

Unlocking the Potential of Watercress By-Products: A Solvent-Free and Effective Approach for Phenylethyl Isothiocyanate Extraction

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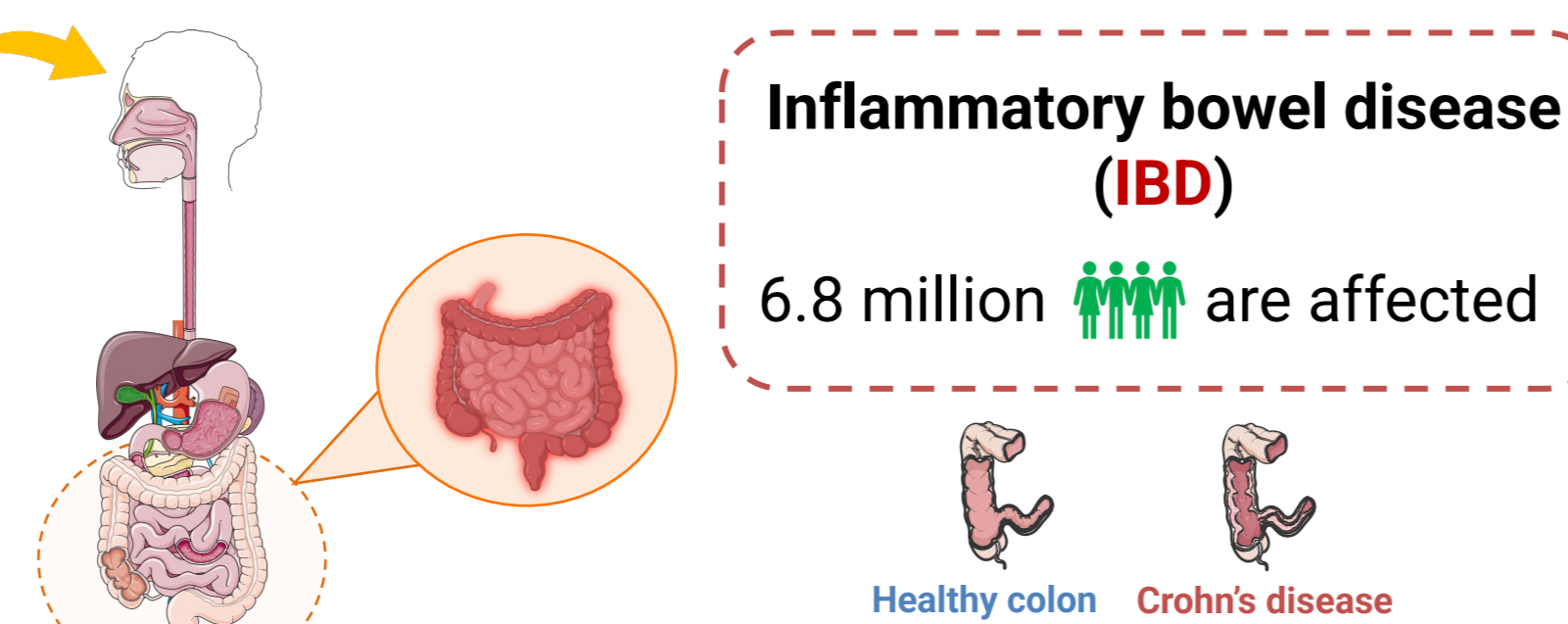
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CONTEXT

