



HAL
open science

Highlighting the antioxidant and hypocholesterolemic properties of peptides from spirulina (*Arthrospira platensis*)

Barbara Deracinois, Camille Dugardin, Ikram Benneceur, Esteban Marechal, Jacques Neuray, Philippe Jacques, Christophe Flahaut, Rozenn Ravallec

► To cite this version:

Barbara Deracinois, Camille Dugardin, Ikram Benneceur, Esteban Marechal, Jacques Neuray, et al.. Highlighting the antioxidant and hypocholesterolemic properties of peptides from spirulina (*Arthrospira platensis*). 8th International Conference on FOOD DIGESTION, Apr 2024, Porto, Portugal. hal-04551664

HAL Id: hal-04551664

<https://hal.science/hal-04551664>

Submitted on 18 Apr 2024

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Highlighting the antioxidant and hypocholesterolemic properties of peptides from *spirulina* (*Arthrospira platensis*).

Deracinois Barbara^{1*}, Dugardin Camille^{1*}, Ikram Benneceur², Esteban Marechal³, Jacques Neuray³, Philippe Jacques², Christophe Flahaut¹ and Rozenn Ravallec¹. * These two authors contributed equally to this work

¹ UMRt BioEcoAgro-INRAE 1158, Univ. Lille, Univ. Artois, Univ. Liège – Institut Charles Viollette, F-62300 Lens and F-59000 Lille, France

² UMRt BioEcoAgro-INRAE 1158, Terra Teaching and Research Centre, Univ. Liège Gembloux Agro-Bio-Tech, B-5030 Gembloux, Belgique

³ Biores s.a., Harzé, Belgique

Hypercholesterolemia, a pivotal public health concern, propels the collaborative international project CASH (*Chromophores Actifs en Santé Humaine*) to explore natural alternatives to statins for blood cholesterol level reduction, aiming to prevent cardiovascular risks. This project, cofounded by Walloon Region through the WAGRALIM Competitiveness Cluster, is a partnership between the University of Liège, the University of Louvain-La-Neuve, the University of Lille, Tilman sa and Biores sa (two Wallonia-based companies).

Spirulina, a blue-green alga of the Cyanophyceae family, is celebrated for its exceptional nutritional properties and well-established antioxidant, antidiabetic, and anti-inflammatory activities [1, 2]. *Spirulina* contains the phycocyanin, a chromoprotein used as natural blue pigment in food.

The CASH project unfolds through diverse tasks ranging from: (i) assessing the bioactive potential of peptides from *spirulina* proteins through an *in silico* approach, (ii) developing bioactive *spirulina* protein hydrolysates, (iii) evaluating the antioxidant, hypocholesterolemic and DPP-IV inhibition activities before and after simulated *in vitro* static INFOGEST gastrointestinal digestion [3, 4], and (iv) identifying peptides and chromopeptides responsible for the studied bioactivities through a peptidomic approach.

In silico approach

Number of bioactive di-, tri-, tetra- and penta-peptides from the BIOPEP-UWM database present in the four major polypeptides of *Arthrospira platensis*

| Proteins | Number of BIOPEP-UWM peptides found in the major protein sequence | | | N° of bioactivity |
|-------------------------|---|-------------|-------------------------|------------------------------|
| | Dipeptides | Tripeptides | Tetra and pentapeptides | |
| Allophycocyanin α chain | 129 | 10 | 0 | 1-2-4-5-7-8-9-10-11-12-14 |
| Allophycocyanin β chain | 158 | 10 | 0 | 1-2-4-6-7-8-9-10-11-12-13-14 |
| C-phycocyanin α chain | 60 | 14 | 1 | 1-2-3-4-7-8-9 |
| C-phycocyanin β chain | 149 | 4 | 2 | 1-2-4-7-8-9-10-11-12-14 |
| Total | 496 | 38 | 3 | 1 to 14 |

