Subcritical water extraction and identification of phenolic compounds from Brewer’s Spent Grain (BSG)

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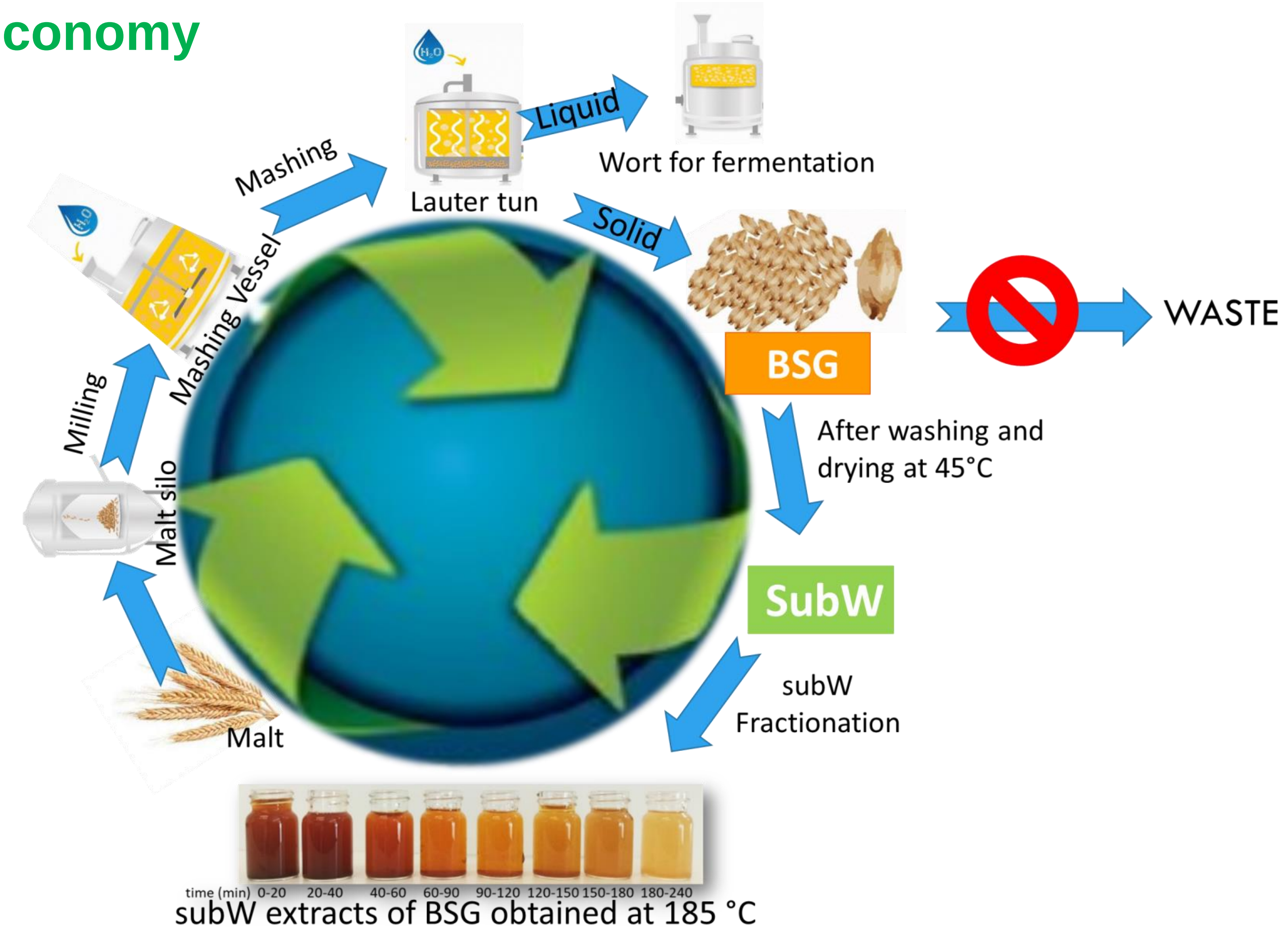
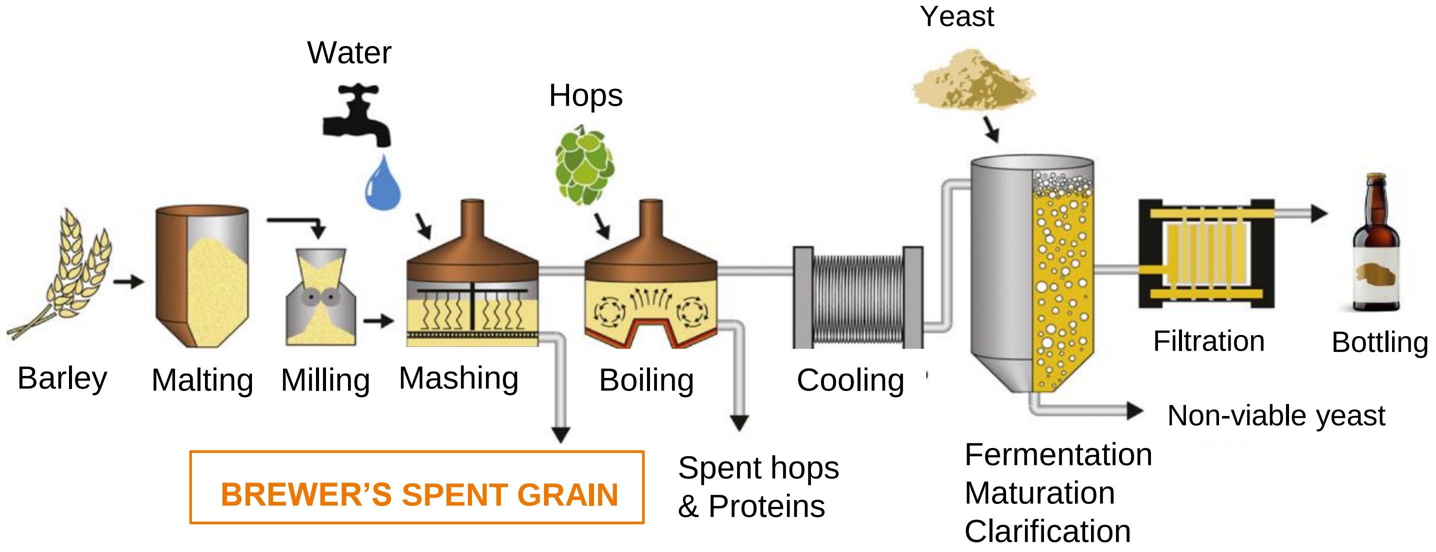
Circular Economy



