroyl deoxyhexosyl-hexose	454.14 Da (C ₂₁ H ₂₆ O ₁₁)
	di- <i>p</i> -coumaroyl hexose	454.12 Da (C ₂₄ H ₂₂ O ₉)



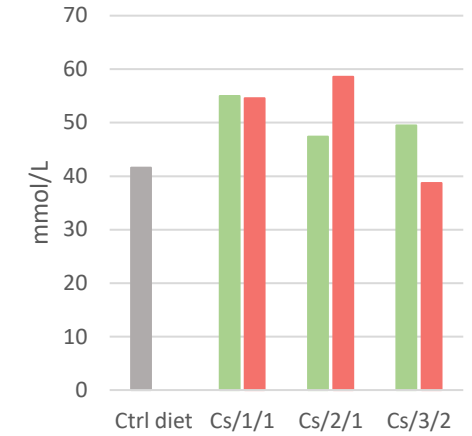
In vitro fermentation analysis



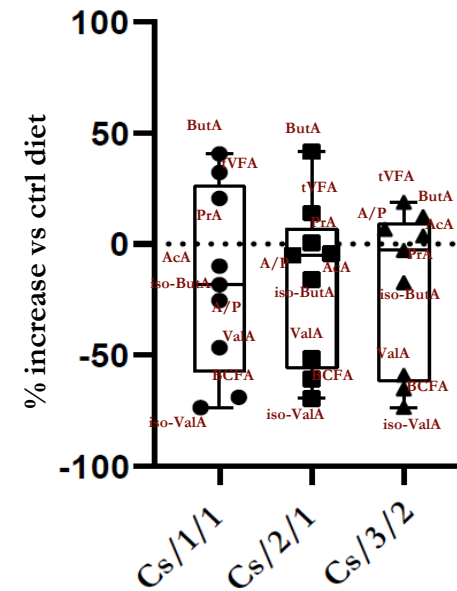
