HYDROLYSIS OF THE PROTEIN FRACTION OF THE INDUSTRIAL SOLID RESIDUE FROM RED ALGAE AFTER AGAR EXTRACTION

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GENERAL INDEX

INDUSTRIAL AGAR EXTRACTION PROCESS

MACROALGA
Gelidium sesquipedale

BASIC TREATMENT
Na_2CO_3
NaOH

WASHING

FILTRATION

SECOND EXTRACTION

AGAR-AGAR EXTRACTION

MACROALGA RESIDUE

MACROALGA RESIDUE

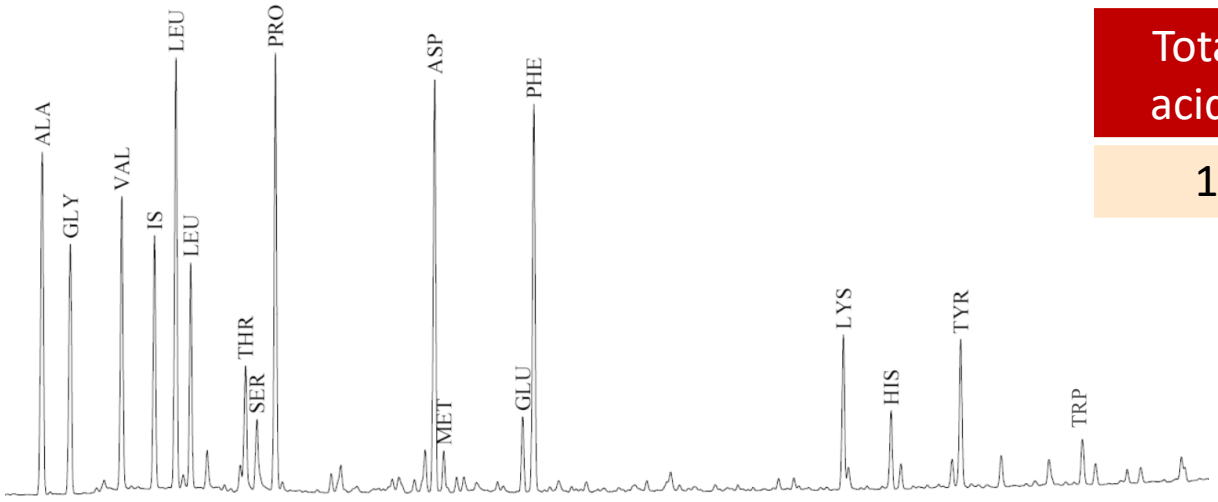
Residual agar = 6.8%

	G. Sesquipedale	Macroalga Residue
CARBOHYDRATES	**38 ± 1**	**42 ± 2**
Glucans	10.7 ± 0.3	23.4 ± 0.9
Galactans	**21.3 ± 0.5**	**10.9 ± 0.5**
Arabinans	1.4 ± 0.1	2.9 ± 0.2
Uronic acids	4.3 ± 0.1	3.8 ± 0.1
LIGNIN	**11.3 ± 1**	**12 ± 1**
Soluble	11 ± 0.1	8.7 ± 0.1
Insoluble	0.3 ± 0.1	3 ± 1
PROTEINS	**14.9 ± 0.3**	**21 ± 1**
LIPIDS	**0.7 ± 0.2**	**0.87 ± 0.09**
ASHES	**14.9 ± 0.3**	**22 ± 2**

MACROALGA RESIDUE

21% PROTEINS: *(mg/g dry macroalga residue)*

Ala	Gly	Val*	Leu*	Ile*	Thr*	Ser	Pro	Asp	Met*	Glu	Phe*	Lys*	His*	Tyr	Trp
19.9	8.7	14	16.3	9.4	7.5	8	15.4	20.4	1.7	16.6	10.6	12	3.4	7.3	0.6

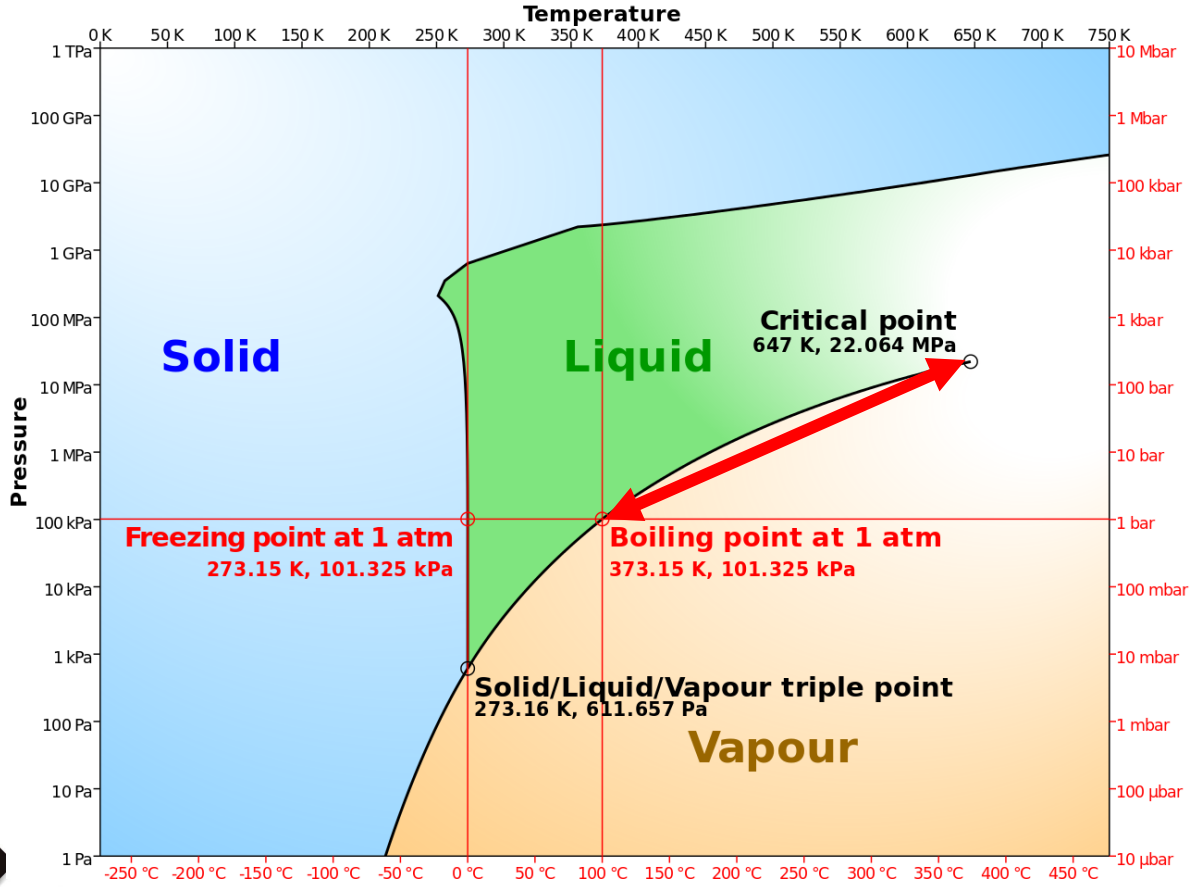


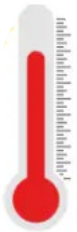
Total amino acids (TAAs)	Essential amino acids (EAAs)*
172 ± 9	76 ± 5

Nitrogen Factor[a] = 4.9

[a]Estimated by calculation spreadsheets provided by NREL according to amino acids sample profile (https://www.nrel.gov/).

