Exploring the bioavailability of phenolic compounds through *in vitro* simulated gastrointestinal digestion - INFOGEST

Adriana C.S. PAIS[1], Ezequiel R. COSCUETA[2], Maria Manuela PINTADO[2], Armando J.D.SILVESTRE[1], Sónia A.O. SANTOS[1]

[1]CICECO-Aveiro Institute of Materials, Chemistry Department, University of Aveiro, 3810-193 Aveiro, Portugal;

[2]Universidade Católica Portuguesa, CBQF - Centro de Biotecnologia e Química Fina -Laboratório Associado, Escola Superior de Biotecnologia,4169-005 Porto, Portugal *a.c.p.s@ua.pt

INTRODUCTION

NATURAL PHENOLIC COMPOUNDS

- Widely distributed in the plant kingdom
- Commonly present in human diet
- Vast range of biological activities (antioxidant, anti-inflammatory and/or antiproliferative)
- Two-way relationship with gut microbiota

These **human health beneficial effects** could be influenced by phenolic compounds **bioavailability.**

Dependent on:

- Phenolic compound's structure,
- Human digestive system enzymatic activity
- Human gut microbiota activity

WHAT IS THE BIOAVAILABILITY OF THE INDIVIDUAL PHENOLIC COMPOUNDS IN ABSENCE OF A COMPLEX FOOD MATRIX?

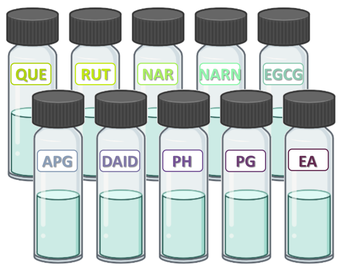
STUDIED COMPOUNDS:

QUERCETIN (QUE) · RUTIN (RUT) · NARINGENIN (NAR) · NARINGIN (NARN) · EPIGALLOCATECHIN-3-O-GALLATE (EGCG)

APIGENIN (APG) · DAIDZEIN (DAID) · PHLORETIN (PH) · PHLOROGLUCINOL (PG) · ELLAGIC ACID (EA)

METHODS

PHENOLIC COMPOUNDS SOLUTIONS
(EtOH:H_2O, 15% v/v)

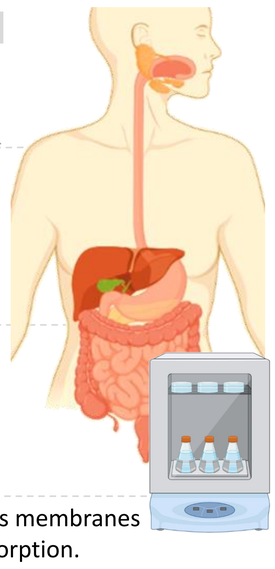


INFOGEST
(*IN VITRO* SIMULATED GASTROINTESTINAL DIGESTION)

Oral phase
- Simulated salivary fluid (SSF)
- α-amylase
- 2 min, 37 °C, under agitation

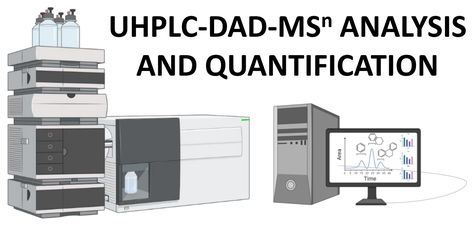
Gastric phase
- Simulated gastric fluid (SGF)
- pepsin
- 2 h, 37 °C, under agitation

Intestinal phase
- Simulated intestinal fluid (SIF)
- pancreatin
- bile salts solution
- 2 h, 37 °C, under agitation



Then, samples were placed in dialysis membranes to simulate small intestine absorption.

