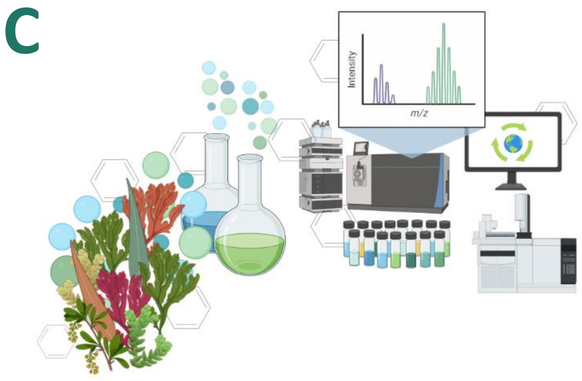
GUT MICROBIOTA MODULATION INDUCED BY PURE PHENOLIC COMPOUNDS: AN *IN VITRO* FECAL FERMENTATION STUDY



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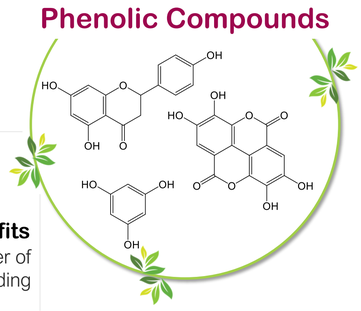
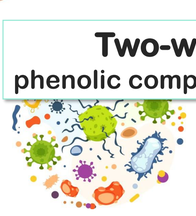
BACKGROUND AND AIM



Bioactive compounds

Health benefits Influenced by a number of factors, including

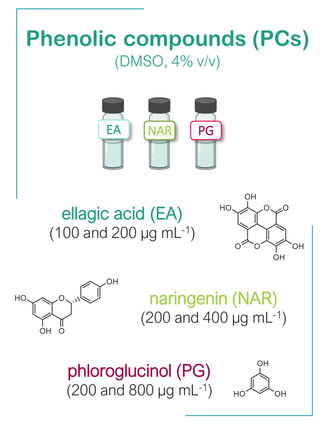
Two-way relationship:
phenolic compounds and gut microbiota

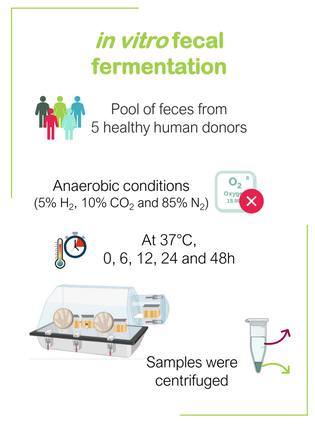


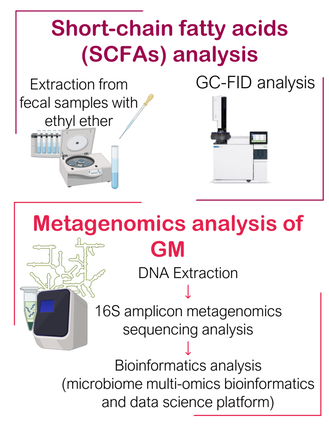
Phenolic compounds are metabolized by these microorganisms, and, simultaneously, modulate their composition.

How these dietary compounds impact individually the human gut microbiota composition?