SUBCRITICAL WATER TECHNOLOGY





Temperature
100 – 374°C

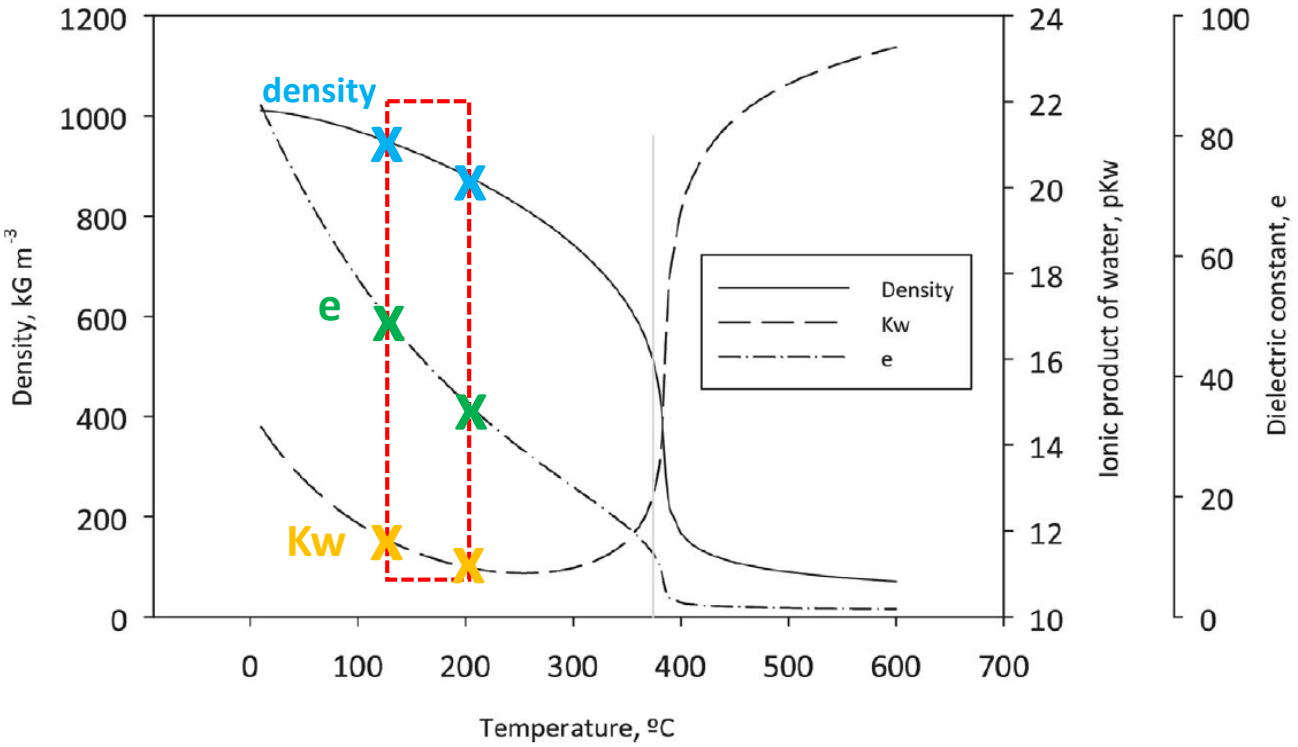
Pressure
> 50bar

SUBCRITICAL WATER TECHNOLOGY

↓ density

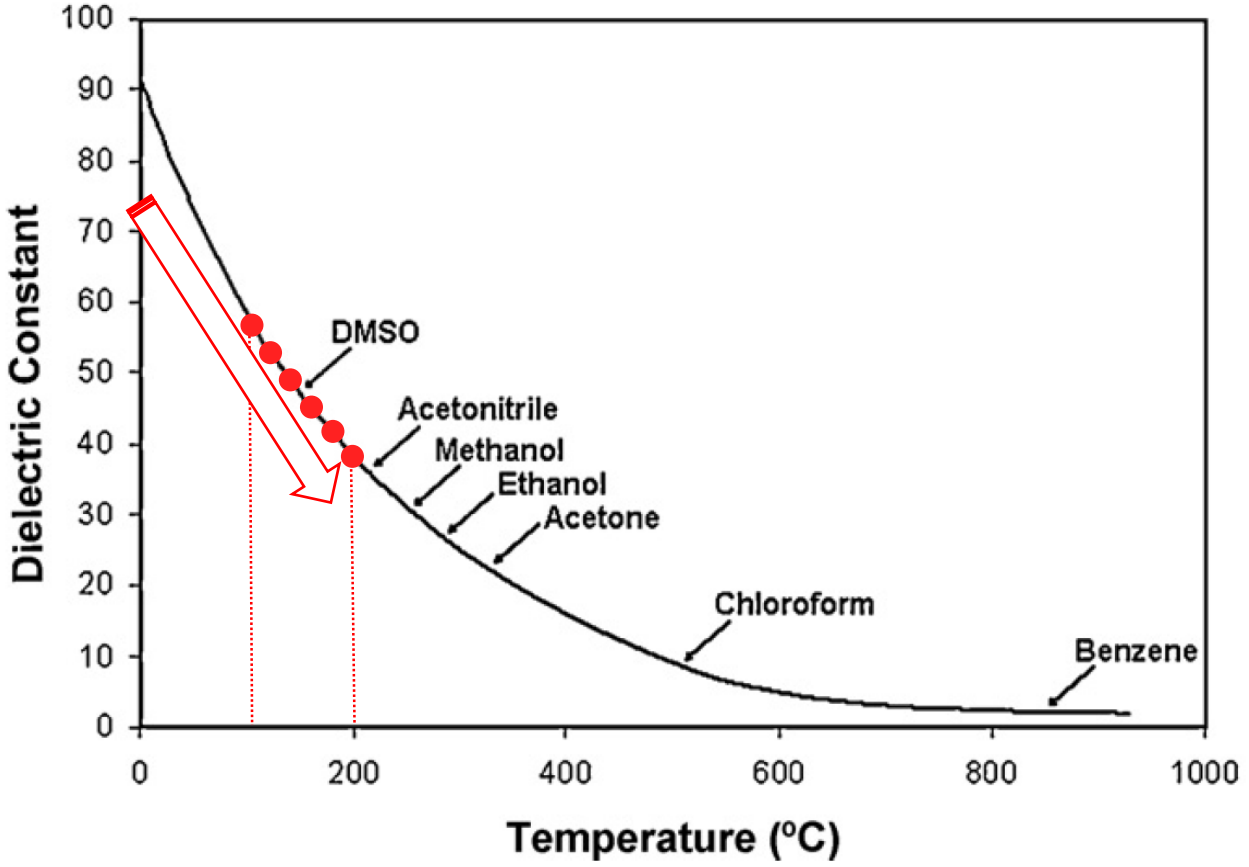
↓ Kw

↓↓ e



*Cocero et al., *The Journal of Supercritical Fluids*, **2018**, 133, 550-565

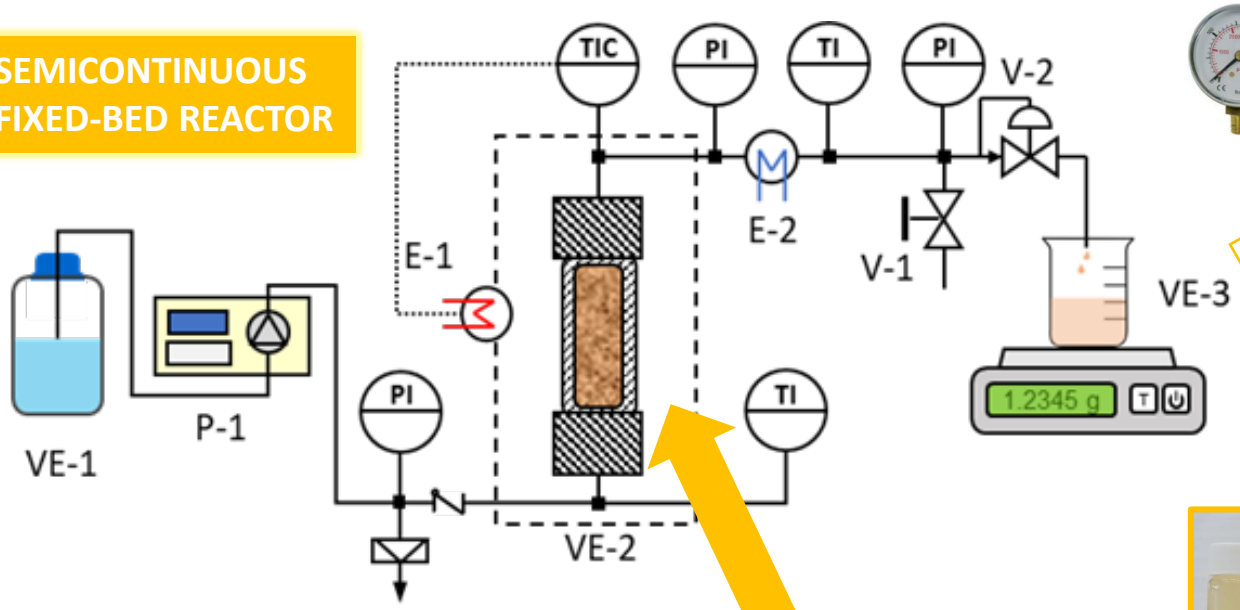
