EVALUATING SUBCRITICAL WATER EXTRACTION FOR RED ALGAE RESIDUE VALORIZATION: CHEMICAL COMPOSITION AND BIOLOGICAL ACTIVITY

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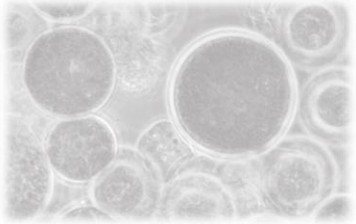
LAQV requimte LABORATÓRIO ASSOCIADO PARA A QUÍMICA VERDE

i4HB Institute for Health and Bioeconomy

UCIBIO Applied Molecular Biosciences Unit

Contextualization…

Gelidium sesquipedale



Microalgae
Unicellular microorganisms

Macroalgae
"Seaweeds"

PIGMENTS

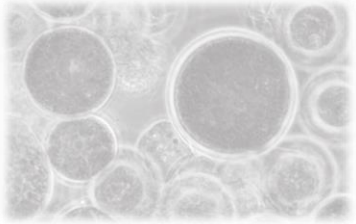
Phaeophyta
brown

Rodophyta
red

Chlorophyta
green

Contextualization…

Gelidium sesquipedale



Microalgae
Unicellular microorganisms

Macroalgae
"Seaweeds"

STRUCTURAL CARBOHYDRATES

Agarophytes
AGAR

Carrageenophytes
Carrageenans

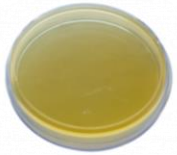
AGAR

Contextualization…

INCREASING AGAR DEMAND

CAGR = 4.2%
(2023-2028)*

Food and beverage



Pharmacy

Cosmetic

Gelidium sesquipedale → **AGAR** - - - - - - - - - → MACROALGA RESIDUE

INCREASING ALGA RESIDUE GENERATION = ACUMMULATION

Leaders of the European agar market*

HISPANAGAR

Setexam
THE AGAR SPECIALISTS

ROKO

TIC GUMS

Foodchem

*www.mordorintelligence.com/es/industry-reports/europe-agar-market

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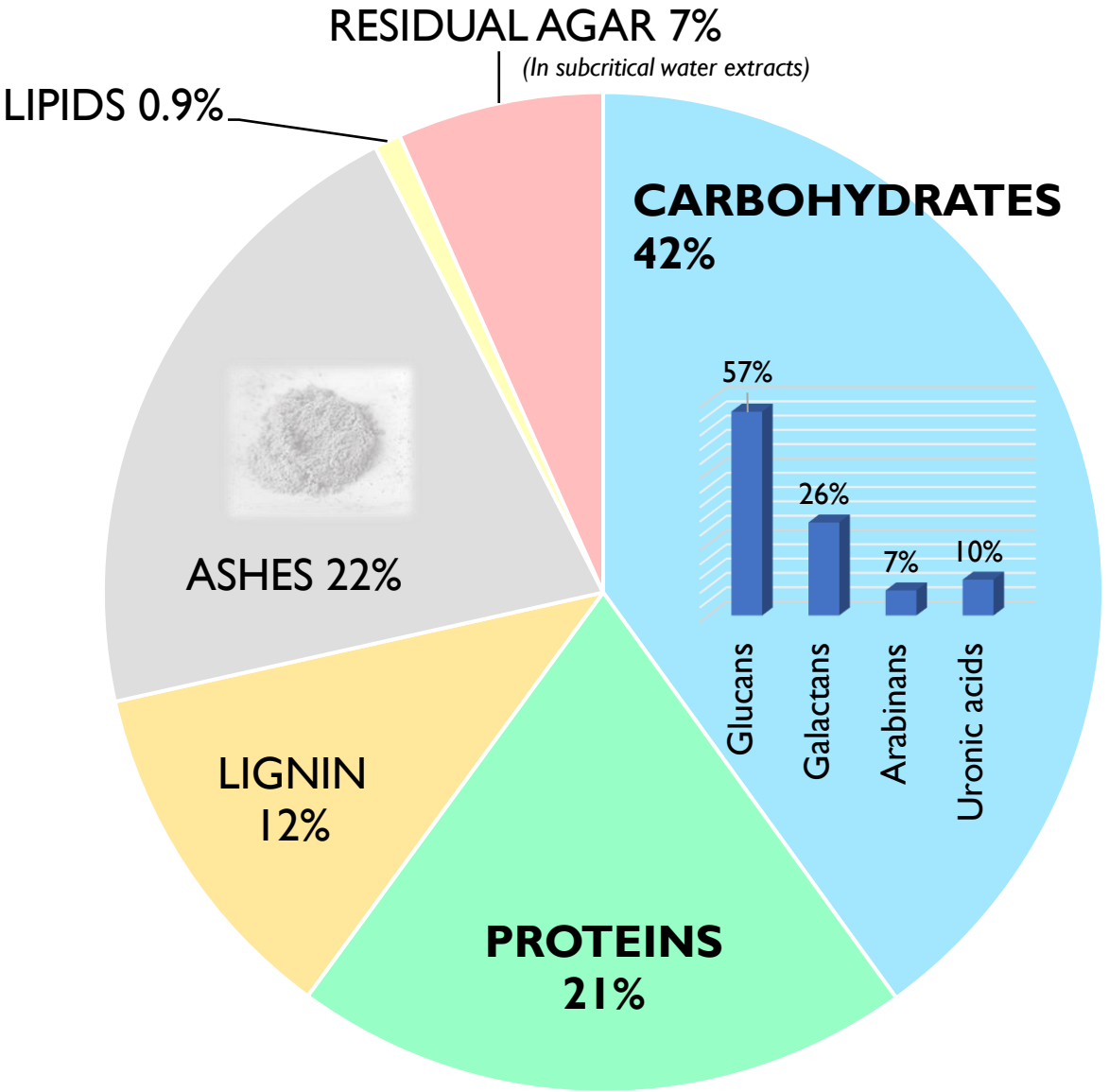
MACROALGA RESIDUE
CHEMICAL COMPOSITION



Contextualization…

MACROALGA RESIDUE
VALORIZATION ⟹ **BIOREFINERY CONCEPT**

Residues are treated as new raw materials to be reincorporated into industrial processes

TRADITIONAL EXTRACTION METHODS

EMERGING TECHNOLOGIES
(GREEN CHEMISTRY)

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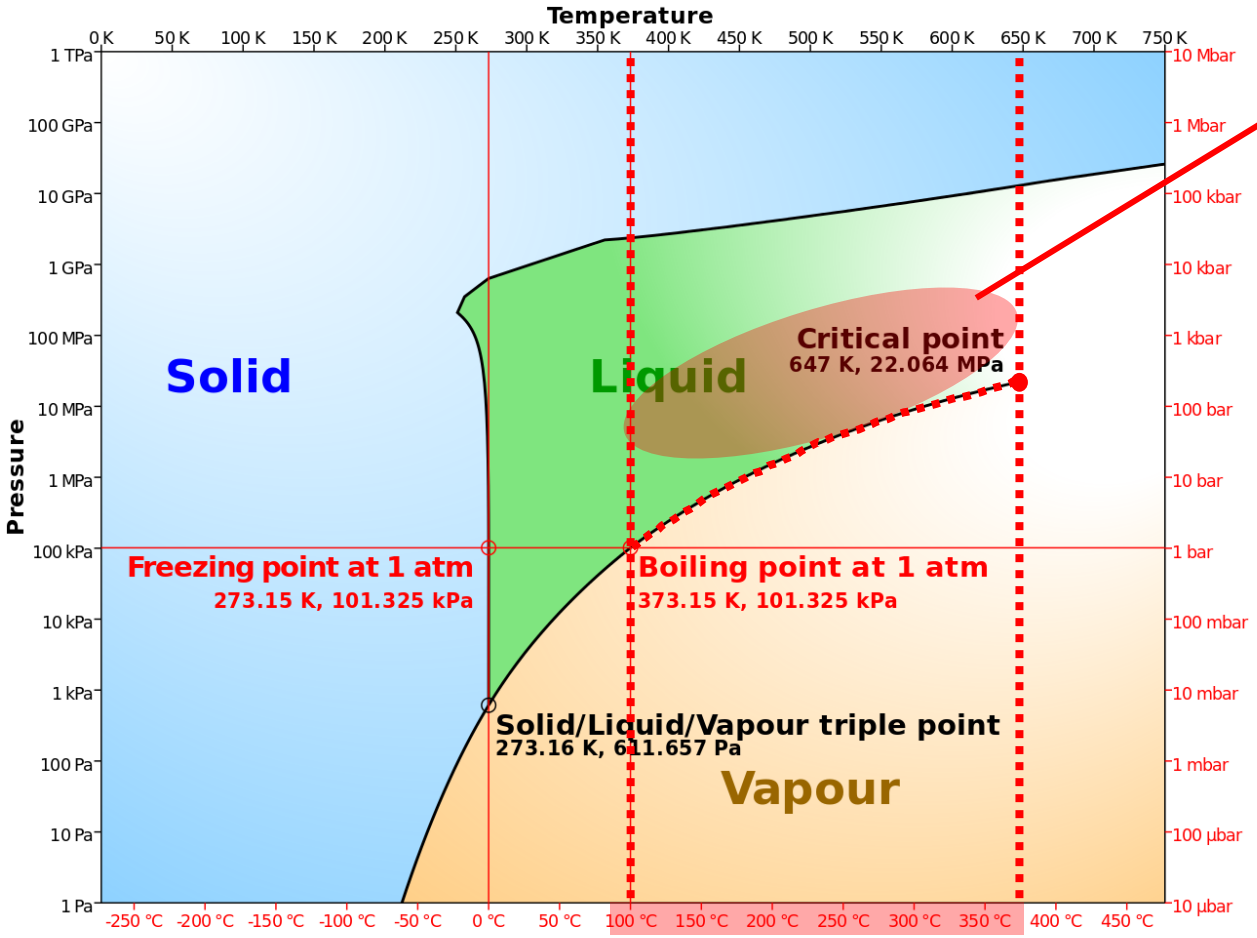
SUBCRITICAL WATER EXTRACTION (SWE)