METHODS







RESULTS

1. SCFAs profile

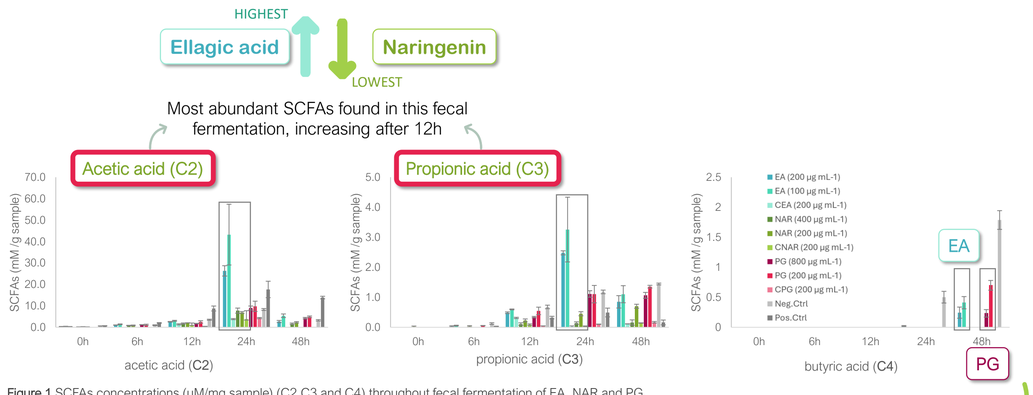


Figure 1 SCFAs concentrations (μM/mg sample) (C2,C3 and C4) throughout fecal fermentation of EA, NAR and PG.

Lower amounts of iso-butyric (iC4), iso-valeric acid (iC5) and caproic (C6) acids were found mainly at 48h PG samples

EA and PG could have potential beneficial effects for host gut health

Acetic acid (C2)
Energy homeostasis
Inflammatory status
Anxiety

Propionic acid (C3)
Appetite regulation
Colon cancer prevention
Primary producers Bacteroidetes

Butyric acid (C4)
Immunomodulatory role
Regulating intestinal homeostasis
Primary producers Firmicutes

2. Relative abundance

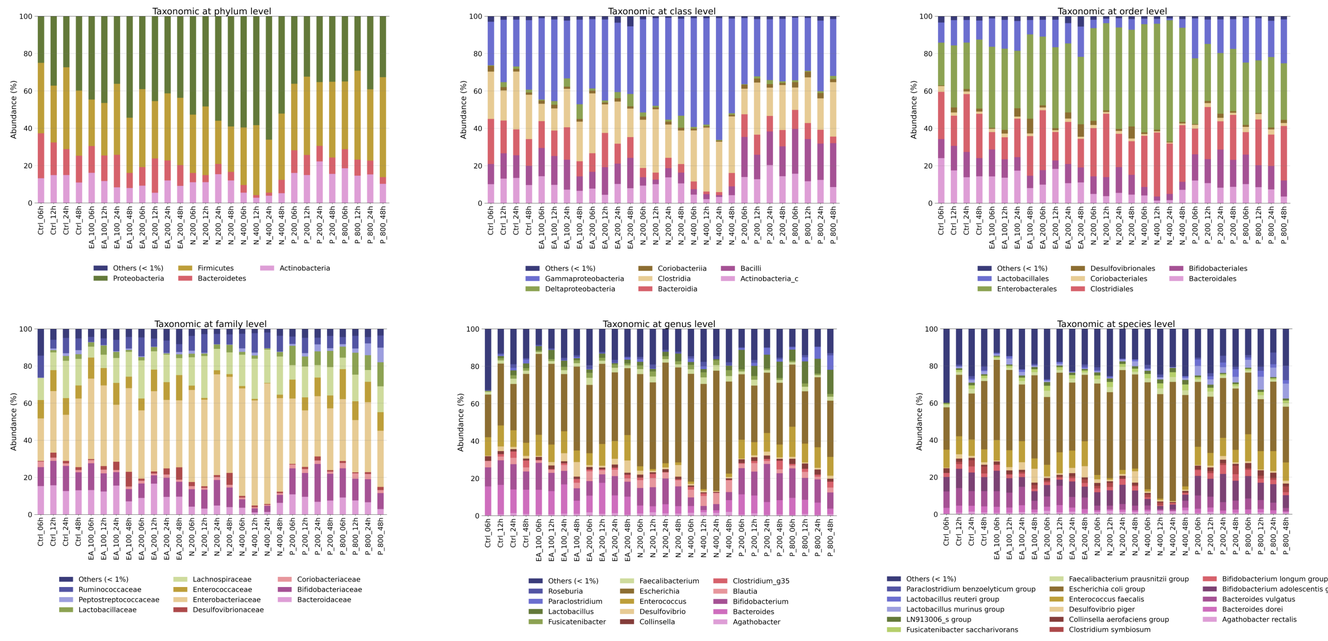


Figure 2 Microbial relative abundances at phylum, class, order, family, genus, and species levels during the 48h of in vitro batch fecal fermentation with ellagic acid (EA, at 100 and 200 μg mL^{-1}), naringenin (N, at 200 and 400 μg mL^{-1}) and PG (P, at 200 and 800 μg mL^{-1}).