SUBCRITICAL WATER TECHNOLOGY



*Carr et al., *Chemical Engineering Journal*, **2011**, 172, 1-17

EXPERIMENTAL SET-UP

SEMICONTINUOUS FIXED-BED REACTOR



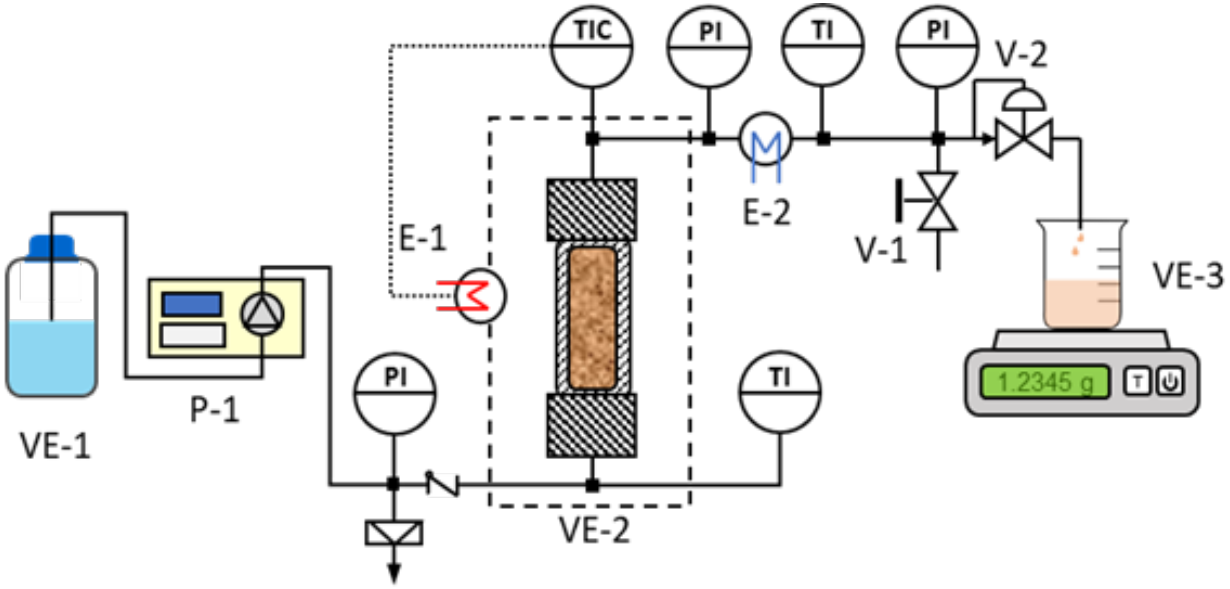
50 bar

Solid residue after SWE

Subcritical water extracts

RAW MATERIAL

EXPERIMENTAL SET-UP



EXPERIMENTAL VARIABLES:

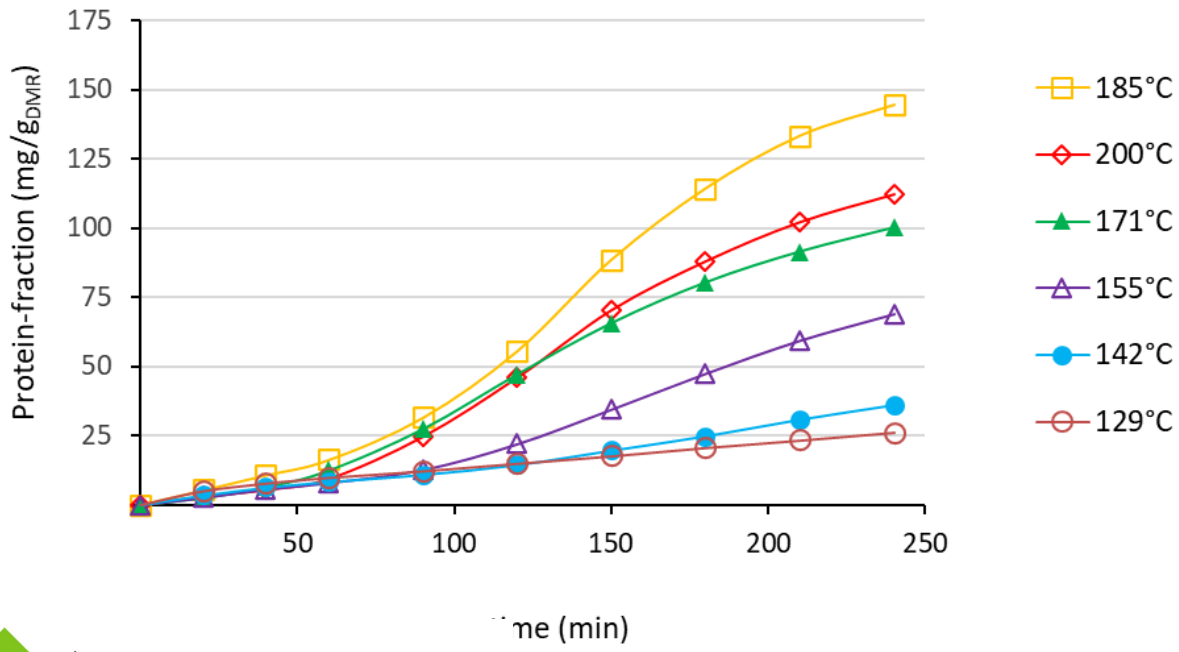
129, 142, 155, 171, 185 and 200°C

2 and 6 ml/min

240 minutes

PROTEIN SW EXTRACTION

PROTEIN: **Temperature effect**

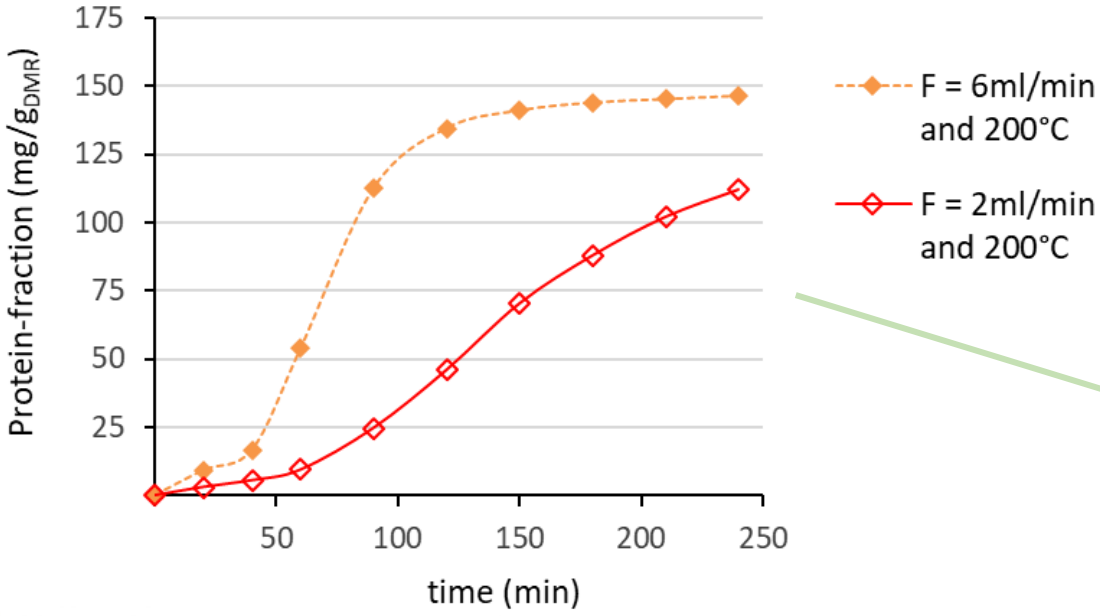


A maximum is observed at 185°C (68.5% yield)

Higher temperatures led to protein degradation at this residence time.

PROTEIN SW EXTRACTION

PROTEIN: **Flow rate effect**



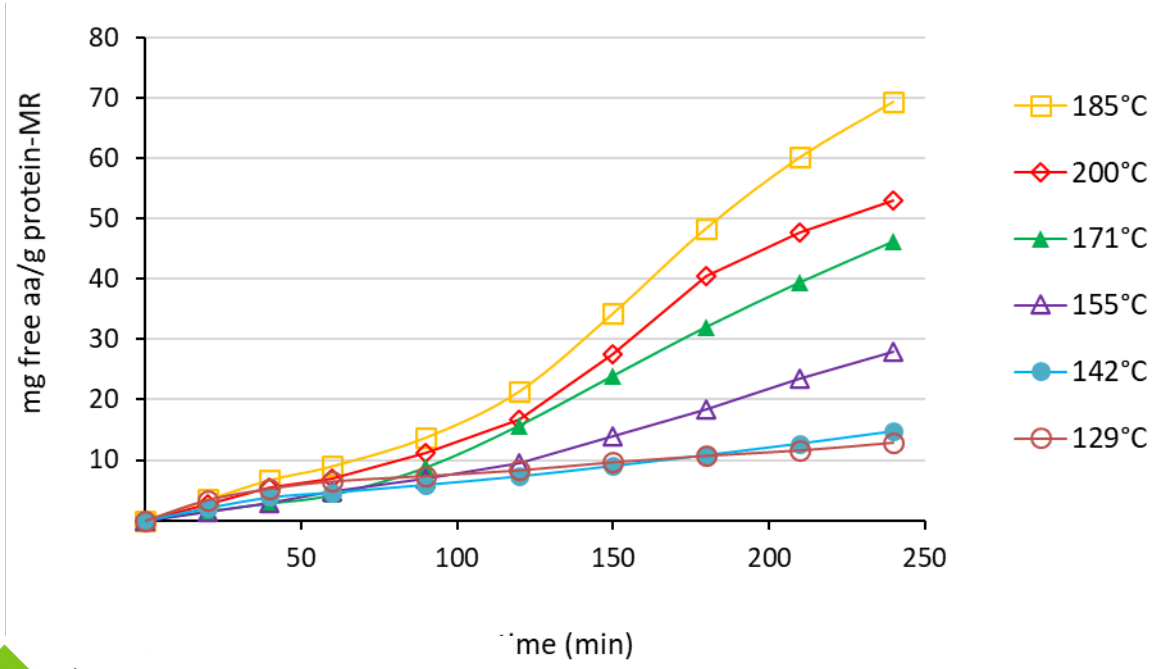
Faster and greater extraction is achieved by working at higher flow rate at the same temperature (200°C) due to a residence time reduction.