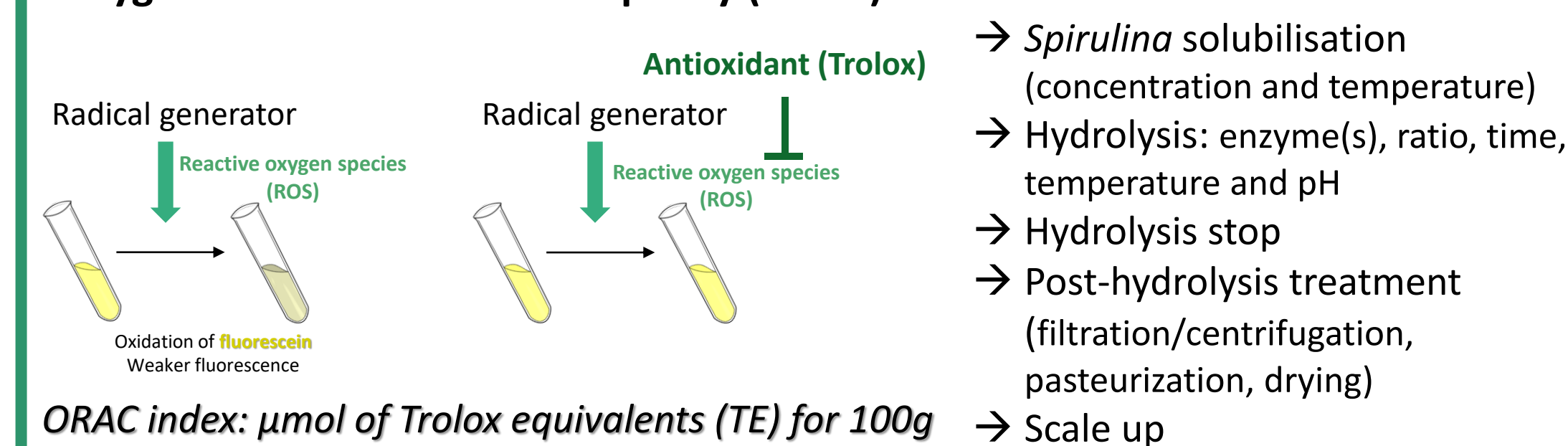
List of bioactivity

| N° | Bioactivity |
|----|---|
| 1 | Antiamnestic or Antithrombotic |
| 2 | ACE inhibitor |
| 3 | Opioid |
| 4 | Stimulating |
| 5 | Immunomodulating |
| 6 | Immunostimulating |
| 7 | Neuropeptide |
| 8 | Regulating |
| 9 | Antioxidative |
| 10 | Activating ubiquitin-mediated proteolysis |
| 11 | Dipeptidyl peptidase III inhibitor |
| 12 | Dipeptidyl peptidase IV inhibitor |
| 13 | CaMPDE inhibitor |
| 14 | Renin inhibitor |

Spirulina protein hydrolysis

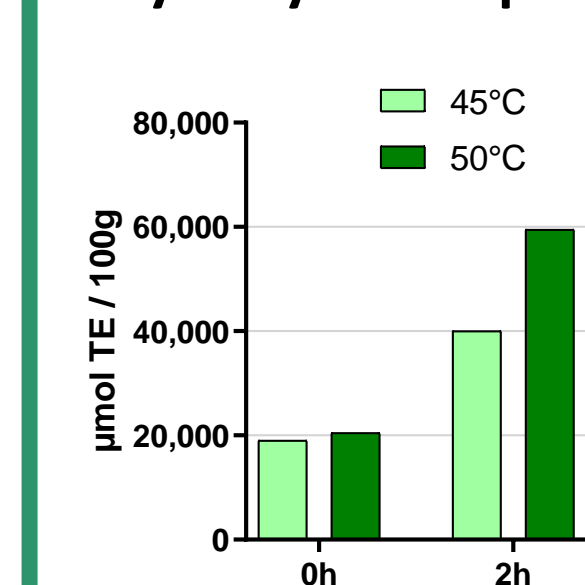
Hydrolysis optimisation with analysis of antioxidant potential