EA and PG
Potential prebiotic effect, since during fecal fermentation, they seemed to positively influence beneficial microorganisms for human health.

NAR
The complex interactions within microbial communities can significantly influence both pathogenicity and sulfur metabolism. NAR has increased in the bacterial orders Enterobacterales and Desulfovibrionales, both of which are associated with intestinal inflammation and various enterohepatic conditions.

3. α-diversity

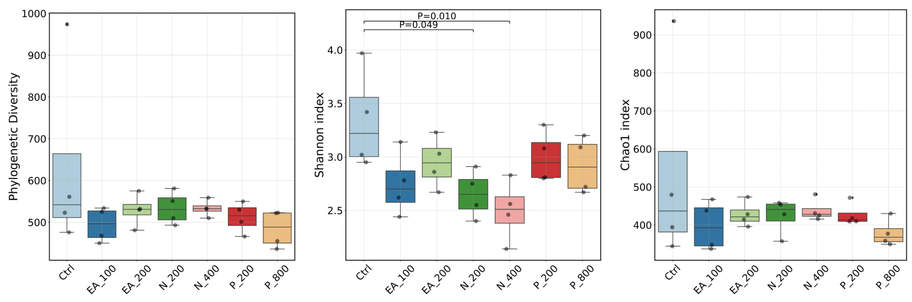


Figure 3 α-diversity indices – phylogenetic diversity, Shannon index, and Chao1 index, of the samples of different PCs – ellagic acid (EA, at 100 and 200 μg mL^{-1}), naringenin (N, at 200 and 400 μg mL^{-1}) and phloroglucinol (P, at 200 and 800 μg mL^{-1}). *p*-values indicate the statistical significance in comparison with the control sample.

NAR may selectively **reduce microbial community diversity.** However, these PCs did not seem to disturb the microbiota ecosystem's equilibrium significantly, which is a **crucial influence on the host's health.**

4. β-diversity

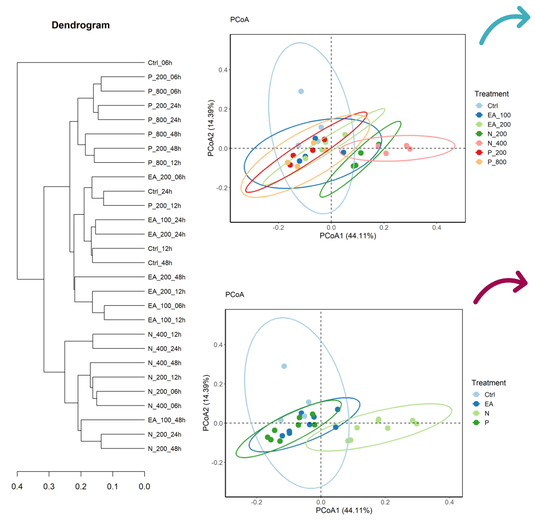


Figure 4 β-diversity analysis - dendrogram resultant from unweighted pair-group method with an arithmetic mean (UPGMA) clustering based on the generalized UniFrac distance, and principal coordinates analysis (PCoA) of the samples of different PCs – ellagic acid (EA, at 100 and 200 μg mL^{-1}), naringenin (N, at 200 and 400 μg mL^{-1}) and phloroglucinol (P, at 200 and 800 μg mL^{-1}) along 48h of fermentation.

EA and PG
no significant difference between concentrations

NAR
concentrations formed significantly distinct clusters

Microbial composition of all samples treated with PCs significantly differ from the control, particularly NAR, and among themselves.

Complementing UPGMA and PCoA, ADONIS (PERMANOVA) evidenced that the influence of PCs on microbial diversity and structure was significant different between all PCs and the control and among the PCs themselves.

PCs showed significant and distinct impact on the β-diversity of the microbiota, influencing the microbial community structure in unique ways

EA
Cluster more closely with the control than the other PCs

NAR
Distinct cluster from that of control, diverging more at 400 μg mL^{-1} and later time points

PG
Distinct cluster away from the control, especially at the earlier time points

Different PCs and concentrations can distinctly influence the gut microbiome

Table 1 Pairwise comparison using analysis of similarities (ANOSIM).