F = 2ml/min; τ = 55.2 min

F = 6ml/min; τ = 18.4 min

PROTEIN SW EXTRACTION

Free amino acids: Temperature effect

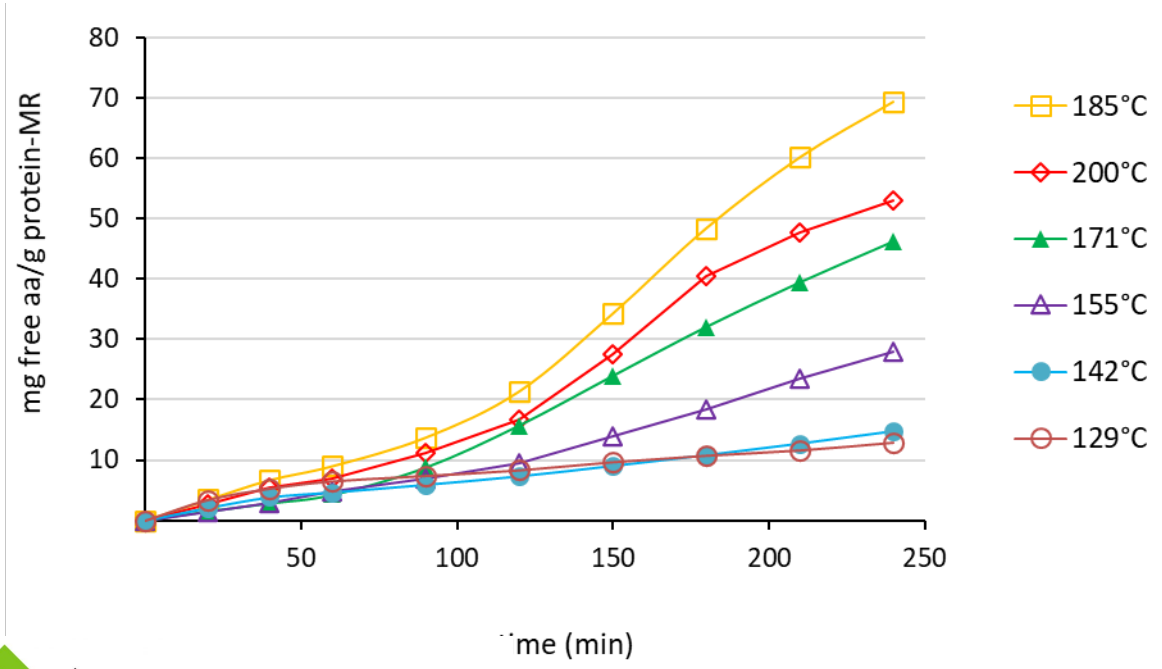


At a constant flow rate of 2ml/min, a maximum at 185°C was observed.

Lower content of free amino acids was detected at 200°C because of amino acid degradation.

PROTEIN SW EXTRACTION

Free amino acids: Temperature effect

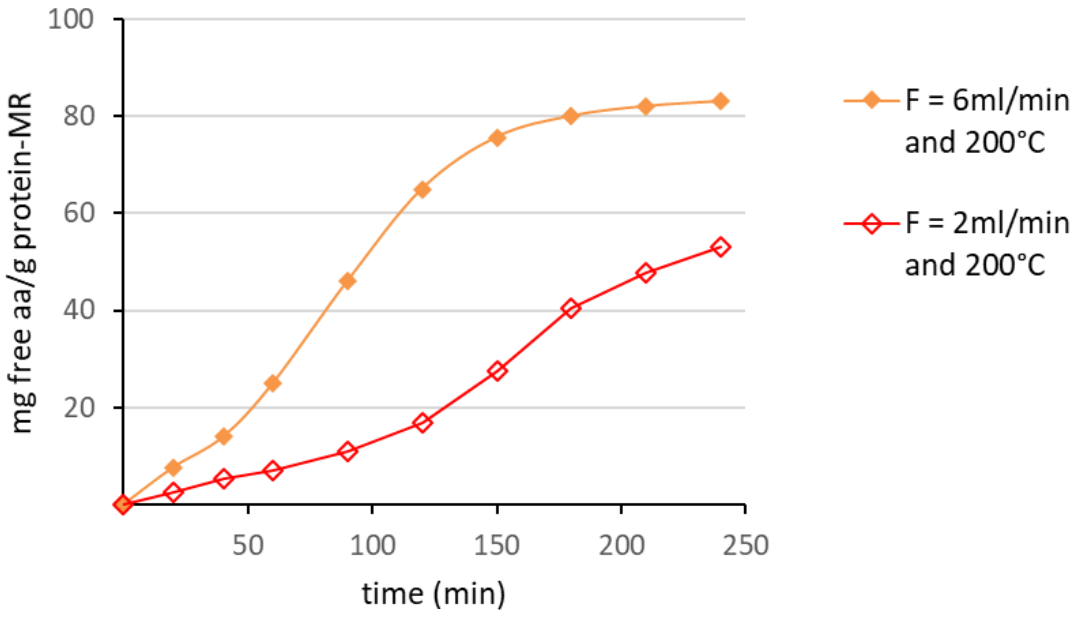


YIELDS (%):

T (°C)	EAAs	TAAs
129	1.3	1.4
142	1.2	1.5
155	1.9	3.0
171	3.8	5.3
185	7.3	8.1
200	6	6

PROTEIN SW EXTRACTION

Free amino acids: Flow rate effect



YIELDS (%):

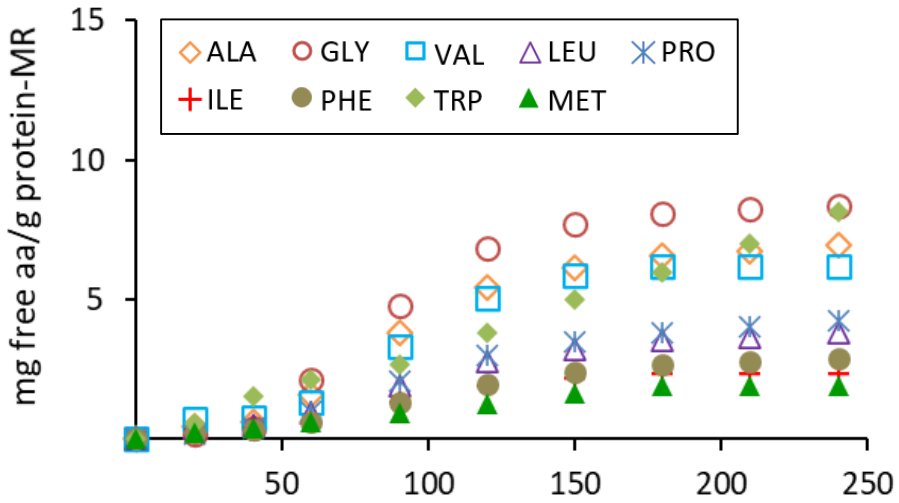
Flow rate (ml/min)	R.T. (min)	EAAs	TAAs
2	55.2	6	6
6	18.4	11	11

Decreasing the residence time by working at higher flow rate, led to faster and higher amino acids yields.

