IMPACT OF GASTROINTESTINAL DIGESTION ON BIOACTIVE PROPERTIES OF ALGAE EXTRACTS

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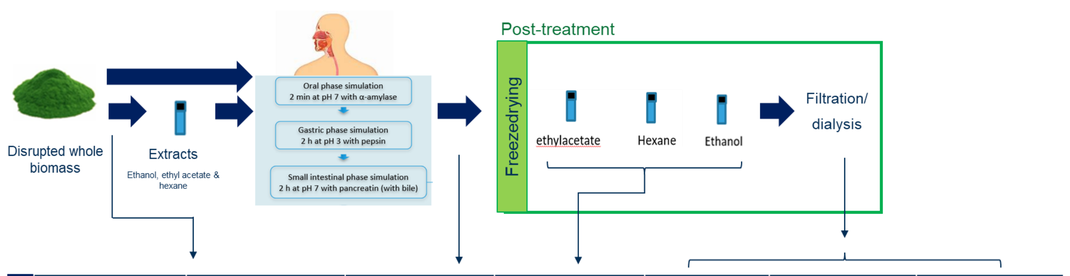
Introduction

- Algae biomass is a source of multiple known and unknown bioactive compounds.
- Key-question: Are bioactive compounds still bioactive after gastrointestinal digestion?

Conclusions

- A test procedure was elaborated
  - taking into account the hydrophobic nature of the algae extracts;
  - while minimizing the risk that compounds added during the digestion trial impact the subsequent bioactivity trial.
- No toxicity was observed in the samples nor the blanks.
- Digestion did impact the bio-activity level in some extracts - reductions as well as increases were observed.
- The first results indicated that most bioactive extracts remained bioactive after the digestion.
- Results are encouraging for the use of algae extracts as bioactive ingredients for functional food & pharma applications.

Procedure establishment



Check bioactive property	Start Vito-1/VITO-10	Digested VITO-11	Re-extract VITO-12/17/18	< 1 kDa VITO-14	1-10 kDA VITO-15	10 kDa VITO-16
Whole algae	31/54	60				
Ethanol extract	82; ++; 36		76; +/-; 61	86; +/-; 52	58; +/-; TBD	25; ++; TBD
EA extract	86; ++;45		87; +/-; 56			
Hexane extract	86; ++;43		37; +/-; 47			

COX-2 % inhibition(VITO); DPPH (VITO); Cell based anti-inflammation – m-IL-6 inhibition (MIGAL)

Figure 2: Simplified schematic overview of the procedure to evaluate the impact of gastrointestinal digestion on the bioactivity in algae biomass and extracts (Top) en first bioactivity results obtained via digestion of the whole disrupted algae biomass (bottom),

Bioprospecting within algae4IBD

- Aim: finding bioactive compounds (small sized – 0.1-10 kDa) that can be useful to prevent and cure IBD (Inflammatory Bowel Disease)

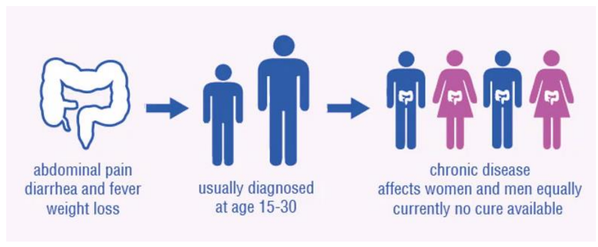


Figure 1: Inflammatory Bowel disease (IBD).

- How? Screening of water, ethanol, ethyl acetate and hexane extracts from > 150 algae strains
- Bioactivity assays used:

Table 1: Bioactivity assay.

Property	Test approach	Reporters
Anti-inflammatory; Anti-pain	High throughput screening (HTS) with cellular reporter gene assays	Cytokines IL-6 & TNF-α
	Validation in THP-1 & RAW cell cultures	Cytokines IL-6 & TNF-α
	Spectrophotometry based	Cyclooxygenase 1 &2 (COX-1; COX-2)
Anti-oxidant	Spectrophotometry based	ABTS and DPPH scavenging, Ferric Iron Reducing, Oxygen Radical Absorbance Capacity (ORAC); iron and coper chelating assays

- Procedures were elaborated to 1) digest whole algae biomass as well as ethanol, ethyl acetate and hexane extracts of algae; and 2) to post-treat the digested biomass for bioactivity testing (Figure 2).
- Considered: oral, gastric and small intestinal phase.
- Post-treatment is required to remove salts, glucose and enzymes from the digested material that might impact bioactivity assays.
- Results with whole disrupted biomass:
  - Extraction of freeze-dried digest was identified as a suitable post-treatment approach.
  - Additional filtration (dialysis, performed on ethanol extract only) did not have added value and led to losses via the <1 kDa fraction.
  - Majority of bioactive compounds in the digest are sized < 10 kDa.
  - Anti-inflammatory properties were well preserved in digested material; while antioxidant properties were reduced.
- Digestion of hydrophobic extracts was enabled via dilution of the substrate and addition of lipid solubilizing compounds.

First validation of the procedure – in vitro bioactivity tests

- The procedures that were established for algae extracts were validated on extracts from different microalgae species.
- Samples before and after digestion were distributed among project partners for extensive in vitro bioactivity testing - Results are summarized in Table 2.
- In general, bioactive properties were mostly preserved after digestion, for anti-inflammatory as well as anti-oxidant properties.

Table 2: Overview of bio-activity verification results (dark green: very bioactive; light green: good bioactivity ; orange: some bioactivity; Red: very low bioactivity).

	Sample type	Reporter inhibition hTNFα [THP-1] AUC	Reporter inhibition hIL6 [RAW 264.7] AUC	Reporter inhibition mTNFα [RAW 264.7] AUC	Reporter inhibition NFkB [THP-1] AUC	Toxicity % viable cells in RAW264.7 cells	Inhibition of IL-6 in RAW264.7 cells (% inhibition) 100 µg/mL	Inhibition of TNFα in RAW264.7 cells (% inhibition) 100 µg/mL	Cox-2 (% inhibition) 10 mg/mL	Ferric Iron Reduction Activity (%)	Copper chelating activity (%)	COX-1 % inhibition	COX-2 % inhibition	ORAC TE value Trolox/g extract	ABTS IC50 (µg/mL)
		Cell-based assays							Spectrophotometry based assays						
	Performed by	IMG	IMG	IMG	IMG	MIGAL	MIGAL	MIGAL	VITO	CCMAR	CCMAR	TEAGSC	TEAGASC	UNINA	UNINA
Microalgae species 1	Ethanol extract before digestion					No									
	Ethanol extract after digestion					No									
	Ethyl acetate extract before digestion					No									
	Ethyl acetate extract after digestion					No									
	Hexane extract before digestion					No									
	Hexane extract after digestion					No									
Microalgae species 2	Ethanol extract before digestion					No									
	Ethanol extract after digestion					No									
	Ethyl acetate extract before digestion					No									
	Ethyl acetate extract after digestion					No									
	Hexane extract before digestion					No									
	Hexane extract after digestion					No									
Blanc	Blancs – 1 (Ethanol)					No									
	Blanc -1 (ethyl acetate)					No									
	Blancs – 2 (hexane)					No									
Microalgae species 3	Ethanol extract before digestion					No									
	Ethanol extract after digestion					No									
	Ethyl acetate extract before digestion					No									
	Ethyl acetate extract after digestion					No									
Positive control															

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