1º Encuentro Ibérico de Fluidos Supercríticos
Encontro Ibérico de Fluidos Supercríticos
EIFS
Santiago de Compostela (Spain), 18-19/2/2020

Recovery of proteins and free amino acids from *Gelidium sesquipedale* alga residue by subcritical water extraction (SWE).

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UNIVERSIDAD DE BURGOS
Biotechnology and Food Science Department

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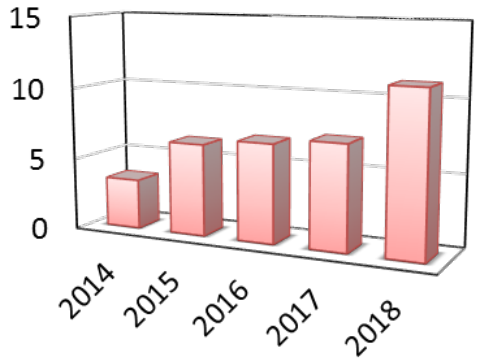
ALGA RESIDUE OBTAINING

SPANISH ALGA PRODUCTION (TN)*



SPANISH AGAR PRODUCTION = 890 TN**

AGAR – AGAR

MACROALGA *G. sesquipedale*

BASIC TREATMENT Na_2CO_3 NaOH

Colorants Salts

WASHING

Colorants

AGAR-AGAR EXTRACTION acid and boiling water bath

FILTRATION

SECOND EXTRACTION boiling water bath

Alga residue **BY-PRODUCT**

Ministerio de Agricultura, Pesca y Alimentación, **2019** (http://www.mapa.gob.es)
**FAO (http://www.fao.org)

BY-PRODUCT CHARACTERIZATION

CARBOHYDRATES 40.0%

- 65.5% Glucans
- 27.3% Galactans
- 7.2% Arabinans

PROTEINS 20.1%

- Soluble 13.0%
- Insoluble 87.0%

LIGNIN 13.2%

- 76.5% Soluble
- 23.5% Insoluble

POLYPHENOLS 4.4%

- Free 34.1%
- Linked 65.9%

ASHES 21.8%

LIPIDS 0.9%

Residual agar = 6.8%

(In subcritical water extracts)

BY-PRODUCT CHARACTERIZATION

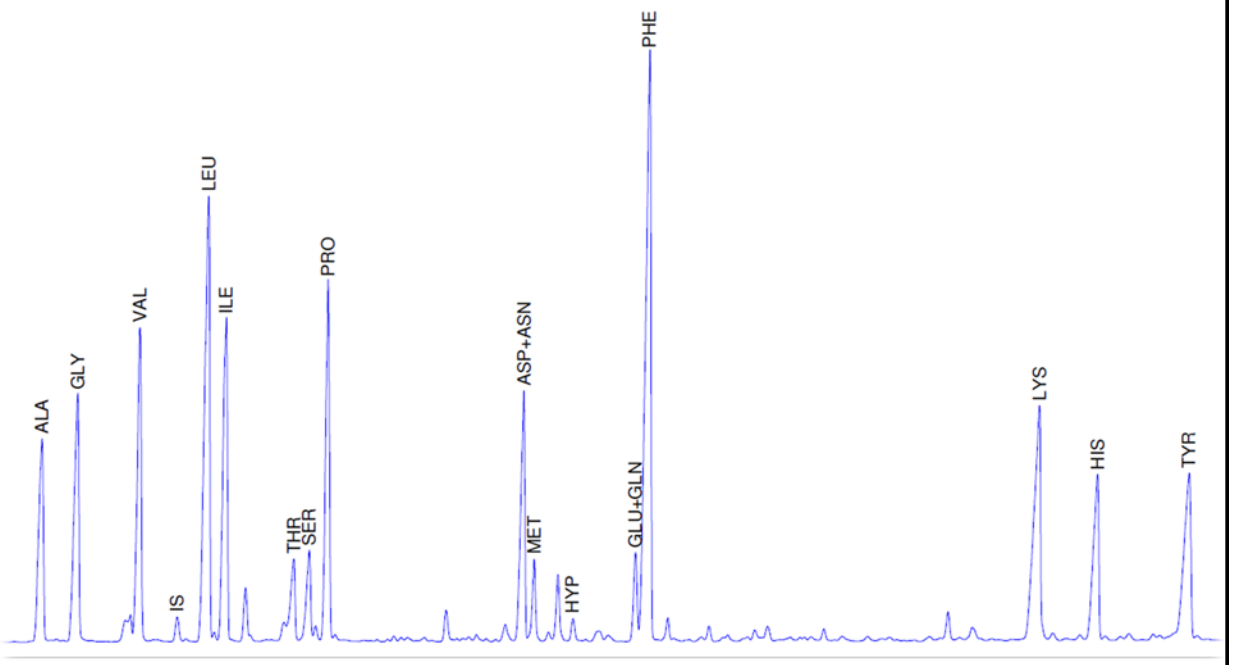
Minerals abundance (ppm):

	G. sesquipedale	By-product
Na	34484,4	874,9
Mg	12763,9	8917,5
S	11710,7	4992,6
K	29352,5	863,3
Al	174,8	8156,5
Si	190,0	109,0
P	955,7	5786,1
Ca	6583,8	449,1
Mn	149,0	385,6
Fe	250,5	6329,8
Co	0,6	2,4
Ti	0,7	7,3
Pb	3,8	10,9
Cu	5,6	8,8
Ni	4,5	10,2
Pd	0,0	0,0
Cr	1,3	13,6
V	2,1	6,2
As	5,7	7,6
Cd	0,5	1,3
Se	0,0	0,3
Zn	246,0	246,5
Sr	35,1	239,2
Total minerals	96921,2	37418,7

ASHES 21.8%

BY-PRODUCT CHARACTERIZATION

FREE AMINO ACIDS



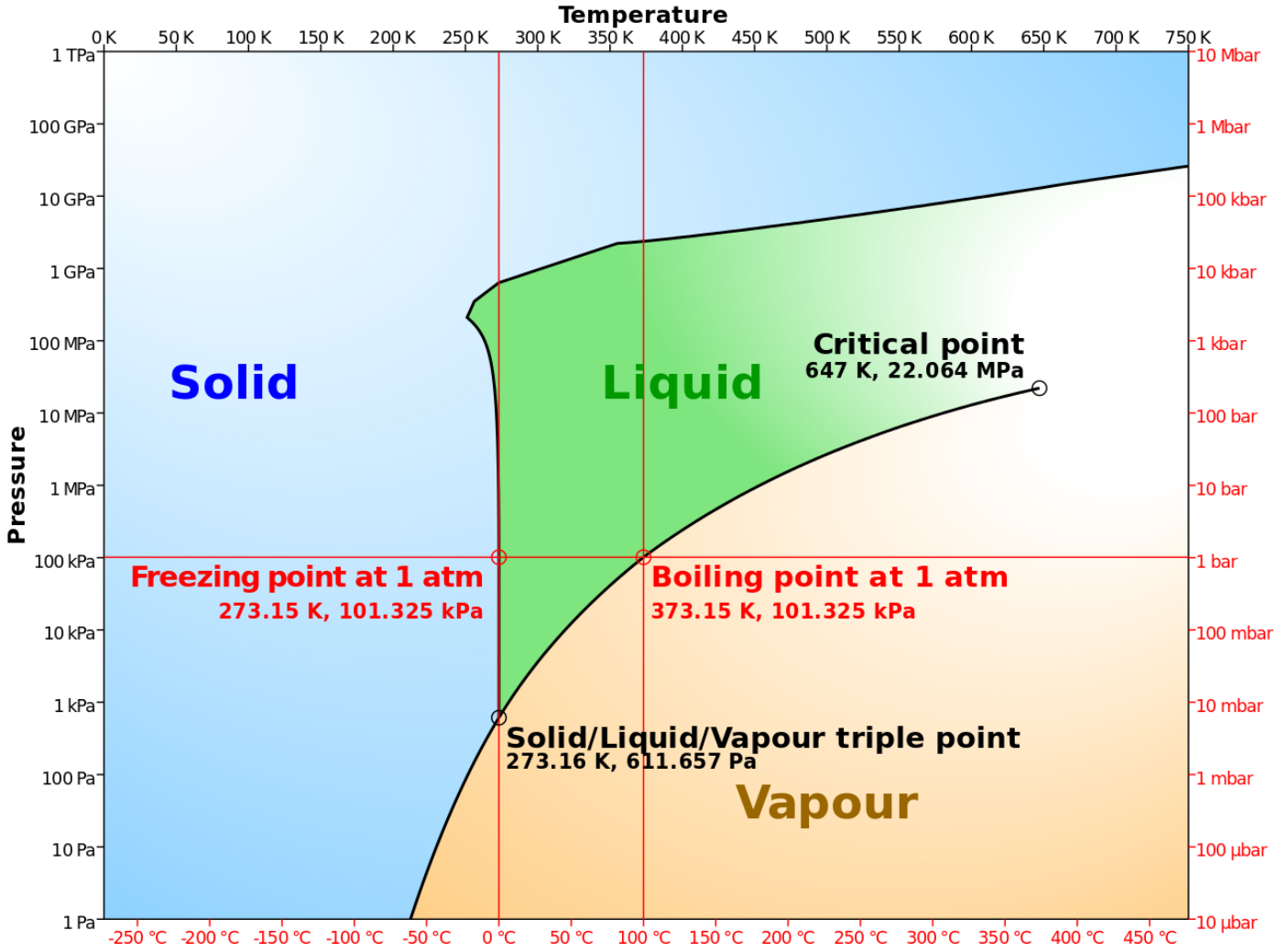
Nitrogen Factor, NF* = 4.8

code	amino acid	original sample (mg/g alga dry)
Ala	alanine	6,0 ±0.1
Gly	glycine	7,2 ±0.2
Val*	valine	22,4 ±0.6
Leu*	leucine	17,4 ±0.5
Ile*	isoleucine	17,3 ±0.6
Thr*	threonine	3,5 ±0.0
Ser	serine	3,4 ±0.0
Pro	proline	10,2 ±0.2
Asp + Asn	aspartic acid + asparagine	7,1 ±0.3
Met*	methionine	2,6 ±0.2
Glu + Gln	glutamic acid + glutamine	4,3 ±0.4
Phe*	phenylalanine	19,0 ±0.8
Lys*	lysine	12,9 ±0.6
His*	histidine	9,9 ±0.6
Tyr	tyrosine	6,6 ±0.0
Total amino acids		149,7 ±1.6