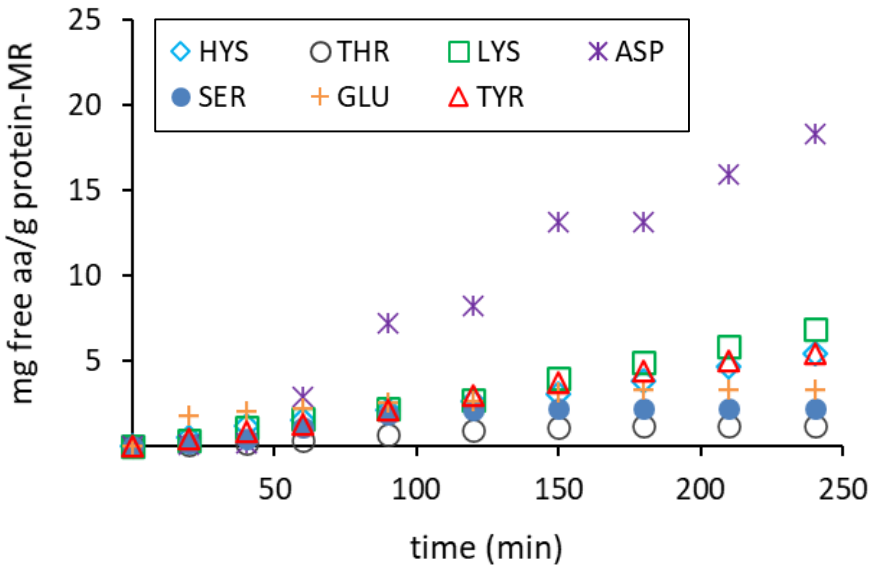
PROTEIN SW EXTRACTION

F = 6ml/min T = 200°C

APOLAR amino acids



POLAR amino acids



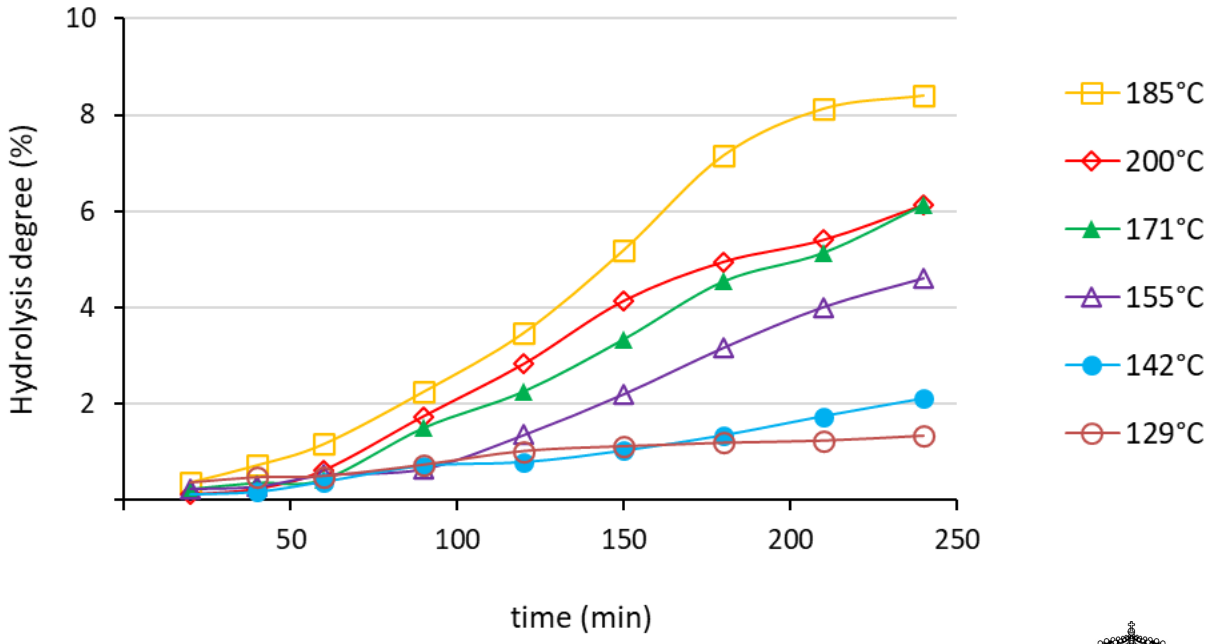
PROTEIN SW EXTRACTION

$$DH(\%) = \left(\frac{h}{h_{tot}}\right) \times 100$$

h = number of equivalent peptide bonds hydrolyzed

h_{tot} = mmol of individual amino acids per gram in the unhydrolyzed protein

Temperature effect



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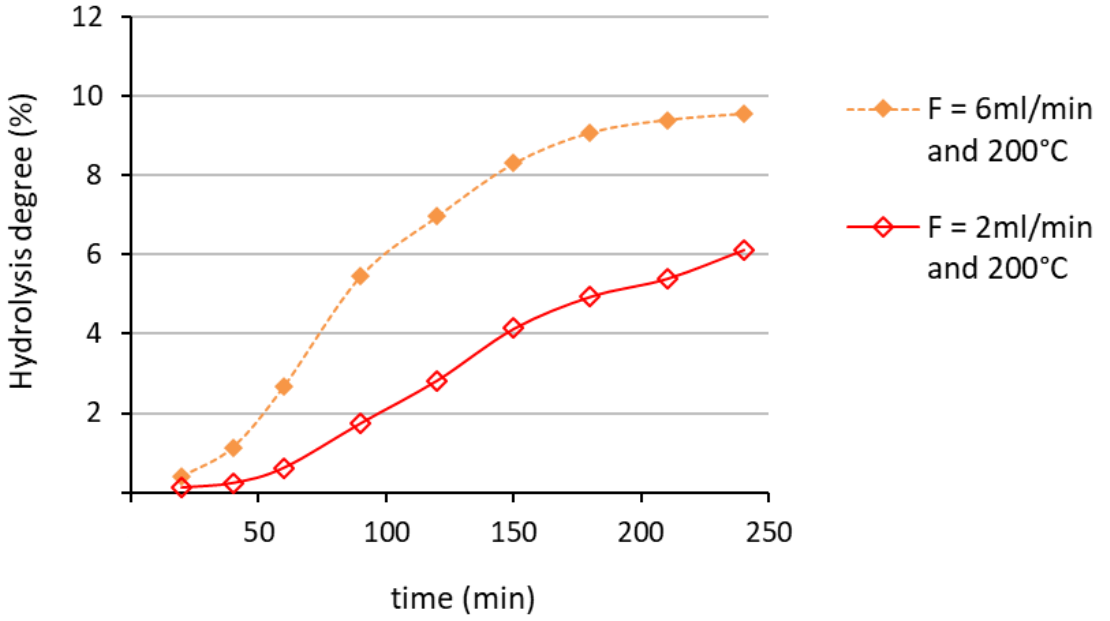
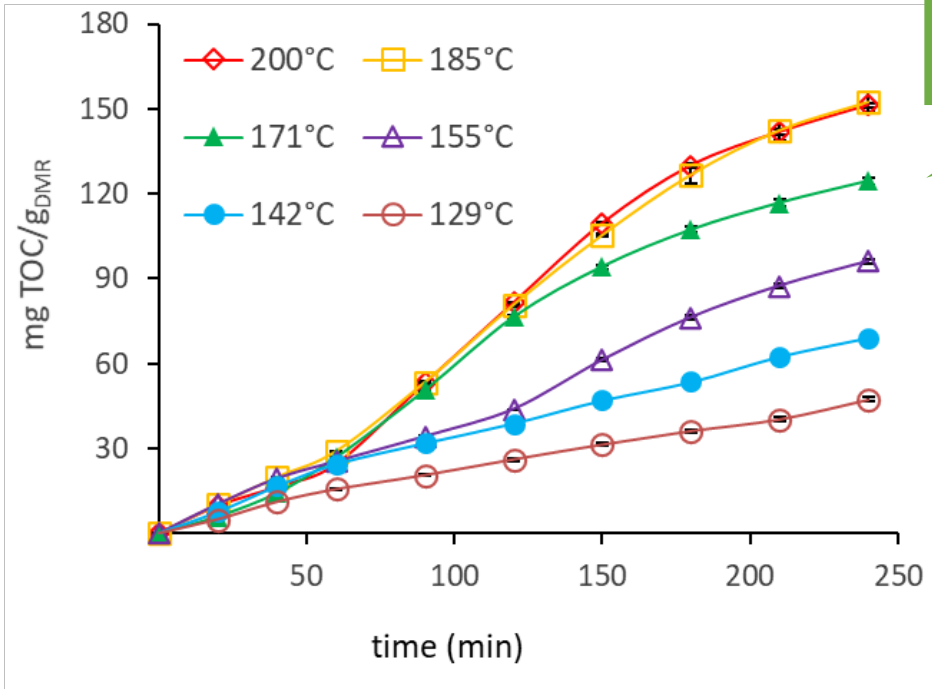
Flow rate effect

