Screening of Algae Processing Technology for Easily Digestible Bioactive Hydrolysates

L. Bím[1], M. Jelínek[1], L. Šánek[2], V. Matůšů[2], P. Švestková[2] & J. Pecha[2]

[1]KORTAN, spol. s r.o., Hrádek nad Nisou – Loučná, Czech Republic

[2]Faculty of Applied Informatics, Tomas Bata University in Zlín, Zlín, Czech Republic

kortanh@seznam.cz

Tomas Bata University in Zlín
Faculty of Applied Informatics

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Introduction

Microalgae are a prospective alternative source of proteins and bioactive compounds in human and animal nutrition.[1] However, their utilisation is often limited by low digestibility due to robust cell walls.[2] Protein extracts in the form of protein hydrolysates can improve digestibility. These hydrolysates possess promising bioactive properties and may act as antioxidants or ACE inhibitors.[2,3]

Challenge: The processing method significantly influences the final hydrolysate's properties, bioactivity, purity, and overall production costs.



Figure 1 Spirulina (left) and Chlorella (right) algae tested

Research Objective

Comprehensive analysis of protein extraction from microalgae *Chlorella* and *Spirulina,* comparing:

- Enzymatic hydrolysis with different enzymes
- Subcritical water extraction (SWE)
- Alkaline and aqueous extraction
- Combined two-step procedures

Methods Tested

All experiments: 5% algae dry matter in reaction mixture

1. Aqueous Extraction

Water extraction at elevated temperatures (60-100°C) for 3 hours with constant stirring. Used as control method.

2. Alkaline Extraction

0.2-0.4 M NaOH at room temperature or boiling, 5-17 hours.

3. Enzymatic Hydrolysis

Alcalase: pH 8.5-9.0, 62.5°C, 2-4h
PROMOD 295L: pH 8.4, 60°C, 6h
Pancreatin: pH 7.5, 40°C, 6h

4. Subcritical Water Extraction (SWE)

Hydrothermal extraction in PARR pressure reactor at 140-180°C, 40-300 min under inert atmosphere.

5. Two-Step Procedures

A) Enzymatic (Alcalase) → SWE (180°C, 40 min)
B) SWE (180°C, 40 min) → Enzymatic (Alcalase)
C) Alkaline extraction (0,4M NaOH) → Enzymatic (Promod 295L)

Analytical Methods:

- Dry matter yield and nitrogen content (Flash2000, Thermo Scientific)
- Antioxidant activity: DPPH radical scavenging and FRAP (ferric reducing power)
- All separations by centrifugation (6000 rpm, 20 min) or vacuum filtration

Results and discussion

Enzymatic Hydrolysis

Enzyme	Microalgae	Protein yield [%]
PROMOD 295L	***Spirulina***	**83.5**
	Chlorella	64.5
Pancreatin	*Spirulina*	80.3
	Chlorella	**69.0**
Alcalase	*Spirulina*	66.2
	Chlorella	62.0

Two-Step Extraction

Procedure	Microalgae	Protein yield [%]		Increase
		Step 1	Step 2	
Enzymatic → SWE	*Spirulina*	62.3	71.4	+9.1
Enzymatic → SWE	*Chlorella*	**47.3**	**66.8**	**+19.5**
SWE → Enzymatic	*Spirulina*	65.3	70.0	+4.7
SWE → Enzymatic	*Chlorella*	59.5	62.2	+2.7
Alkali → Enzymatic	*Spirulina*	50.4	61.6	+11.2

Observation: *A two-step hydrolysis significantly enhanced nitrogen extraction. Enzymatic pretreatment cleaves proteins and disrupts cell structures, increasing the accessibility of intracellular compounds for the subsequent SWE step, which likely further disrupts residual polysaccharide layers and mobilises remaining intracellular substances. The synergistic effect was particularly pronounced for Chlorella (+20 percentage points), despite the enzymatic treatment lasting only 2 hours.*