Oxygen radical absorbance capacity (ORAC) test

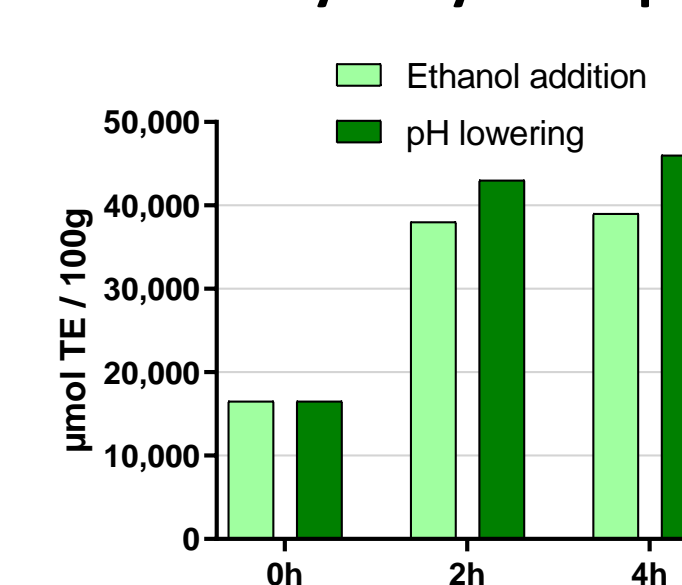


ORAC index: μmol of Trolox equivalents (TE) for 100g

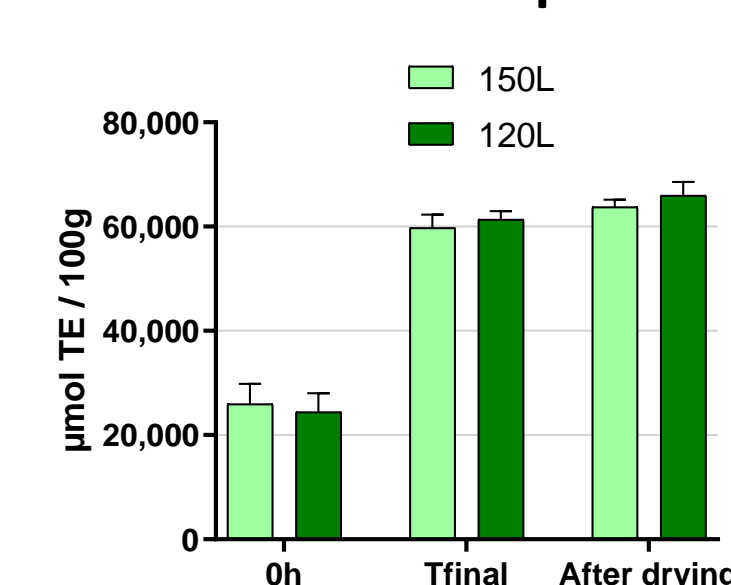
Hydrolysis temperature



Hydrolysis stop

