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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-04551664v1>

Submitted on 18 Apr 2024

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

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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

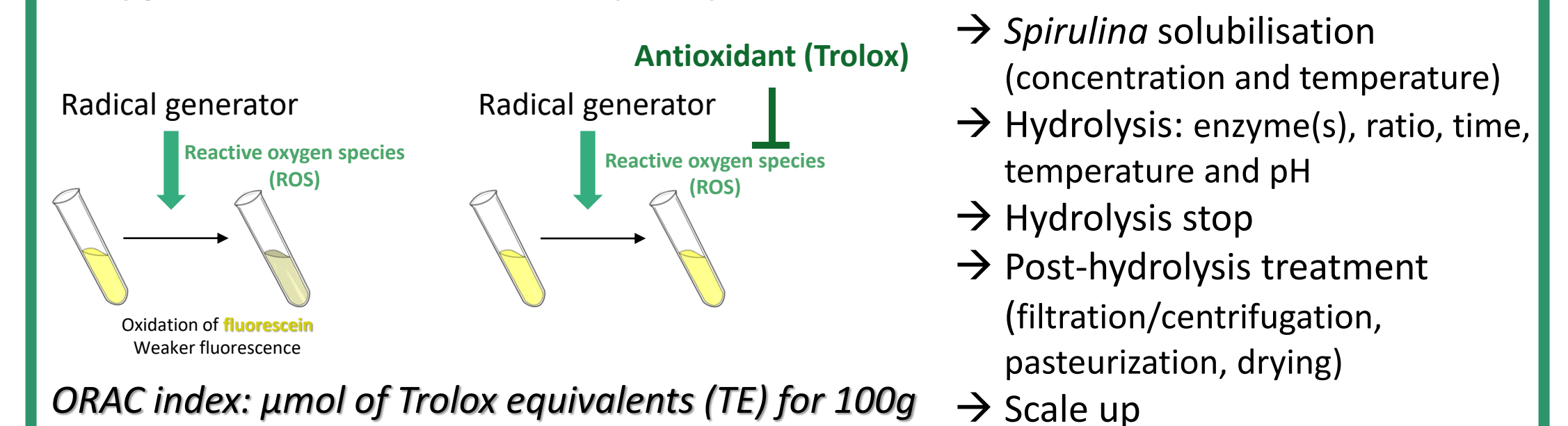
List of bioactivity

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

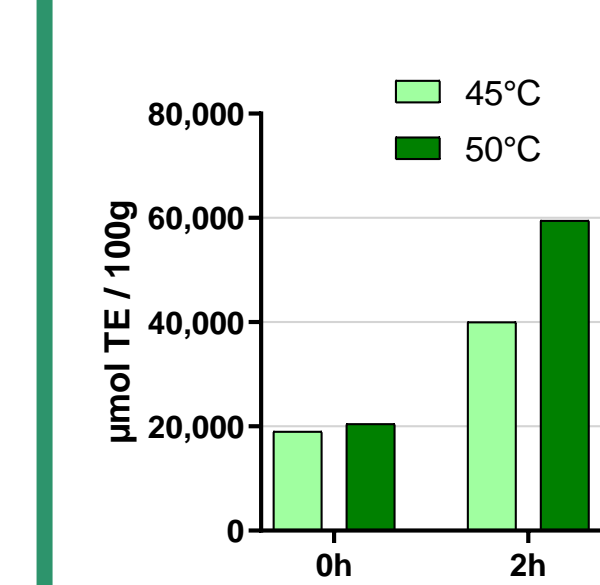
Spirulina protein hydrolysis

Hydrolysis optimisation with analysis of antioxidant potential

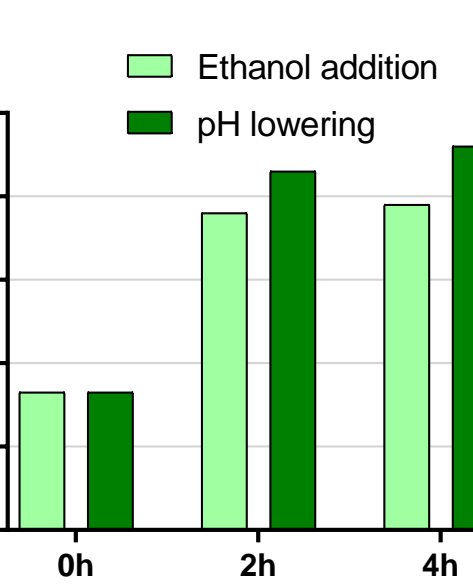
Oxygen radical absorbance capacity (ORAC) test