Non-toxic

Non-flammable

Inexpensive

Safe



SUBCRITICAL WATER

100 – 374°C

> Vapour phase

*Adapted from Plaza & Turner et al. (2015)

Objectives:

- Investigate **Subcritical Water Extraction** as a novel technology to **valorize alga residue** after agar extraction

  EVALUATION OF
  - **Chemical composition**
  - **Biological activity**

- **Compare** the extract obtained by SWE with other obtained by **ethanol conventional extraction**

Raw material

HISPANAGAR

Burgos, Castilla y León, Spain

https://www.hispanagar.com

Oven-drying (45°C)
24 hours

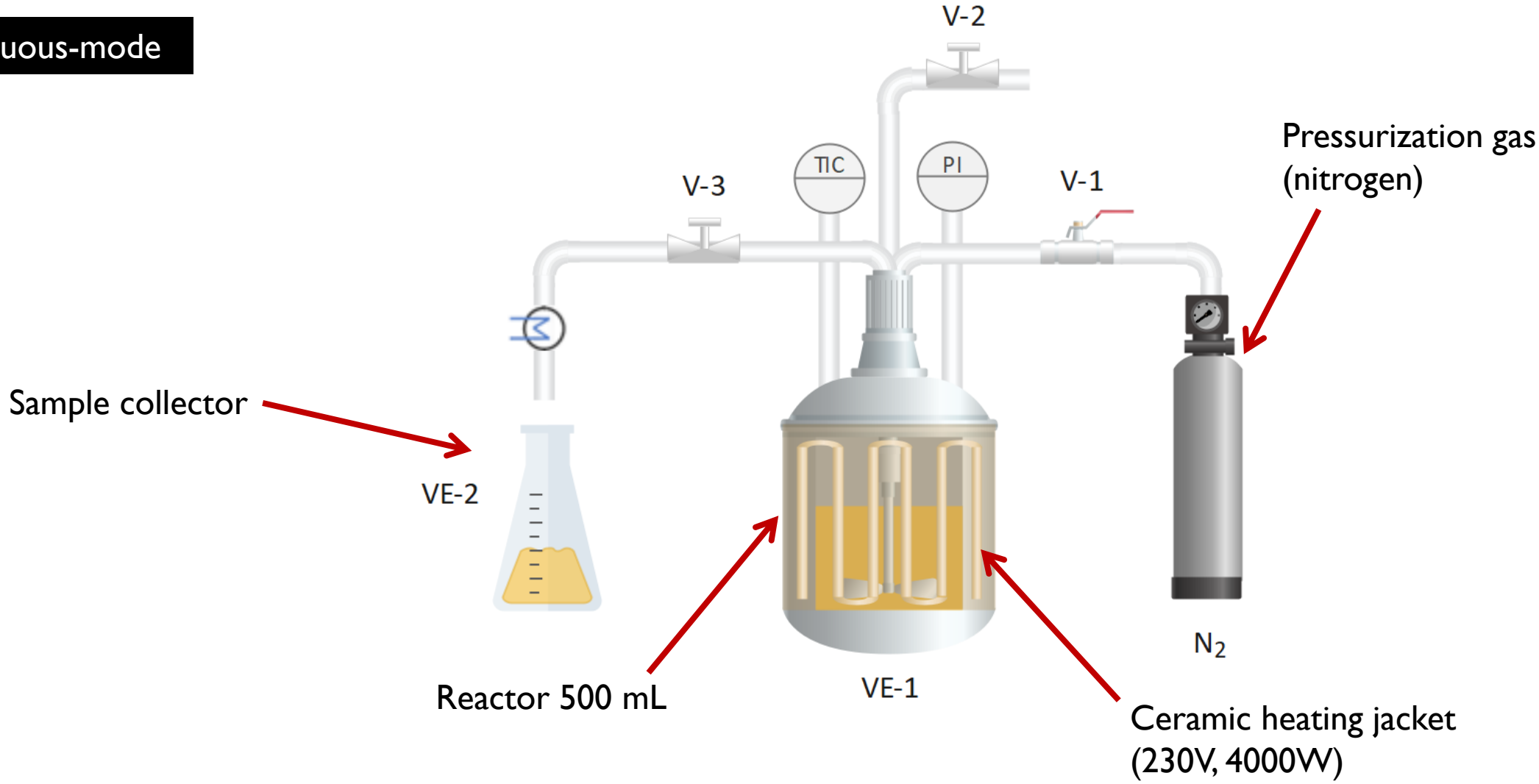
DRY MACROALGA RESIDUE (DMR)

Milling and Sieving

Fraction particle size
< 500 µm

Subcritical Water Extraction (SWE)

Discontinuous-mode



Subcritical Water Extraction (SWE)

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Discontinuous-mode

Optimal conditions

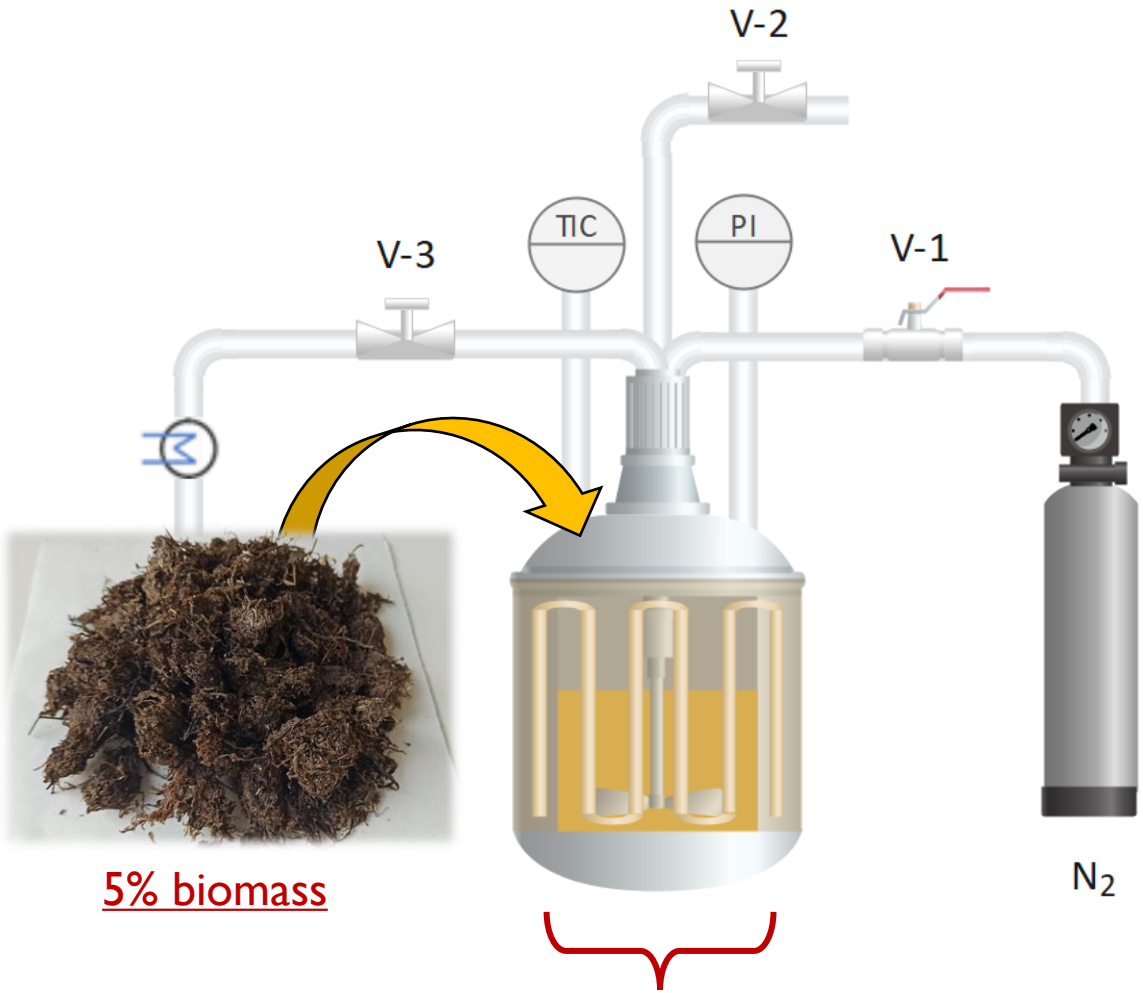
(*Trigueros et al. 2023*)

