A green snack for a greener planet: Bite-sized functional cookies supplemented with chestnut shell antioxidants

Diana Pinto[1], Manuela M. Moreira[1], Elsa F. Vieira[1], Tanja Brezo-Borjan[2], Anna Vallverdú-Queralt[3,4], Jaroslava Švarc-Gajić[2], Cristina Delerue-Matos[1], Francisca Rodrigues[1,*]

[1] - REQUIMTE/LAQV, ISEP, Polytechnic of Porto, Rua Dr. António Bernardino de Almeida, 4249 015 Porto, Portugal; [2] - Faculty of Technology, University of Novi Sad, Bulevar cara Lazara 1, 21000 Novi Sad, Serbia; [3] - Nutrition, Food Science and Gastronomy Department, School of Pharmacy and Food Science, University of Barcelona, Barcelona, Spain; [4] - Consorcio CIBER, M.P. Fisiopatología de la Obesidad y la Nutrición (CIBERObn), Instituto de Salud Carlos III (ISCIII), Madrid, Spain.

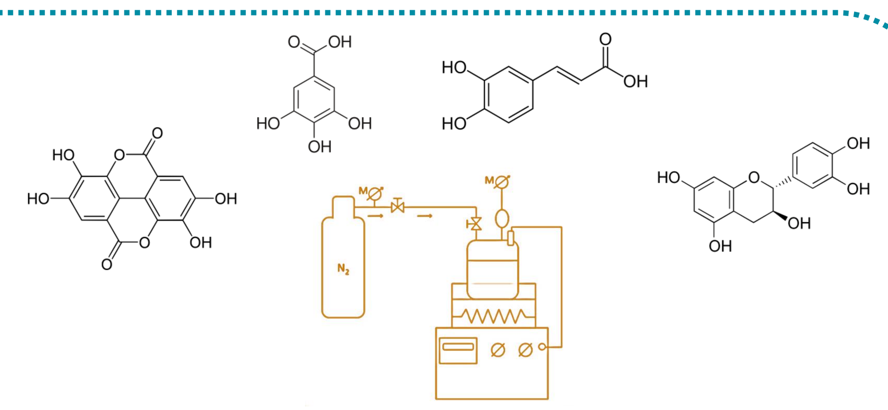
* francisca.rodrigues@graq.isep.ipp.pt

SCIENTIFIC BACKGROUND

- Bioactive molecules play a pivotal role in combating oxidative stress and inflammation associated with several chronic diseases [1-3].
- The extraction of bioactive molecules from natural sources faces severe challenges, seeking more promising and sustainable technologies [1].
- The use of food by-products as sources of bioactives is a concern to create eco-friendly products, contributing to the sustainability of agri-food chain complying with the Sustainable Development Goals and European Green Deal.

Chestnut shells (CS)

- Undervalued & plentiful by-product
- Rich in phenolics & vitamin E [1]
- Antioxidant, antimicrobial & anti-inflammatory effects [1,2]
- Potential use as nutraceutical ingredient [5]



Subcritical Water Extraction (SWE)

A nutraceutical extract from CS rich in polyphenols recovered by this eco-friendly technology was recently validated by ***in vitro*** & ***in vivo*** assays [2-4]

AIMS & RATIONALE

To appraise the effects of *in vitro* gastrointestinal simulated digestion on the bioaccessibility & bioactivity of functional cookies enriched with chestnut shells (CS) extract prepared by SWE and previously validated by *in vitro* & *in vivo* assays.