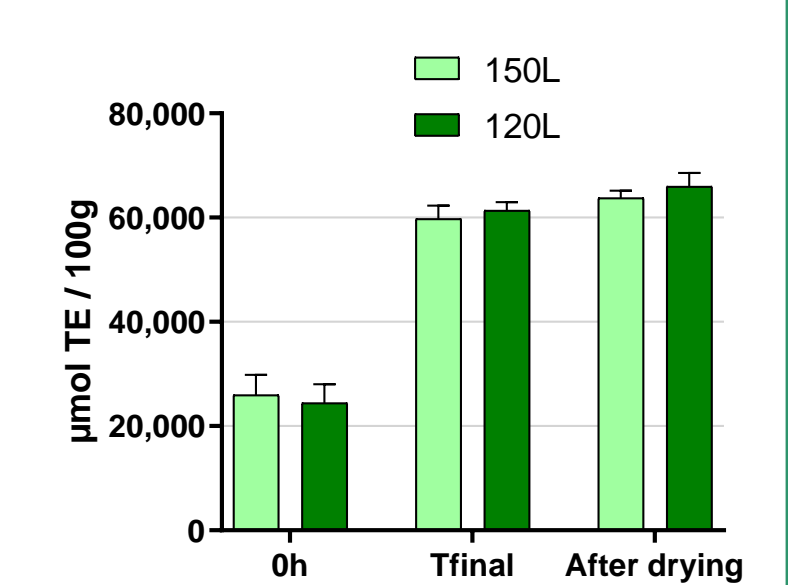
Hydrolysis temperature



Hydrolysis stop

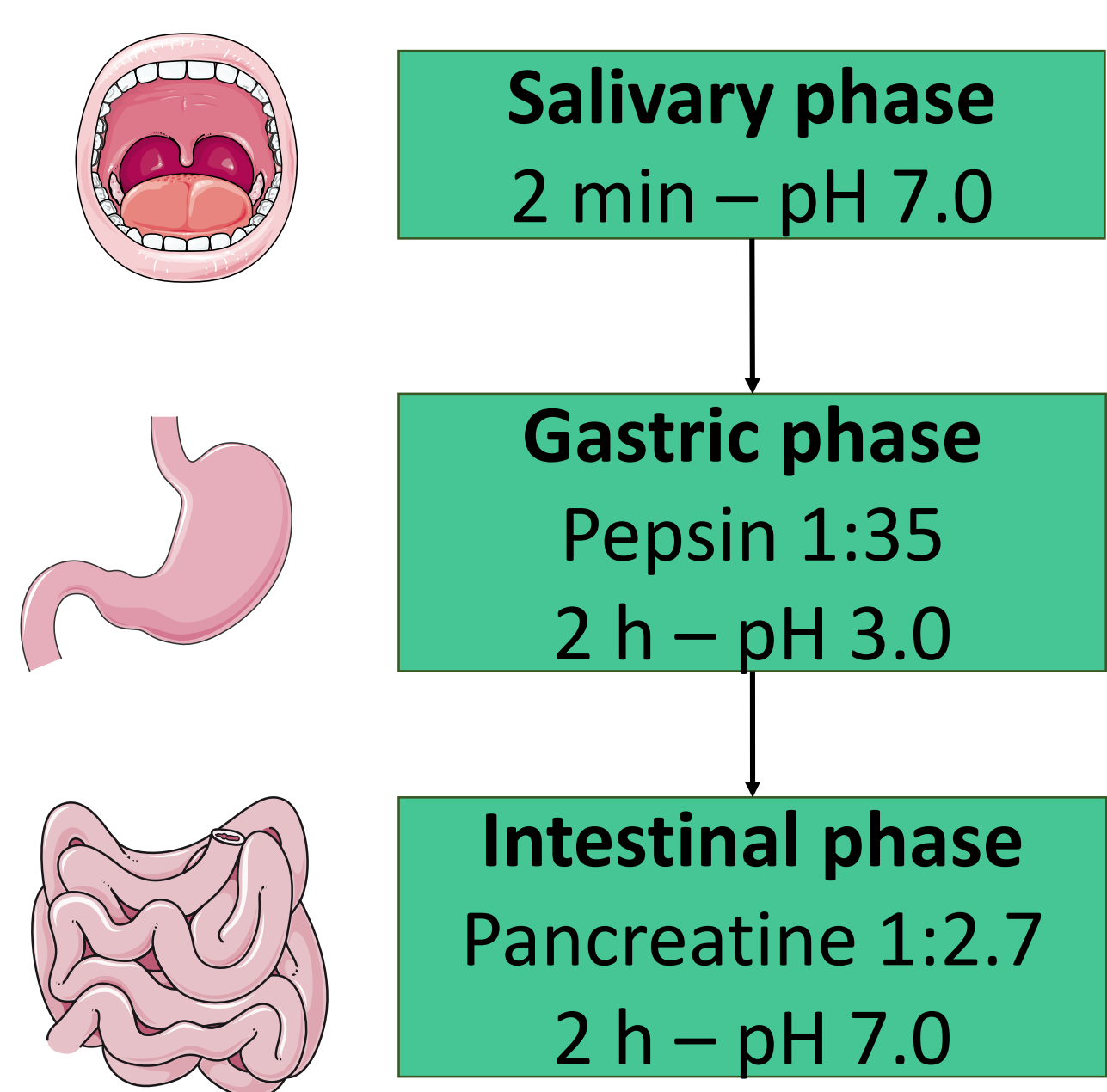