TEMPERATURE
175°C

PRESSURE
50 bar

TIME
130 minutes

MECHANICAL STIRRING
500 rpm



Dry-powder SWE extract
(**DMR-SWE**)

Ethanol Extraction (EE)

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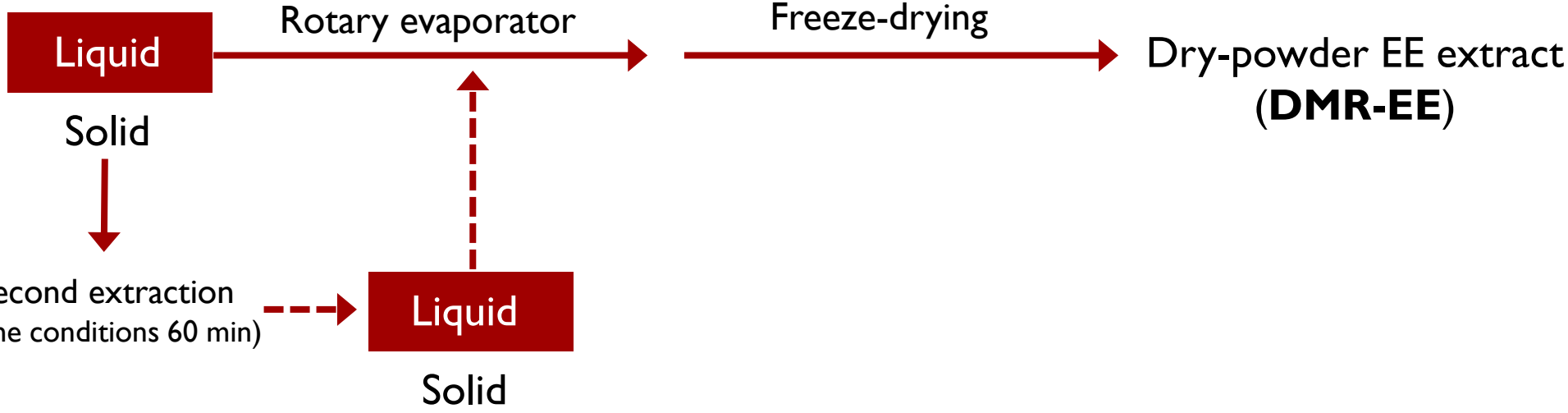
5% biomass:

10 g DMR (<500 µm) + 200 mL solvent

Ethanol 80% in water

25°C, 130 min



Chemical Composition

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Compound ($mg/g_{dry\text{-}extract}$)	**DMR-SWE**	**DMR-EE**
Total Carbohydrates	164 ± 10^{a}	19 ± 2^{b}
Total Protein	11.2 ± 1.0^{a}	1.24 ± 0.06^{b}
Total Phenolic compounds	57 ± 7^{a}	8.0 ± 0.3^{b}

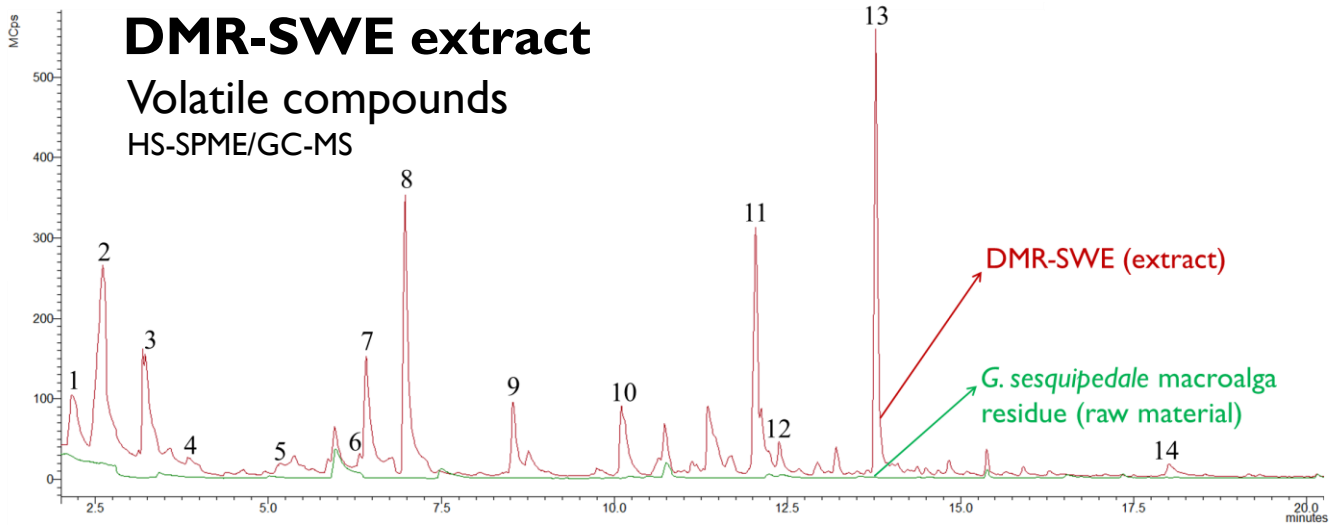
Values expressed as mean ± SEM.
Values with different letters in each row are significantly different, $p<0.01$

Chemical Composition

DMR-SWE extract

Volatile compounds

HS-SPME/GC-MS



	Compounds	RT	Most abundant ions (m/z)	Rmatch	Area/10^7 ± SEM	Concentration (mg/$kg_{dry\text{-}extract}$)
1	Formic acid	2.17	45/46	967	30.7 ± 0.2	697 ± 6
2	Acetic acid	2.60	43/45/60	913	87.5 ± 1.3	504 ± 7
3	1-Hydroxy-2-propanone	3.22	43	889	78 ± 2	527 ± 7
4	Acetoin	3.84	43/45	894	4.9 ± 0.2	-
5	Acetamide	5.17	43/44/59	944	10.1 ± 1.0	386 ± 5
6	Methylpyrazine	6.32	67/94	924	3.1 ± 0.2	0.31 ± 0.00
7	Furfural	6.41	95/96	947	71 ± 2	3.05 ± 0.11
8	2-Furanmethanol	6.97	41/53/98	971	48.4 ± 1.4	95 ± 5
9	1-(2-Furanyl)-ethanone	8.54	95/110	897	26.61 ± 0.08	0.49 ± 0.00
10	5-Methyl-2-furfural	10.11	109/110	947	23.9 ± 1.0	0.31 ± 0.01
11	3-Methyl-1,2-cyclopentanedione	12.04	41/55/69/112	947	24.5 ± 0.7	56 ± 2
12	Benzyl alcohol	12.38	77/79/108	890	7.17 ± 0.14	1.56 ± 0.02
13	Furyl hydroxymethyl ketone	13.78	95	945	134 ± 8	-
14	5-Hydroxymethylfurfural	18.01	41/97	862	2.7 ± 0.6	92.7 ± 0.5