dCH₄ after 24h



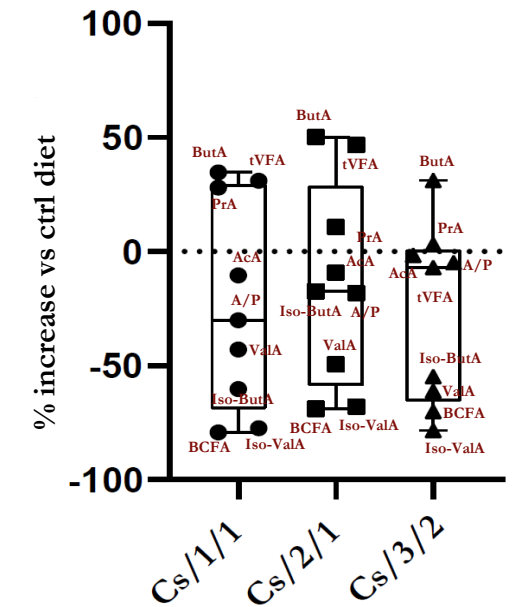
Total VFA after 24h



50 mg



200 mg



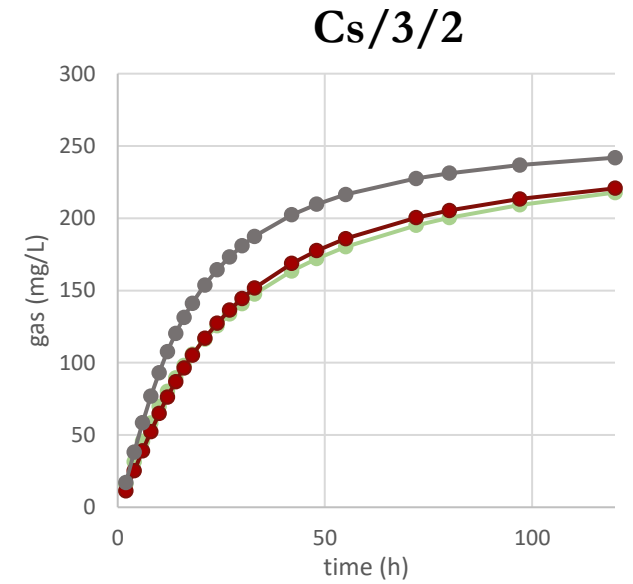
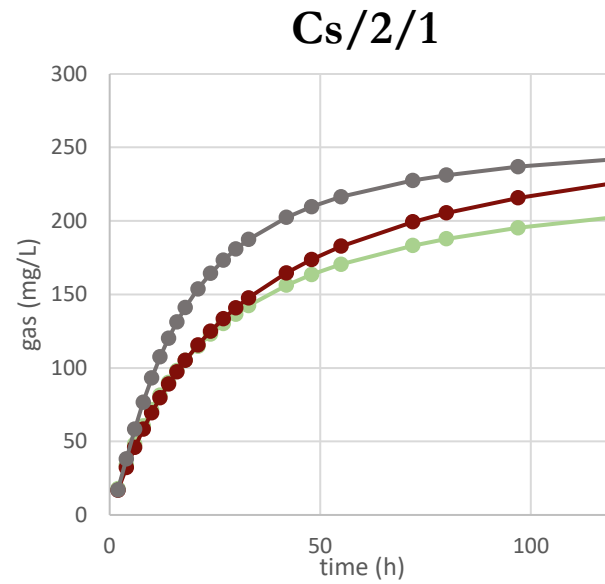
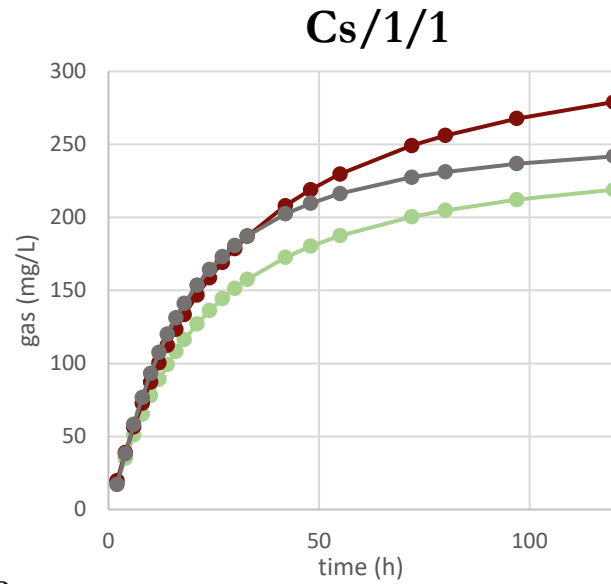