Scale up



Bio-activities and proteomics

Simulated gastro-intestinal digestion

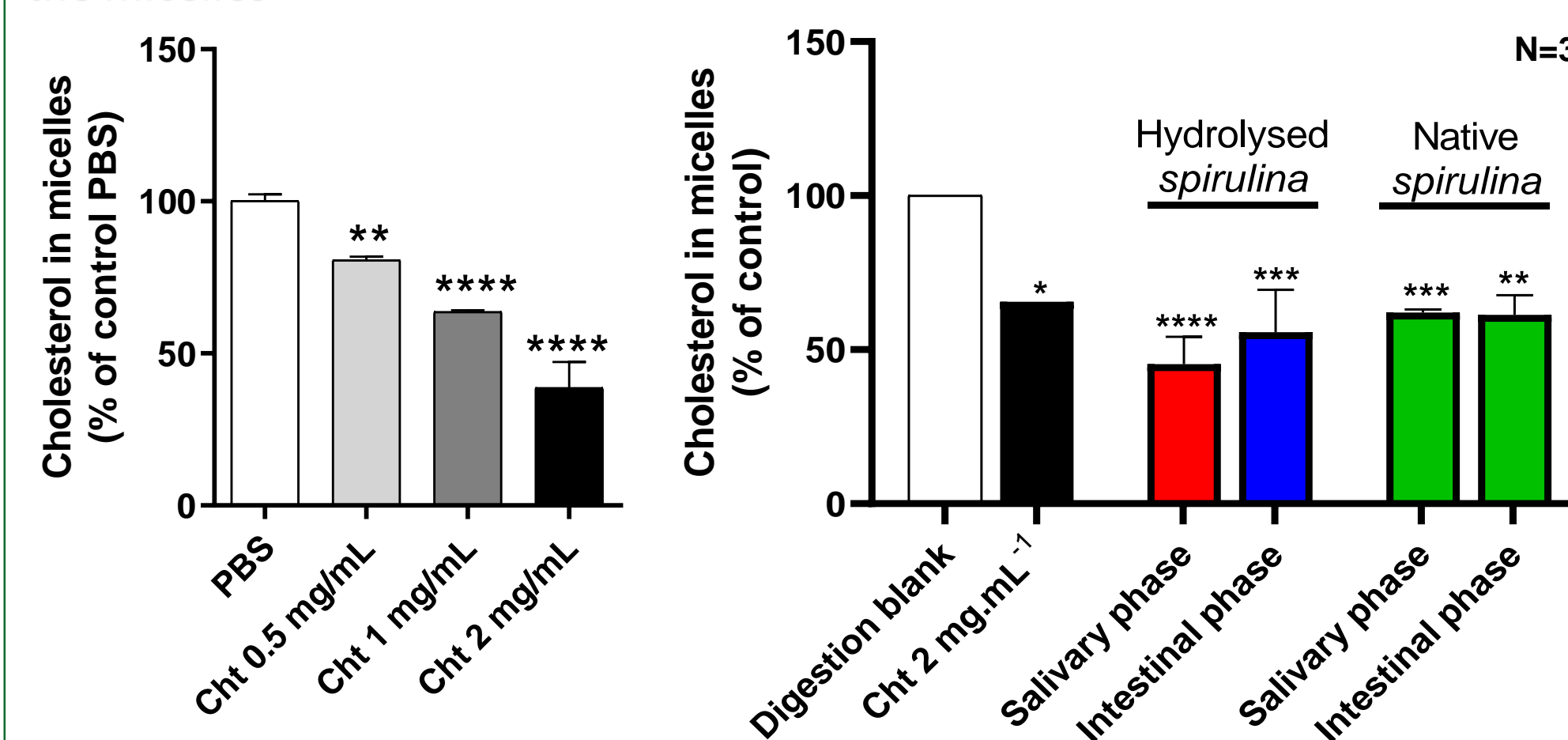