**Essential amino acids (EEAs)*

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

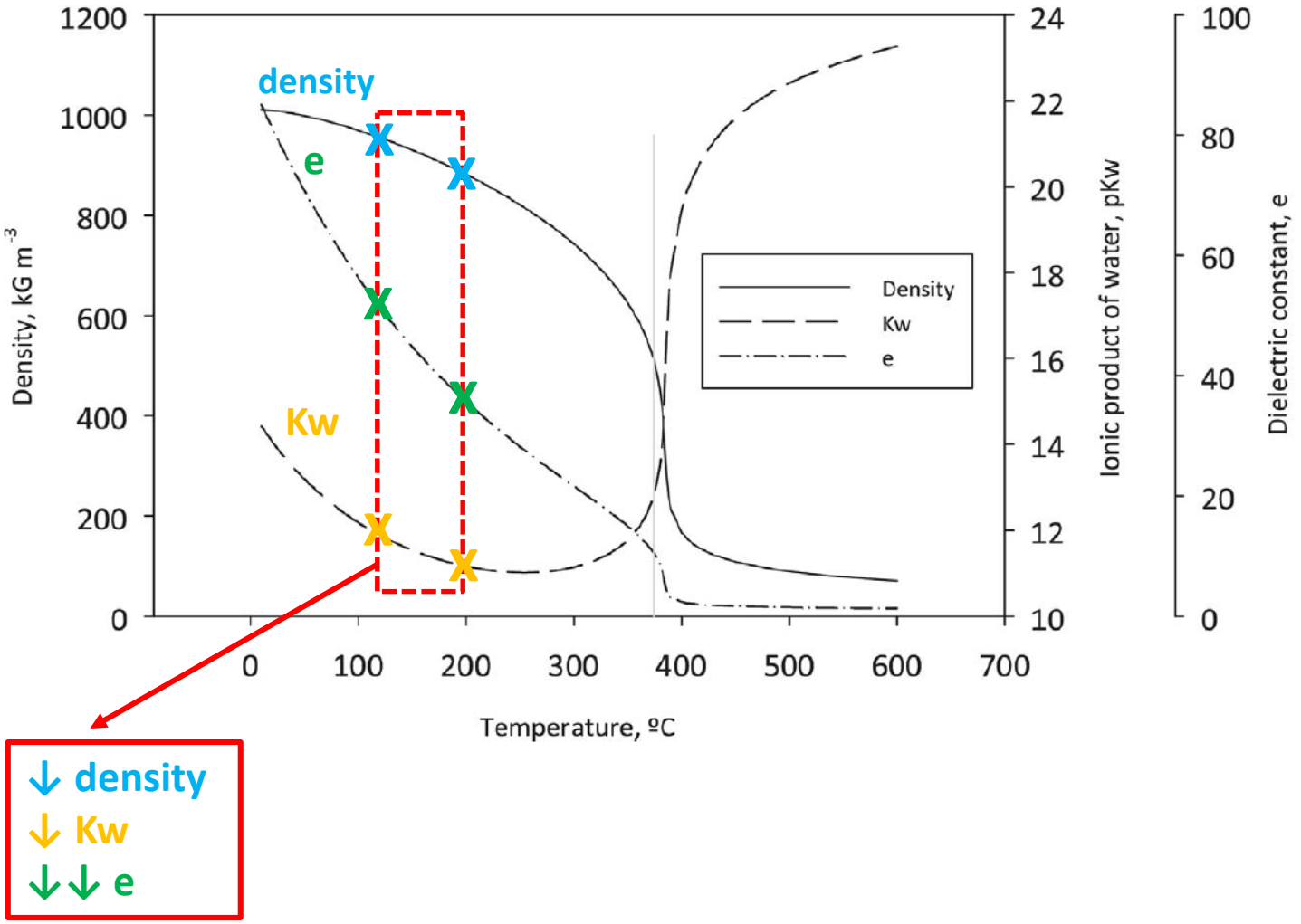
SUBCRITICAL WATER PROPERTIES



Temperature
100 – 374°C

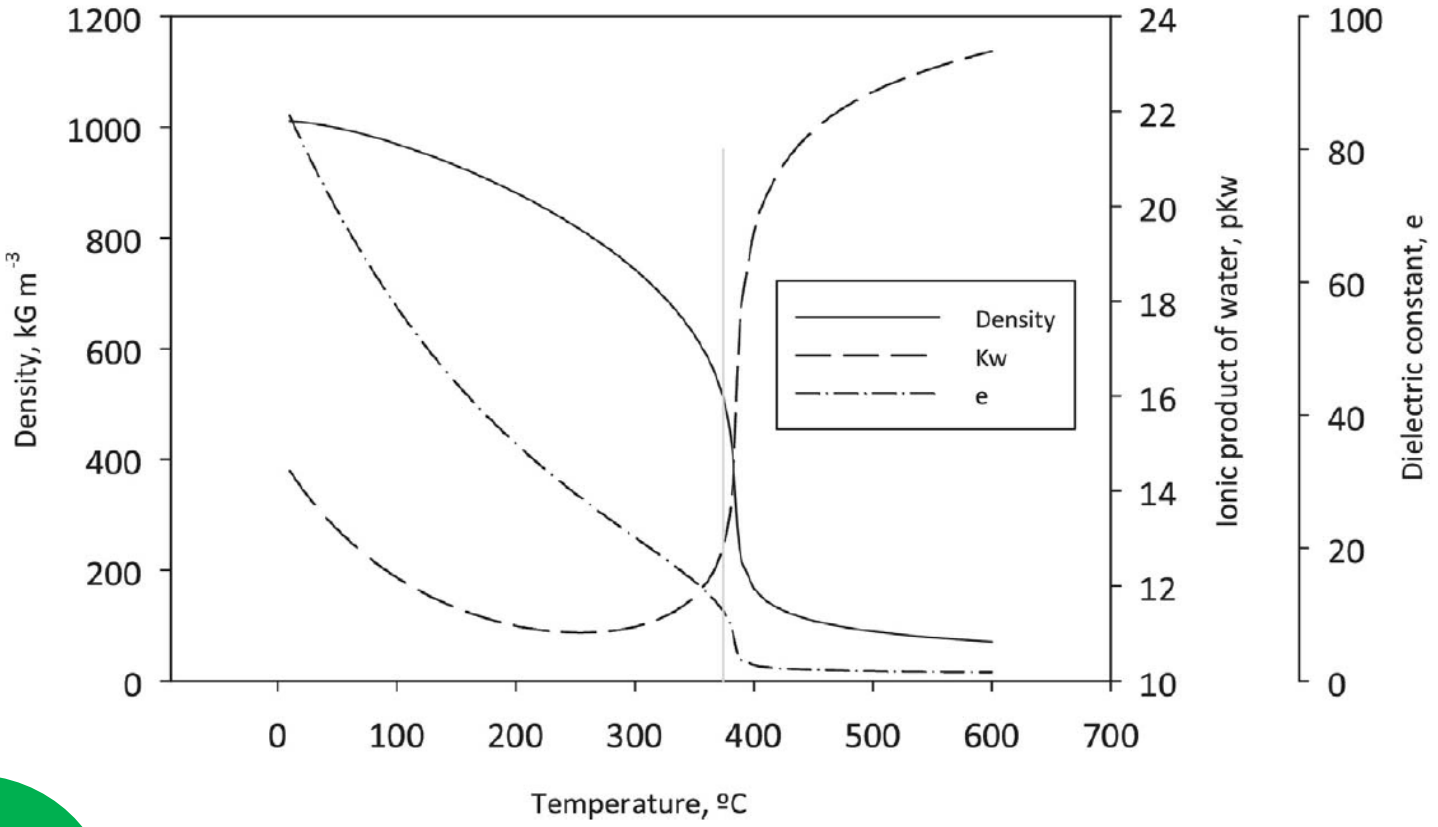
Pressure
> 50bar

SUBCRITICAL WATER PROPERTIES



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

SUBCRITICAL WATER PROPERTIES



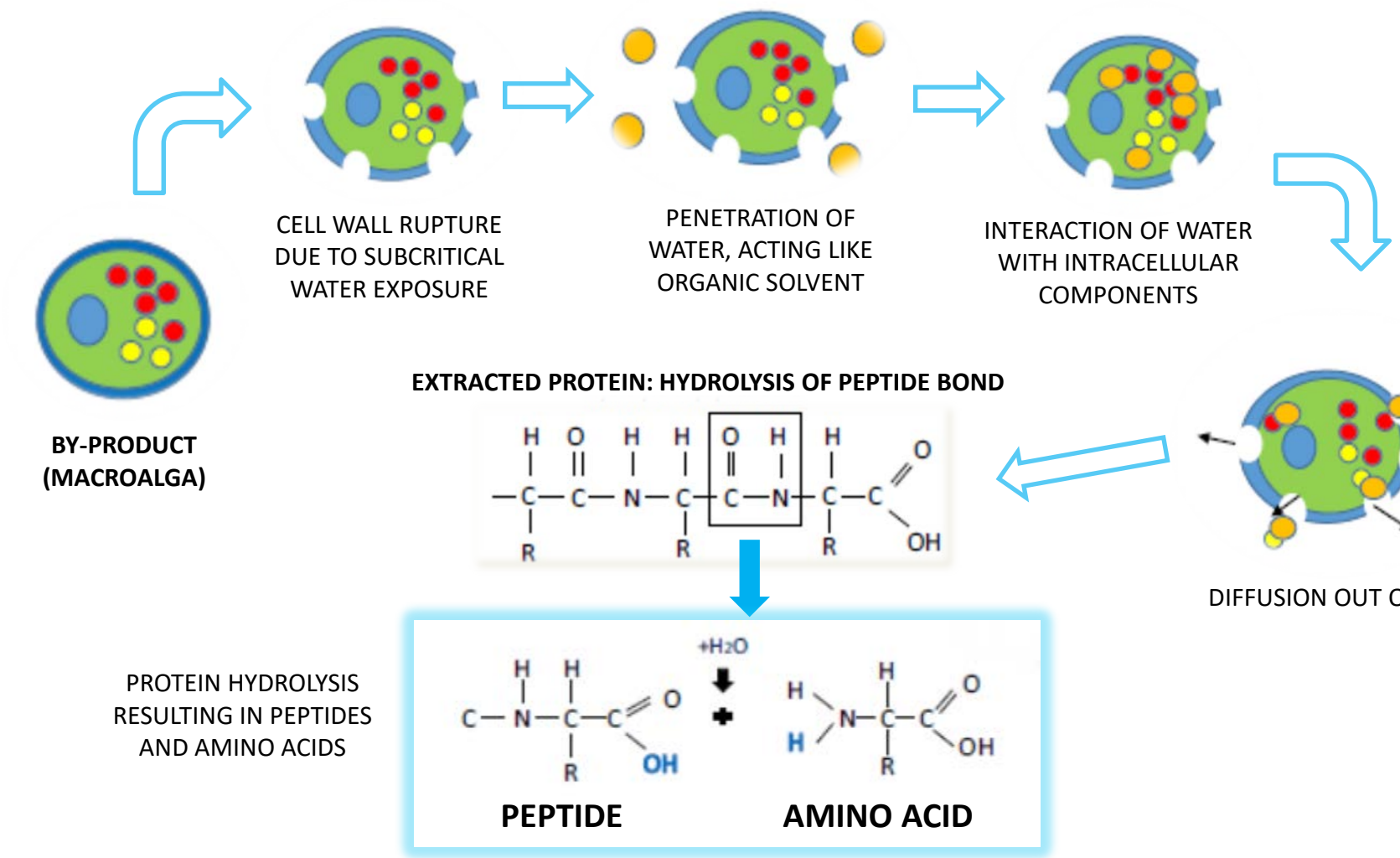
GREEN SOLVENT

→ Non-toxicity
→ Non-contamination
→ Safe to work with

UNIQUE PROPERTIES AS SOLVENT

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

PROTEIN HYDROLYSIS BY SUBCRITICAL WATER

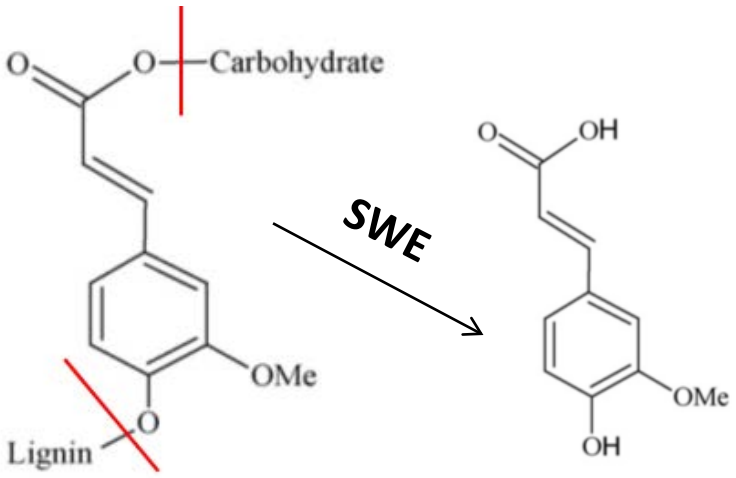