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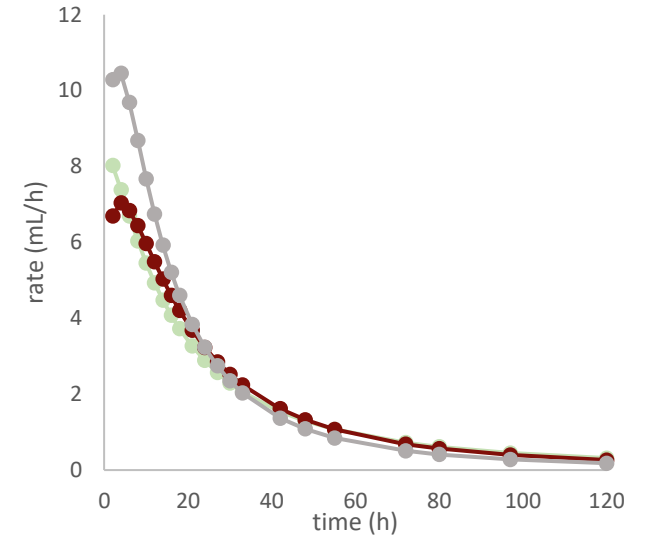
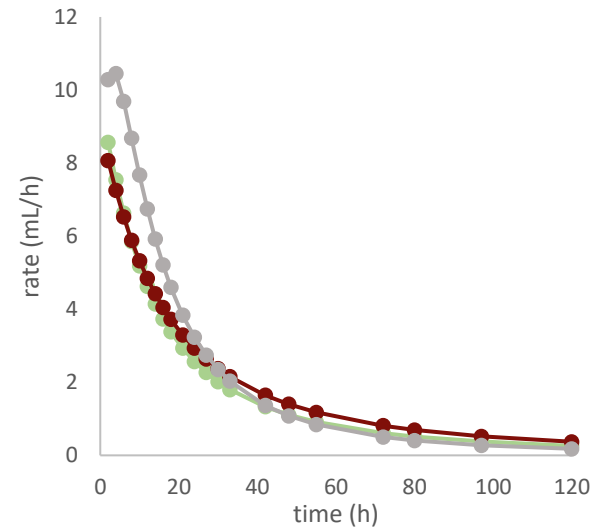
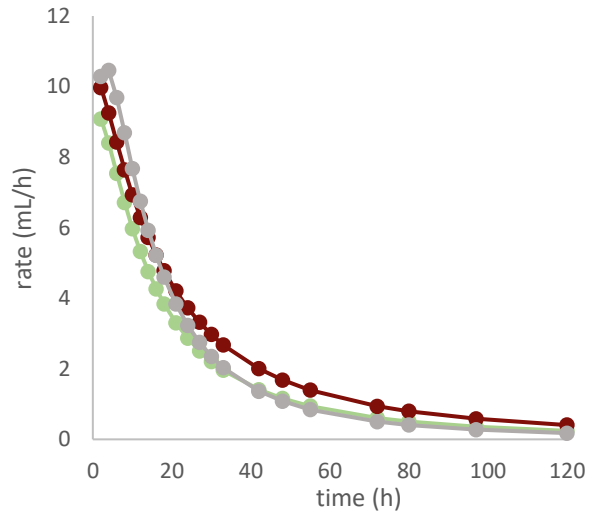
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In vitro gas production