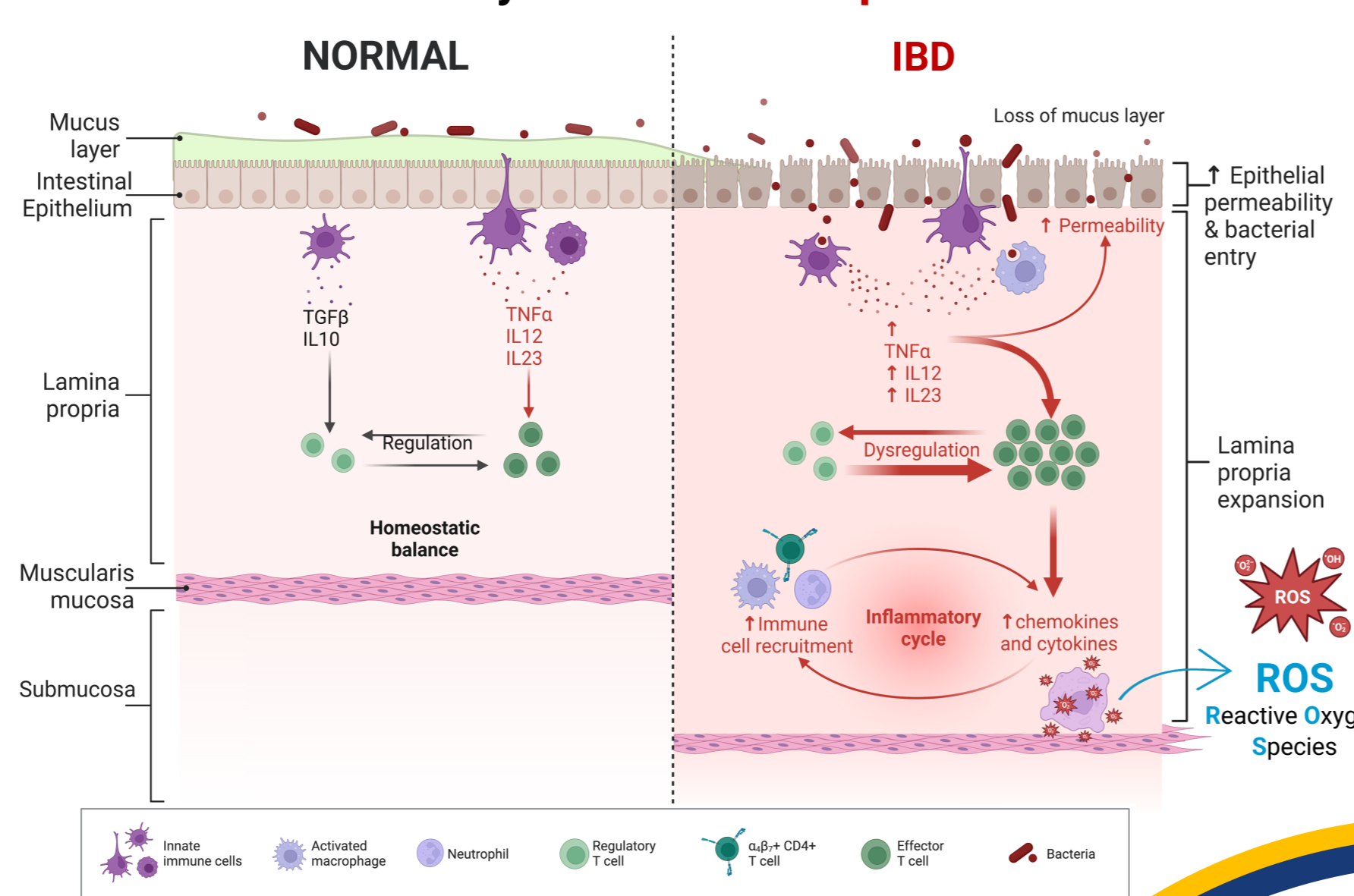
Gastrointestinal diseases have become increasingly **incidence** and **prevalent** over the past decade, affecting up to 40% of individuals worldwide. This has resulted in significant **morbidity**, **mortality**, and **burden on healthcare systems**.

IBD is characterised by chronic **intestinal inflammation**, especially in the colon

