ENHANCE MICROALGAE

Interreg Atlantic Area
European Regional Development Fund

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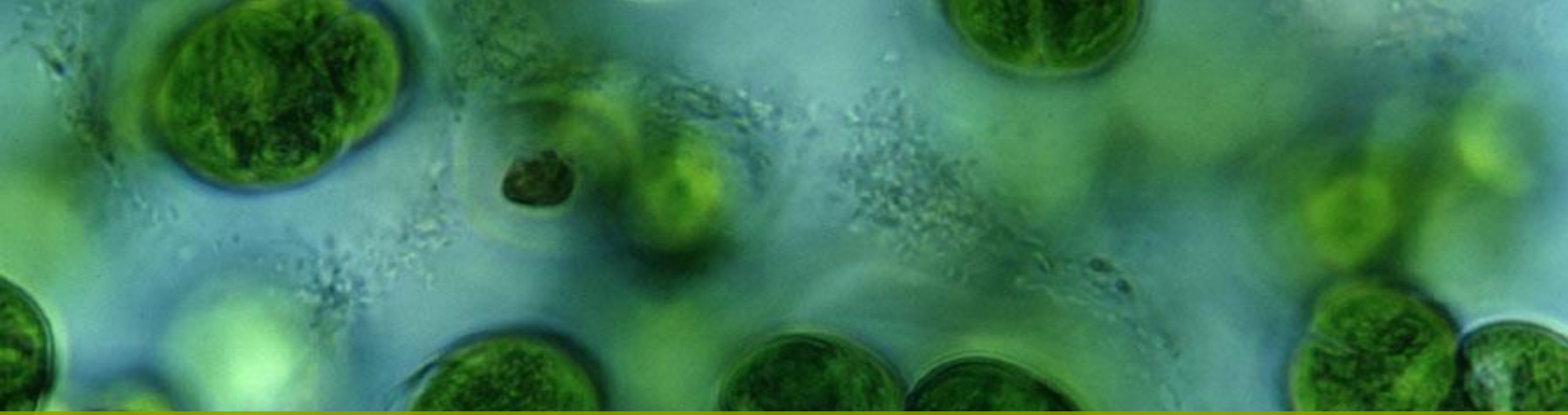
Ultrasound-assisted processing strategies for the generation of protein from *Chlorella vulgaris*