*Adapted from: Zainan et al., *Biomass Conversion and Biorefinery*, **2019**.

POLYPHENOLS EXTRACTION BY SUBCRITICAL WATER

1) RELEASE: BOND HYDROLYSIS

Ether bond hydrolysis > 170°C

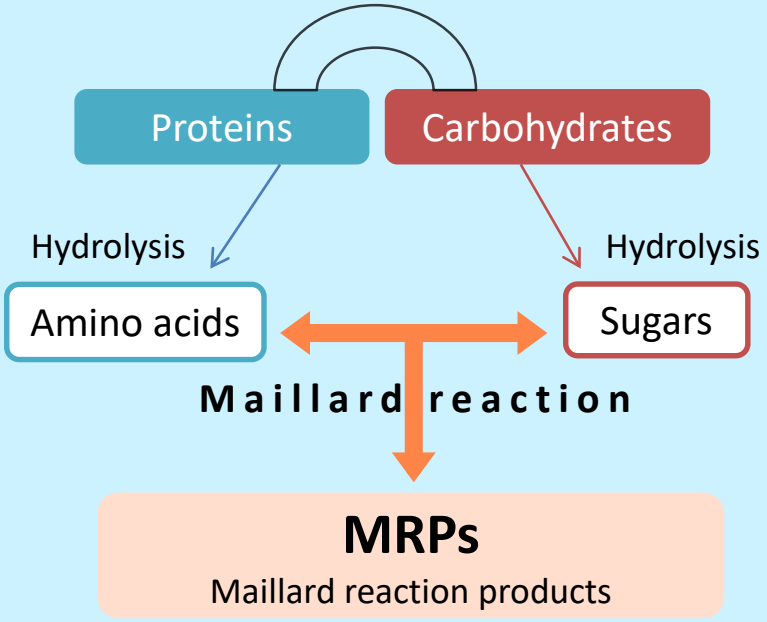


Lignin/phenolics-carbohydrate complex

Phenolic compounds

+ Lignine
+ Carbohydrates

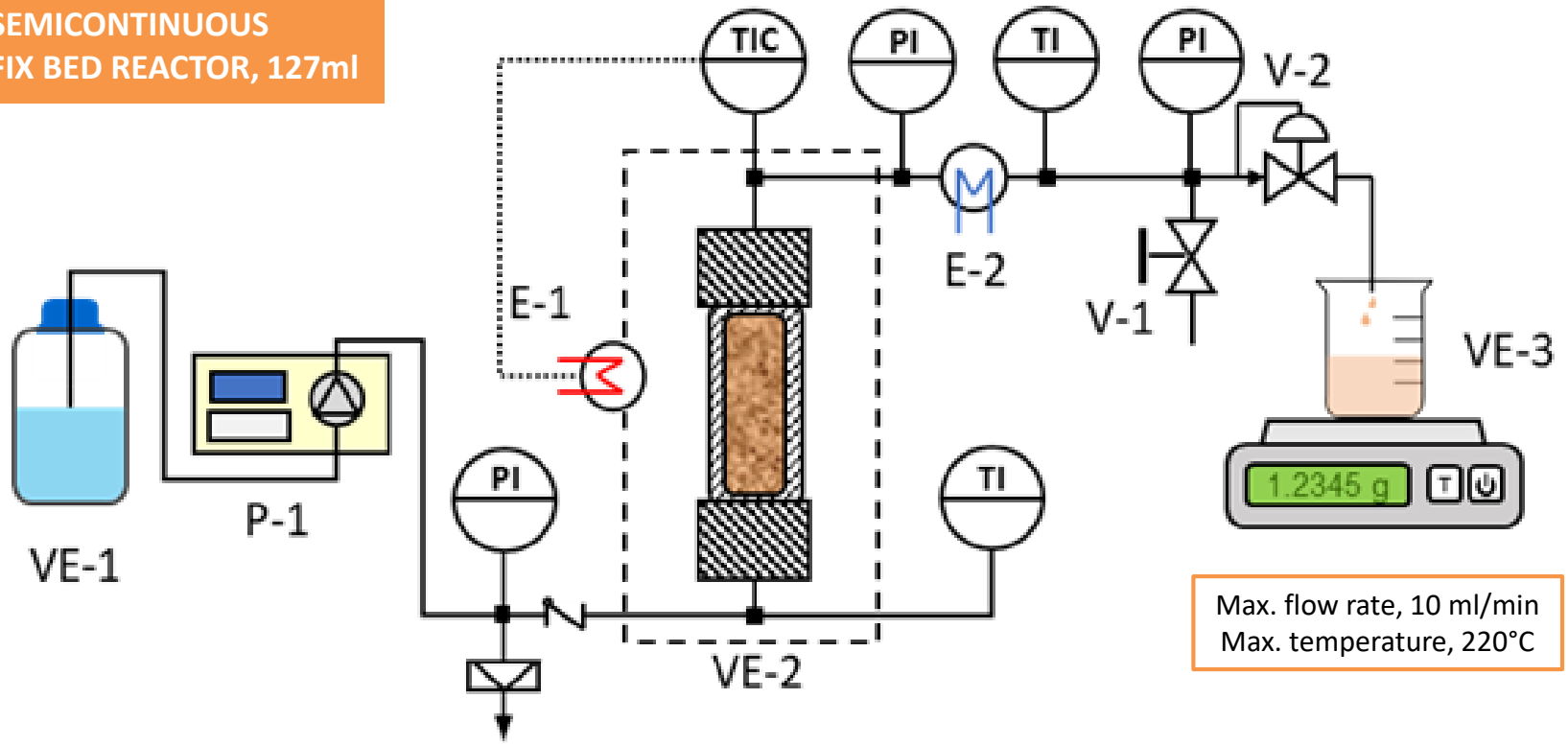
2) NEOFORMATION: MAILLARD REACCTION



→ Influence in the overall **antioxidant activity**

SUBCRITICAL WATER EXTRACTION

SEMICONTINUOUS
FIX BED REACTOR, 127ml



Max. flow rate, 10 ml/min
Max. temperature, 220°C

STUDY PARAMETHERS:

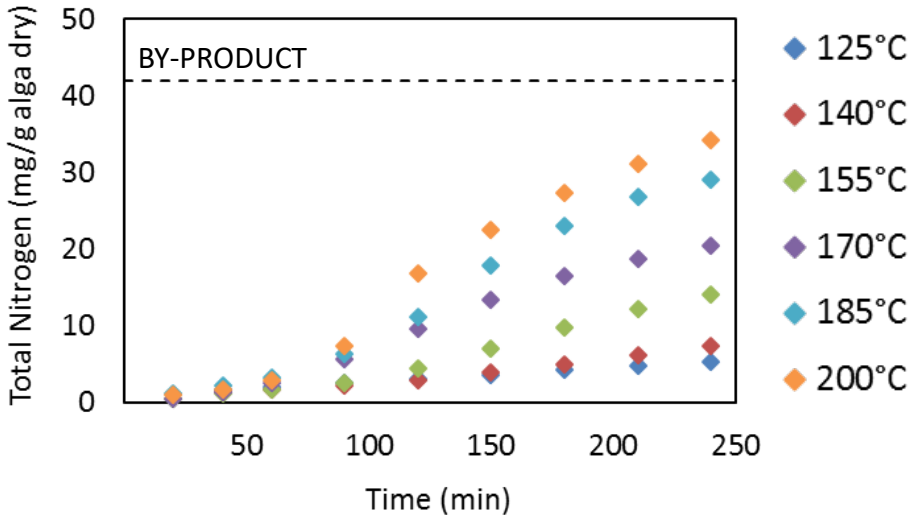
→ Temperatures: 125, 140, 155, 170, 185 and 200°C (2ml/min).