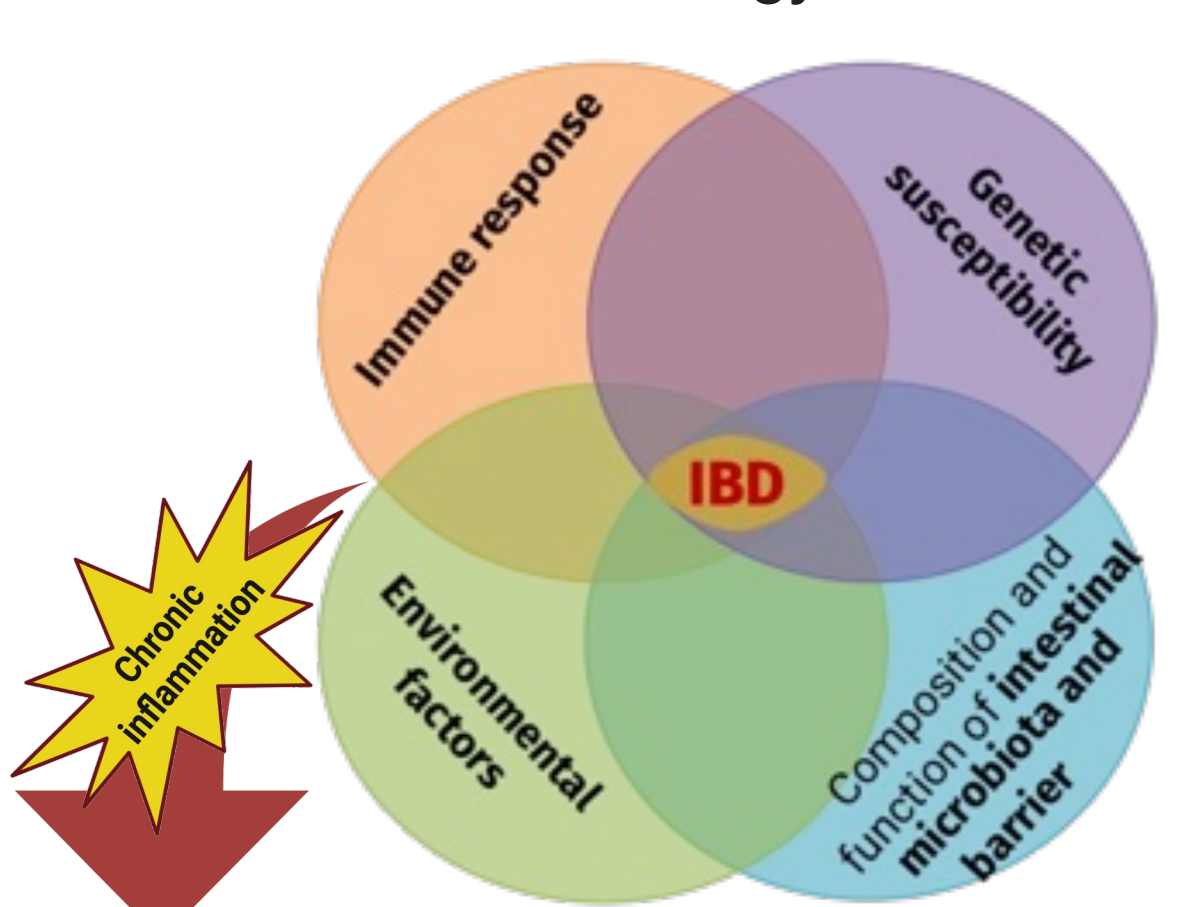
Crohn's Disease
IBD
Ulcerative Colitis



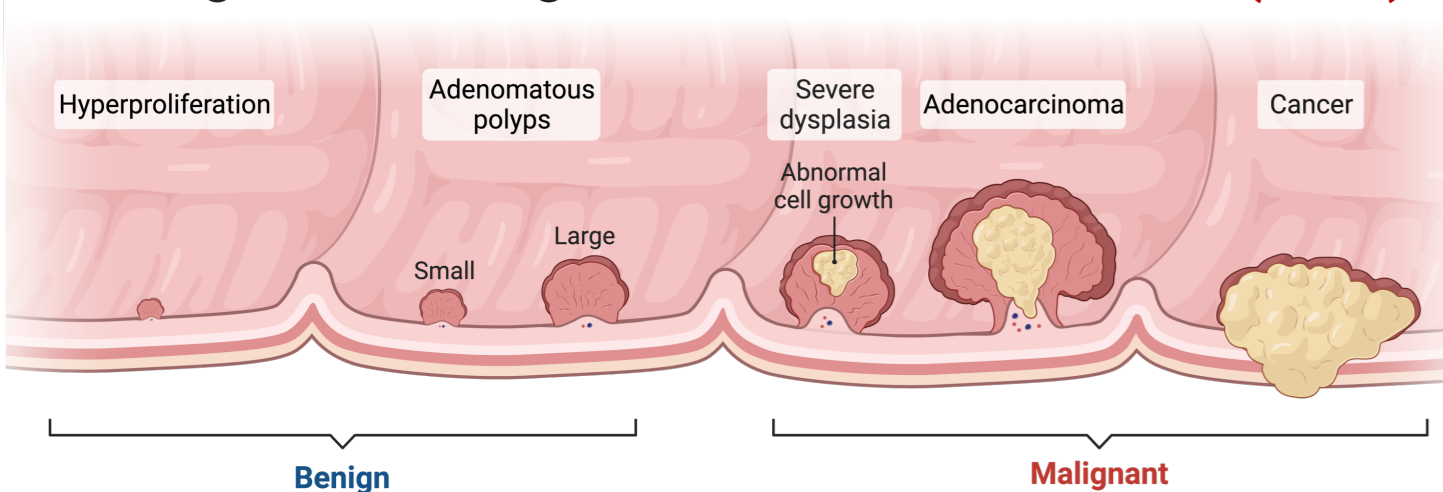
Healthy and Inflamed Epithelium



What is the aetiology of IBD?



Benign and Malignant Colorectal Cancer (CRC)



How common is CRC?