METHODOLOGY

1 Extraction of phenolic compounds from CS by SWE

Chestnut shells

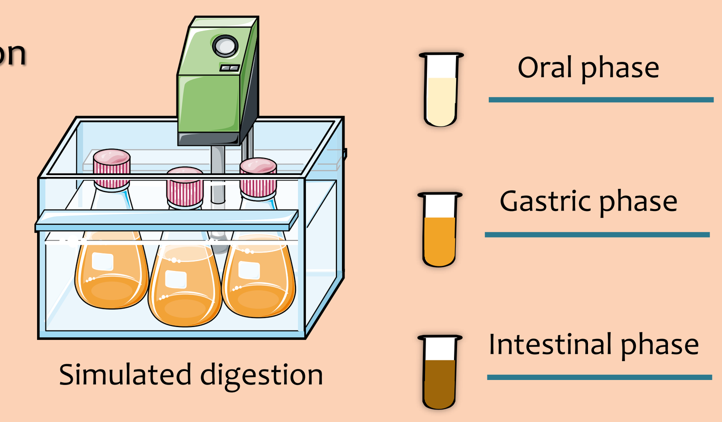
Subcritical Water Extraction

2 Preparation & Characterization of functional cookies

Sugar (32.20%)
White flour (29.40%)
Butter (20.70%)
Eggs (2 = 7.80%)
Cocoa powder (7.08%)
Lyophilized CS extract (1.77%)
Sodium bicarbonate (0.71%)
Sodium chloride (0.34%)

- ✓ Nutritional composition
- ✓ Bioactivity
- ✓ Phenolic composition
- ✓ Sensory evaluation

3 *In vitro* gastrointestinal digestion



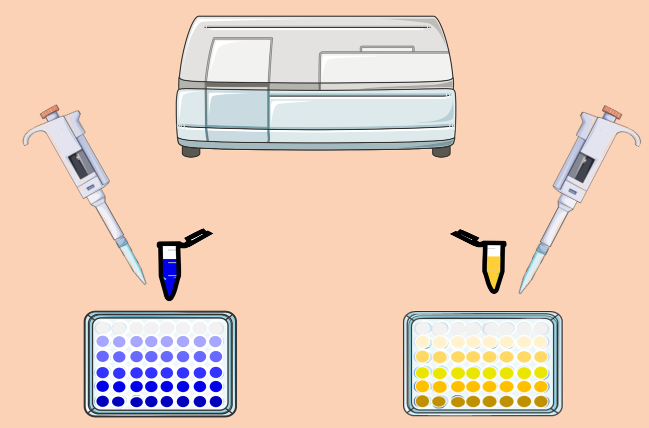
Oral phase

Gastric phase

Intestinal phase

Simulated digestion

4 Bioactivity



Phenolic content & antioxidant assays

α-amylase inhibitory assay

5 Phenolic composition

Phenolic profiling by HPLC-DAD

RESULTS & DISCUSSION

1 Nutritional composition

Proximate composition	Content (% dw)
Carbohydrates	53.9
Fat	32.6
Protein	6.2
Fiber	5.2
Moisture	4.8
Ash	2.1

- ✓ Functional cookies were mainly composed of **carbohydrates** (>50%) and fat (≈30%)
- ✓ Moderate contents of fiber (≈5%) and minerals (≈2%)
- ✓ Low contents of protein (≈6%) and moisture (≈5%)