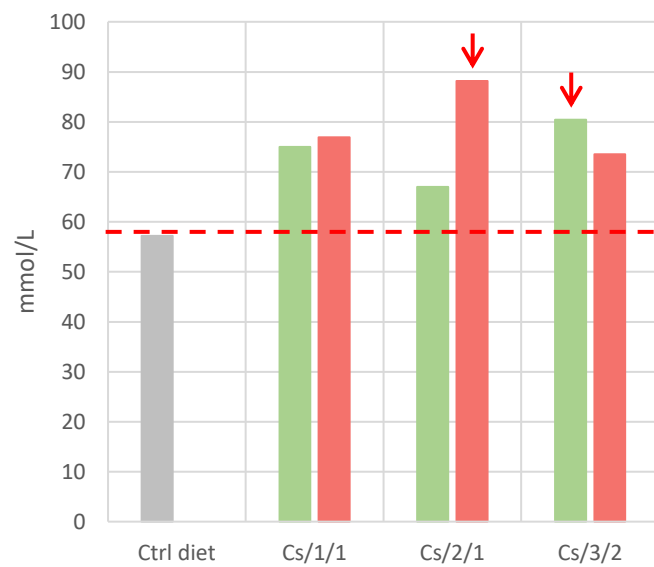


In vitro fermentation rate

● Ctrl diet
 ● 50 mg
 ● 200 mg



Total VFA after 120h

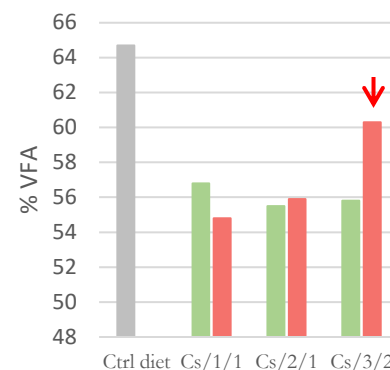


50 mg

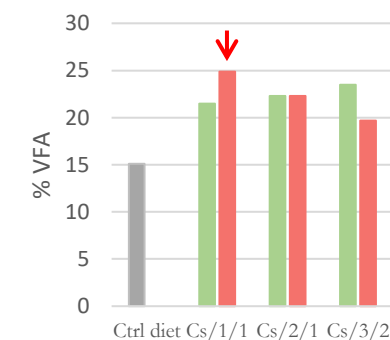
	Ctrl diet	Cs/1/1	Cs/2/1	Cs/3/2
pH	6.20	6.46	6.49	6.34
BCFA (%VFA)	7.42	3.95	3.08	2.56
A/P	4.27	2.64	2.50	2.38

200 mg

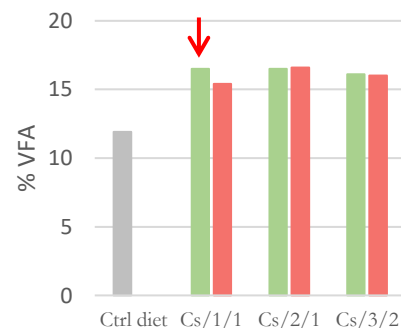
	Ctrl diet	Cs/1/1	Cs/2/1	Cs/3/2
pH	6.20	6.43	6.35	6.31
BCFA (%VFA)	7.42	3.80	3.28	3.09
A/P	4.27	2.20	2.50	3.06