CRC is the **3rd most common cancer type** worldwide & the **2nd most deadly cancer**
Data source: Globocan 2020

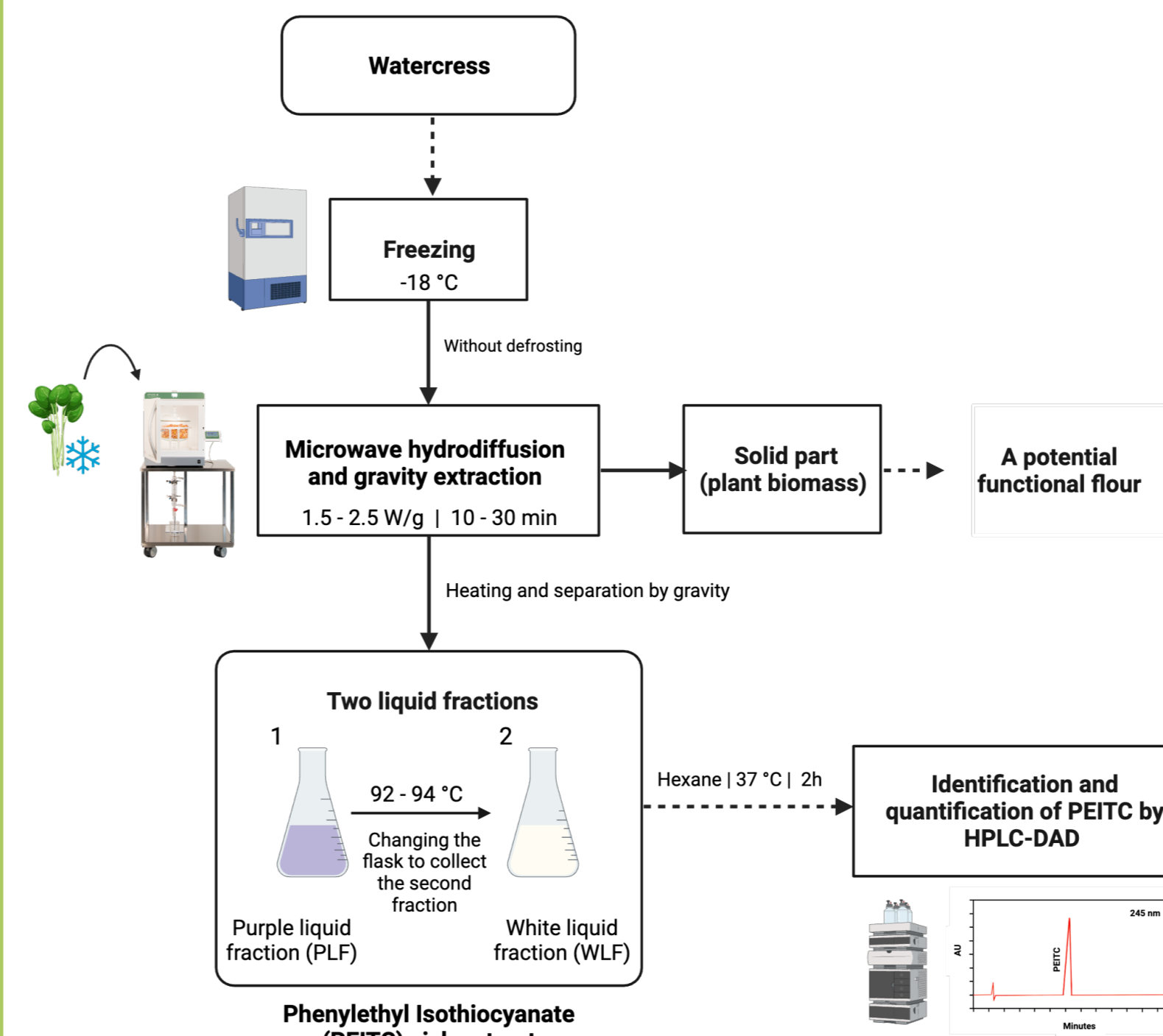
PHENYLETHYL ISOTHIOCYANATE (PEITC)

Looking for a green and reliable method to extract PEITC from watercress?

LOOK HERE

METHODS

Flowchart of the PEITC MHG extraction method



MHG experimental design

Three-level response surface design

Independent factors, respective levels and responses evaluated on MHG process optimisation.

Factors	-1	0	+1
X ₁ : Microwave irradiation power (W/g)	1.50	2	2.50
X ₂ : Microwave irradiation time (min)	10	20	30
Response	Units		
Final temperature of the MHG process	°C		
Extraction yield	% (g extract 100 g ⁻¹ watercress - WC fresh weight)		
PEITC concentration	µg PEITC g ⁻¹ watercress dry basis (DB)		
Total Phenolic Content	mg gallic acid equivalent (GAE) g ⁻¹ WC DB		
ABTS	µmol Trolox Equivalents (TE) g ⁻¹ WC DB		
ORAC	µmol TE g ⁻¹ WC DB		

The design resulted in 24 runs of experiments according to the 3³+3 design in two blocks on successive days, which includes 9 factorial runs and 3 central runs. The ANOVA test was applied to assess the quality of the regression models.