Chemical Composition

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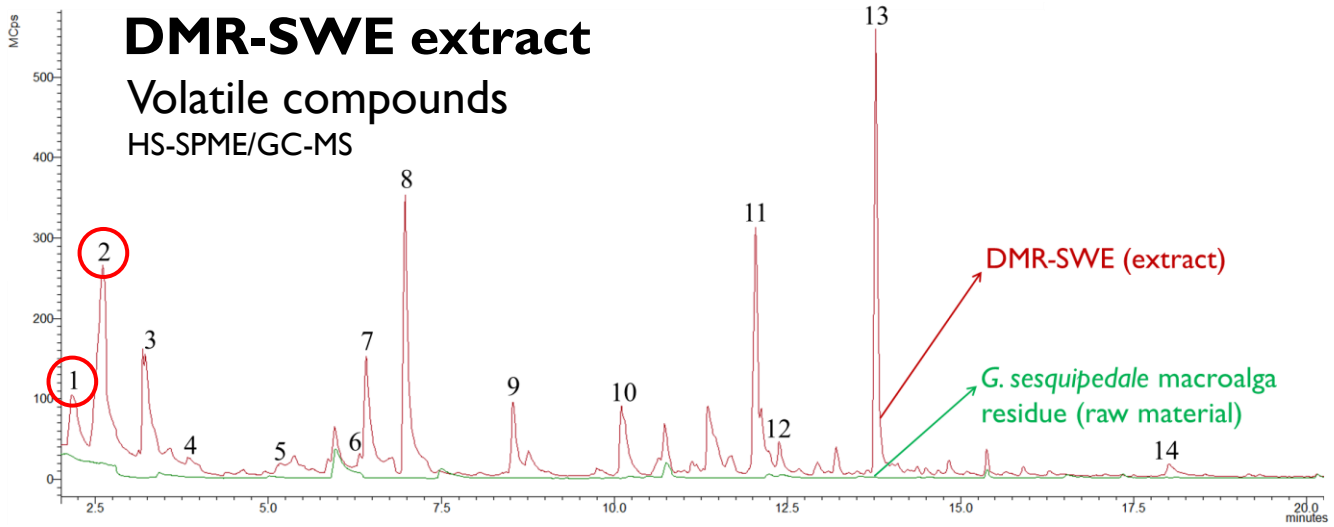
DMR-SWE extract

Volatile compounds

HS-SPME/GC-MS



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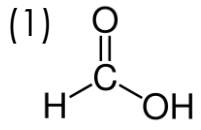
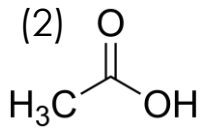
(1) 

(2) 

SUGAR/AMINO ACIDS **DEGRADATION** COMPOUNDS

$pH_{SWEextract}$ = 5.86 ± 0.01

Chemical Composition

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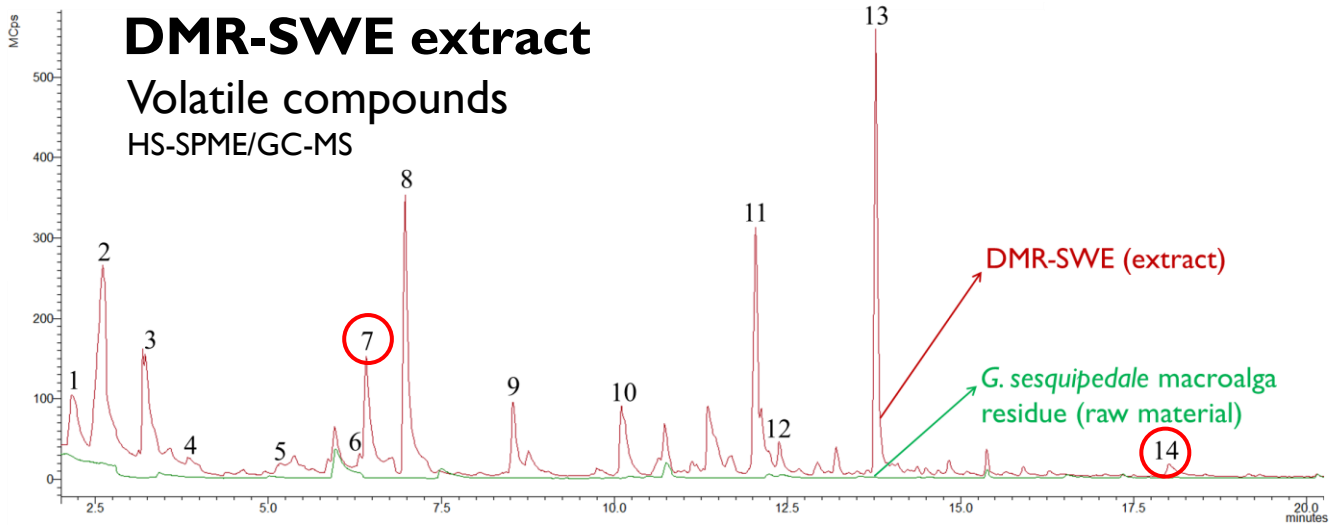
DMR-SWE extract

Volatile compounds

HS-SPME/GC-MS



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MAILLARD REACTION

Non-Enzymatic Browning

\+

(7) Furfural ↓ — Pentoses (↓)

(14) 5-HMF ↑ — Hexoses (↑)

SUGAR/AMINO ACIDS **DEHYDRATION** COMPOUNDS

Chemical Composition

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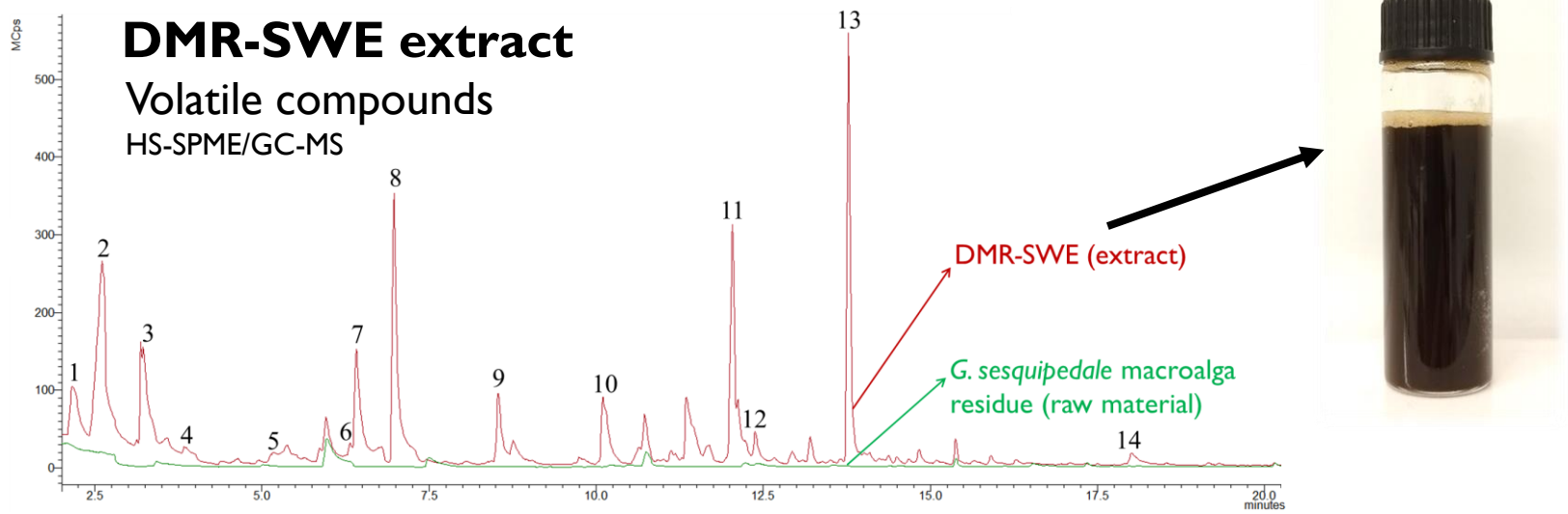

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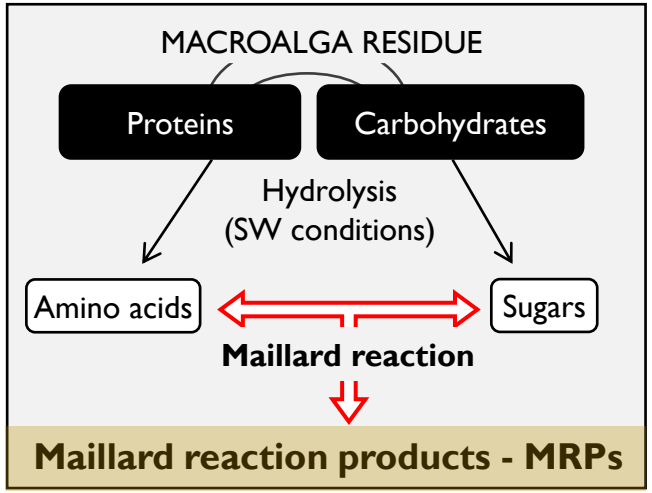
Rate/extent Maillard reaction