Adapted from 3 and 4

Hypocholesterolemic activity

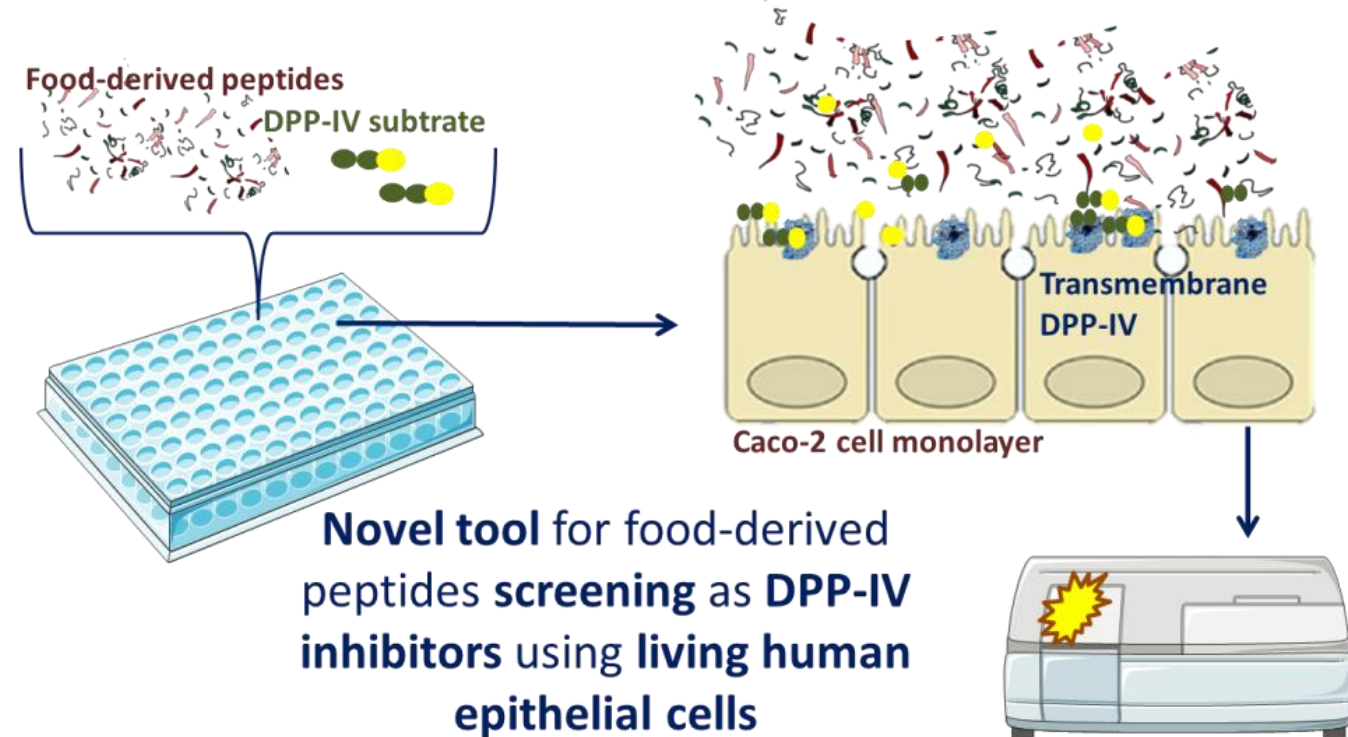
Effect on cholesterol micellar solubilisation