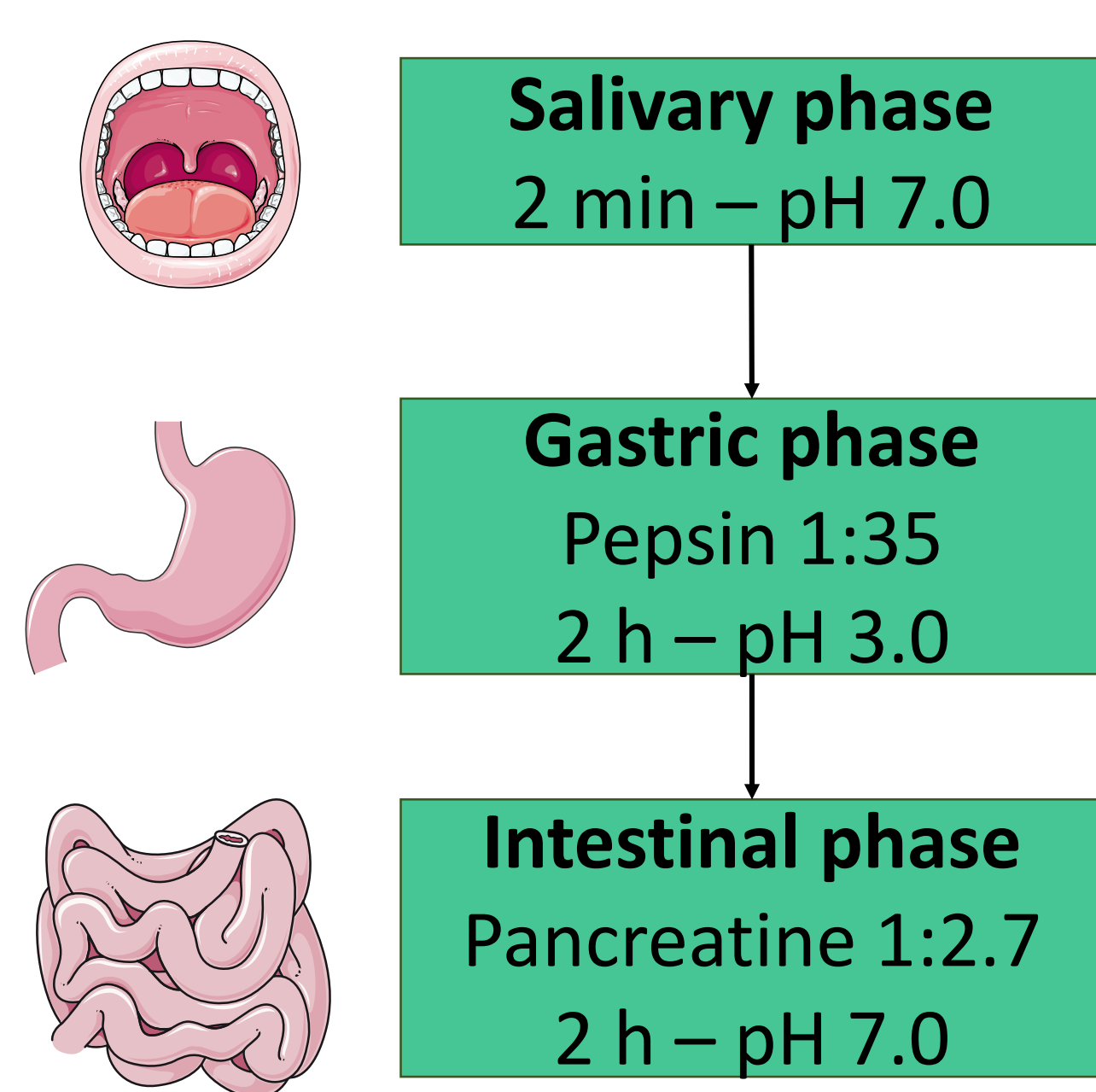


Scale up



Bio-activities and proteomics

Simulated gastro-intestinal digestion

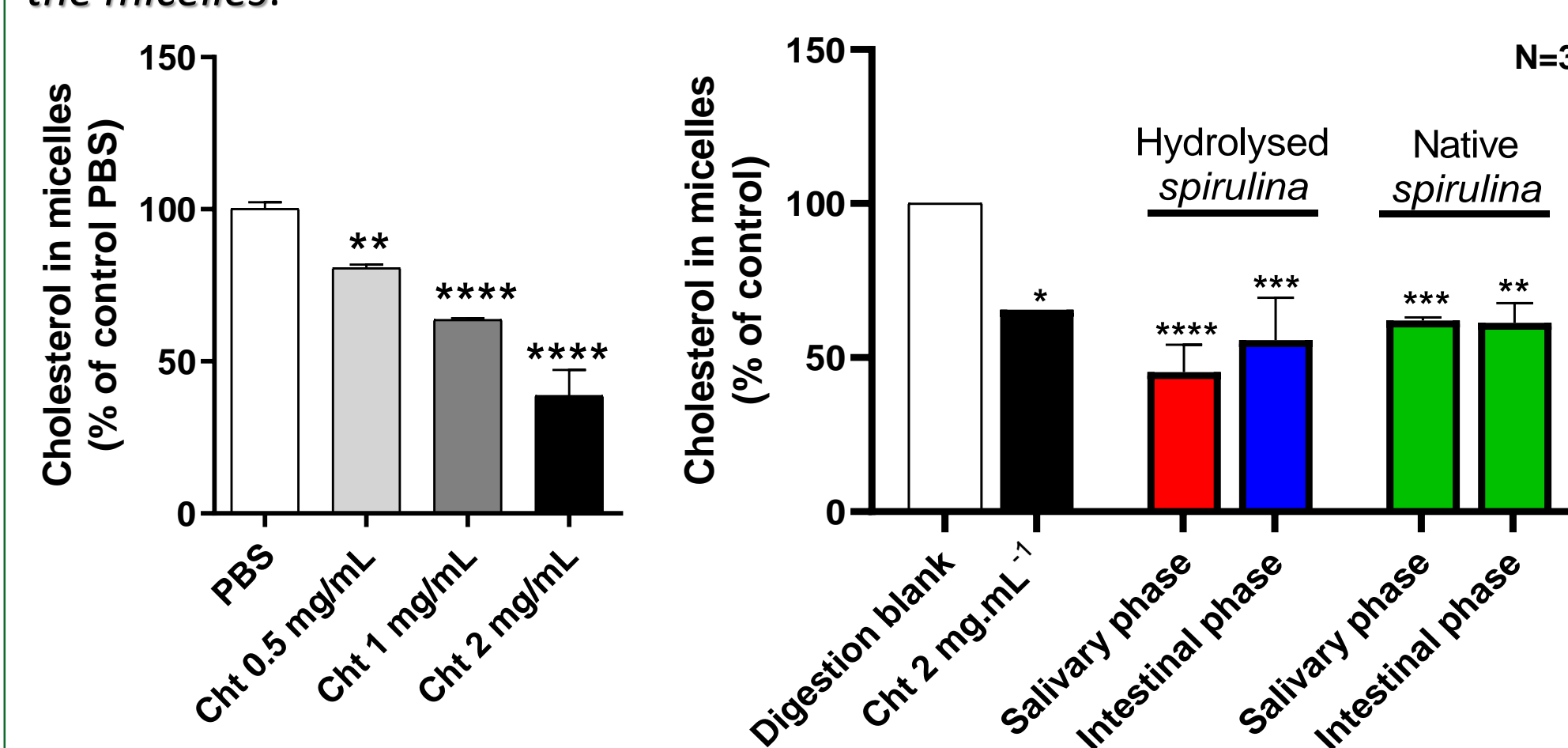


Adapted from 3 and 4

Hypocholesterolemic activity

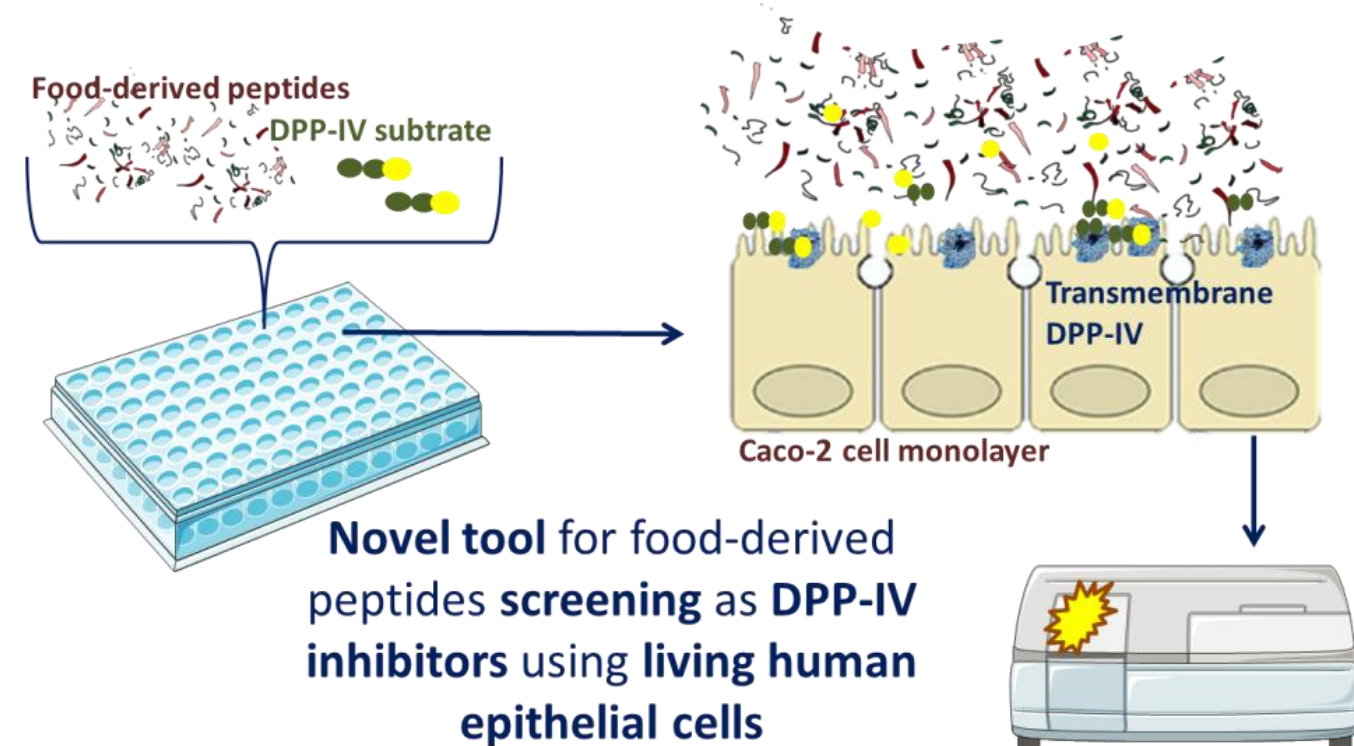
Effect on cholesterol micellar solubilisation

- Incubation 1h at 37°C of samples with micelles containing cholesterol
- Centrifugation at 100,000 xg during 60 minutes at 37°C
- Cholesterol measurement in supernatant (fluorimetric assay kit)
- The less cholesterol there is in the supernatant, the less it is solubilized in the micelles.



Method development with cholestyramine (Cht - Bile salt chelating) and hypocholesterolemic effect of hydrolysates (Concentration = 6,25 mg/mL)