- A previous test extraction was carried out with fresh watercress (not frozen), and PEITC was not detected. The **matrix freezing process appears essential for obtaining PEITC**, so all extractions were performed with frozen watercress.
- At the end of the extraction, two liquid fractions (PLF and WLF) were obtained, but **responses of the crude extract were analysed using the total extract (PLF + WLF) for optimisation**.



Total Phenolic Content: Folin-Ciocalteu method

Antioxidant activity: ABTS scavenging and ORAC assay

PEITC quantification and Polyphenols profile (HPLC-DAD)

RESULTS AND DISCUSSION

The model was statistically significant (p<0.0001), providing a valuable tool to optimise watercress extraction yield, PEITC and TCP extraction, and antioxidant activity.

Model's equation coefficients.

	Final temp.	Extraction yield	PEITC concentration	TPC	ABTS	ORAC
Intercept	-5.25500	137.37500	-5372.42536	-4.52765	-29.01050	-54.19291
X ₁	-2.82500	-32.00000	3492.63868	3.02108	19.28857	37.84801
X ₂	-0.884167	-2.13333	227.91792	0.386111	1.99132	4.29008
X ₁ X ₂	1.73825	0.850000	-1.08725	-0.161206	-0.386071	-1.73618
X ₁ ²		5.50000	-734.04574		-2.38786	
X ₂ ²		0.018750	-4.03796		-0.023845	

PEITC concentration: power and extraction time, along with the quadratic term for extraction time, significantly influenced the Total PEITC. The model was statistically significant (p<0.0001) and a dependable tool for navigating the design space to optimise PEITC concentration during extraction. (R²= 91.35%; predicted R²= 78.85%; lack of fit P= 0.1277).

The data shows that the interaction between power and extraction time influences the efficiency of extracting bioactive compounds. A synergistic effect between these parameters can be exploited to enhance yield and quality.

Derringer optimisation of MHG extraction and validation

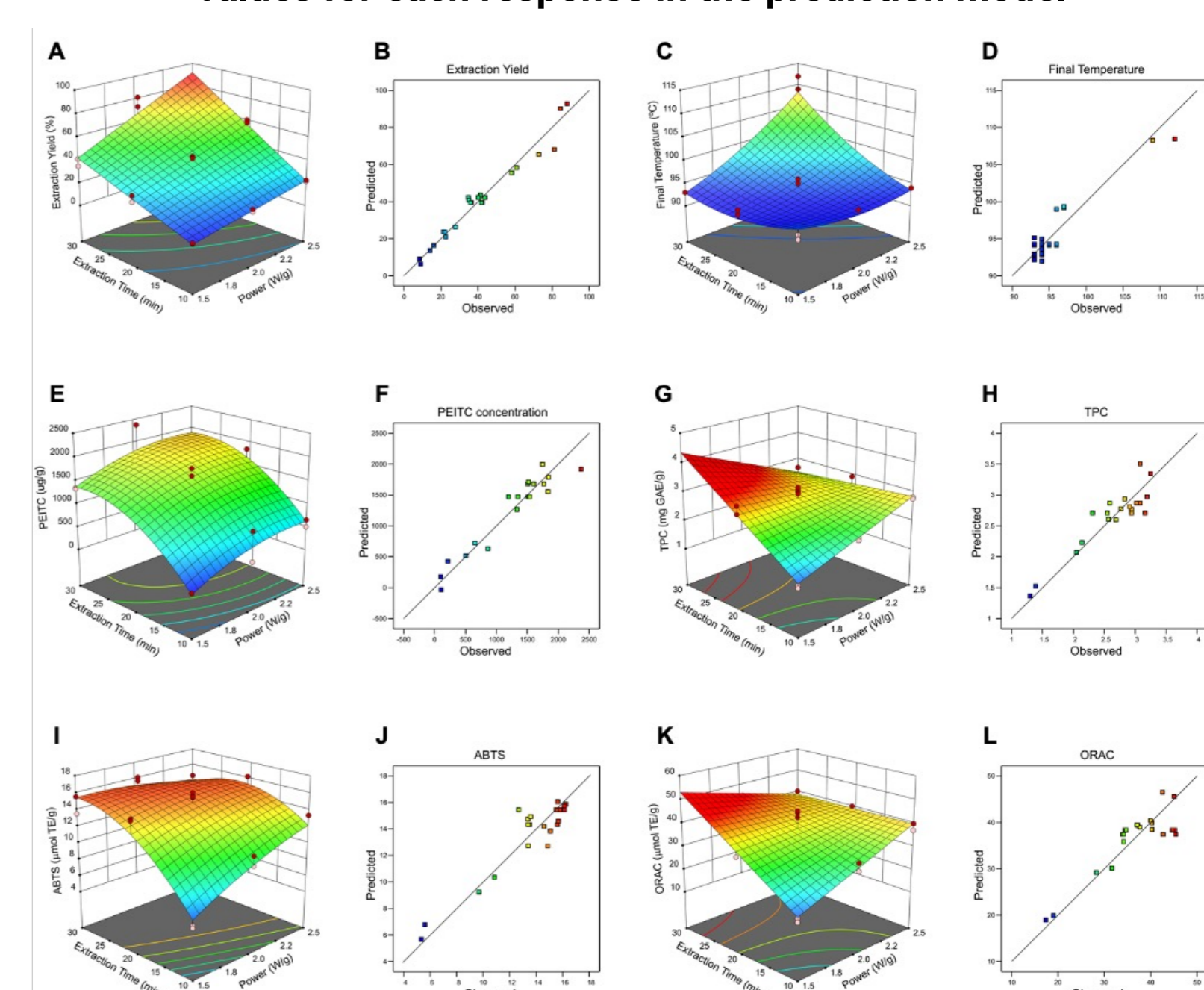
Factors	Factors values			Levels
	Optimum	Low	High	
X ₁ : Microwave irradiation power (W/g)	1.91	1.50	2.50	
X ₂ : Microwave irradiation time (min)	29	10	30	
Optimal desirability: 0.871				
Response	Values	Values	Values	
Final temperature of the MHG process	96.99 ± 1.89	97.25 ± 0.50		
Extraction yield	58.68 ± 4.87	70.25 ± 5.61		
PEITC concentration	1775.61 ± 225.34	1818.26 ± 375.61		
Total Phenolic Content	3.48 ± 0.24	3.63 ± 0.50		
ABTS	15.49 ± 1.34	14.26 ± 1.96		
ORAC	45.89 ± 4.13	40.88 ± 6.51		

