Foods 2022

Metabolomic fingerprinting of phenolic compounds in blood serum from rats treated with chestnut shells extract

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State of art

- ✓ 1/3 of food production is wasted

- ✓ 88 M tonnes are generated annually
- ✓ Associated costs estimated at 143 billion euros

Principal causes

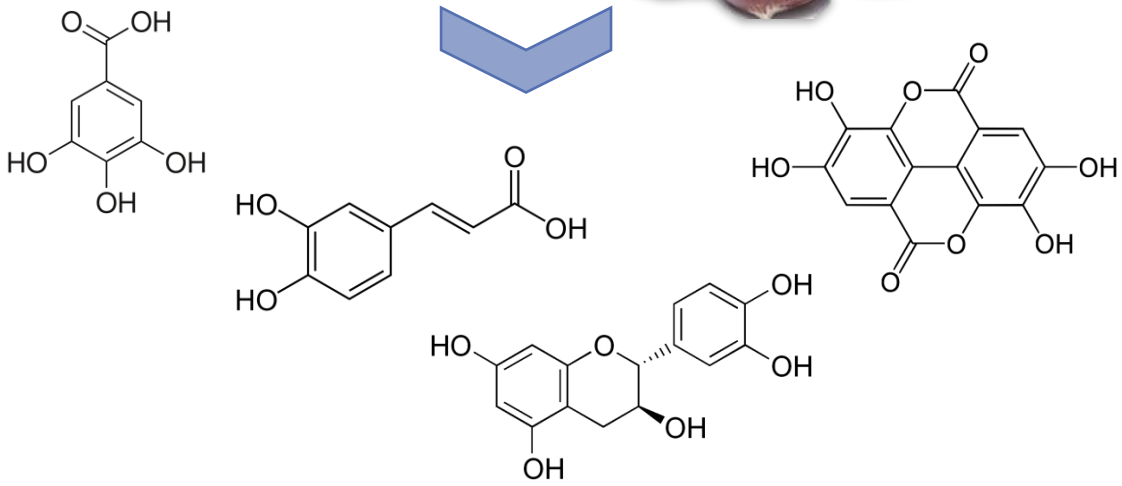
Sustainability Challenge

State of art

Macronutrients
&
Micronutrients

Vitamins
Mineral
Polyphenols

Chestnut shells



In vitro
Antioxidant
Anti-inflammatory
Hypoglycemic
Hypolipidemic

In vivo
Anti-obesity
Anti-inflammatory
Hepatoprotective

Objectives

- Investigate the targeted metabolomic profile of polyphenols in blood serum from rats orally treated with an eco-friendly chestnut shells extract

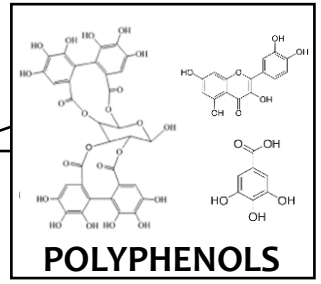
LC-ESI-LTQ-Orbitrap-MS

This is the **first study** that proposes a comprehensive analysis of the **metabolomic fingerprinting of phenolics-enriched chestnut shells** extract in **blood serum** after oral treatment of rats.

Experimental design

Extraction of polyphenols

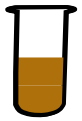
Chestnut shells (CS)



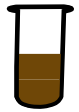
POLYPHENOLS

Subcritical Water Extraction (SWE)

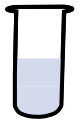
In vivo studies



CS extract 50 mg/kg b.w.



CS extract 100 mg/kg b.w.