1 Total phenolic content, antioxidant/antiradical properties & bioaccessibility

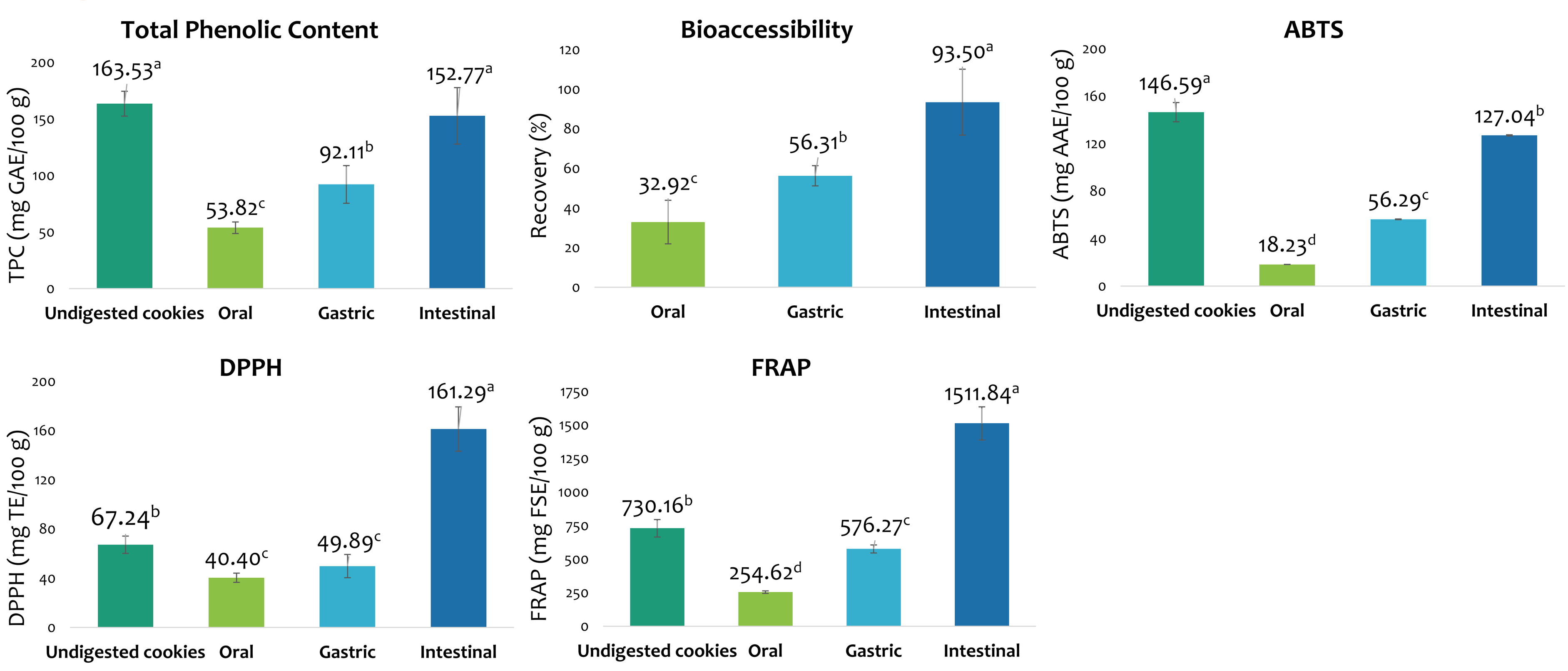


Figure 1. Total phenolic content (TPC) & antioxidant/antiradical activity of functional cookies and digested samples. Different letters (a–c) indicate significant differences (*p*<0.05). AAE, ascorbic acid equivalents. FSE, ferrous sulfate equivalents. GAE, gallic acid equivalents. TE, Trolox equivalents.

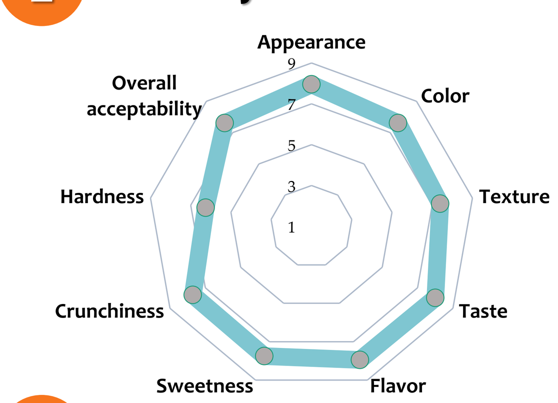
- ✓ Higher phenolic concentrations were retained after intestinal digestion, endorsing its better antioxidant & antiradical properties
- ✓ Phenolics recovery improved as follows: oral < gastric < intestinal digests, reaching 94% of maximum bioaccessibility

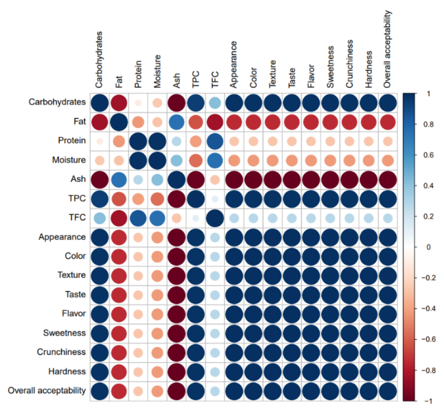
3 Scavenging activity against reactive oxygen and nitrogen species

Table 1. Scavenging activity of functional cookies against reactive oxygen and nitrogen species (ROS & RNS, respectively). Different letters (a–c) indicate significant differences (*p*<0.05). * results indicated in inhibition percentage (%) tested directly in digests.