Cellular DPP-IV inhibition activity

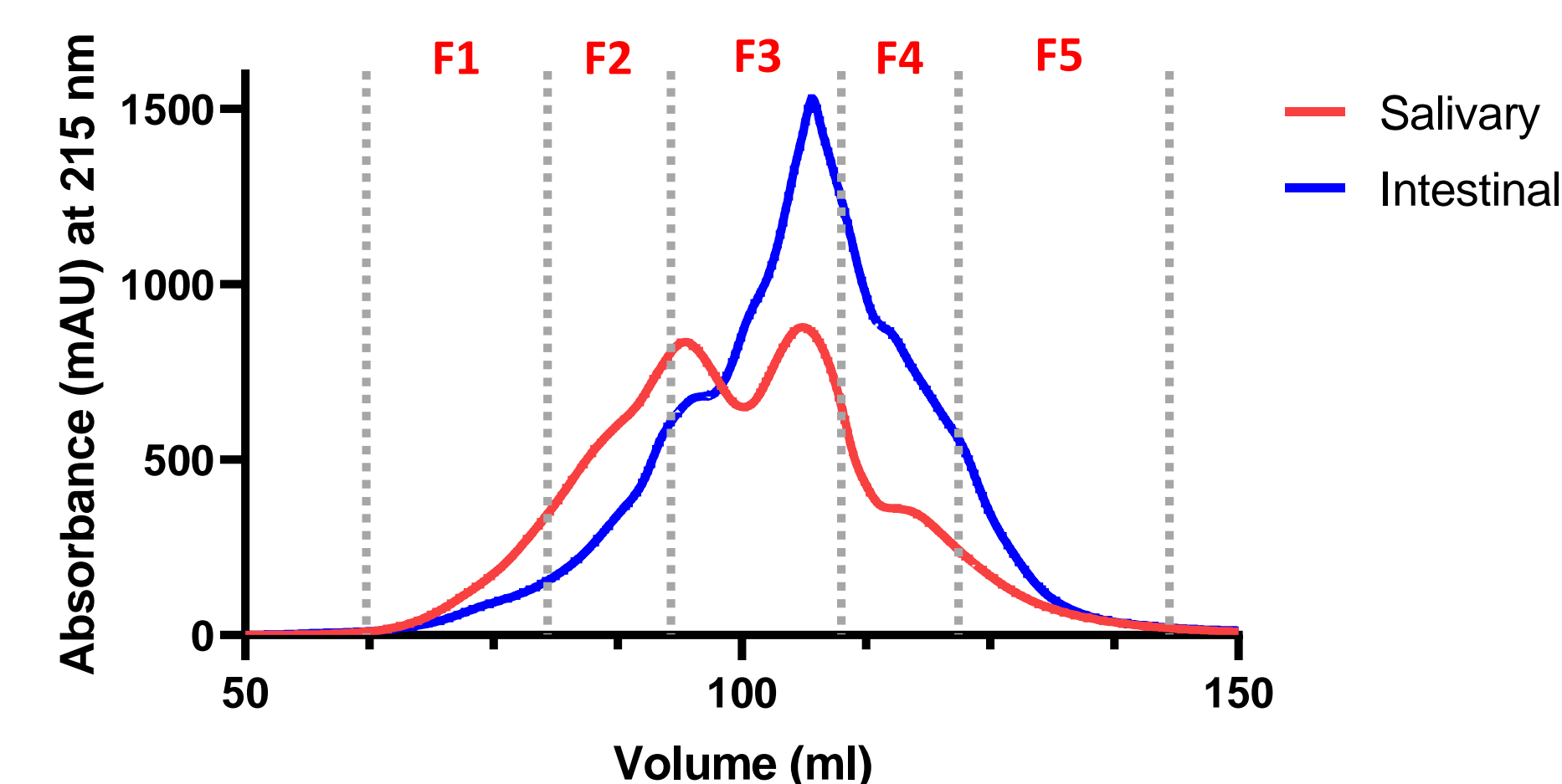


The more the activity of DPP-IV is inhibited, the less it degrades GLP-1.
→ Hypoglycemic effect

DPP-IV IC₅₀ of hydrolysates (mg.mL⁻¹)