→ Flow rates: 2 and 6 ml/min (200°C) with residence times of 55.2 and 18.4min, respectively.

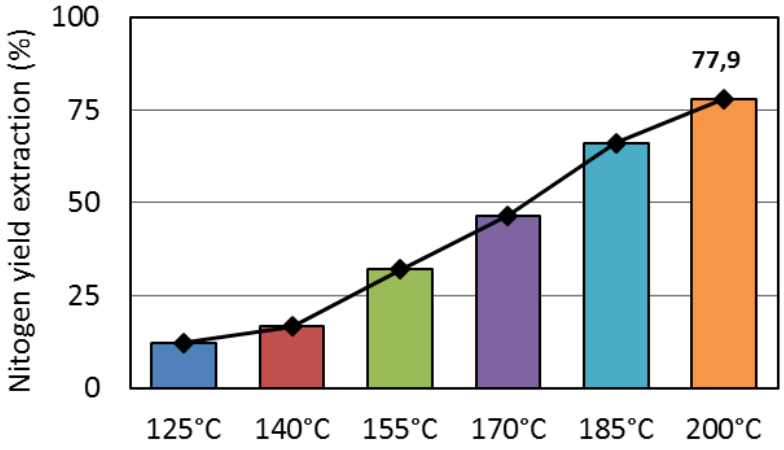
Time: 240 minutes; Pressure: 50 bar

NITROGEN EXTRACTION

TEMPERATURE EFFECT

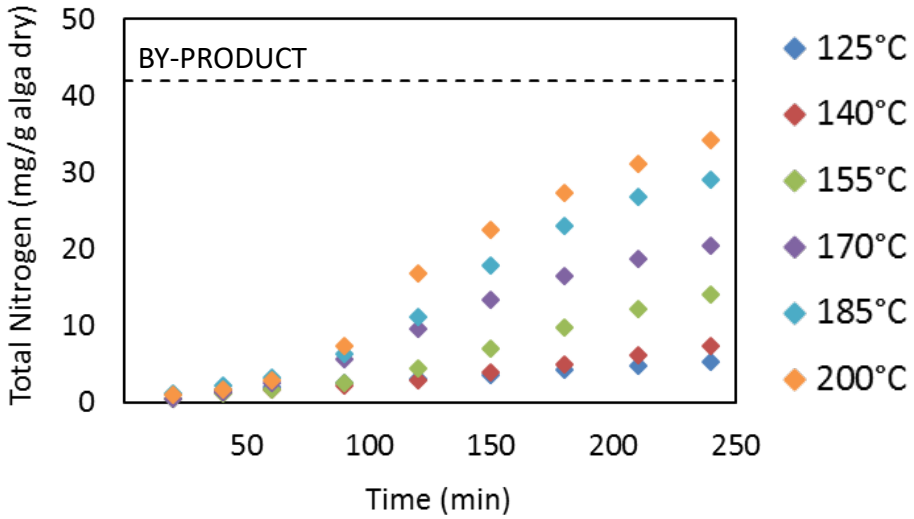


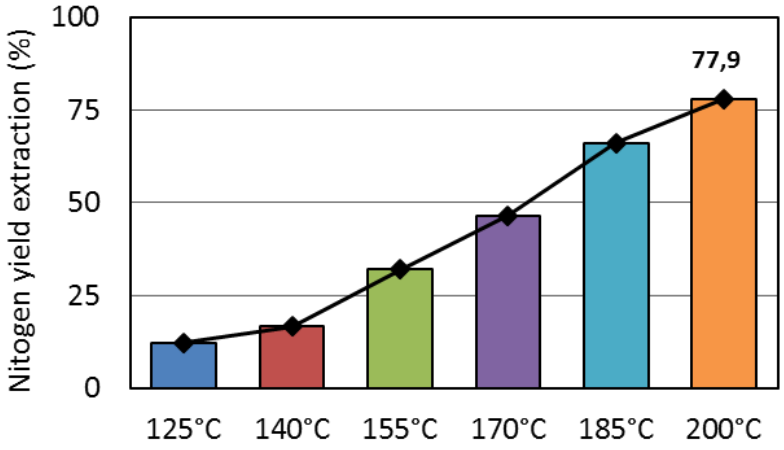
Increasing temperaure means increasing nitrogen extraction



NITROGEN EXTRACTION

TEMPERATURE EFFECT





Maximum nitrogen extraction

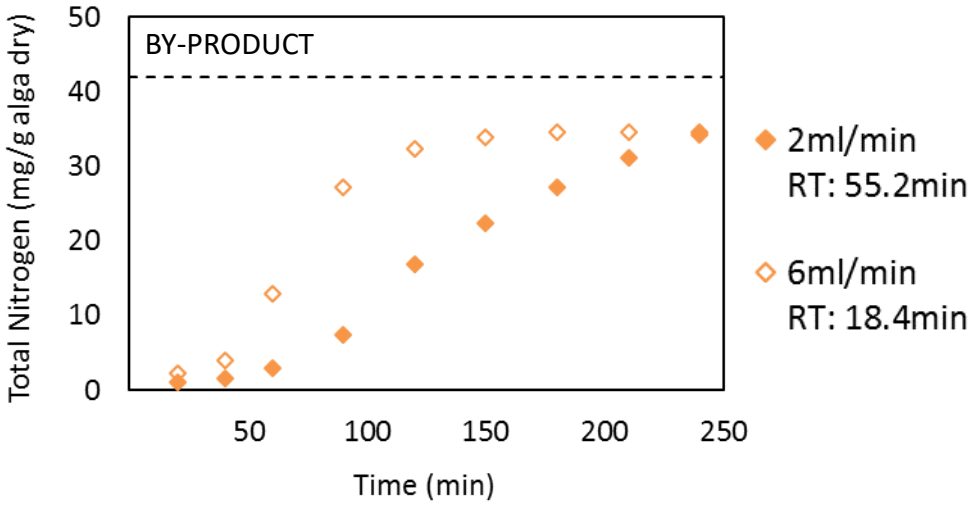
200°C = 77,9%

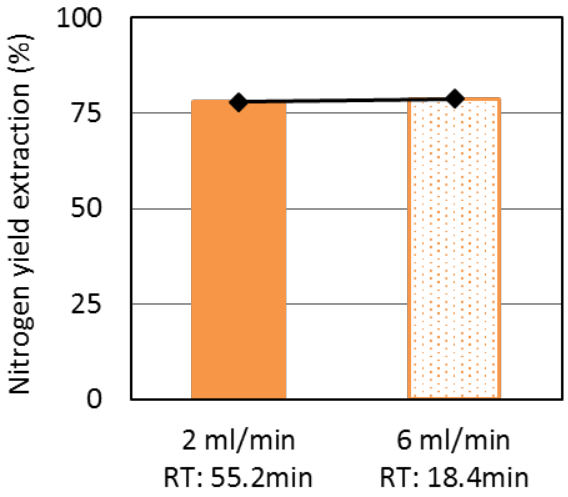
$$\% \, Extraction \, Yield = \frac{TN \, in \, SW \, extract}{TN \, in \, by-product} \times 100$$

NITROGEN EXTRACTION

Total nitrogen recovered at 6 ml/min is not significatively different in compare to 2ml/min

FLOW RATE EFFECT

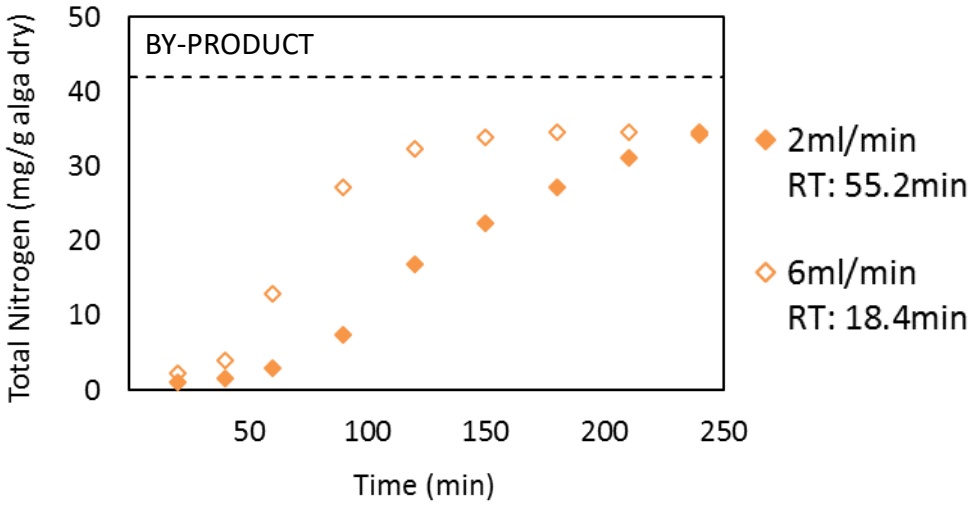




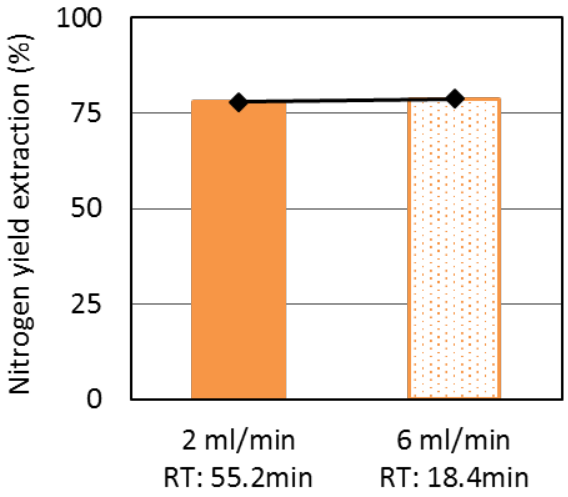
$$\%\ Extraction\ Yield\ = \frac{TN\ in\ SW\ extract}{TN\ in\ by-product} \times 100$$