ROS & RNS	$O_2^{\bullet-}$	H_2O_2	HOCl	$ONOO^-$	$ROO^\bullet$
	IC_{50} (µg/mL)				µg TE/mg DW
Oral	n.d.	n.d.	13.23 ± 0.43*,c	10.57 ± 0.67*,c	0.19 ± 0.02d
Gastric	10.19 ± 1.86*,c	n.d.	33.16 ± 1.25*,b	26.38 ± 0.53*,b	1.06 ± 0.07c
Intestinal	39.13 ± 1.53*,a	14.34 ± 2.28*,b	43.55 ± 1.16*,a	45.19 ± 1.12*,a	2.52 ± 0.13b
Undigested cookies	35.85 ± 1.04 *,b	28.23 ± 0.56 *,a	81.81 ± 5.01	115.00 ± 3.59	5.81 ± 0.59a

2 Sensory evaluation





- ✓ **Acceptable sensory attributes** (84% of acceptability)
- ✓ Carbohydrates & polyphenols contribute to the sensory properties

2 Phenolic profile upon *in vitro* gastrointestinal digestion

- ✓ Oral digest: 17 phenolic compounds identified = 47.92 mg/100 g cookies
- ✓ Gastric digest: 25 phenolic compounds identified = 65.83 mg/100 g cookies
- ✓ Intestinal digest: 24 phenolic compounds identified = 127.09 mg/100 g cookies
- ✓ Undigested cookies: 25 phenolic compounds identified = 154.09 mg/100 g cookies

67% phenolic acids
26% hydrolysable tannins
5% flavonoids
2% caffeine

Gallic acid, ellagic acid & catechin were the major phenolic compounds

Table 2. Bioaccessibility of phenolic compounds from functional cookies after *in vitro* gastrointestinal digestion. Different letters (a–c) indicate significant differences (*p*<0.05).

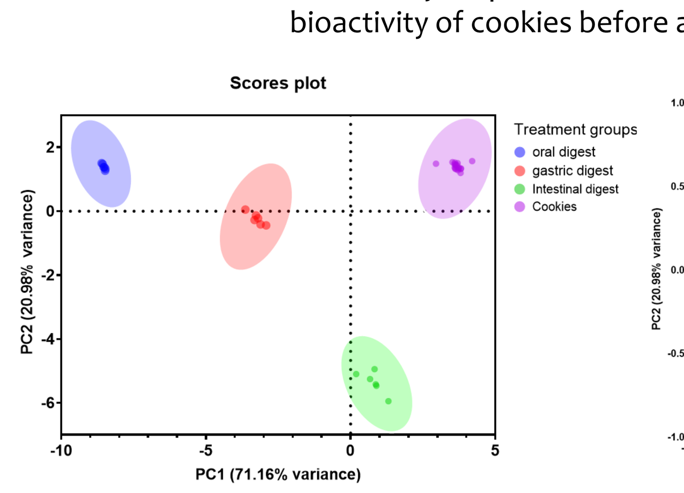
Bioaccessibility (%)	Hydroxybenzoic acids	Hydroxycinnamic acids	Flavanols	Flavonols	Flavones	Hydrolysable tannins	Alkaloids
Oral	45.12 ± 2.26c	46.44 ± 2.32c	4.84 ± 0.24c	2.50 ± 0.13c	15.17 ± 0.76c	0.29 ± 0.02a	25.55 ± 1.28c
Gastric	56.24 ± 2.81b	72.57 ± 3.63b	21.23 ± 1.06b	33.36 ± 1.67b	73.21 ± 3.66a	0.64 ± 0.03a	129.44 ± 6.47b
Intestinal	78.12 ± 3.91a	105.33 ± 5.27a	80.47 ± 4.02a	69.16 ± 3.46a	40.60 ± 2.03b	82.78 ± 4.14b	149.37 ± 7.47a