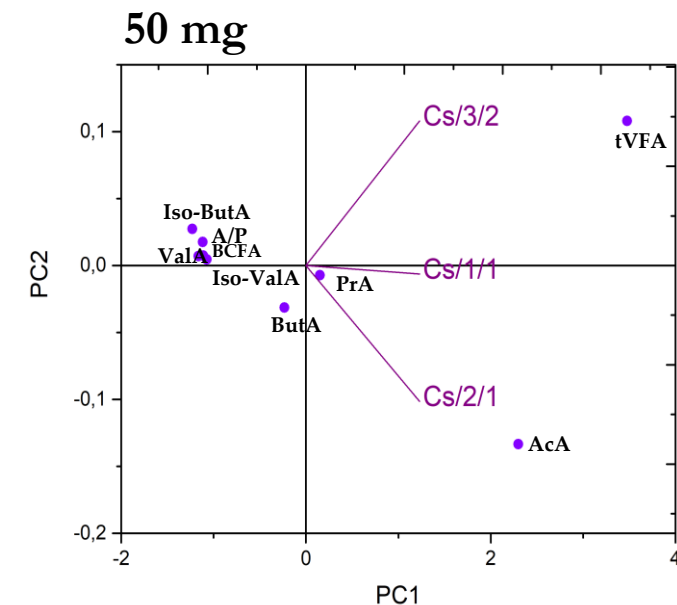
Acetic acid



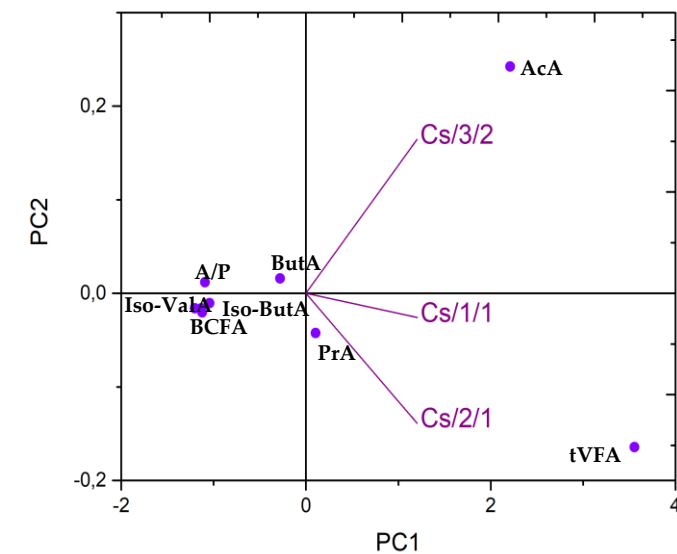
Propionic acid



Butyric acid



200 mg



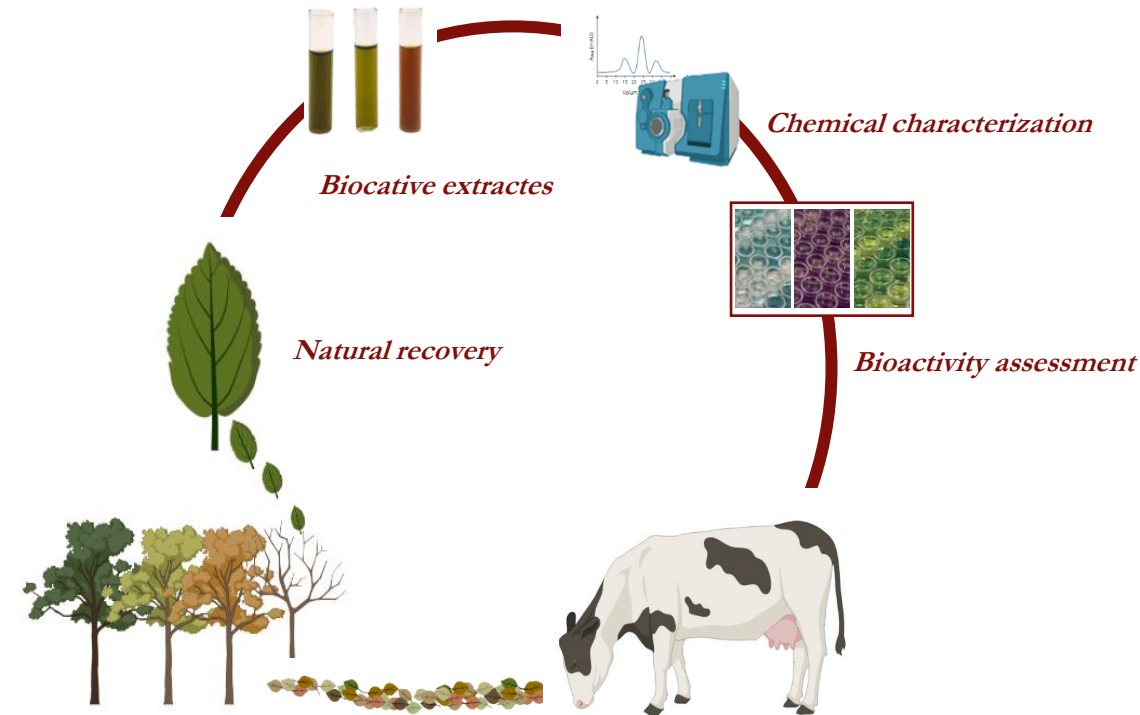
Conclusions

Chestnut leaves appear as a promising material affecting ruminal fermentation parameters and methanogenesis probably with effects on **ruminal microbiome**.

All tested fraction reduce the **CH₄ emission** after 24 h of incubation, mainly **Cs/2/1**.

The addition of **50 mg** of the **Cs/3/2** and **200 mg** of the **Cs/2/1** mainly increased the production of VFAs.

Acetic acid is decreased by all tested fractions whereas **propionic** and **butyric acid** are increased by **Cs/1/1** at 200 and 50 mg, respectively.



FOOD CHEM LAB *research group*



**THANKS FOR
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