The optimal condition chosen maximises PEITC concentration and bioactive markers without using organic solvents.

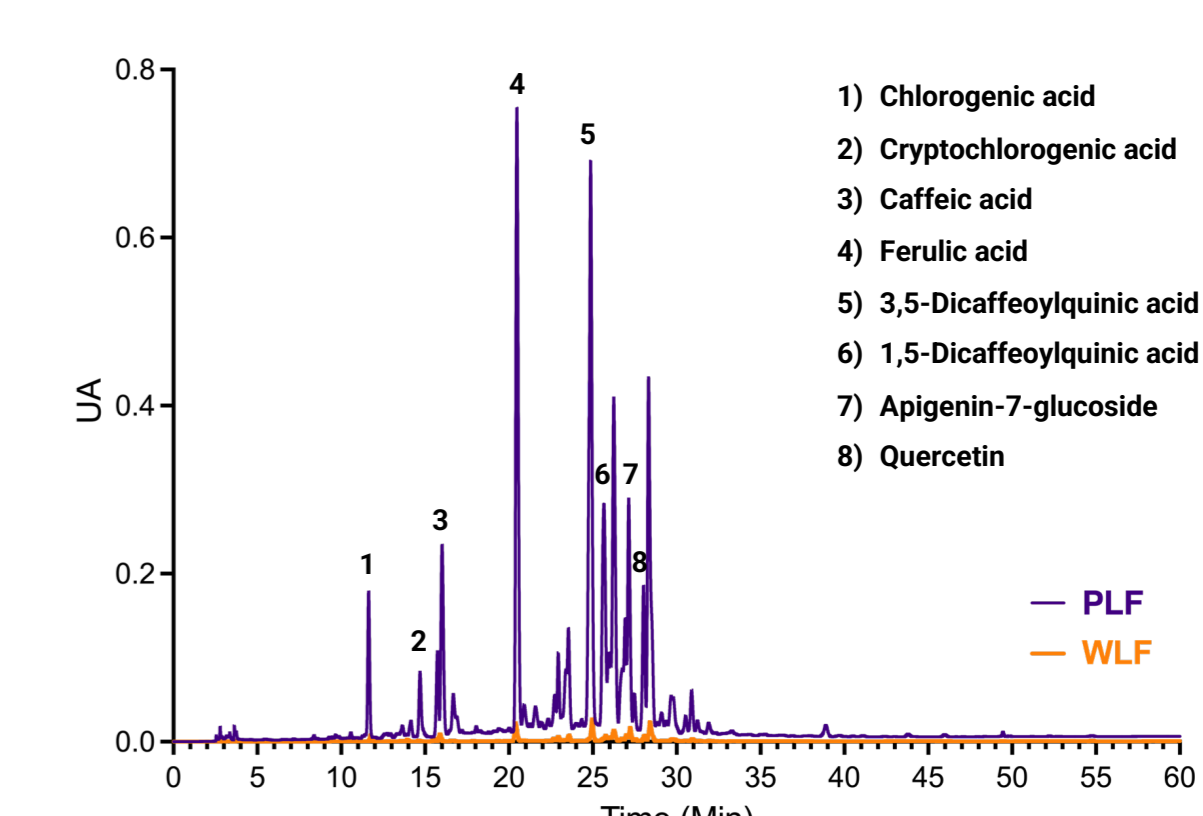
The developed work allows PEITC extraction, resulting in 1818 µg PEITC/g WC DB, compared to 1682 µg PEITC/g WC DB using n-hexane (Coscueta et al. 2020). Optimising the extraction process of PEITC unlocks its immense potential as a health-promoting agent.