NITROGEN EXTRACTION

FLOW RATE EFFECT

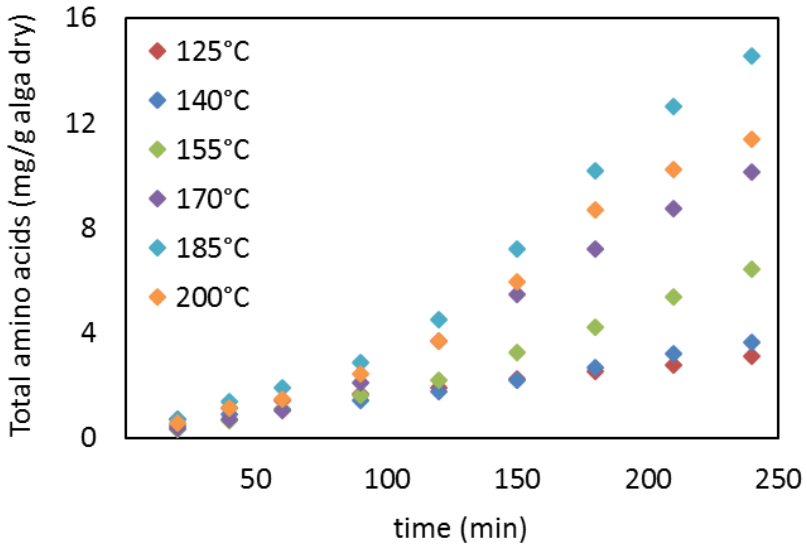


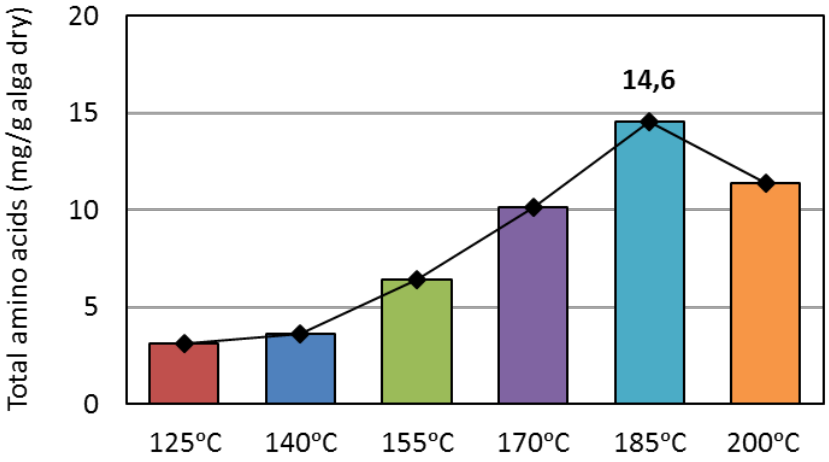
Lower RT, consequence of greater flow rate, makes nitrogen extraction faster



AMINO ACIDS EXTRACTION

TEMPERATURE EFFECT

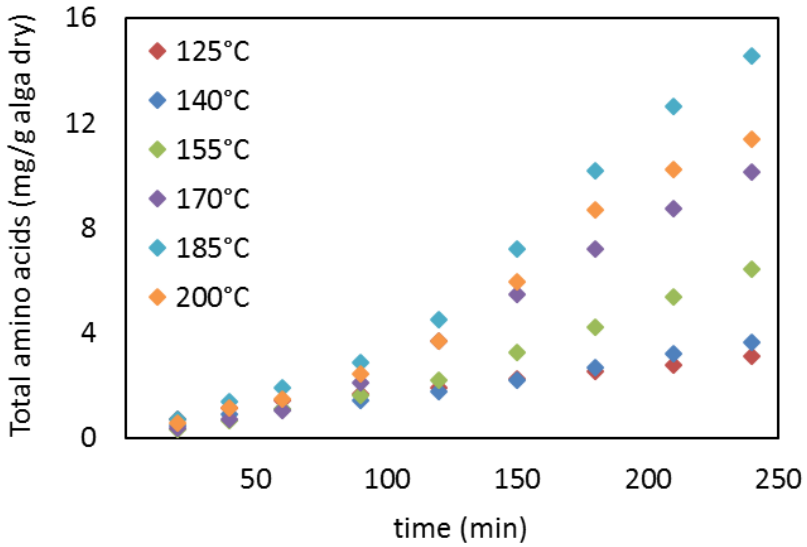


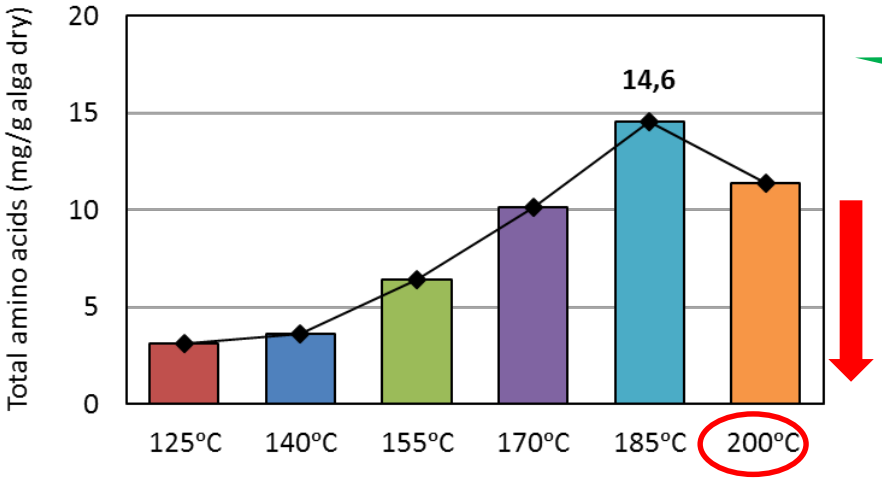


- 125°C - 185°C: Greater extraction with increasing temperature
- Maximum at 185°C
- Decrease at 200°C

AMINO ACIDS EXTRACTION

TEMPERATURE EFFECT





Maximum amino acids extraction

185°C = 14.6 mg/g alga dry

AMINO ACIDS EXTRACTION

		SWE (constant flow rate = 2ml/min)					
code	amino acid	125°C	140°C	155°C	170°C	185°C	200°C
Ala	alanine	2,8	3,3	8,2	14,6	**24,0**	21,7
Gly	glycine	2,7	4,2	10,0	20,3	**29,4**	21,6
Val*	valine	0,8	0,5	1,0	2,9	6,4	6,3
Leu*	leucine	1,0	0,9	1,4	2,6	4,6	3,6
Ile*	isoleucine	0,7	0,4	0,7	1,5	3,6	3,0
Thr*	threonine	1,8	1,7	3,0	5,2	6,2	4,8
Ser	serine	4,6	6,0	10,9	15,2	**21,8**	11,1
Pro	proline	1,3	2,0	3,2	5,2	7,6	6,3
Asp	aspartic acid	1,2	2,7	15,8	30,0	**30,3**	13,3
Met*	methionine	1,0	1,3	4,2	6,8	**10,6**	8,0
Glu	glutamic acid	7,3	5,1	4,4	5,7	**12,2**	5,9
Phe*	phenylalanine	0,6	0,5	0,9	1,8	3,3	2,6
Lys*	lysine	2,0	2,1	2,3	2,8	4,6	3,1
His*	histidine	0,5	0,9	1,7	2,9	4,9	3,3
Tyr	tyrosine	4,3	6,0	7,4	7,8	**10,6**	9,4

**Essential amino acids (EEAs)*

INDIVIDUAL AMINO ACIDS

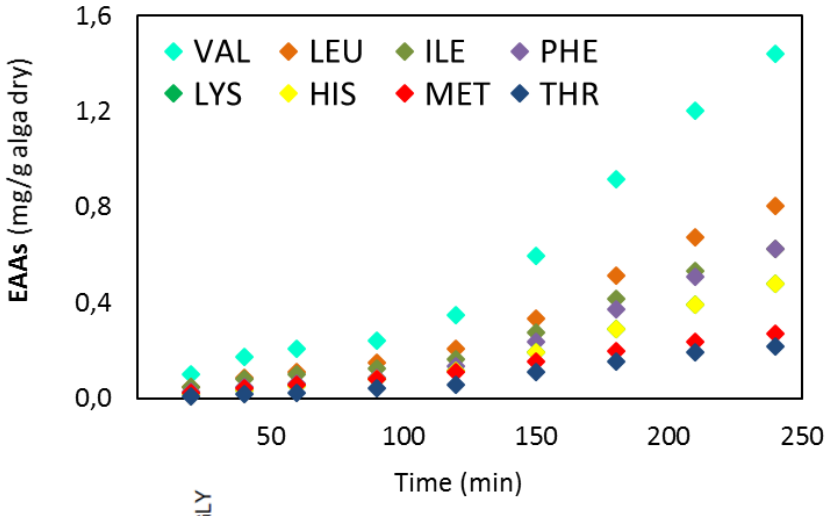
Yield extraction (%)

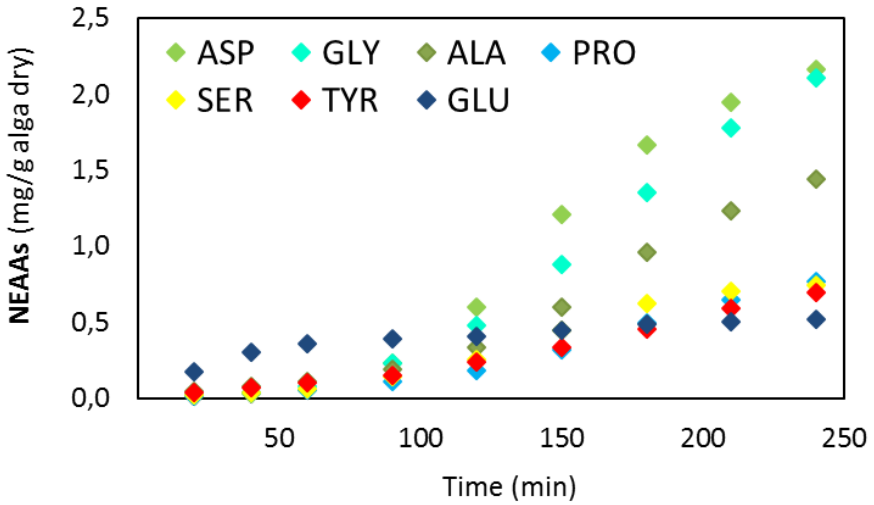
Maximum yield extraction at 185°C for all the amino acids, decreasing at 200°C