Marco Garcia-Vaquero – University College Dublin

Brijesh Tiwari – TEAGASC

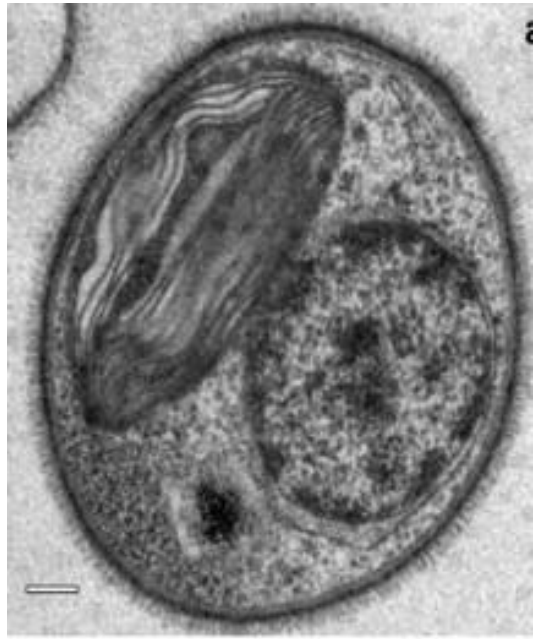
La Rochelle, 20th October 2022



01 Introduction

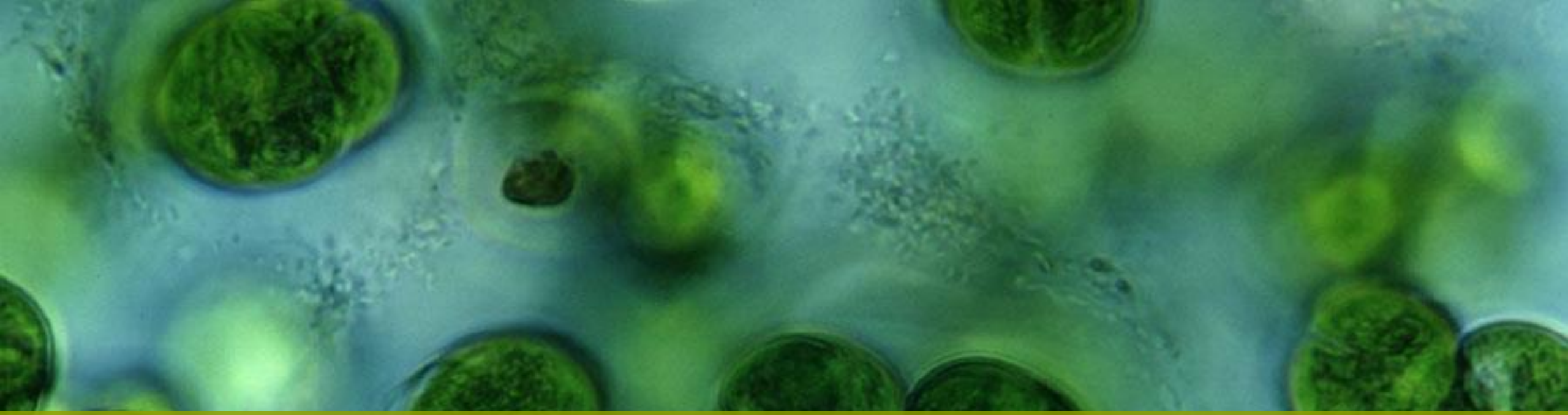
Chlorella sp. as source of protein and free amino acids

- *Chlorella vulgaris* is one of the major green microalgae species currently commercialized for bioenergy, feed and food applications.
- *C. vulgaris* accumulates a remarkable amount of protein (about **40–58% dry weight (DW)**) rich in essential amino acids compared with terrestrial plants (Safi et al. 2013; Hayes et al. 2019).
- The presence of a **rigid cell wall** in *C. vulgaris* hinders the extraction of intracellular proteins (Ursu et al. 2014).
- The **conventional extraction** of proteins from *Chlorella* spp. is time consuming as prolonged agitation is needed to achieve an appreciable recovery of compounds.
- Lysis of the cell wall by mechanical, thermal, chemical, ultrasound and enzymatic treatments could significantly enhance the extraction efficiency of proteins from the biomass (Ursu et al. 2014; Zhang et al. 2018a).
- There is also an increased interest in the food industry for alternative and sustainable sources of **food flavouring agents**, such as flavour-active free amino acids (FAAs) from multiple sources (Poojary et al. 2017a), although the potential for microalgae as a source of food flavouring agents has not yet been explored.



Objectives

This study aims to investigate the effect of multiple ultrasonic processing strategies to recover **protein** and **umami flavouring amino acids** from *C. vulgaris* by exploring:
(1) the use of a single solvent (ultrasound-assisted single solvent extraction or **UASE**),
(2) the sequential application of solvents (ultrasound-assisted sequential solvent extraction or **UASSE**)
(3) the enzymatic extraction (ultrasound-assisted enzymatic extraction or **UAEE**) of protein using food grade enzymes (lysozyme and protease).
The efficiency of these procedures to break down the cell wall of *C. vulgaris* was also evaluated using scanning electron microscopy (SEM).



02 Material and methods

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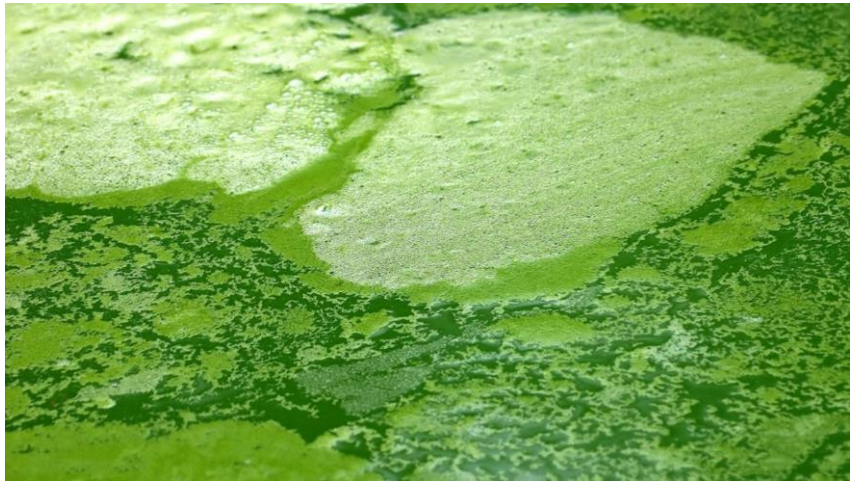
Biomass and processes