Abs 360 = 65.1

Abs 420 = 26.8

MAILLARD REACTION
Non-Enzymatic Browning



Chemical Composition

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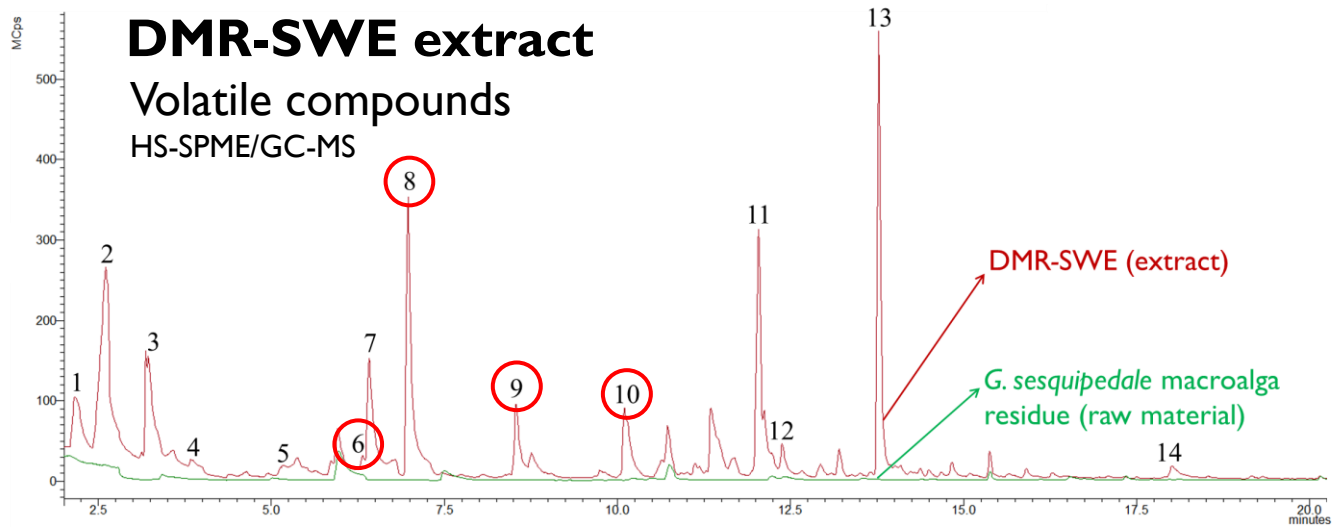

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Roasted, burnt, sweet (OT=60-105000)

Weak, fermented, creamy, caramel (OT=2000)
Smoky, roasty (OT=10000)
Almond, sweet, bitter (OT=500)

OT = Odor threshold in ppb

*Bento-Silva et al. (2022)

Biological Activity

Antioxidant activity

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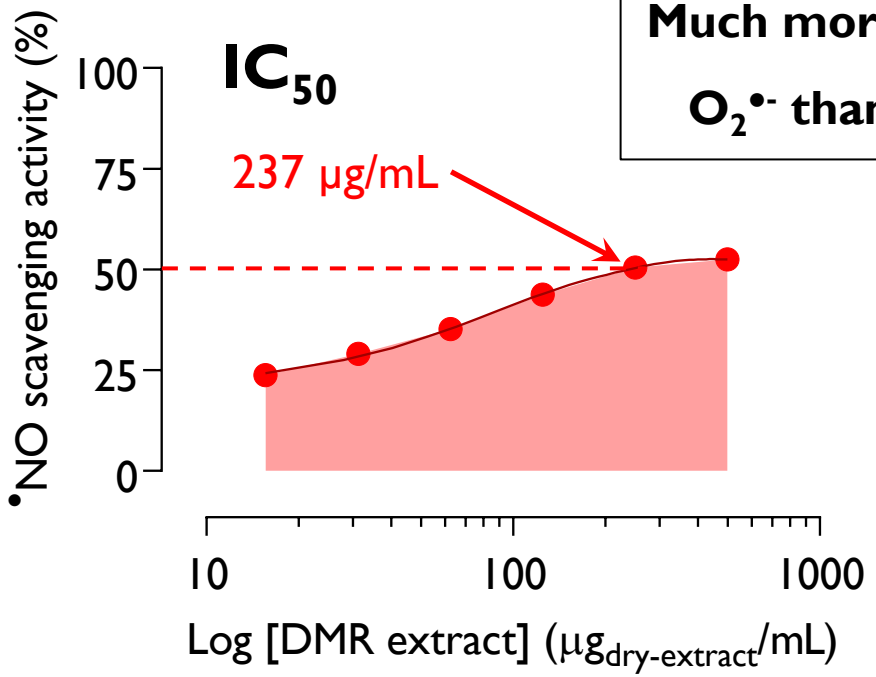
DMR-SWE extract

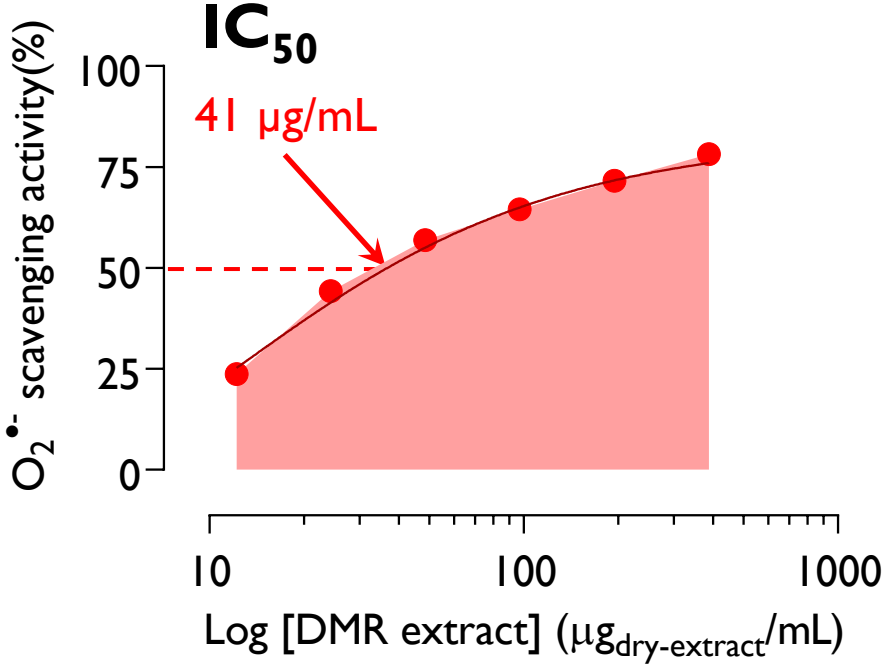
Scavenging potential = Concentration dependent manner

Much more active for $O_2^{\bullet-}$ than for $^{\bullet}NO$

Nitric Oxide ($^{\bullet}NO$)



Superoxide ($O_2^{\bullet-}$)

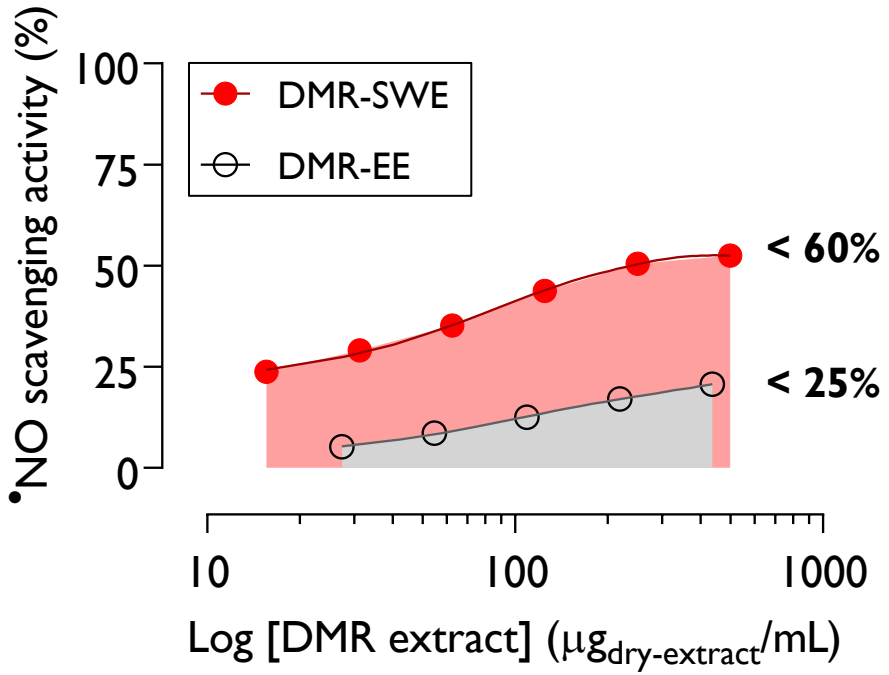


Biological Activity

Antioxidant activity

DMR-SWE / DMR-EE extracts

Nitric Oxide ($^{\bullet}NO$)