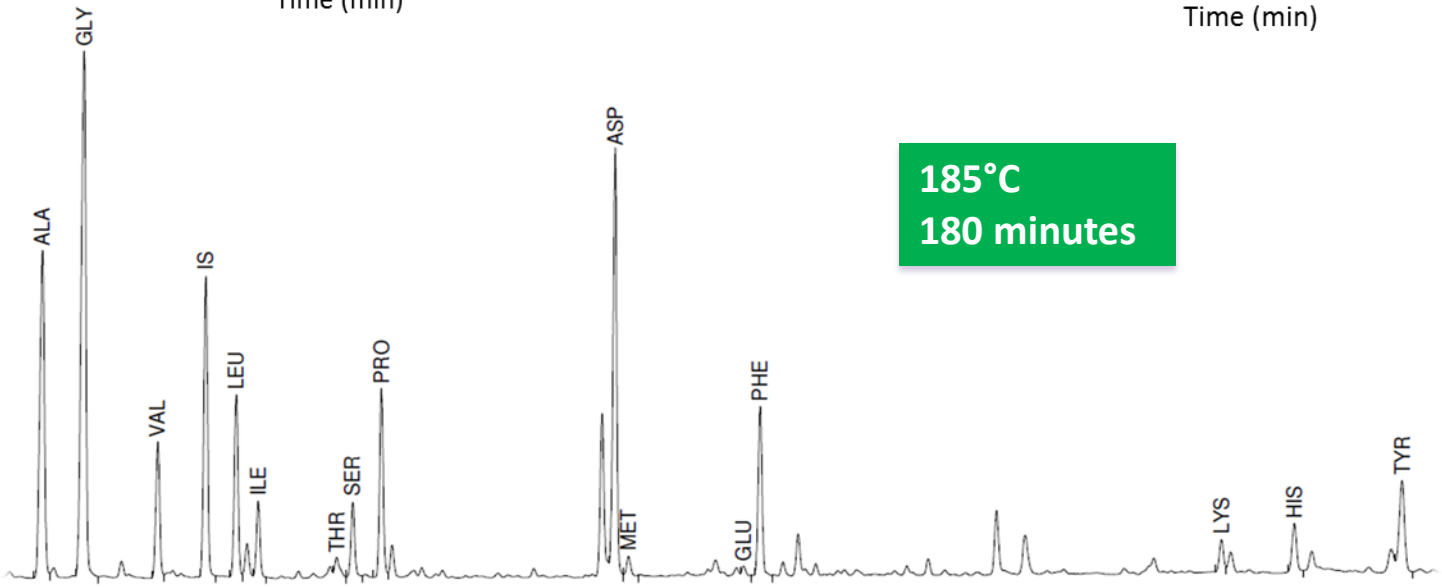
$$\%\ Extraction\ Yield = \frac{aa\ in\ SW\ extract}{aa\ in\ by-product} \times 100$$

AMINO ACIDS EXTRACTION

Maximum extraction at 185°C (2ml/min):



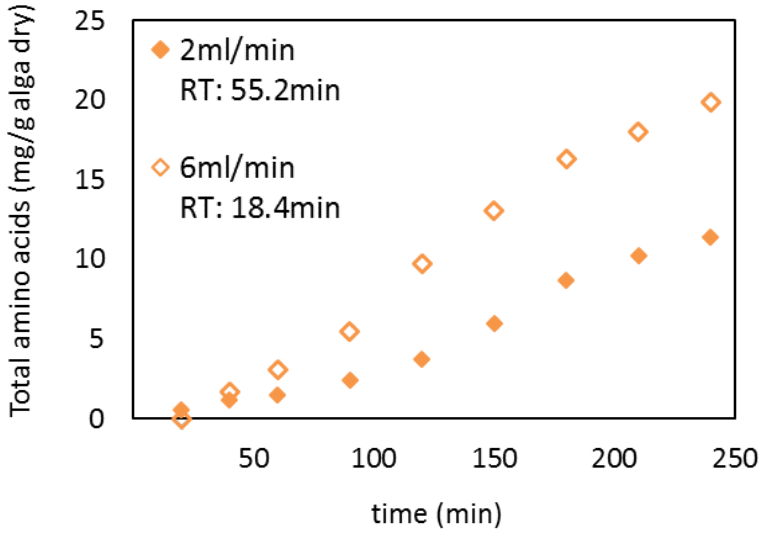


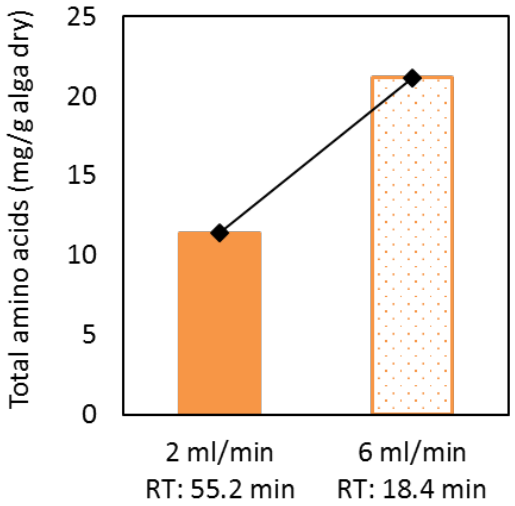


185°C
180 minutes

AMINO ACIDS EXTRACTION

FLOW RATE EFFECT

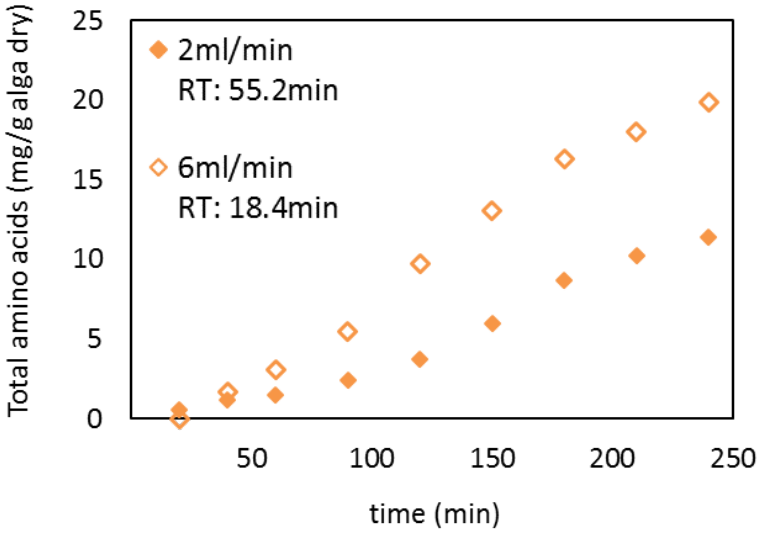


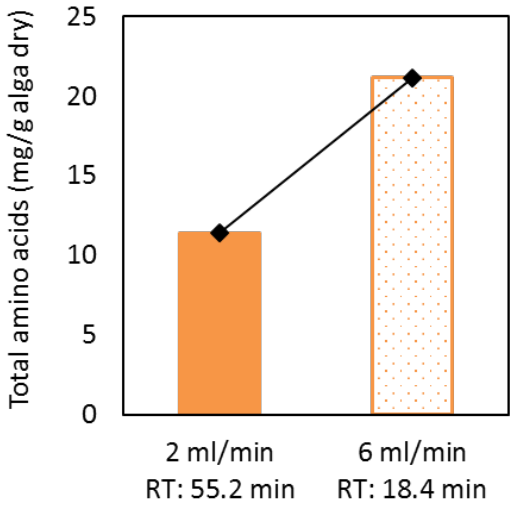


Lower RT, consequence of greater flow rate, makes higher and faster amino acids extraction

AMINO ACIDS EXTRACTION

FLOW RATE EFFECT





Maximum amino acids extraction

6ml/min RT: 18.4min = 21.1 mg/g alga dry

AMINO ACIDS EXTRACTION

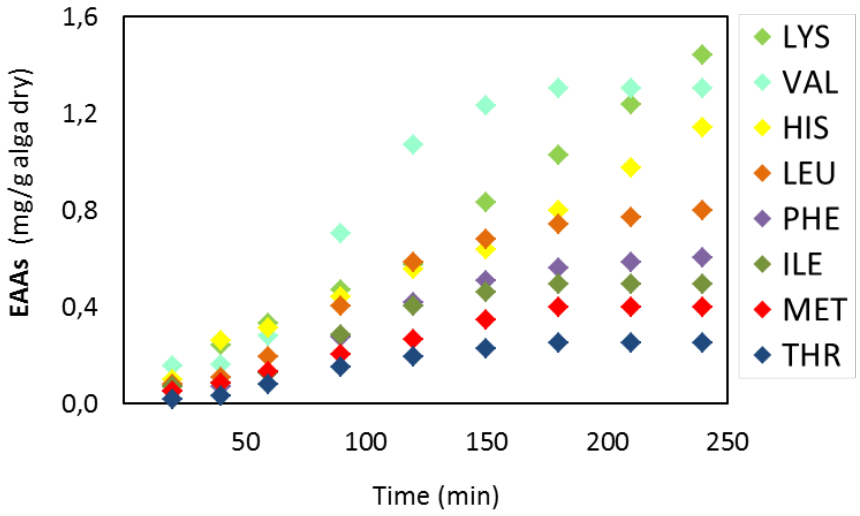
INDIVIDUAL AMINO ACIDS

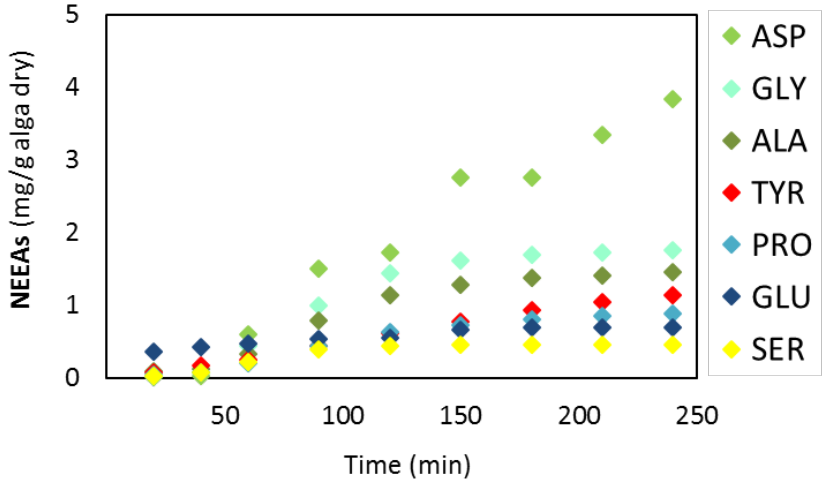
Yield extraction (%)