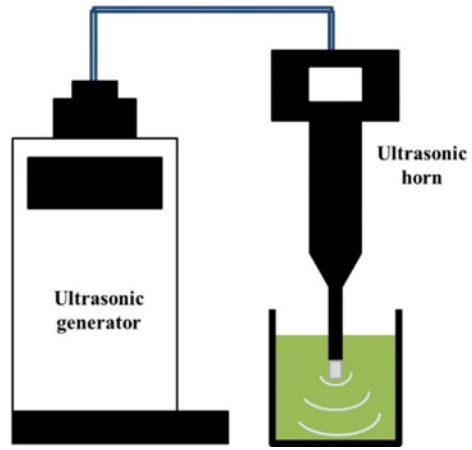
Microalgae

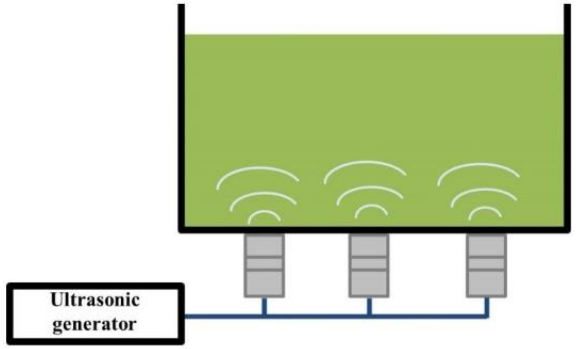
- Axenic *Chlorella vulgaris* with a dry matter of 95.5% w/w.
- Supplied by Nutress B.V., Phycom (Nijkerk, The Netherlands).
- Biomass dried in a steam heated drum dryer (135 °C), monitoring the microalgal drying film to not exceed 100 °C.



US processing

- USE
  - Water (pH 7), HCl (0.4 M, pH 0.4) and NaOH (0.4 M, pH 13.6).
  - UIP500hdT (50–60 Hz, Hielscher Ultrasound Technology, Germany).
  - US maximum power (100%), 10 min.
- UASSE
  - UIP500hdT (50–60 Hz, Hielscher Ultrasound Technology, Germany)
  - 0.4 M NaOH (1:10 w/v) and then treated with US for 10 min followed by a centrifugation step (8000×g, 15 min) to separate the pellet. In the second stage, this pellet was re-suspended in 0.4 M HCl (1: 10 w/v) and shaken for 1 h (170 rpm, 20 °C) followed again by centrifugation.
- UAEE
  - lysozyme (0.02% w/v) pH 7.3 or protease (0.1% v/v) pH 3.6.
  - UIP500hdT (50–60 Hz, Hielscher Ultrasound Technology, Germany). Power 0-100%, 10 min followed by 1 h incubation.
  - US bath (Fisherbrand Transsonic TI-H). Frequencies 35 to 130 kHz during 1, 2 and 6 h.



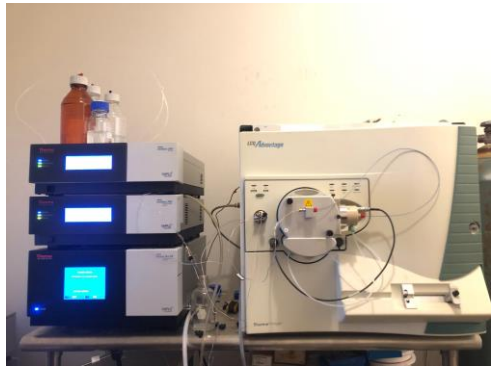


02 Material and methods

Chemical measurements, SEM and statistical analyses

Chemical measurements

- Protein determination: Nitrogen analyser (FP628, LECO Corp., USA). All the measurements were performed in duplicate. Protein recovery (%) is expressed as the percentage yield of protein extracted per total protein present in the biomass.
- Free amino acids: UHPLC-FLD instrument (Thermo Ultimate 3000 RS, Thermo Scientific, USA) equipped with an Agilent AdvanceBio AAA column (100 mm length × 3.0 mm internal diameter × 2.7 µm particle size, Agilent Technologies, USA) fitted to a guard cartridge. Full details at Hildebrand et al (2020)