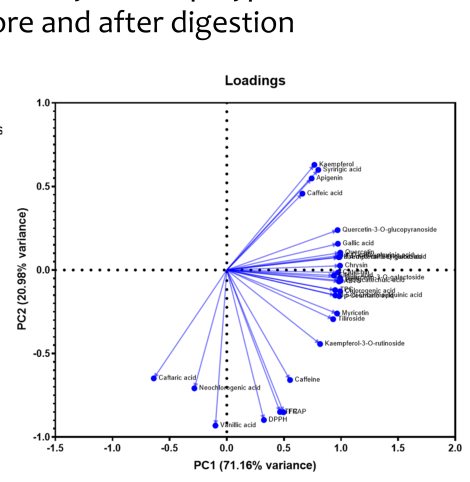
4 Hypoglycemic properties

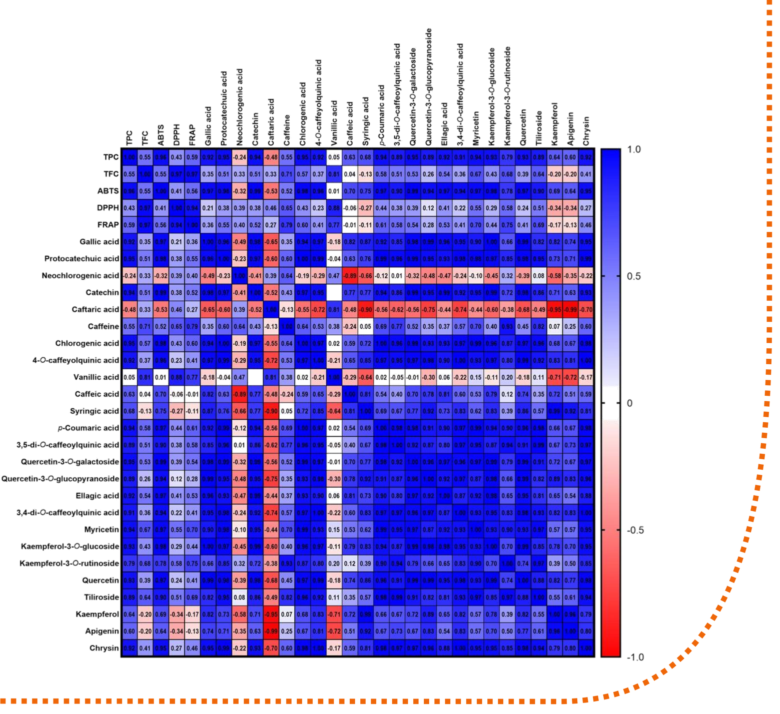
Oral 3.84% α-amylase inhibition › **Gastric** 18.86% α-amylase inhibition › **Undigested extract** 21.88% α-amylase inhibition › **Intestinal** 71.27% α-amylase inhibition

5 Multivariate data analysis

- ✓ Multivariate analyses predicted the key role of polyphenols in the bioactivity of cookies before and after digestion







CONCLUDING REMARKS

These findings highlighted the sustainable employment of antioxidants-rich CS extract as an active nutraceutical ingredient in functional cookies, proposing a novel approach to valorize this agro-industrial waste and contribute to the sustainability of agri-food chain.

References:
[1] D. Pinto et al., Food Res. Int. 144 (2021) 110364. [2] D. Pinto et al., Food Chem. 334 (2021) 127521. [3] D. Pinto et al., Food Chem. 404 (2023) 134546. [4] D. Pinto et al., Food Res. Int. 170 (2023) 112963. [5] D. Pinto et al., Foods 12 (2023) 640.

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