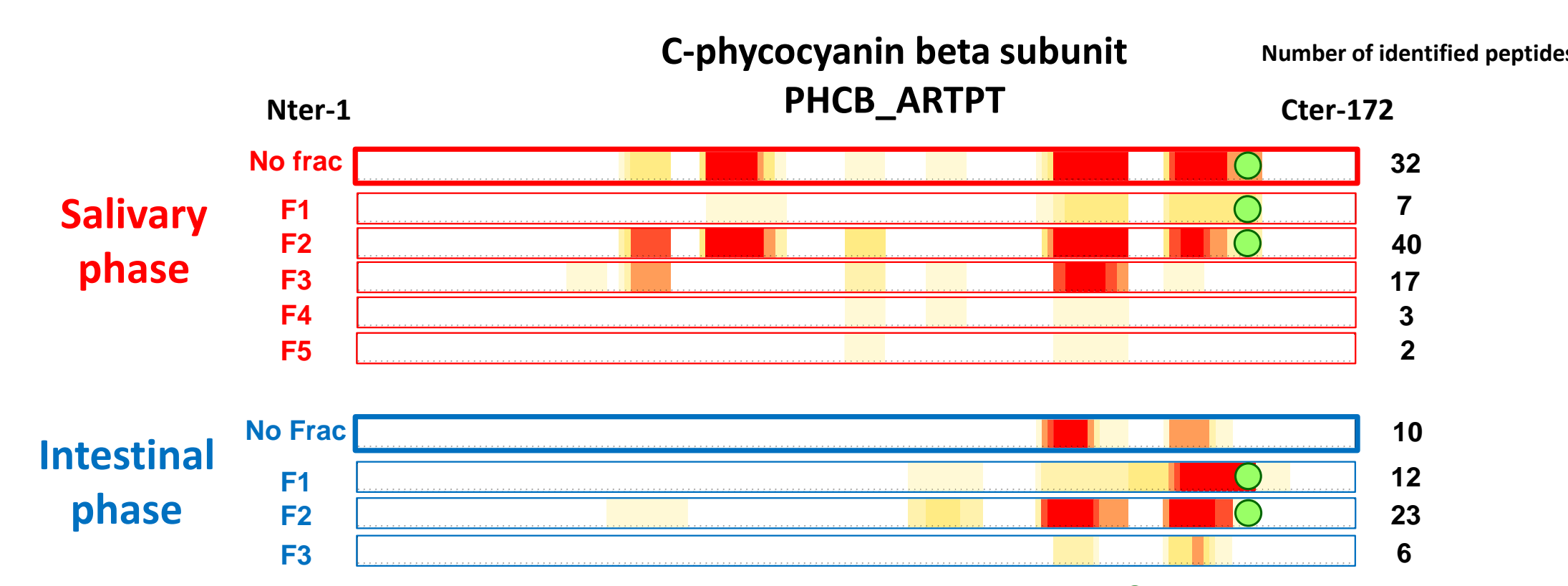
| Sample | DPP-IV IC ₅₀ of hydrolysates (mg.mL ⁻¹) | |
|-----------------------------|--|------------------|
| | Salivary phase | Intestinal phase |
| Hydrolysed <i>spirulina</i> | 1,59 | 1,20 |
| Native <i>spirulina</i> | 5,39 | 1,55 |
| Digestion blank | 1709681 | 358,23 |

SEC-Fractionation



Summary of results obtained on SEC subfractions

| | Salivary phase | | | | | Intestinal phase | | | | |
|----------------------------------|----------------|------|-----|----|----|------------------|-----|----|----|----|
| | F1 | F2 | F3 | F4 | F5 | F1 | F2 | F3 | F4 | F5 |
| Number of identified peptides | 430 | 1046 | 135 | 2 | 1 | 88 | 338 | 31 | 0 | 0 |
| PCB (Cys 153) identification | ✓ | ✓ | | | | ✓ | ✓ | | | |
| Chromopeptide detection (672 nm) | +++ | ++ | + | | | +++ | +++ | + | | |
| Antioxidant activity | | ✓ | ✓ | | | | | ✓ | ✓ | |
| Hypocholesterolemic activity | | ✓ | ✓ | | | | | ✓ | ✓ | |



Heat maps: Representation of the distribution of identified peptides along the primary sequence of the phycocyanin C beta chain

The project led to the identification of fractions obtained by gel filtration of phycocyanin hydrolysates with high antioxidant activity. This innovative study promises to redefine preventive approaches against cardiovascular diseases by harnessing the bioactive potential of *spirulina* peptides, paving the way for natural and sustainable solutions to enhance cardiovascular human health.

- Abreu AP, Martins R, Nunes J (2023) Emerging Applications of Chlorella sp. and Spirulina (Arthrospira) sp. Bioengineering
- Gentscheva G, Nikolova K, Panayotova V, et al (2023) Application of Arthrospira platensis for Medicinal Purposes and the Food Industry: A Review of the Literature. Life
- Brodkorb A, Egger L, Alminger M, et al (2019) INFOGEST static in vitro simulation of gastrointestinal food digestion. Nat Protoc
- Atallah N, Deracinois B, Boulier A, et al (2020) In Vitro Assessment of the Impact of Industrial Processes on the Gastrointestinal Digestion of Milk Protein Matrices Using the INFOGEST Protocol. Foods