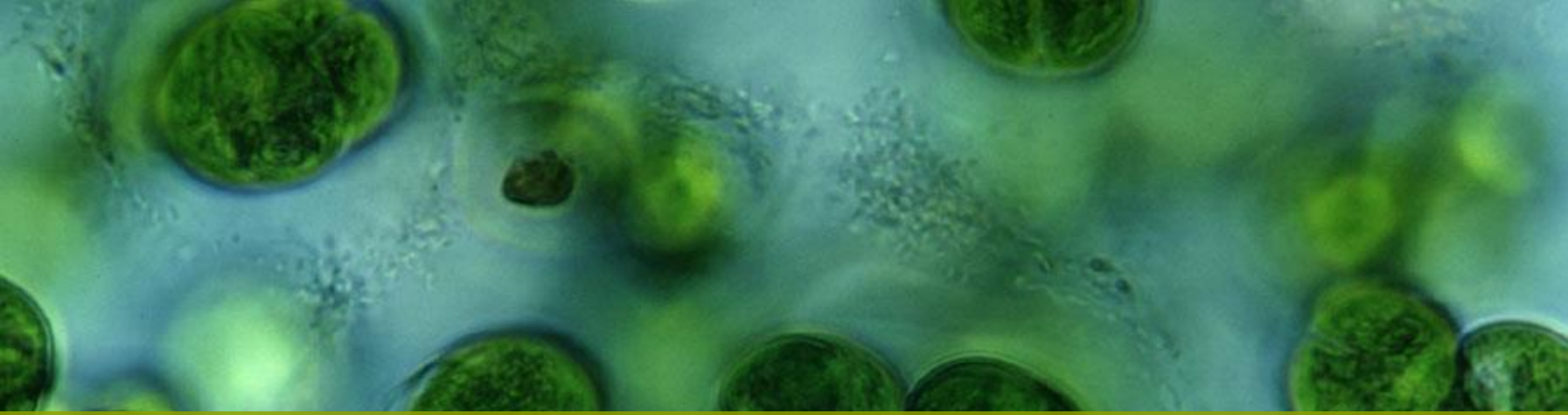


Scanning electron microscopy (SEM)

- Coating: 10 nm layer of gold film. Emitech K575X Peltier cooled sputter coater (Quorum Technologies, UK).
- FEI Quanta 3D FEG Dual Beam SEM (FEI, USA). Accelerating voltage 5.0 kV and current 5.92 pA.

Statistical analyses

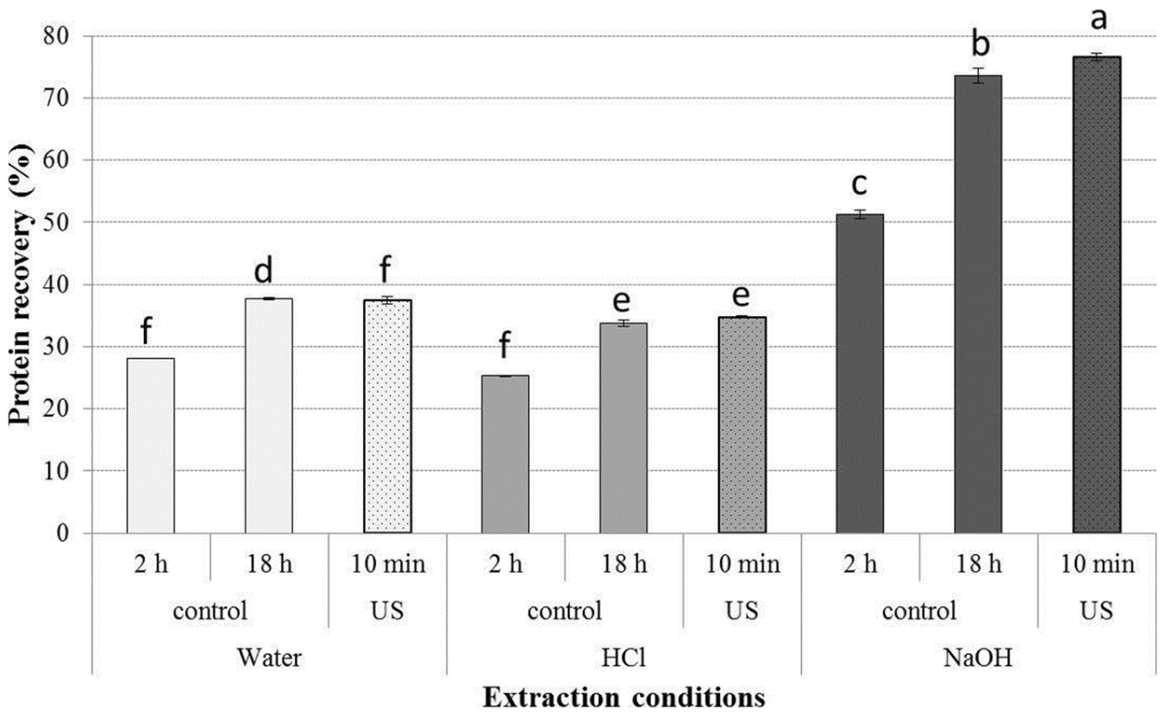
- SPSS version 23.0 (IBM SPSS Statistics).
- General linear model and Tukey post hoc tests.
- Criterion for statistical significance was $P < 0.05$.