UHPLC-DAD-MS^n ANALYSIS AND QUANTIFICATION



For each compound simulated digestion phase, bioaccessibility index (%) was determined through the following equation:

$$Bioacessibility\ index\ (\%) = \frac{Digested\ sample\ (\mu g\ mL^{-1})}{Initial\ solution(\mu g\ mL^{-1})} \times 100$$

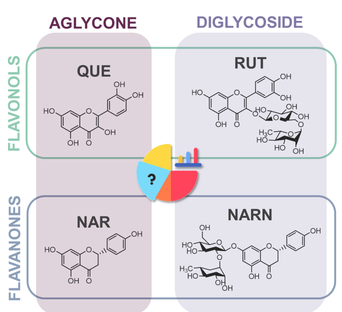
Intestinal absorption (%) was determined through the intestinal sample and those collected inside of dialysis membrane, as follows:

$$Intestinal\ absorption\ (\%) = 100 - (\frac{Dialysis\ sample(\mu g\ mL^{-1})}{Intestinal\ sample(\mu g\ mL^{-1})} \times 100)$$

RESULTS

BIOACCESSIBILITY INDEX (ALONG GASTROINTESTINAL TRACT)

How sugar units influence flavonoids' bioaccessibility?



Studied diglycosides bioaccessibility percentages **after each simulated digestion were higher** than those of the aglycones.

This could be due to the lower solubility of the aglycone forms in aqueous media.

The **most bioaccessible compounds** after gastrointestinal digestion

APG **147.50 %** · DAID **158.08 %** · PG **159.82 %**

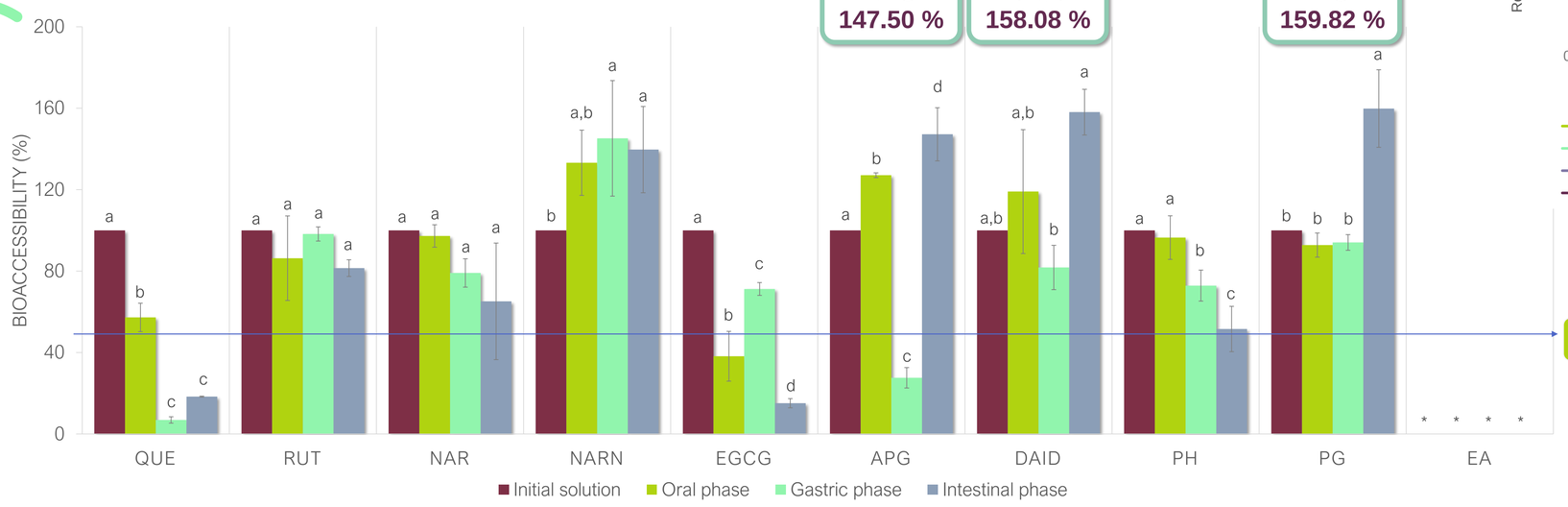


Figure 2 - Bioaccessibility indexes (%) along the INFOGEST for each studied phenolic compound.
* < LOD and/or LOQ; Different letters indicate significant differences ($p < 0.05$), determined by one-way repeated measured ANOVA followed by Tukey's post-hoc test.