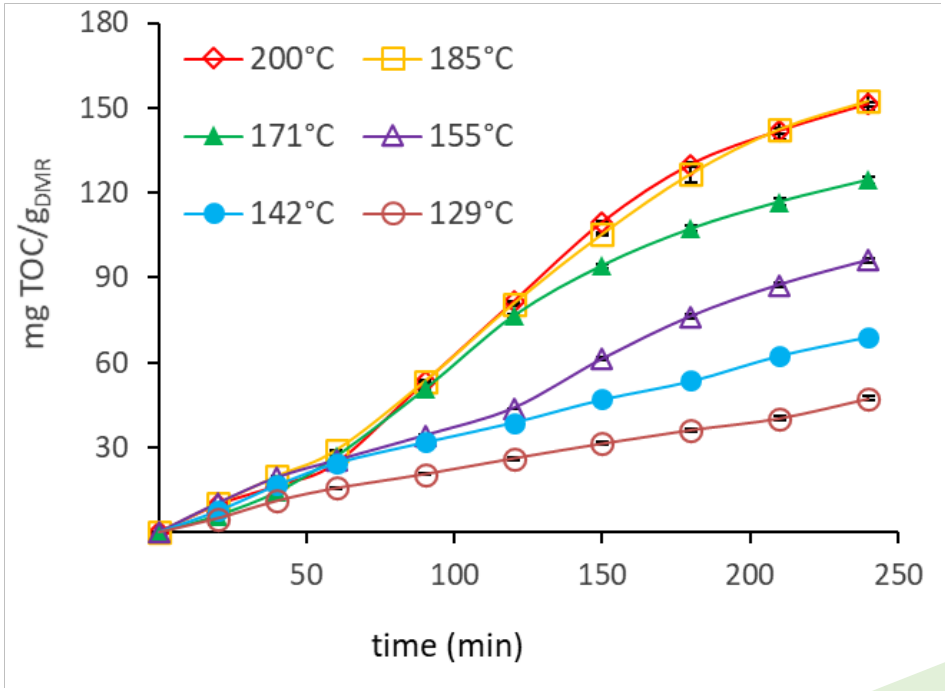


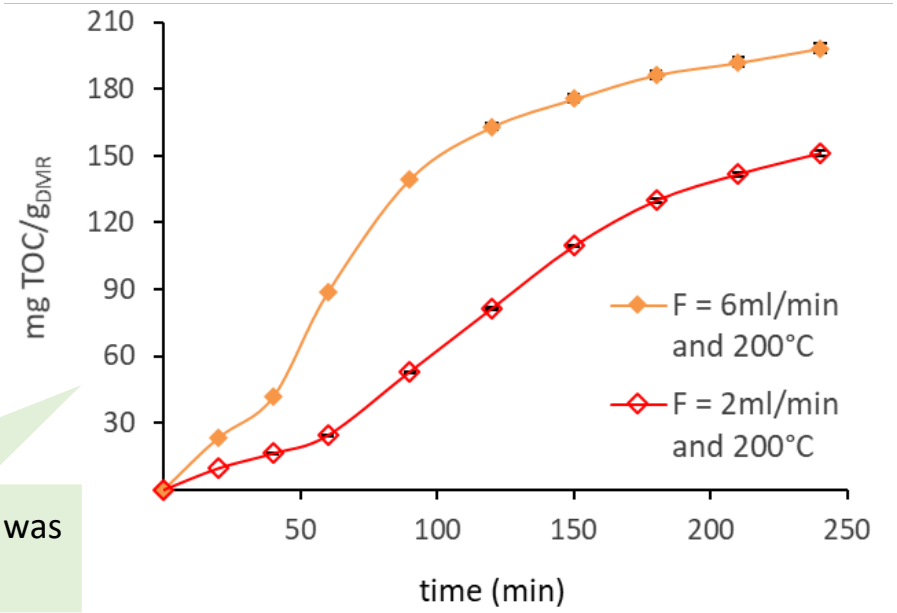
TOTAL ORGANIC CARBON SW EXTRACTION



At lowest flow rate, TOC increased with increasing temperature.

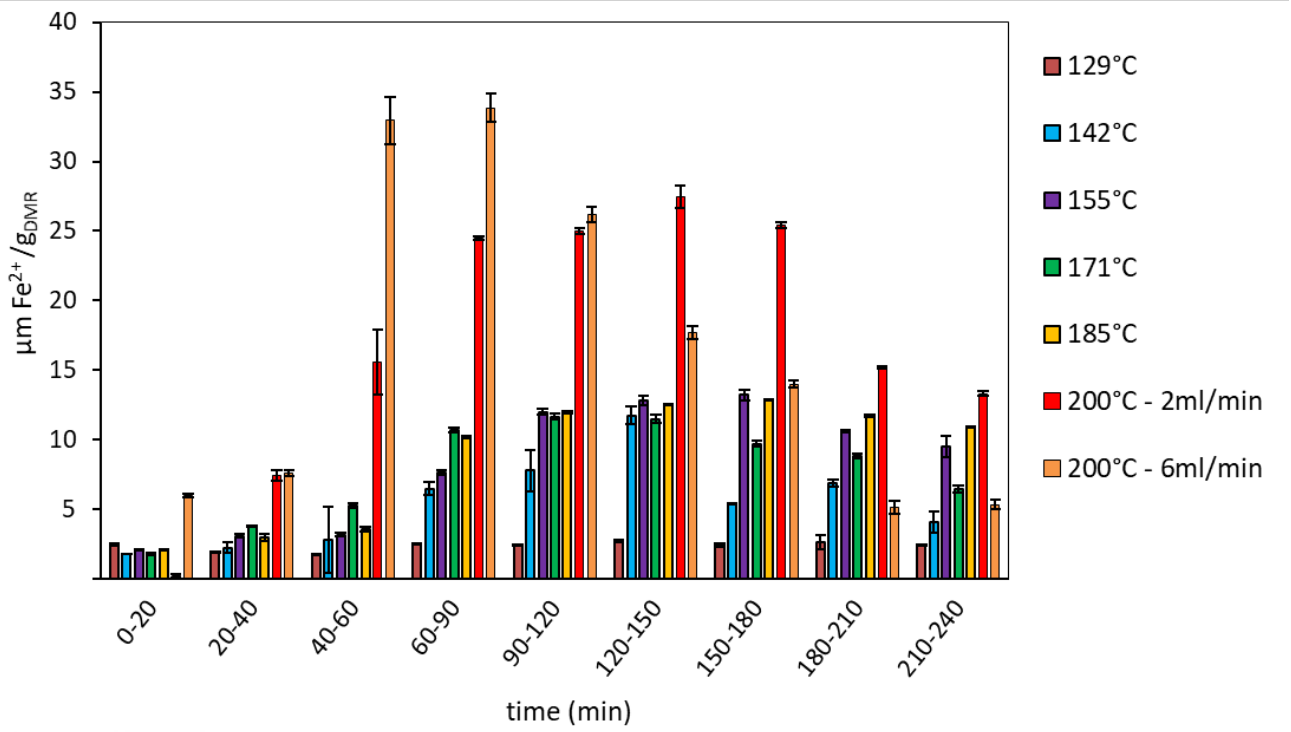
TOTAL ORGANIC CARBON SW EXTRACTION





Faster and higher release was observed at highest flow rate.