- 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

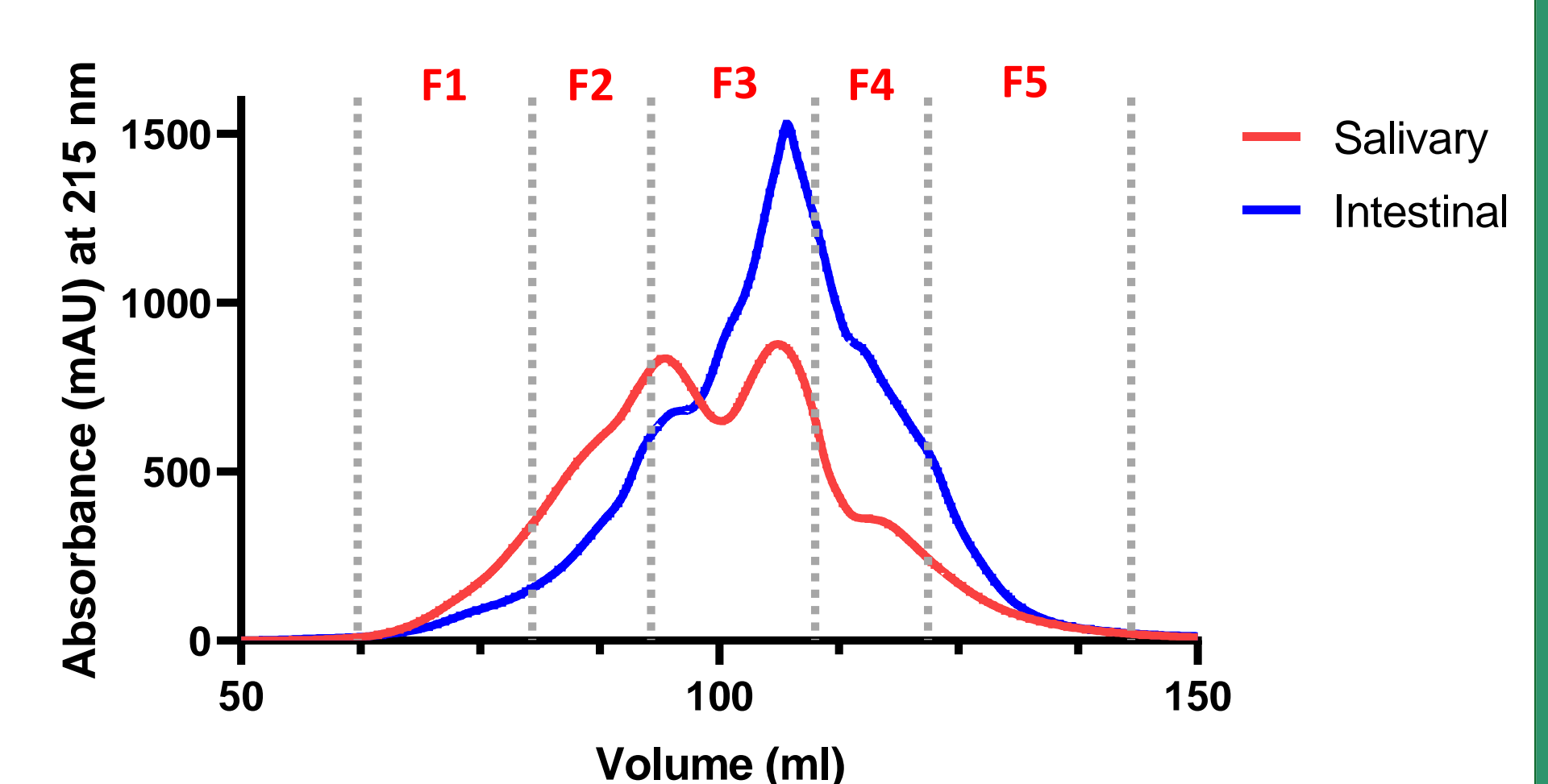


The more the activity of DPP-IV is inhibited, the less it degrades GLP-1. → Hypoglycemic effect