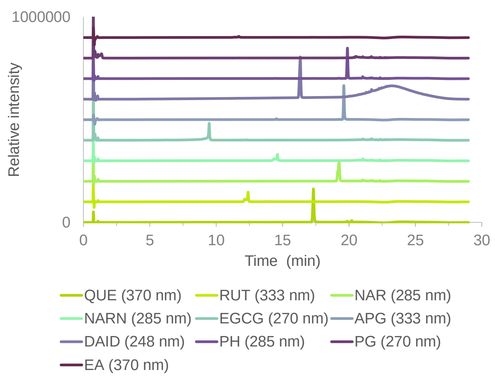


Figure 1 - Chromatogram profile of each studied compound obtained through UHPLC-DAD-MS^n

After simulated intestinal digestion:

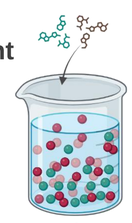
PCs bioaccessibility > 50%

(except for QUE, EGCG, and EA)

Ellagic acid

Most challenging bioaccessibilty screening along the simulated gastrointestinal tract was hindered by its low solubility.

pH-dependent solubility



Bioaccessibility variations along the gastrointestinal digestion of QUE, EGCG, APG, PG and EA

CONCLUSIONS

- Studied phenolic compounds did not undergo enzymatic digestion after INFOGEST, as we did not detect any resultant metabolites.
- Bioaccessibility percentages determined after the intestinal digestion phase showed that most of the studied phenolic compounds were about 50% bioaccessible.
- Absorption rates of all studied phenolic compounds were high (> 80%), suggesting that they can be absorbed into the systemic circulation or follow to the colon, where gut microbiota may metabolize them.
- Solubility of phenolic compounds could limit their bioavailability, as QUE and EA, demonstrated lower bioaccessibility along the simulated gastrointestinal tract due to their low solubility in water.

INTESTINAL ABSORPTION

Highly absorbable in the simulated small intestine

> 80% absorption rates

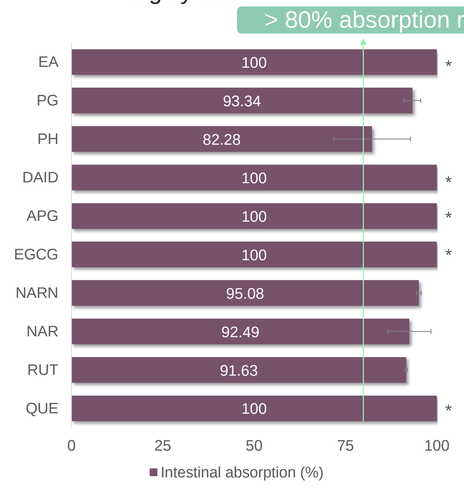
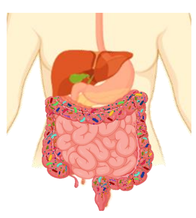
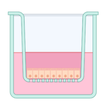


Figure 3 - Intestinal absorption (%) of each studied phenolic compound after the INFOGEST simulation system. Those with an "*" were considered as 100% since the samples collected from inside the dialysis membrane were < LOD and/or LOQ, so not possible to determinate.



These results are different from the reported ***in vivo*** **absorption percentages (5-10%)**

Future work



Intestinal absorption of the studied pure phenolic compound should be evaluated using another simulator system (e.g.: CACO-2 cell monolayers permeability assay).

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