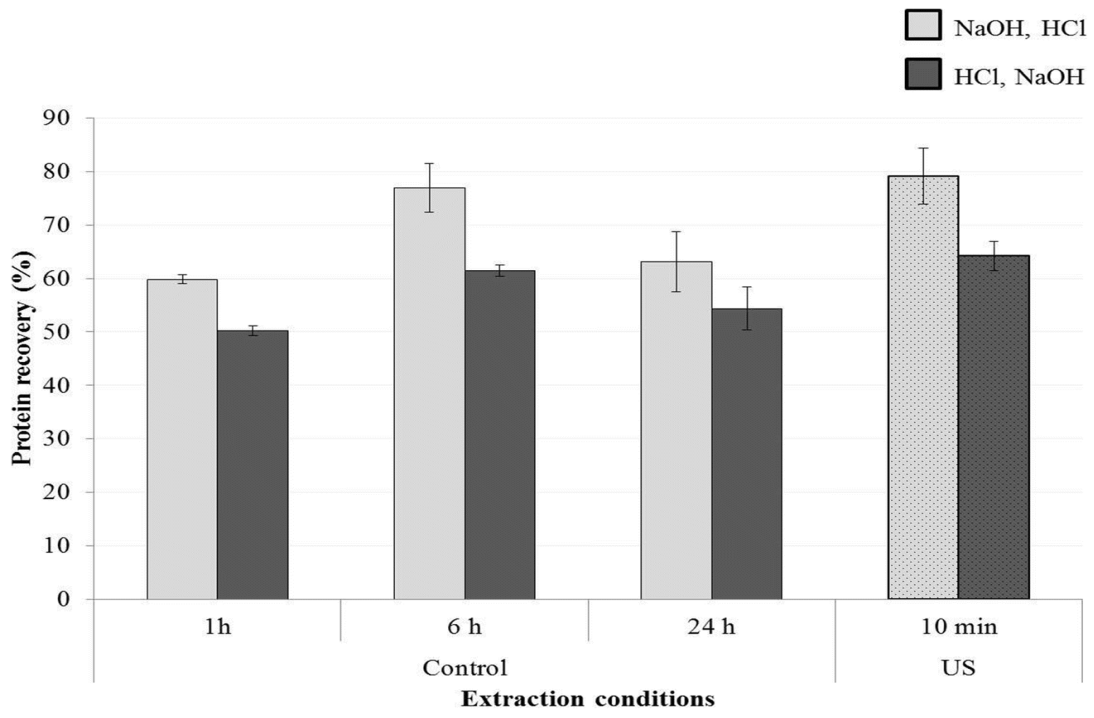
03 Results and discussion

UASE and UASSE on protein recovery

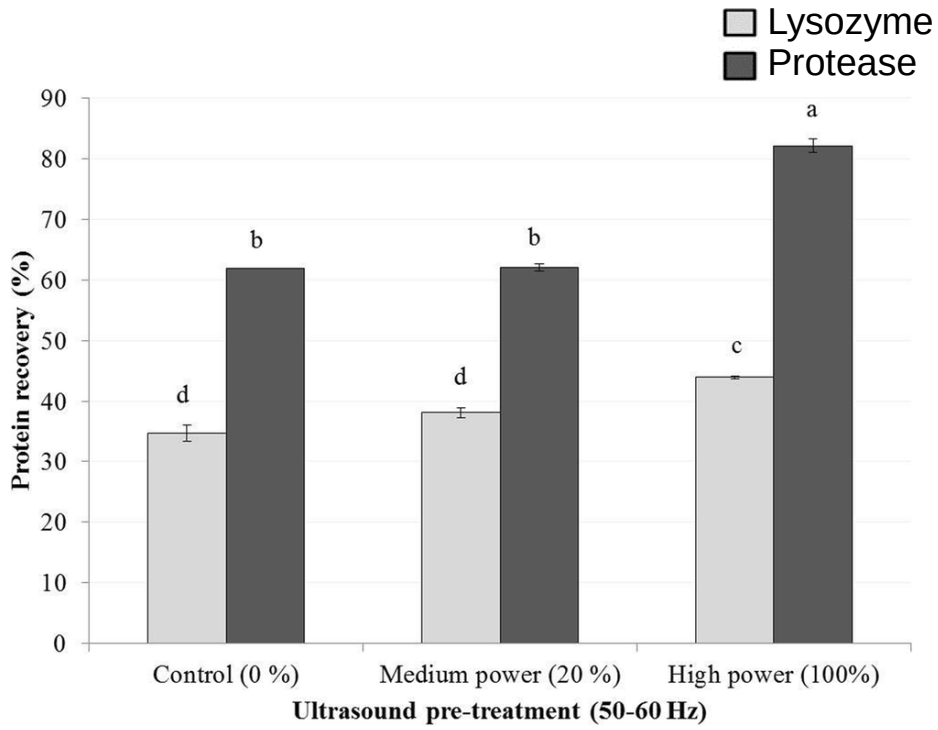
UASE

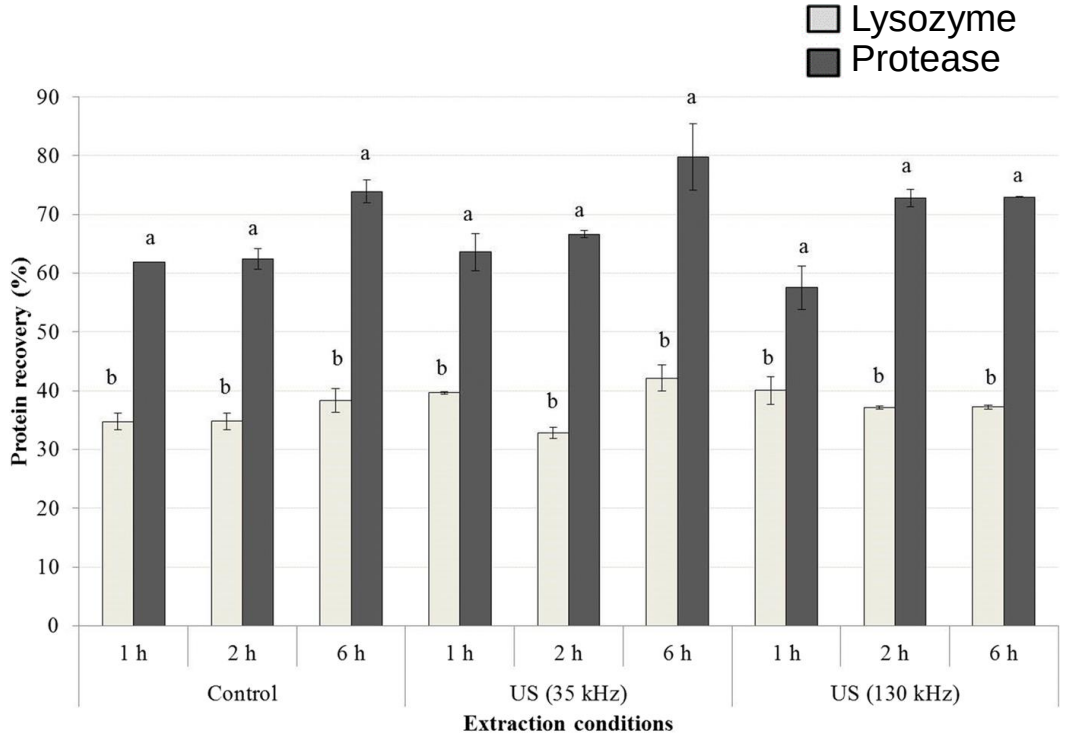


UASSE

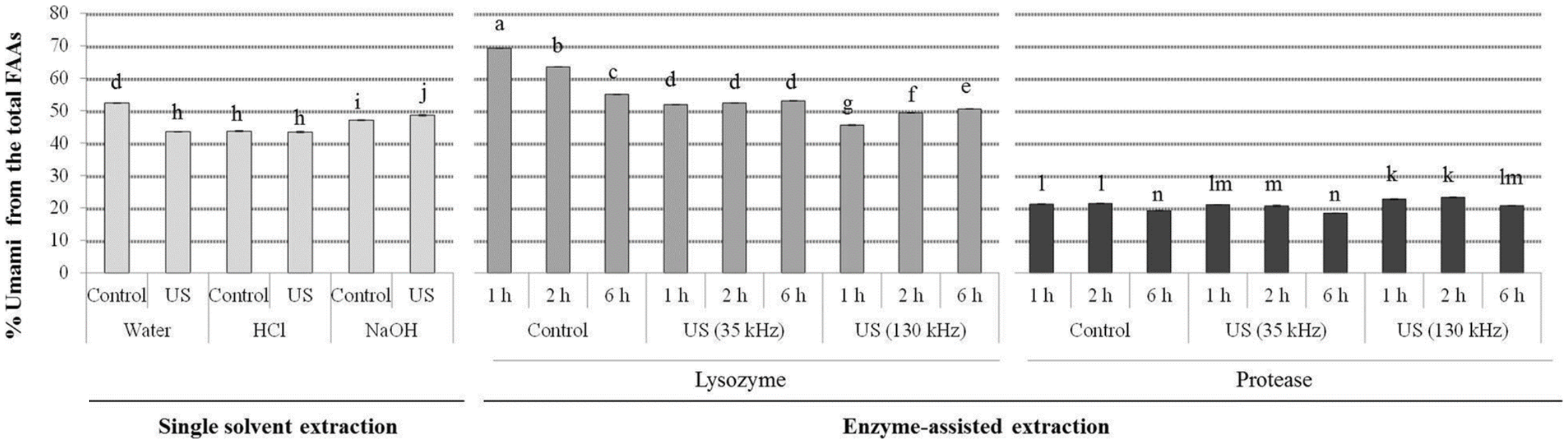


US power and frequency on protein recovery using UAEE



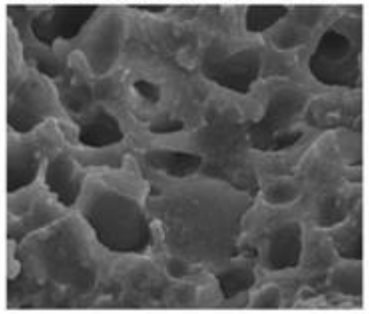


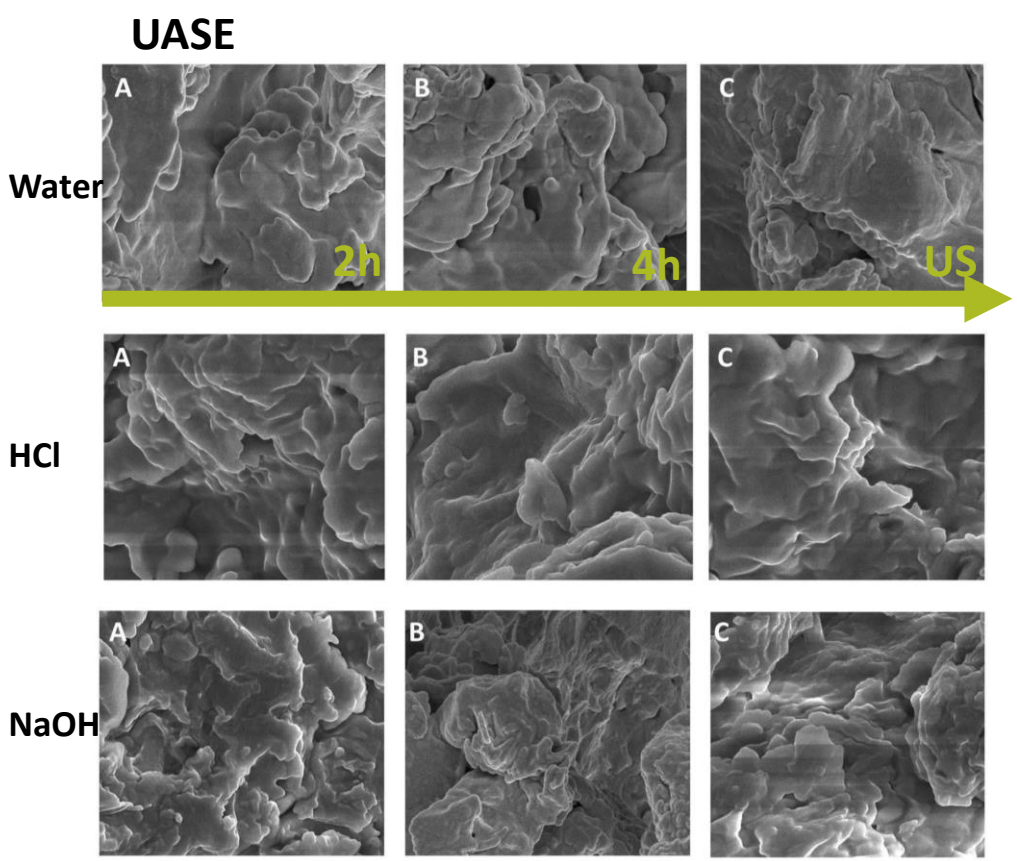
US processing on umami FAA

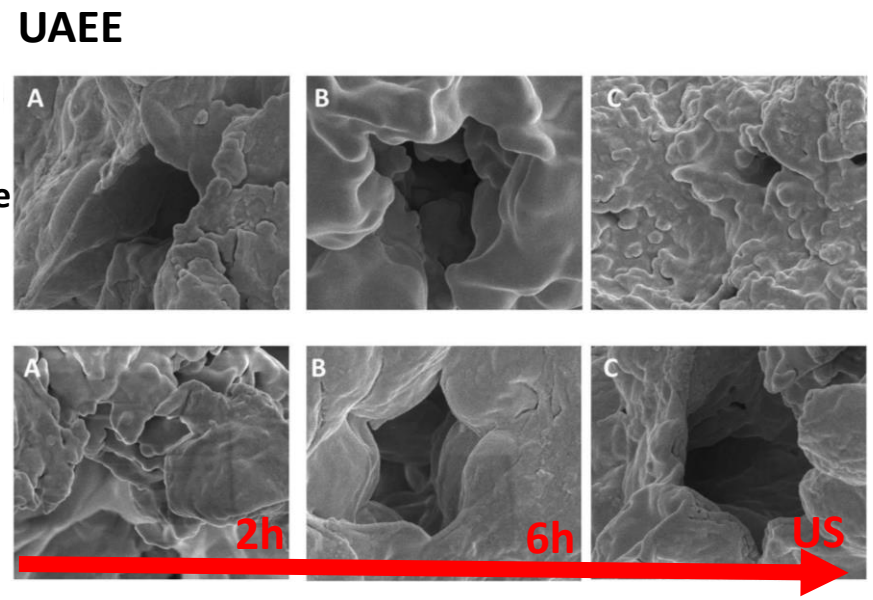


SEM evaluation

Untreated *Chlorella vulgaris*







Conclusions

Overall, this study shows that ultrasound processing is an effective tool for rapid extraction of soluble proteins from *C. vulgaris*.

The **highest protein recoveries were obtained when UAEE** was carried out with proteases. Although ultrasound processing can be combined with **sequential solvent extraction and lytic enzymatic treatments to improve the recovery of proteins, these methods create additional production costs.**

Taking this into account, alkaline **UASE could be the method of choice**, as it is a less complex, and thus less costly, while still providing a comparable protein recovery to the other investigated methods.

Furthermore, the extracts obtained from *C. vulgaris* had relatively high amounts of umami FAAs and could thus be used as a promising source of flavour ingredients for new food product formulations.

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Check for updates

Ultrasound-assisted processing of *Chlorella vulgaris* for enhanced protein extraction

Gunda Hildebrand[1] · Mahesha M. Poojary[2] · Colm O'Donnell[1] · Marianne N. Lund[2,3] · Marco Garcia-Vaquero[4,5] · Brijesh K. Tiwari[1,4]

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Thank you!

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