		SWE (constant temperature = 200°C)	
code	amino acid	2ml/min RT: 55.2 min	6ml/min RT: 18.4 min
Ala	alanine	21,7	**24,2**
Gly	glycine	21,6	**24,5**
Val*	valine	6,3	5,8
Leu*	leucine	3,6	4,6
Ile*	isoleucine	3,0	2,9
Thr*	threonine	4,8	7,1
Ser	serine	11,1	13,6
Pro	proline	6,3	8,8
Asp	aspartic acid	13,3	**53,8**
Met*	methionine	8,0	**15,5**
Glu	glutamic acid	5,9	**16,4**
Phe*	phenylalanine	2,6	3,2
Lys*	lysine	3,1	11,2
His*	histidine	3,3	11,6
Tyr	tyrosine	9,4	**17,5**

**Essential amino acids (EEAs)*

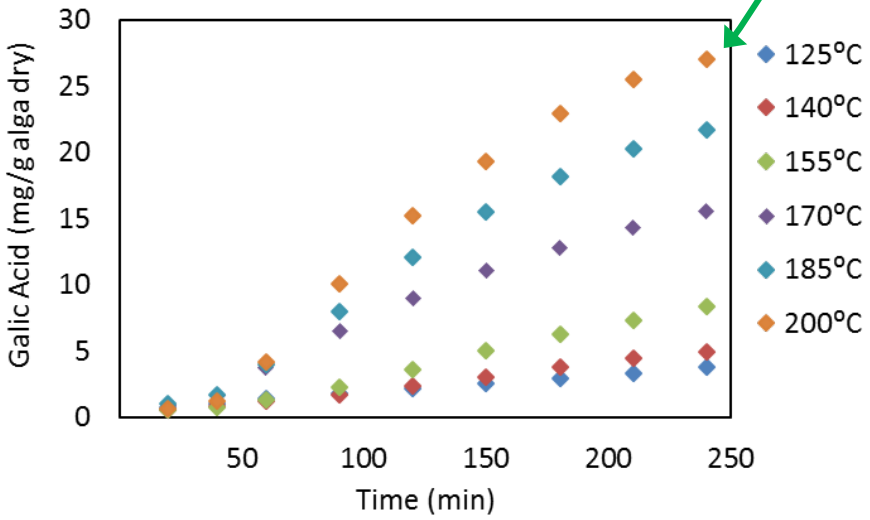
Maximum extraction at 6ml/min RT: 18.4 min:

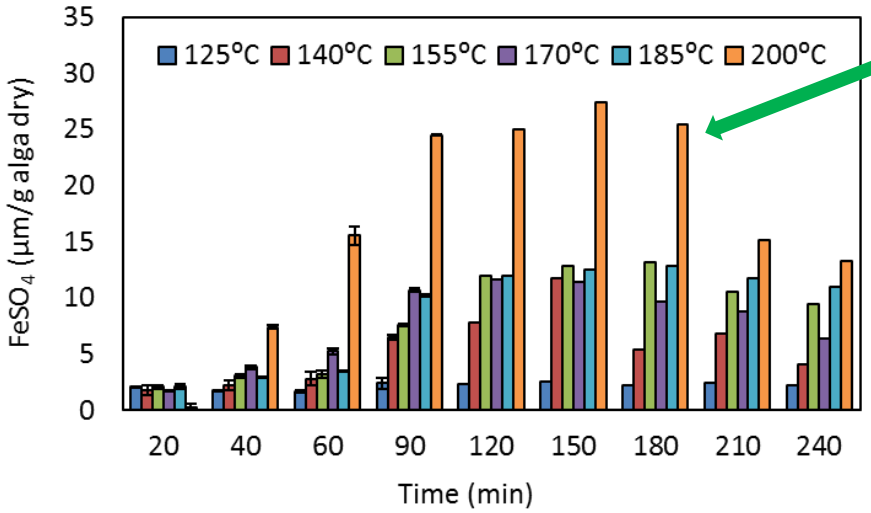




POLYPHENOLS EXTRACTION AND ANTIOXIDANT ACTIVITY

TEMPERATURE EFFECT

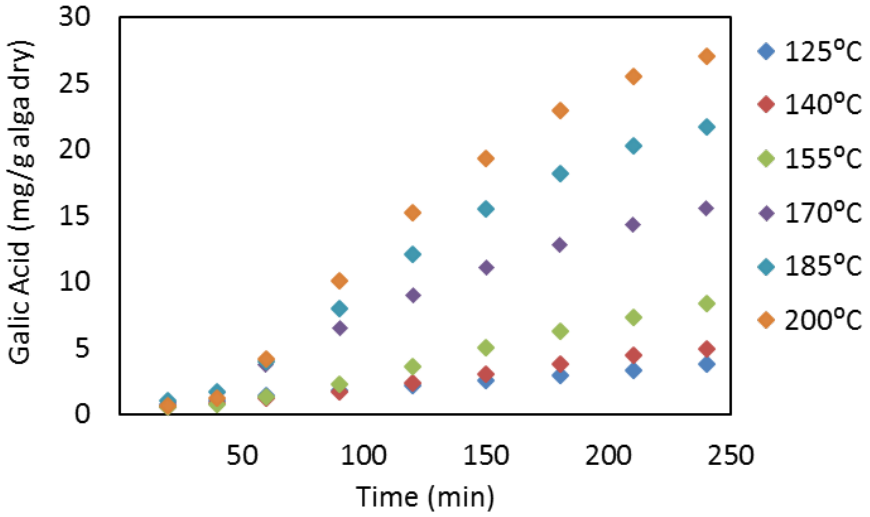


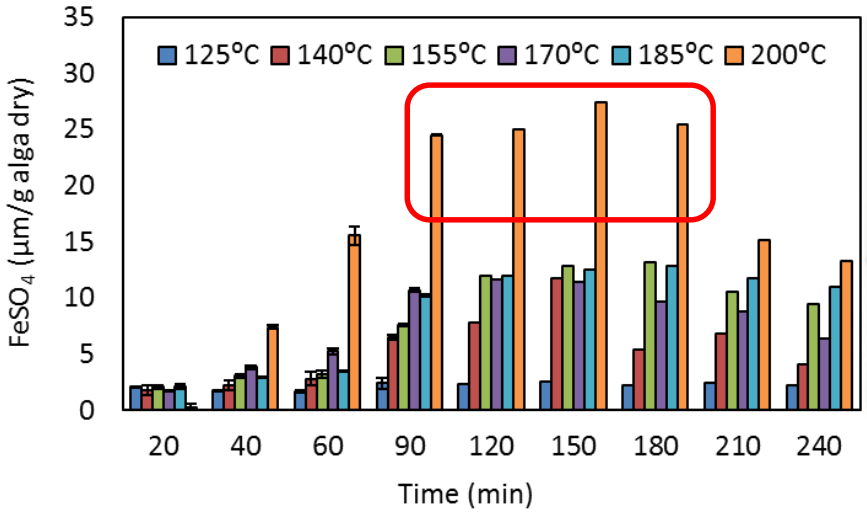


The greater work temperature, the greater total phenolic compounds recovery and antioxidant activity development

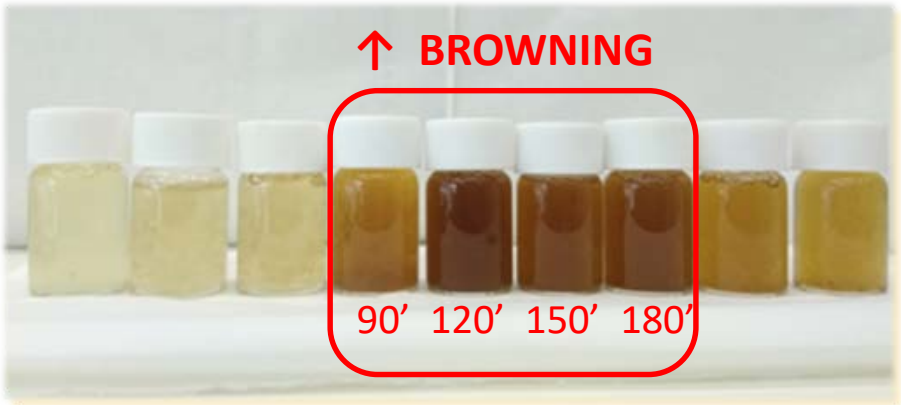
POLYPHENOLS EXTRACTION AND ANTIOXIDANT ACTIVITY