Brewer's Spent Grain (BSG)



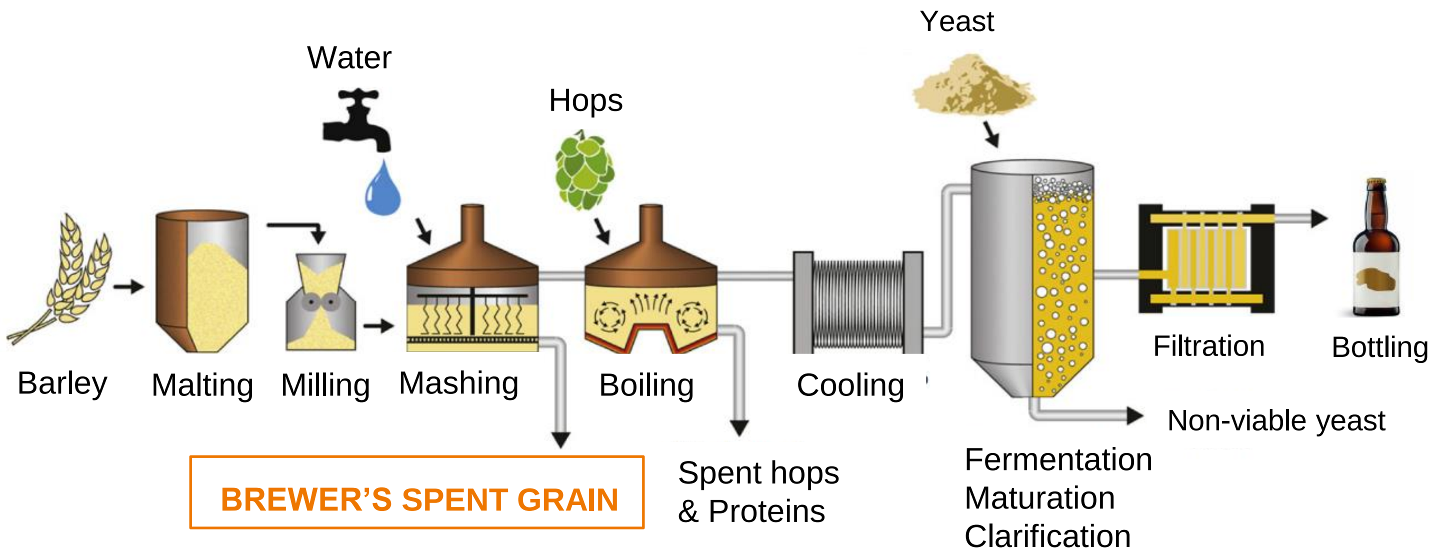
85 % of the total **by-products** generated → 20 kg of BSG/ 100 L of beer produced.