DPP-IV IC₅₀ of hydrolysates (mg.mL⁻¹)

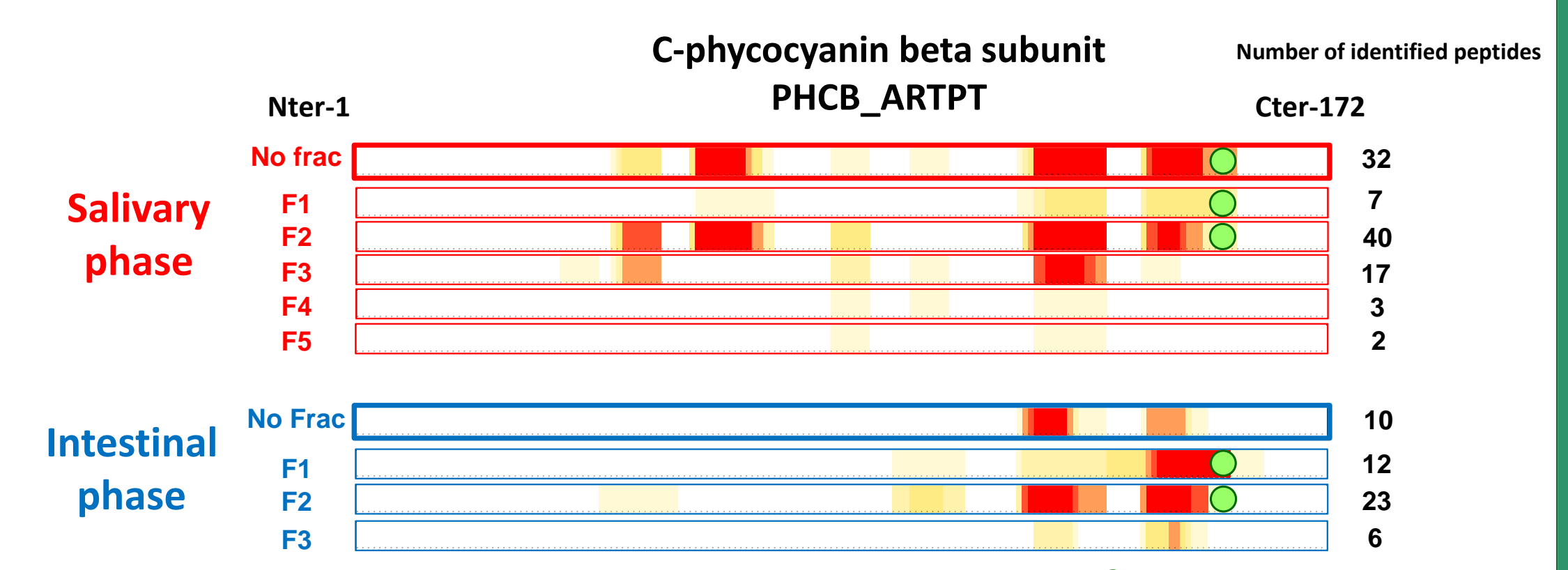
Sample	DPP-IV IC ₅₀ of hydrolysates (mg.mL ⁻¹)	
	Salivary phase	Intestinal phase
Hydrolysed <i>spirulina</i>	1,59	1,20
Native <i>spirulina</i>	5,39	1,55
Digestion blank	1709681	358,23

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.

1. Abreu AP, Martins R, Nunes J (2023) Emerging Applications of Chlorella sp. and Spirulina (Arthrospira) sp. Bioengineering
2. 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
3. Brodtkorb A, Egger L, Alminger M, et al (2019) INFOGEST static in vitro simulation of gastrointestinal food digestion. Nat Protoc
4. 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