TEMPERATURE EFFECT





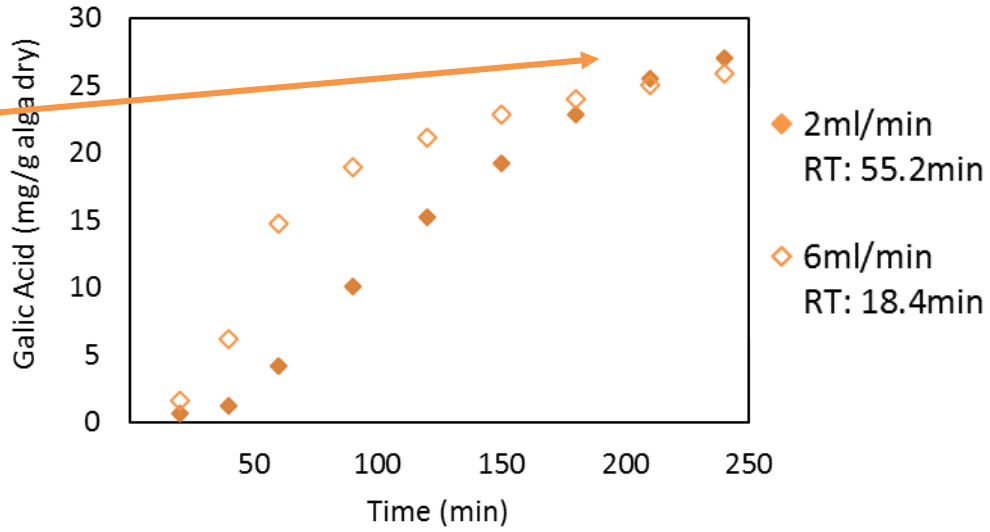
200°C

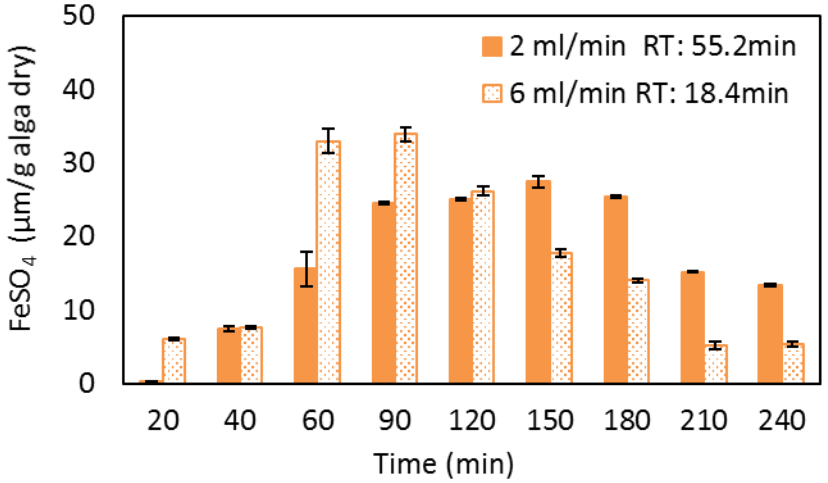


POLYPHENOLS EXTRACTION AND ANTIOXIDANT ACTIVITY

FLOW RATE EFFECT

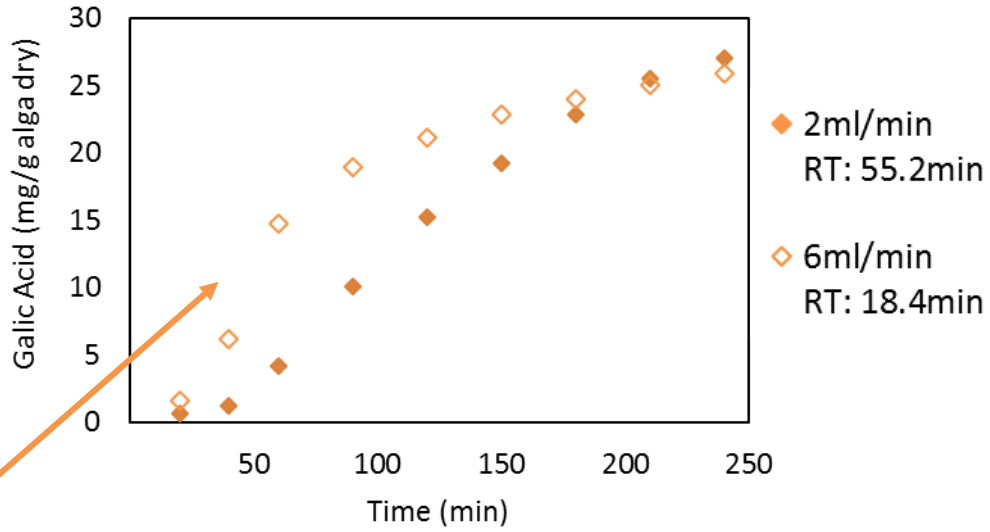
Total polyphenols recovered at different flow rates is not significatively different



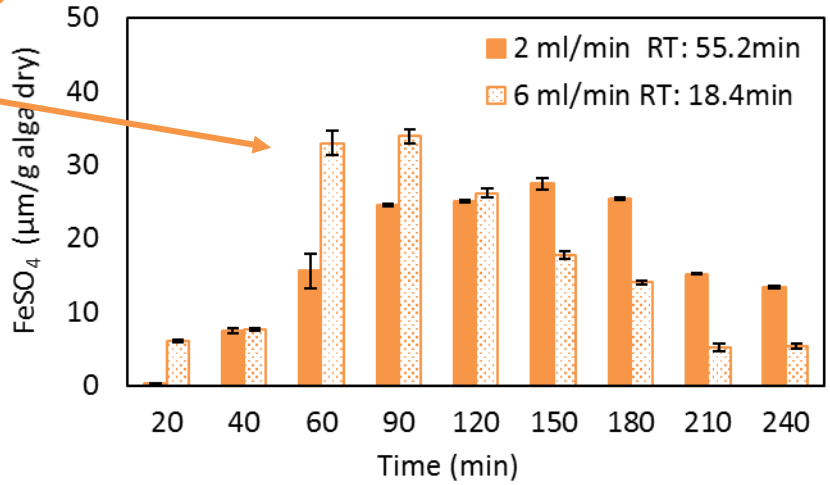


POLYPHENOLS EXTRACTION AND ANTIOXIDANT ACTIVITY

FLOW RATE EFFECT



Faster polyphenols extraction and antioxidant activity development is reached at lowest RT



SOLID RESIDUE

125°C 140°C 155°C 170°C 185°C 200°C

Sample	C (%)	H (%)	N (%)	S (%)	O (%)	Ashes (%)
G. sesquipedale	38,7 ± 0,5	5,4 ± 0,1	3,5 ± 0,3	0,3 ±0,1	36,9 ±1,1	14,9 ±0,3
By-product	42,4 ± 0,9	5,9 ± 0,1	4,4 ± 0,5	0,2 ±0,1	34,3 ±0,3	21,8 ±0.8
125°C	37,9 ± 0,0	5,1 ± 0,1	4,0 ± 0,3	0,2 ±0,0	28,7 ±3,0	30,6 ±0,4
140°C	35,9 ± 2,6	3,6 ± 0,4	3,1 ± 0,2	0,0 ±0,1	25,4 ±2,9	39,5 ±3,2
155°C	37,7 ± 0,7	5,0 ± 0,1	3,5 ± 0,4	0,0 ±0,0	28,9 ±1,8	33,4 ±2,4
170°C	30,4 ± 0,2	3,3 ± 0,4	1,9 ± 0,2	0,0 ±0,0	28,4 ±4,3	32,4 ±5,3
185°C	33,8 ± 0,7	5,1 ± 0,3	1,7 ± 0,1	0,0 ±0,0	28,2 ±7,8	37,9 ±5,2
200°C	19,9 ± 0,2	3,4 ± 0,8	1,5 ± 1,3	0,1 ±0,1	28,2 ±0,8	44,5 ±2,7

NITROGEN

SOLID RESIDUE

125°C 140°C 155°C 170°C 185°C 200°C

Sample	C (%)	H (%)	N (%)	S (%)	O (%)	Ashes (%)
G. sesquipedale	38,7 ± 0,5	5,4 ± 0,1	3,5 ± 0,3	0,3 ±0,1	36,9 ±1,1	14,9 ±0,3
By-product	42,4 ± 0,9	5,9 ± 0,1	4,4 ± 0,5	0,2 ±0,1	34,3 ±0,3	21,8 ±0.8
125°C	37,9 ± 0,0	5,1 ± 0,1	4,0 ± 0,3	0,2 ±0,0	28,7 ±3,0	30,6 ±0,4
140°C	35,9 ± 2,6	3,6 ± 0,4	3,1 ± 0,2	0,0 ±0,1	25,4 ±2,9	39,5 ±3,2
155°C	37,7 ± 0,7	5,0 ± 0,1	3,5 ± 0,4	0,0 ±0,0	28,9 ±1,8	33,4 ±2,4
170°C	30,4 ± 0,2	3,3 ± 0,4	1,9 ± 0,2	0,0 ±0,0	28,4 ±4,3	32,4 ±5,3
185°C	33,8 ± 0,7	5,1 ± 0,3	1,7 ± 0,1	0,0 ±0,0	28,2 ±7,8	37,9 ±5,2
200°C	19,9 ± 0,2	3,4 ± 0,8	1,5 ± 1,3	0,1 ±0,1	28,2 ±0,8	44,5 ±2,7

+ + +

ASHES

SOLID RESIDUE

Sample	C (%)	H (%)	N (%)	S (%)	O (%)	Ashes (%)
G. sesquipedale	38,7 ± 0,5	5,4 ± 0,1	3,5 ± 0,3	0,3 ±0,1	36,9 ±1,1	14,9 ±0,3
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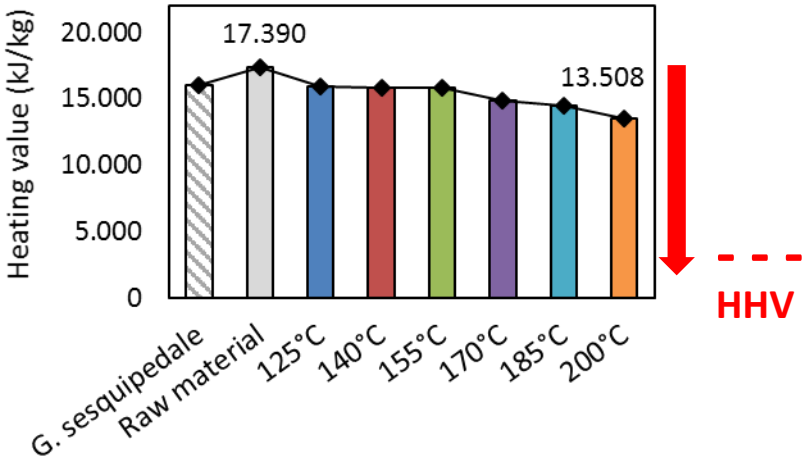
140°C	35,9 ± 2,6	3,6 ± 0,4	3,1 ± 0,2	0,0 ±0,1	25,4 ±2,9	39,5 ±3,2
155°C	37,7 ± 0,7	5,0 ± 0,1	3,5 ± 0,4	0,0 ±0,0	28,9 ±1,8	33,4 ±2,4
170°C	30,4 ± 0,2	3,3 ± 0,4	1,9 ± 0,2	0,0 ±0,0	28,4 ±4,3	32,4 ±5,3
185°C	33,8 ± 0,7	5,1 ± 0,3	1,7 ± 0,1	0,0 ±0,0	28,2 ±7,8	37,9 ±5,2
200°C	19,9 ± 0,2	3,4 ± 0,8	1,5 ± 1,3	0,1 ±0,1	28,2 ±0,8	44,5 ±2,7



$$HHV(kJ/kg)^{*} = 3.55C^{2} - 232C - 2230H + 51.2C \times H + 131N + 20600$$

*Friedl et al., *Analytica Chimica Acta* **2008**, 544, 191-198

CONCLUSIONS

- ✓ *Gelidium sesquipedale* residue after agar-agar extraction is a very **valuable by-product** because of its carbohydrate, protein and bioactive content.
- ✓ SWE is a useful technique to obtain bioactive compounds, such as **proteins**, **free amino acids**, and **polyphenols**.
- ✓ Increasing **temperature** and **solvent flow rate** have much influence on the extraction yield.
- ✓ SWE constitutes an interesting **alternative** to conventional treatments based on organic solvent.

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