ANTIOXIDANT ACTIVITY SW EXTRACTION



Antioxidant capacity increased with temperature and flow rate.

The maximum at 2ml/min was obtained at 60-90 min, while at 6ml/min, maximum was reached at 40-60 min.

SOLID RESIDUE AFTER SWE

$$Hydrolysis\ yield\ (\%) = \left(\frac{W - W_1}{W}\right) \times 100$$

W = *weight of the dry sample introduced in the reactor*

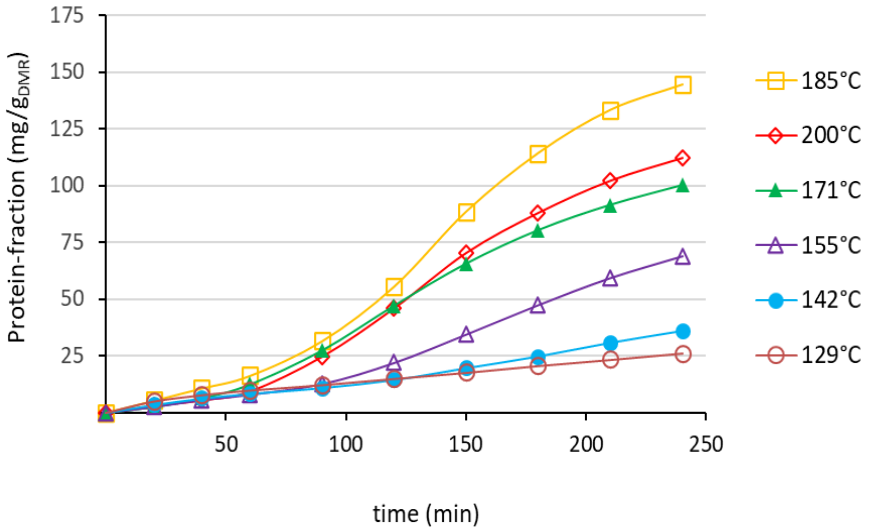
W_1 = *weight of the dried residue after SW extraction*

	Yield (%)	C (%)	H (%)	N (%)	S (%)	O (%)	Ashes (%)
G. sesquipedale	-	36.0 ± 0.3	5.4 ± 0.2	3.5 ± 0.3	0.26 ± 0.07	40 ± 1	14.9 ± 0.9
Macroalga Residue	-	35.6 ± 1.2	5.9 ± 0.2	4.2 ± 0.4	0.21 ± 0.05	32.3 ± 0.3	21.8 ± 1.1
Residue-SW,. 129°C	21.2	33.5 ± 0.9	5.2 ± 0.3	4.2 ± 0.3	0.20 ± 0.03	29 ± 3	29.5 ± 0.8
Residue-SW,. 142°C	26.2	33.3 ± 0.6	4.5 ± 0.4	3.5 ± 0.5	0.04 ± 0.03	27 ± 2	31.9 ± 1.8
Residue-SW,. 155°C	31.5	32.8 ± 1.6	4.7 ± 0.1	3.0 ± 0.2	n.d.	21 ± 4	33.4 ± 2.4
Residue-SW,. 171°C	40.7	34.9 ± 1.1	4.0 ± 0.2	2.2 ± 0.1	n.d.	22 ± 2	36.2 ± 2.6
Residue-SW,. 185°C	44.3	34.6 ± 0.9	4.0 ± 0.3	1.8 ± 0.1	n.d.	22 ± 2	37.9 ± 1.9
Residue-SW,. 200°C	50.7	31.4 ± 1.6	3.1 ± 0.3	1.0 ± 0.2	n.d.	22 ± 3	42.7 ± 2.1

COMPARISON WITH OTHER HYDROLYTIC TECHNIQUES

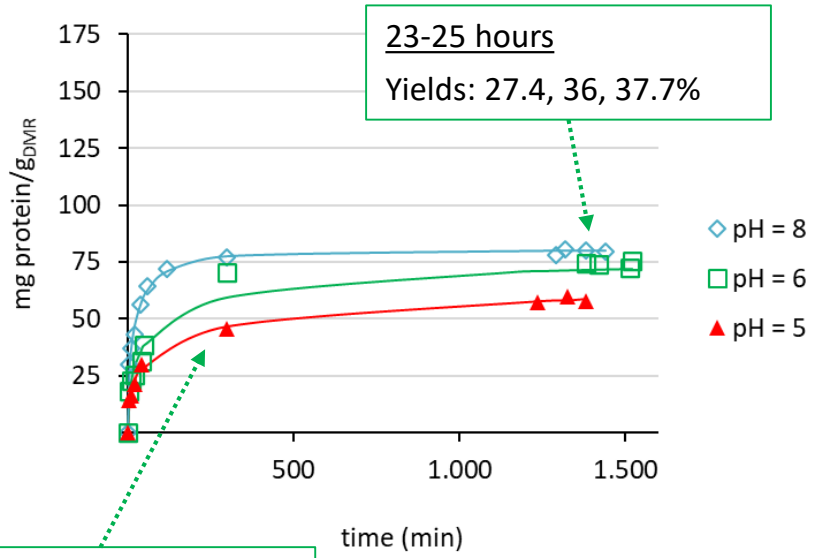
SW extraction

Maximum yield ≈70% in 4 hours



Enzyme assisted extraction

PROTEASE 6% - 50°C



23-25 hours
Yields: 27.4, 36, 37.7%

300 minutes (5 hours)
Yields: 21.6, 33.4, 36.5%

COMPARISON WITH OTHER HYDROLYTIC TECHNIQUES

SW extraction

	mg prot/g_{DMR}	Yield (%)
129°C	26.2	12.4
142°C	35.9	17
155°C	68.9	32.7
171°C	100.1	47.5
185°C	**144.4**	**68.5**
200°C – 2ml/min	112.2	53.2
200°C – 6ml/min	**146.4**	**69.5**

Maximum yield
≈70% in 4 hours

Enzyme assisted extraction

	mg prot/g_{DMR}	Yield (%)
0.25% cellulase	21.5	10.2
0.5% cellulase	21.8	10.4
1% cellulase	24.3	11.6
2% cellulase	53.6	25.5
4% cellulase	62	29.3
6% cellulase	**62**	**29.5**
8% cellulase	**63**	**29.9**
6% xylanase	50.9	24.2
6% protease	59.5	28.3
3% C + 3% P	**64.2**	**30.6**
3% C + 3% X	46	21.8
2%C + 2%P + 2%X	59.2	28.2

Maximum yield
≈30% in 24 hours

CONCLUSIONS

- ✓ Macroalga residue after industrial agar extraction still contains high-value bioactive compounds such as carbohydrates, proteins or amino acids.
- ✓ SW technology led to an efficient extraction of the protein fraction of the macroalga residue after agar extraction.
- ✓ The best experimental conditions were 200°C and 6ml/min with nearly 70% of total protein extracted.
- ✓ The complete valorization of macroalga residue by SW treatment is a very promising strategy in order to reach a circular economy system.

THANKS FOR YOUR ATTENTION

Grupo de Biotecnología Industrial y Medioambiental

https://www.ubu.es/biotecnologia-industrial-y-medioambiental-bioind

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