Control (water)

1x/day for 7 days treatment, *p.o.*

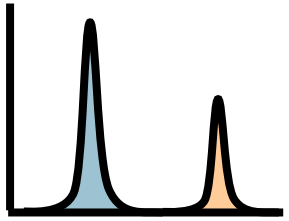
Wistar rats

n = 6 per group

Animal procedures followed the European Directive 2010/63/EU

Metabolomic analysis

Sample pre-treatment
Protein precipitation for the extraction of phenolic compounds & metabolites

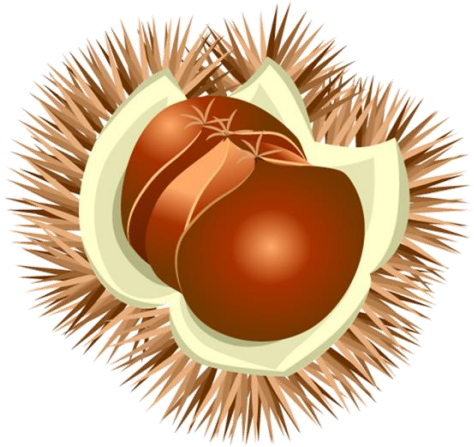


LC-ESI-LTQ-Orbitrap-MS
targeted metabolomic on phenolic compounds

Metabolomic profiling in blood serum

Phenolic acids

Hydroxybenzoic acids
&
Hydroxycinnamic acids



Flavonoids

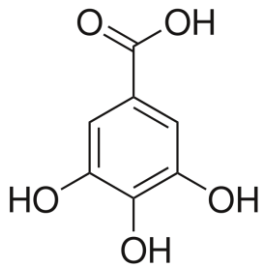
Lignans

Other polyphenols

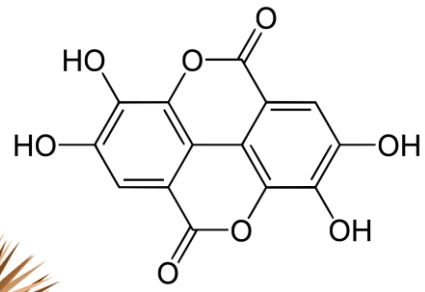
Metabolomic profiling in blood serum

Phase I

- Hydrogenation



Gallic acid

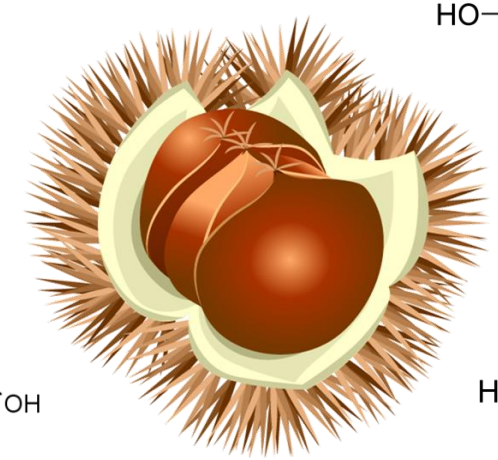


Ellagic acid

Phase II & gut microbiota

- Dimethylation

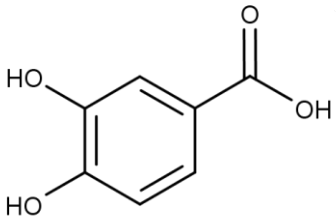
\+ Unmetabolized form & **Urolithin A** metabolites from methylation & sulfation