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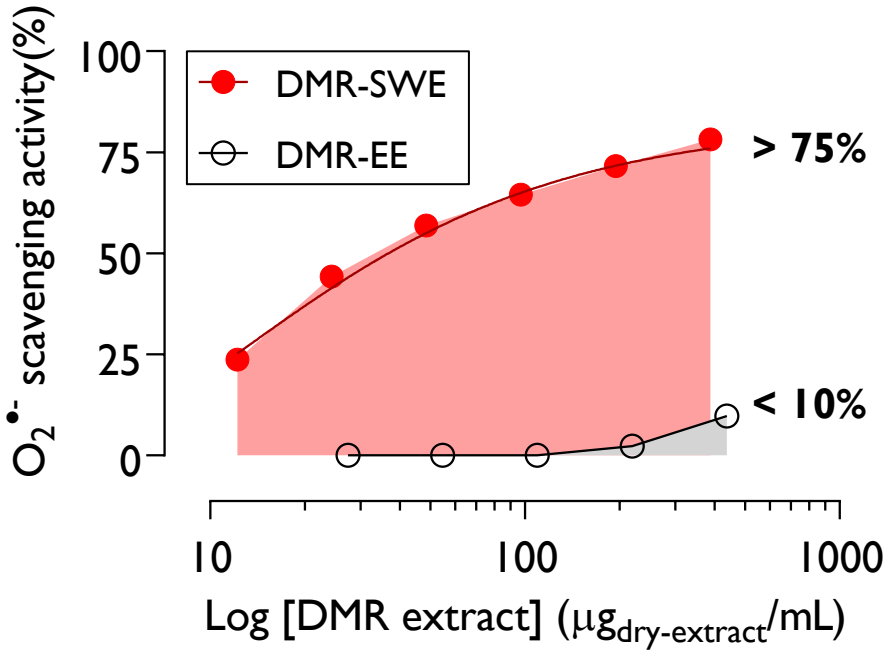

Biological Activity

Antioxidant activity

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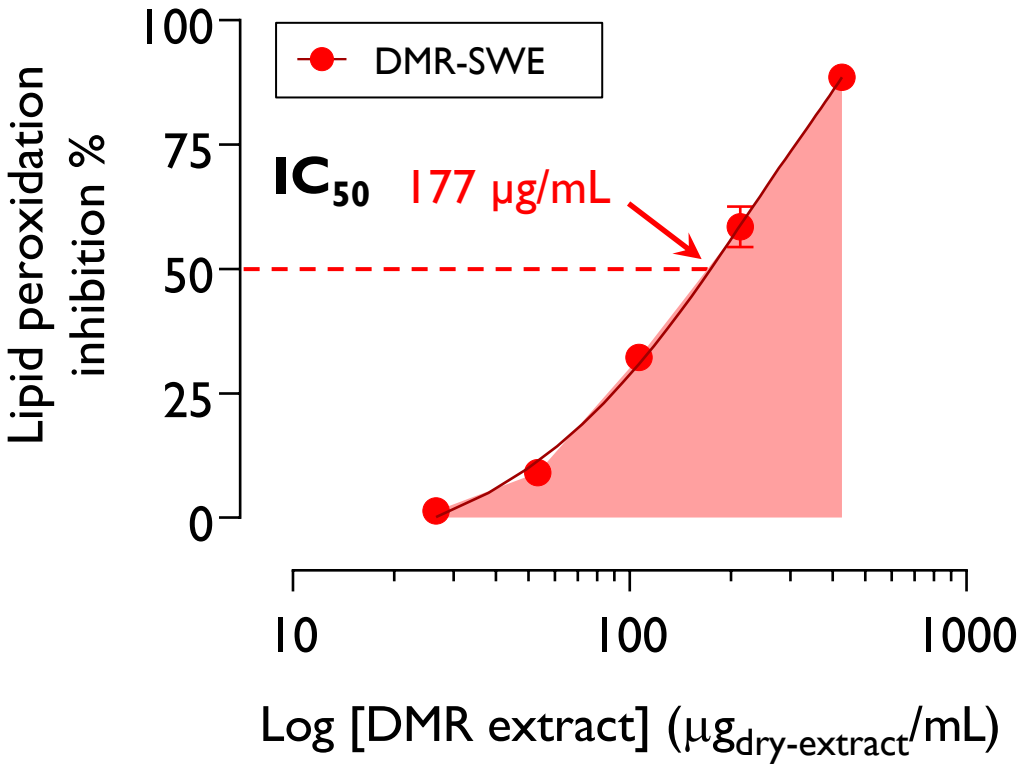
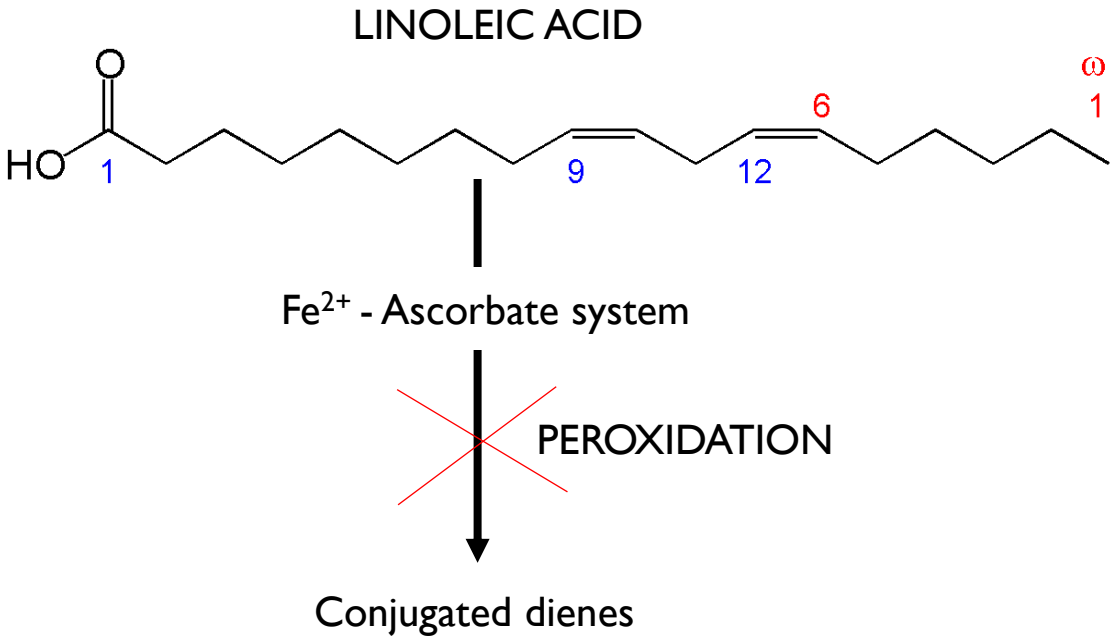
DMR-SWE extract