Comparison	*R*	Significance
EA (100 μg mL^{-1}) - EA (200 μg mL^{-1})	-0.1667	0.882
N (200 μg mL^{-1}) - N (400 μg mL^{-1})	0.5729	0.036
P (200 μg mL^{-1}) - P (800 μg mL^{-1})	-0.05208	0.555

EA and PG different concentrations
can be grouped for further analysis as they are not significantly different

NAR different concentrations
should be considered separately due to significant differences

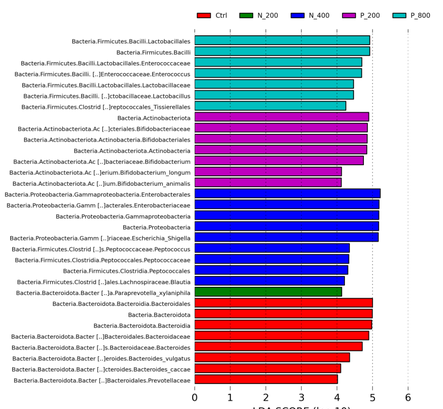
5. Community differences analysis

EA was not included in the graphical representation of LEfSe analysis since it did not yield specific biomarkers at the stringent threshold set in the LEfse analysis. This could be due to:

Lower pronounced or distinctive impact of EA on the microbiota (comparing to those of other PCs)

Uniform distribution of EA effects across the microbial community, affecting many taxa to a lesser degree that falls below the threshold for detection

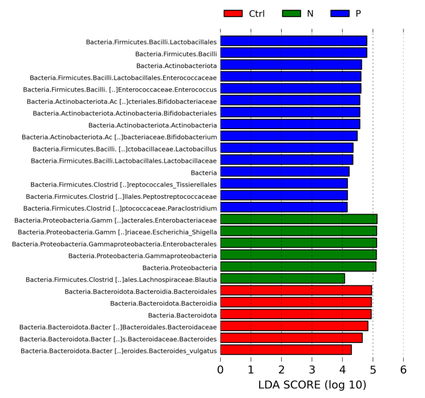
EA neutral impact on the microbial taxa examined.



NAR
200 μg mL^{-1} Increase *Paraprevotella* strain
400 μg mL^{-1} Increase Escherichia/ Shigella genera
Dose-dependent effect

PG
200 μg mL^{-1} Increase Lactobacillaceae
800 μg mL^{-1} Increase Bifidobacteriaceae
Positive effect, enhancing the gut microbiome's resilience and function

Figure 5 LefSe histogram and cladogram of the GM composition after fermentation with naringenin (N, at concentrations 200 and 400 μg mL^{-1}) and phloroglucinol (P, at concentrations 200 and 800 μg mL^{-1}).



NAR
Increased Enterobacteriales, including commensal and pathogenic bacteria
Complex influence on gut health

PG
Increase beneficial taxa, such as Lactobacillus and Bacteroides
Fiber degradation and production of critical metabolic by-products

Figure 6 LefSe histogram and cladogram of the GM composition after fermentation with naringenin (N) and phloroglucinol (P).

FINAL REMARKS

- PCs, mainly EA and PG, led to the production of SCFAs, predominantly acetic acid, propionic acid, and butyric acid, which are well-known for their beneficial effects on human health.
- The 16S amplicon metagenomic analysis of each PC fermentation sample evidenced that both EA and PG could contribute to the growth of beneficial bacteria, namely *Lactobacillus* and *Bifidobacterium* species, that may be responsible for specific functions within the gut ecosystem, such as fermenting dietary fibers, or protecting against pathogens.
- In contrast, NAR altered the gut microbiota composition by influencing other genera, specifically Escherichia and Salmonella, which are known to have potential pathogenic effects on human health
- PCs seemed to individually influence the gut's metabolic landscape, with potential implications for host health. This insight is crucial for developing new dietary supplements or nutraceuticals, which could be designed to modulate GM composition and promote a healthy, balanced gut ecosystem.

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