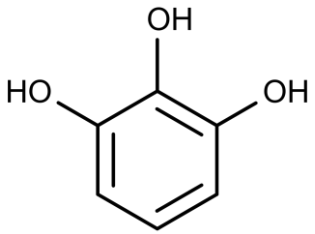
Phase II

- Methylation
- Sulfation
- Methylation + Sulfation

\+ Unmetabolized form



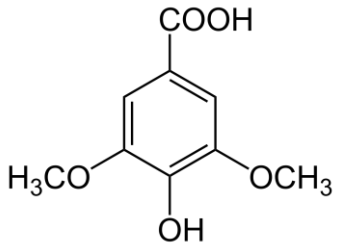
Protocatechuic acid



Pyrogallol

Phase II

- Sulfation
- Glucuronidation
- (Di)methylation + Sulfation or Glucuronidation



Syringic acid

\+ Unmetabolized form

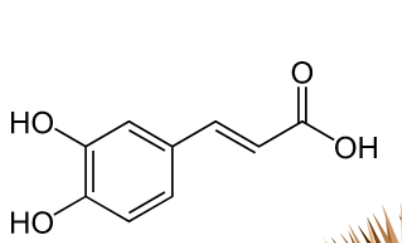
Phase II

- Methylation
- (Di)methylation + Sulfation

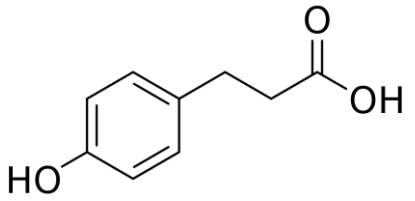
Metabolomic profiling in blood serum

Phase I & II

- Hydrogenation
- Hydrogenation + Sulfation or Glucuronidation
- Sulfation



Caffeic acid

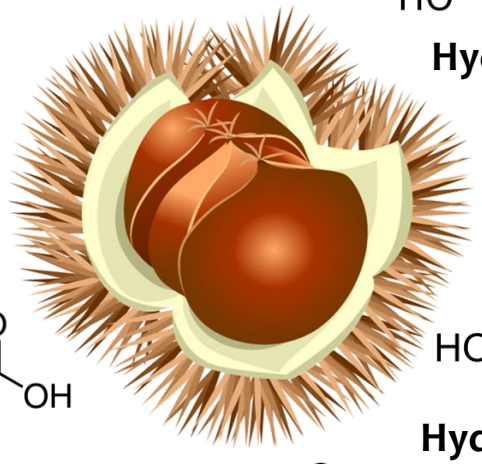


Hydroxyphenylpropionic acid

Phase II

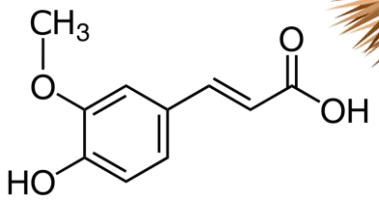
- Sulfation
- Dimethylation + Sulfation

\+ Unmetabolized form

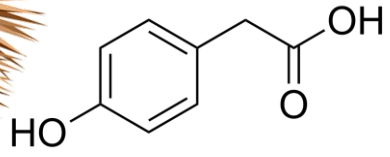


Phase I & II

- Hydroxylation
- Hydrogenation
- Hydrogenation + Sulfation or Glucuronidation
- Methylation + Sulfation or Glucuronidation
- Hydrogenation + Methylation + Sulfation