Lipid Peroxidation



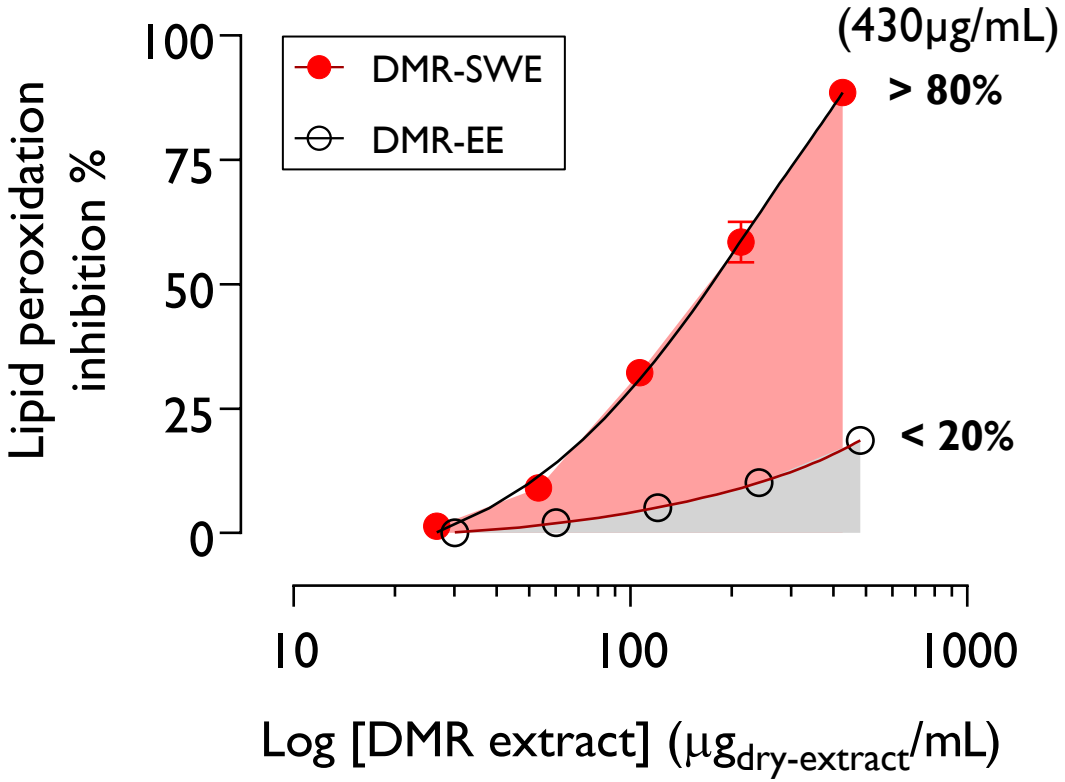


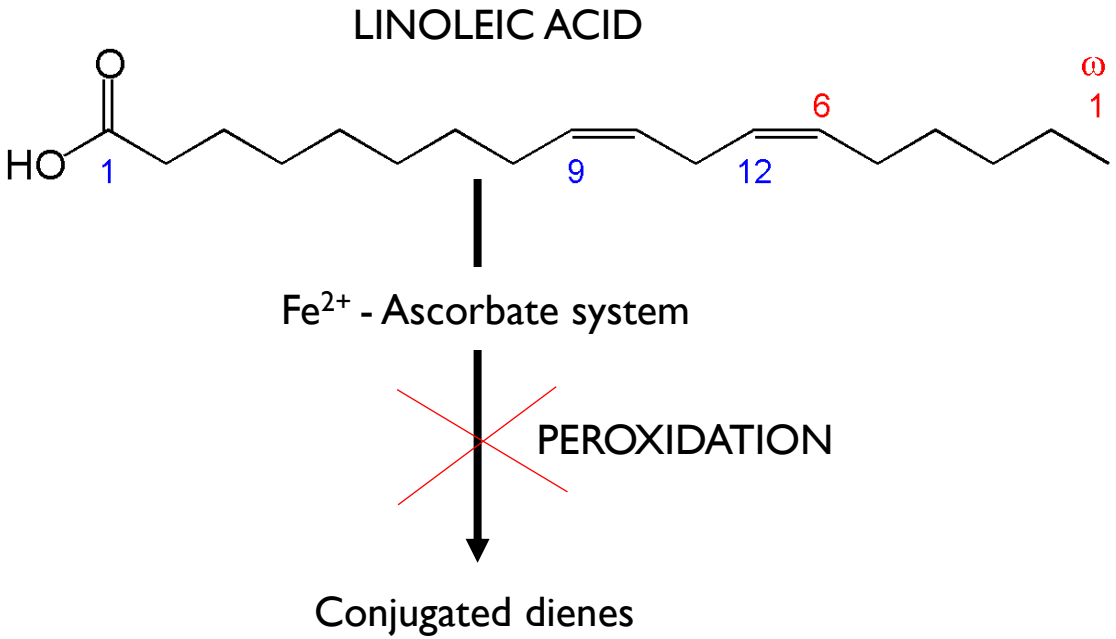
Biological Activity

Antioxidant activity

DMR-SWE / DMR-EE extracts

Lipid Peroxidation





Biological Activity

Anti-inflammatory

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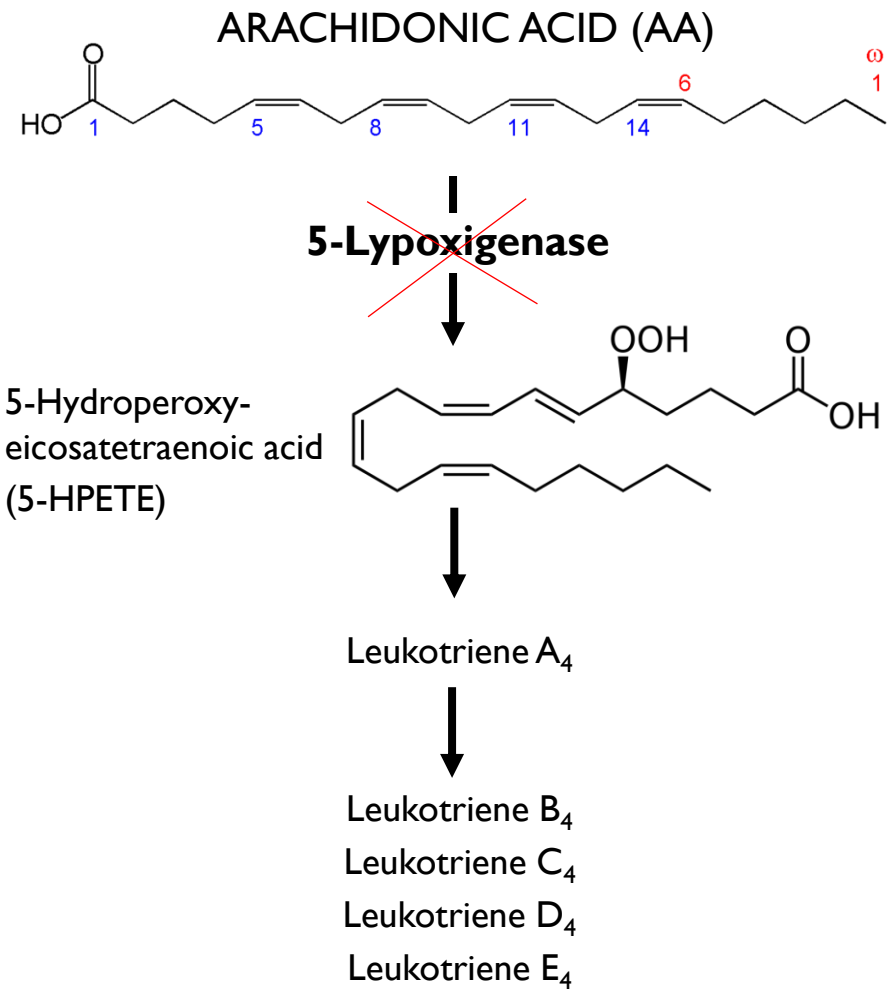
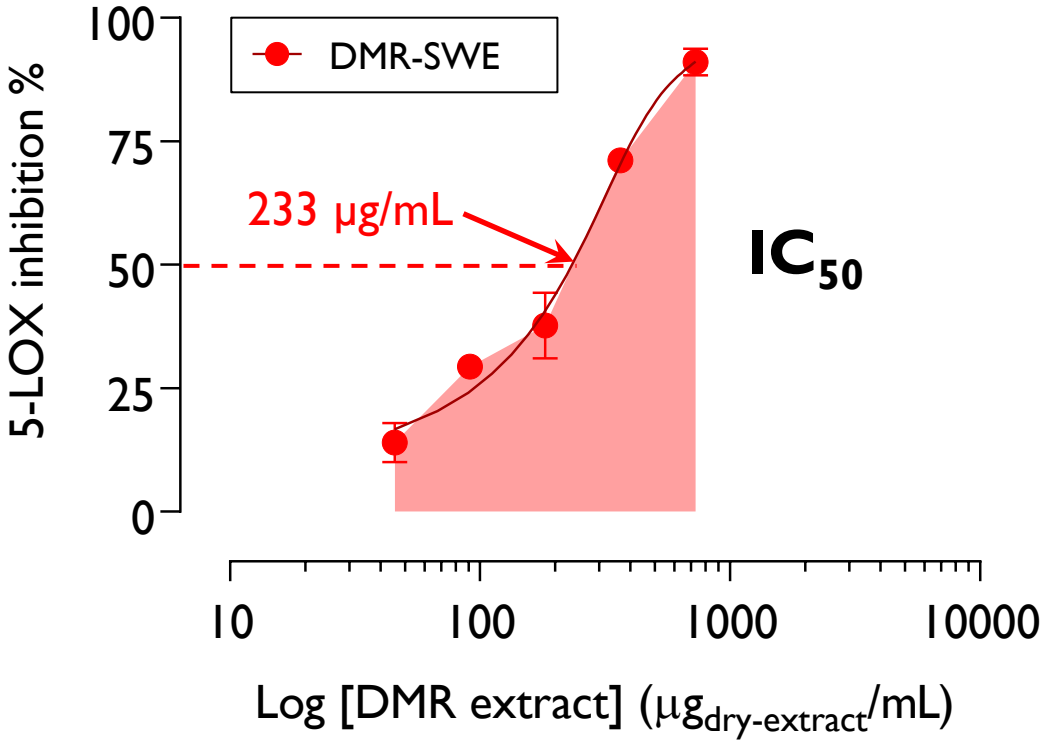
DMR-SWE extract