CONCLUSION: through 3-level factorial optimisation, we validate that MHG is a sustainable and scalable technique for extracting PEITC from watercress. The extracts have antioxidant capacity, which is also important for their possible application in preventing or treating IBD. Ferulic acid is the main phenolic compound identified. Therefore, these PEITC extracts should be further studied for potential biomedical, nutraceutical and food applications.

Optimisation of the MHG process and observed versus predicted values for each response in the prediction model

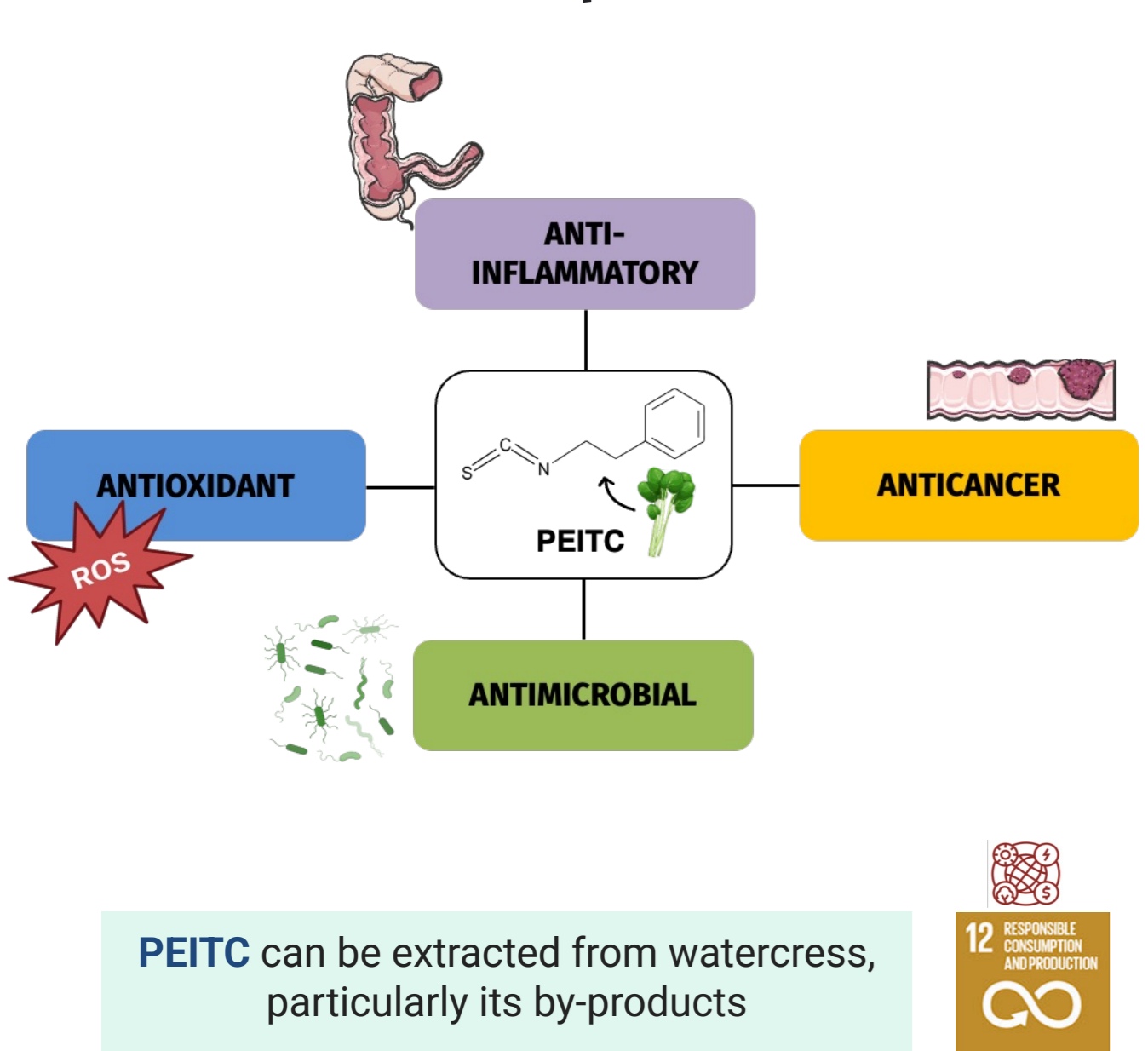


Phenolic compounds profile by HPLC-DAD at 320 nm from a representative replica of optimal extracts