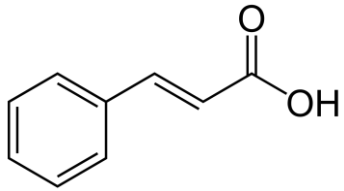
Ferulic acid



Hydroxyphenylacetic acid

Phase II

- Sulfation
- Glucuronidation



Cinnamic acid

Phase II

- Glucuronidation

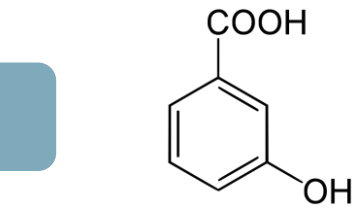
\+ Unmetabolized form

Metabolomic profiling in blood serum

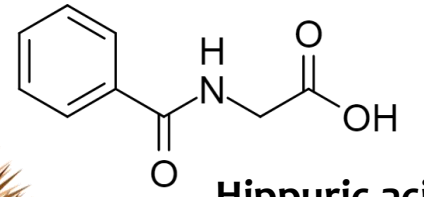
Phase II

- Sulfation

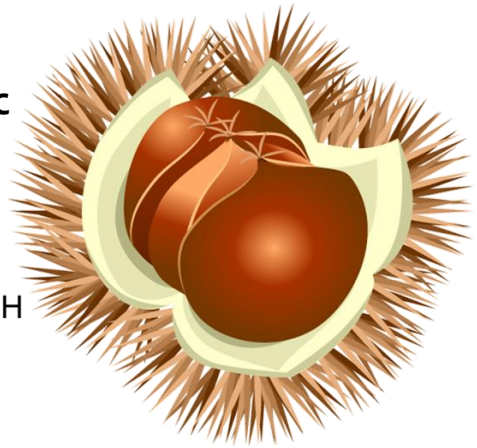
+ Unmetabolized form



Hydroxybenzoic acid

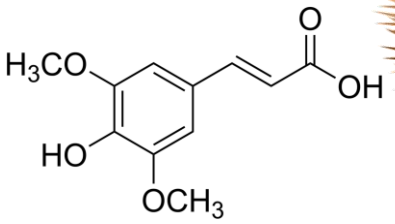


Hippuric acid

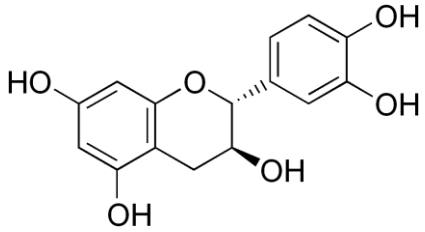


Phase II

- Sulfation



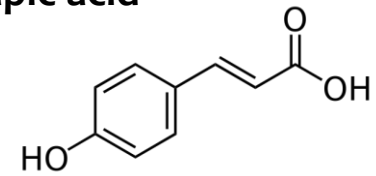
Sinapic acid



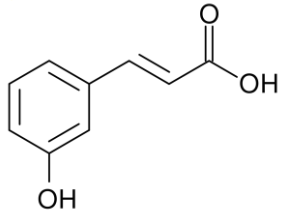
(Epi)catechin

Phase II

- Sulfation



***p*-Coumaric acid**



***m*-Coumaric acid**

Phase II

- Methylation + Sulfation

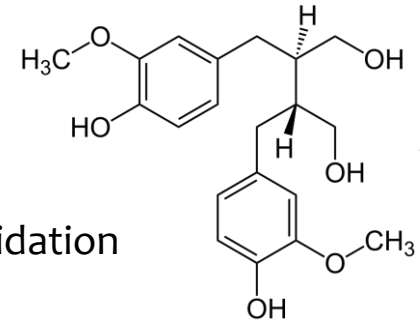
+ Unmetabolized forms

Metabolomic profiling in blood serum

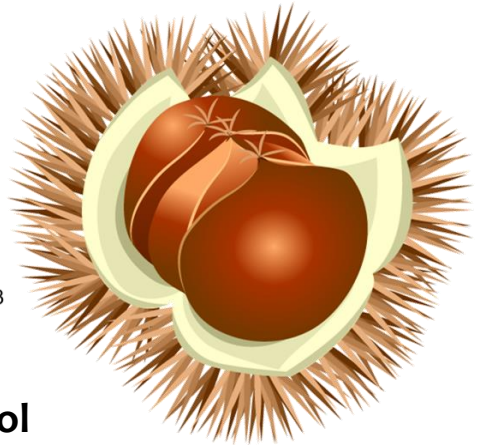
Phase II & gut microbiota

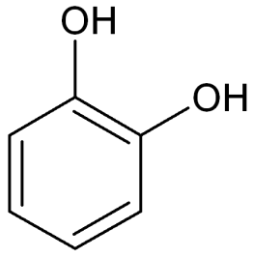
- Enterodiol + Disulfation
- Enterolactone + Glucuronidation

✚ Unmetabolized form



Secoisolariciresinol





Catechol

Phase II

- Methylation
- Glucuronidation
- Sulfation
- Methylation + Sulfation

✓ A total of **52 compounds** were identified, mostly **phenolic acids & metabolites**.

✓ **80%** of the metabolites resulted from **phase II** reactions; the remaining **20%** derived from **phase I**.

✓ **11** compounds correspond to **parent compounds**; the remaining represent their metabolites.

Conclusion

The **detection** of parent compounds in serum **attested their absorption** in **unmetabolized** form.

Phase II metabolites were secreted into circulating blood due to their high polarity and molecular weight.

Identical metabolomic profile for both CS extract groups.

This work validate **for the first time** a new nutraceutical ingredient extracted from chestnut shells.

Acknowledgments

- PTDC/ASP-AGR/29277/2017
- UIDB/50006/2020 & UIDP/50006/2020
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- CEECIND/01886/2020
- RYC-2016-19355

Thank you for the attention!