5-Lipooxygenase



Biological Activity

Anti-inflammatory

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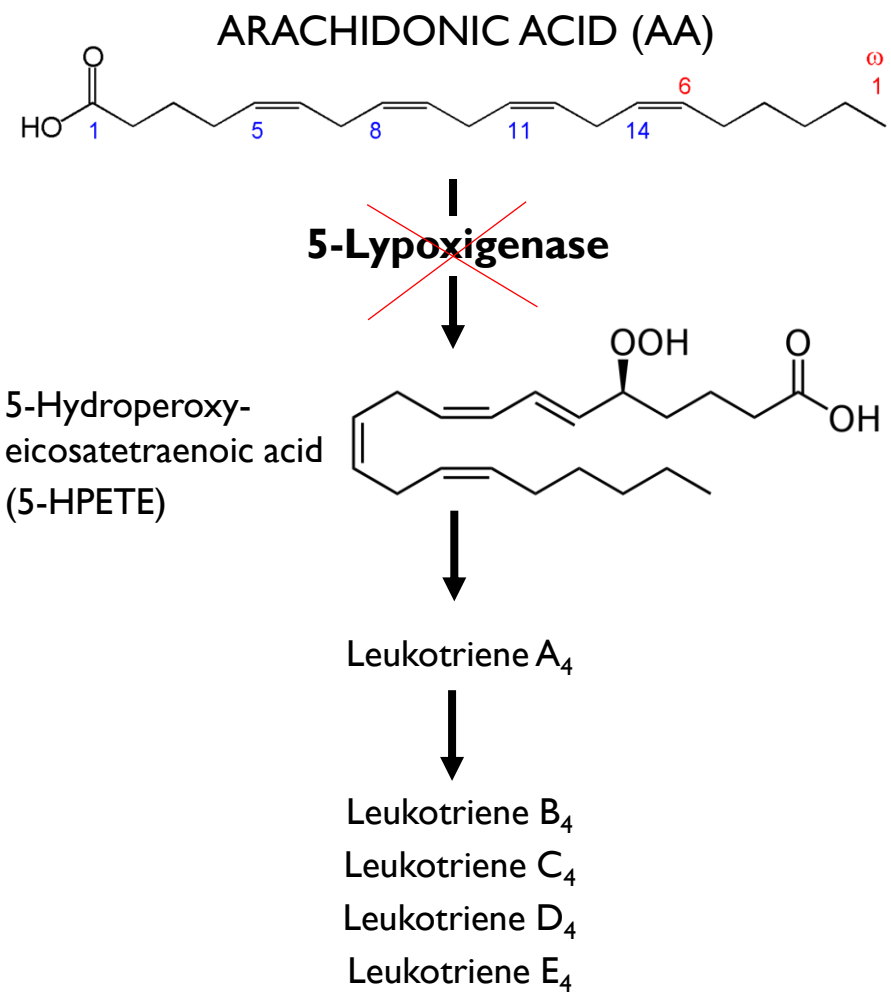
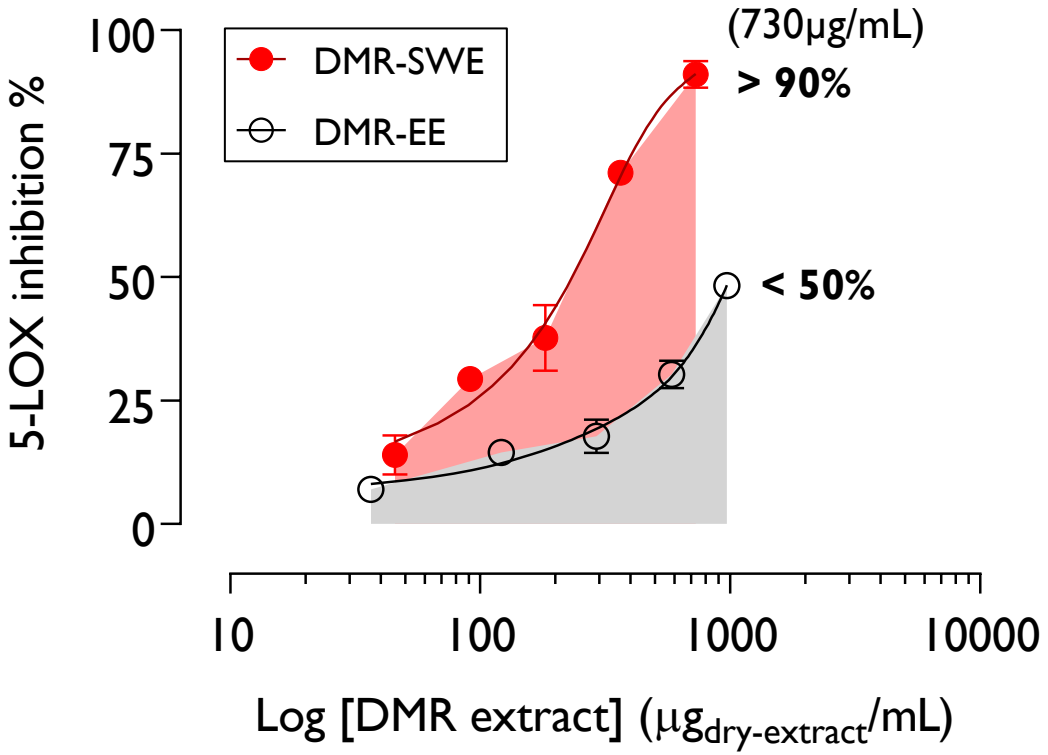
DMR-SWE / DMR-EE extracts



5-Lipooxygenase



Cell viability

XXVII ENCONTRO LUSO GALEGO DE QUÍMICA
22-24 NOVEMBRO 2023
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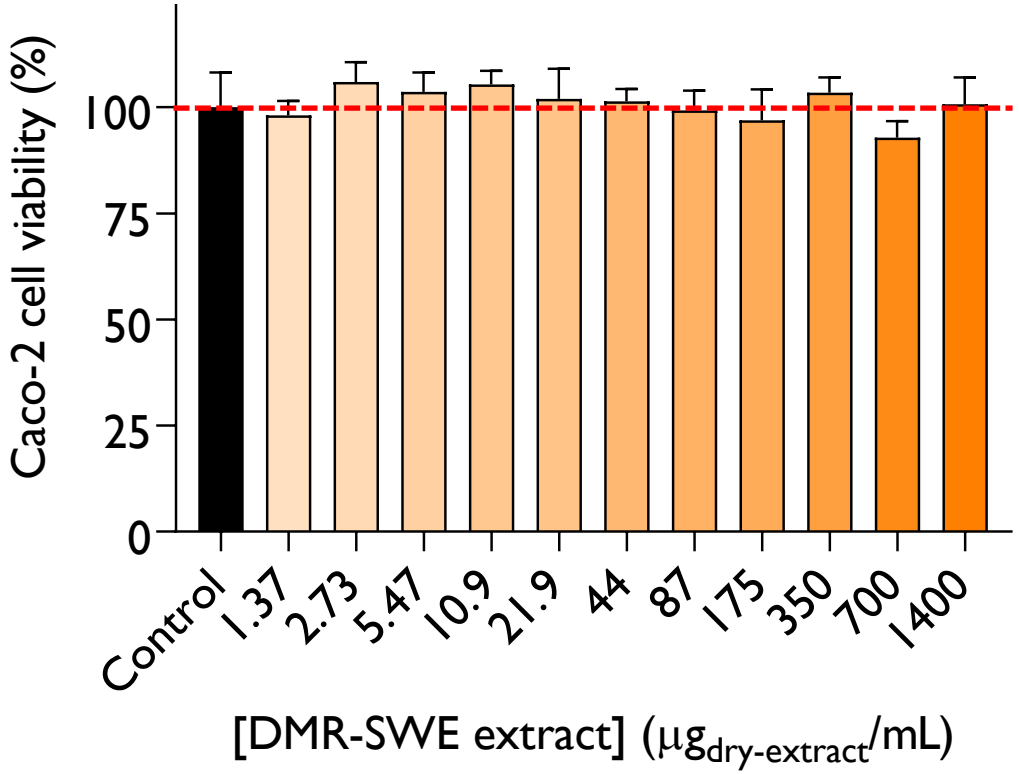
DMR-SWE extract

Caco-2 cells *Human colorectal adenocarcinoma*



The DMR-SWE extract:

→ Does **not cause significant changes** on cell **viability of Caco-2** cell line

→ Shows no cytotoxicity on Caco-2 cells in the concentration range for which the extract exhibits bioactivity:

41-237 µg/mL Antioxidant activity
177 µg/mL Lipid Peroxidation inh. activity
233 µg/mL Anti-inflammatory activity

Final Conclusions…

- The residue after agar extraction from *Gelidium sesquipedale* is a highly **valuable by-product**, owing to its rich content of bioactive compounds.
- SWE is a useful technology for the effective valorization of algal residue, providing extracts with substantial bioactivity, as an **alternative to conventional ethanolic extraction**.
- The SWE extract demonstrates significant **antioxidant capacity** towards nitric oxide and superoxide radical species.
- The efficacy of the SWE extract in inhibiting enzymes associated with various disorders or diseases establishes it as a promising tool in the **management and therapy of diverse pathologies**.

These findings, coupled with the extract's safety profile, underline the effectiveness of SWE extraction as an excellent technology to valorize red alga residue producing extracts that could be incorporated into medical formulations or food products.

THANKS FOR YOUR ATTENTION

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