Antioxidant Activity

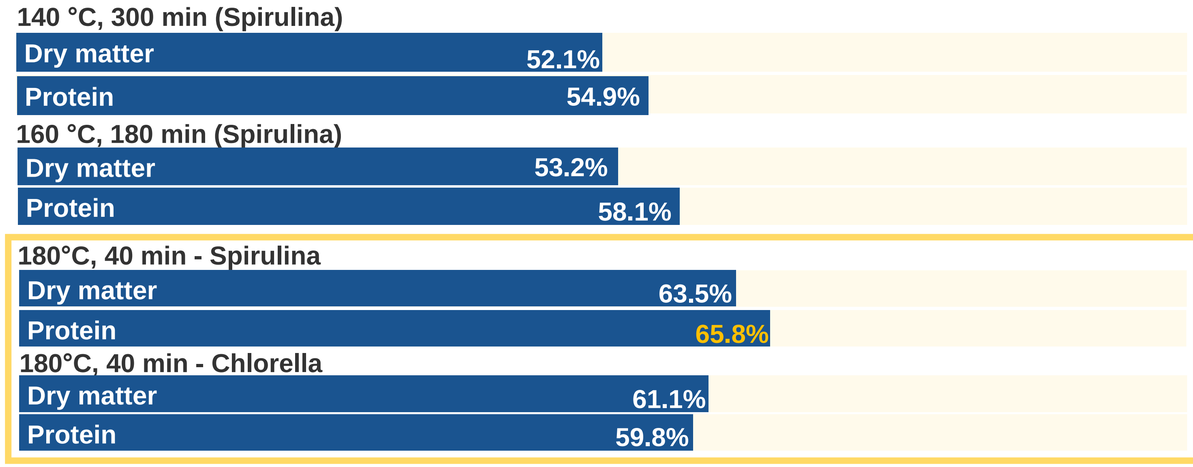
Method	FRAP (µM/g)	DPPH (µM/g)	Microalgae
Two-step (Enzyme+SWE)	**439**	**373**	*Spirulina*
SWE 180°C, 60 min	403	352	*Spirulina*
SWE 180°C, 30 min	402	367	*Chlorella*
SWE 180°C, 40 min	318	301	*Spirulina*
PROMOD 295L	193	74	*Spirulina*
Alcalase	190	111	*Spirulina*
Pancreatin	169	81	*Spirulina*
Aqueous (boiling point)	157	180	*Spirulina*

Values expressed as Trolox equivalents per gram dry matter



Figure 2 Protein hydrolysate from Chlorella – Enzymatic (Pancreatin)

Subcritical Water Extraction



Key Findings

- Spirulina showed better processability than Chlorella due to a less robust cell wall structure.
- Enzymatic hydrolysis proved the most effective: **PROMOD 295L** achieved 79% dry matter and **83.5% protein yield** for Spirulina.
- In the case of Chlorella, the most effective enzyme was **pancreatin → 69 % protein yield**.
- Temperature, not prolonged time, determines **SWE efficiency**; the highest Spirulina protein yield (65.8%) occurred **at 180 °C for 40 min**.
- Two-step extraction of Spirulina combining enzymatic (Alcalase) and SWE processing yielded of 66.6 % of dry matter and 71.4 % of protein
  - Combination of enzymatic and SWE showed the highest antioxidant activity (>350 µM Trolox equiv./g).

Conclusions

Most Effective Method:

- *Spirulina:* Enzymatic hydrolysis with **PROMOD 295L** achieved **83.5% protein yield and 79% dry matter**
  - Thin peptidoglycan layer enables superior enzyme penetration; all methods showed **10-20% higher protein yields vs. *Chlorella***
- *Chlorella*: Robust cellulosic wall requires more aggressive processing; **pancreatin** (broad enzyme spectrum) was the most effective, achieving a **69% protein yield**

Highest Bioactivity:

- Subcritical water extraction consistently yielded high antioxidant activity (>300 µM Trolox/g in both the FRAP and DPPH assays).

Two-step Extraction Synergy:

- Combining enzymatic pretreatment with SWE significantly enhanced nitrogen recovery and antioxidant activity
  - Spirulina: FRAP 439 µM Trolox/g; DPPH 373 µM Trolox/g
  - Further optimisation of reaction time and enzyme selection may improve overall extraction efficiency.

Overall Impact: This comprehensive screening demonstrates that substantial yields of bioactive protein hydrolysates can be obtained from microalgae using straightforward, industrially viable methods. Method selection should be optimised based on target algae species, desired product properties (protein content vs antioxidant activity), and available processing infrastructure.

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