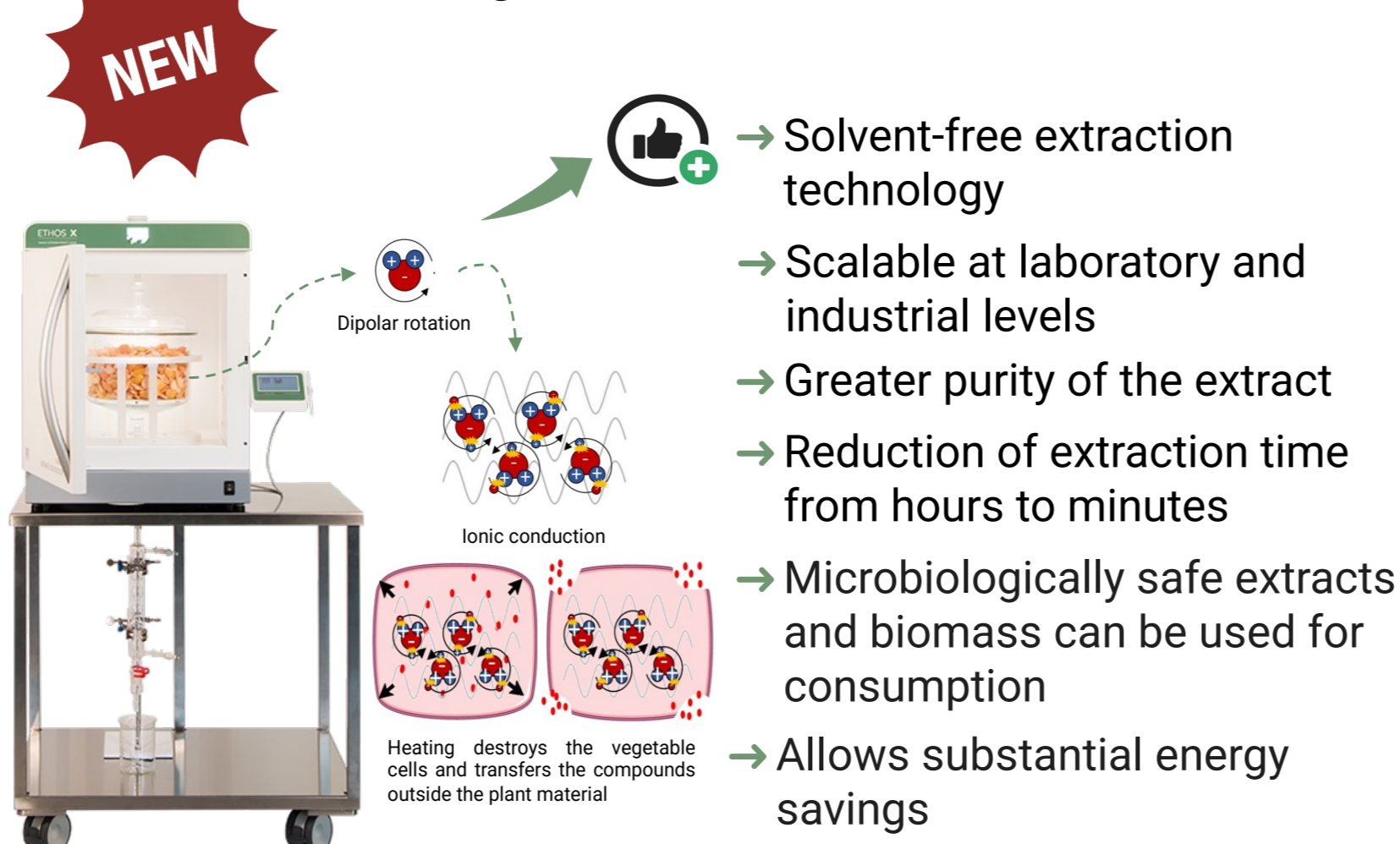
- Prominent peaks were identified.
- High temperatures may change some absorption spectra.
- For better identification, LC/MS analysis is recommended.

How PEITC can help to alleviate IBD?



How can we extract PEITC?

Microwave Hydrodiffusion and Gravity (MHG) as a green tool for PEITC extraction



OBJECTIVES

For the first time, apply the MHG technology to produce extracts enriched with PEITC from watercress by-products.

The present work is based on the following research questions:

- Is MHG suitable for PEITC extraction, comparable to currently used techniques?
- What are the variables that most affect the extractive process?
- What are the optimal extraction conditions?

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