➢ **BSG** generated by a **small local** company → small breweries within the biorefinery concept

✓ Europe craft beer market→ US$ 42.52 million in 2020 → Expected to grow up to US$ 91.26 million by the end of 2025

UBU

Brewer’s Spent Grain (BSG)



85 % of the total **by-products** generated → 20 kg of BSG/ 100 L of beer produced.

➢ **BSG** generated by a **small local** company → small breweries within the biorefinery concept

✓ Europe craft beer market→ US$ 42.52 million in 2020 → Expected to grow up to US$ 91.26 million by the end of 2025

Integration of **BSG** within a **biorefinery** concept is of great interest to obtain different high value **biocompounds**

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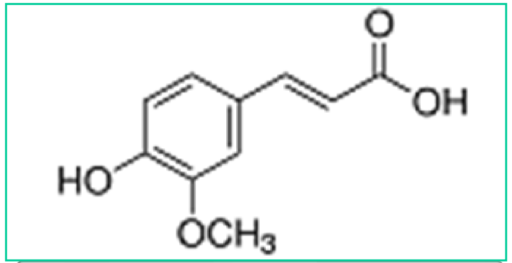
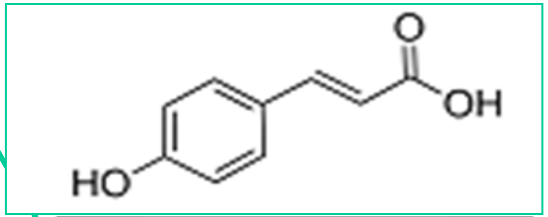
✓ Phenolic Compounds

A valuable source of phenolic compounds

Hydroxycinnamic acids



Ferulic acid



p-Coumaric acid

Antioxidant effect

✓ Extract Phenolic Compounds

Solid–liquid extraction

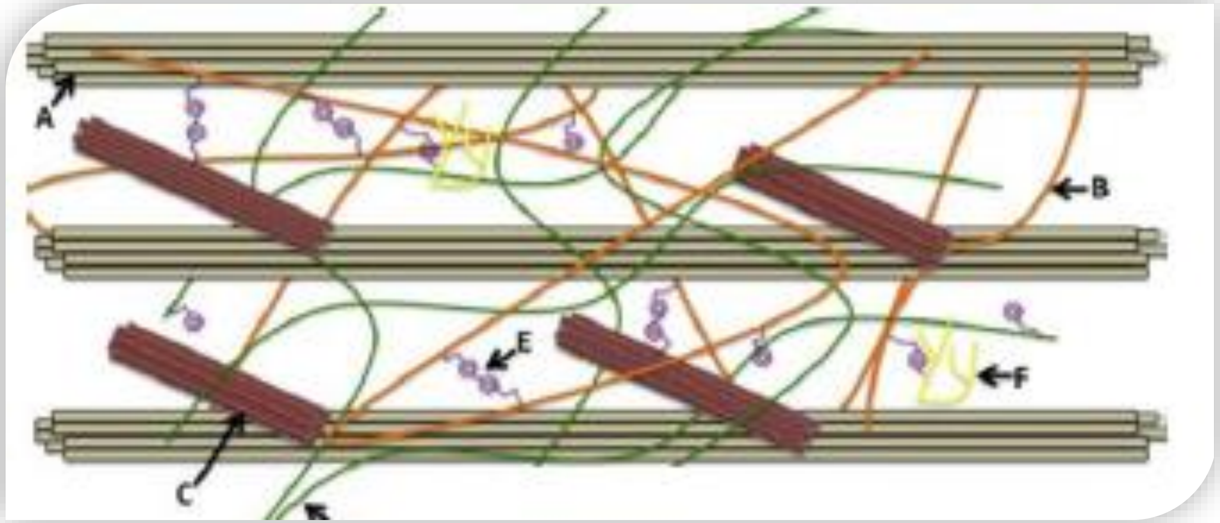
↓

Long extraction times

↓

Low extraction yield

BSG, a lignocellulosic material



Lignin is **connected** to the cell wall polysaccharides by **phenolic acids**, being necessary **a hydrolytic method** to release them

✓ **Extract Phenolic Compounds**

Different hydrolytic techniques

- **Ultrasound assisted extraction (UAE)**
- **Chemical hydrolysis**
- **Enzymatic hydrolysis**

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✓ Extraction of Phenolic Compounds

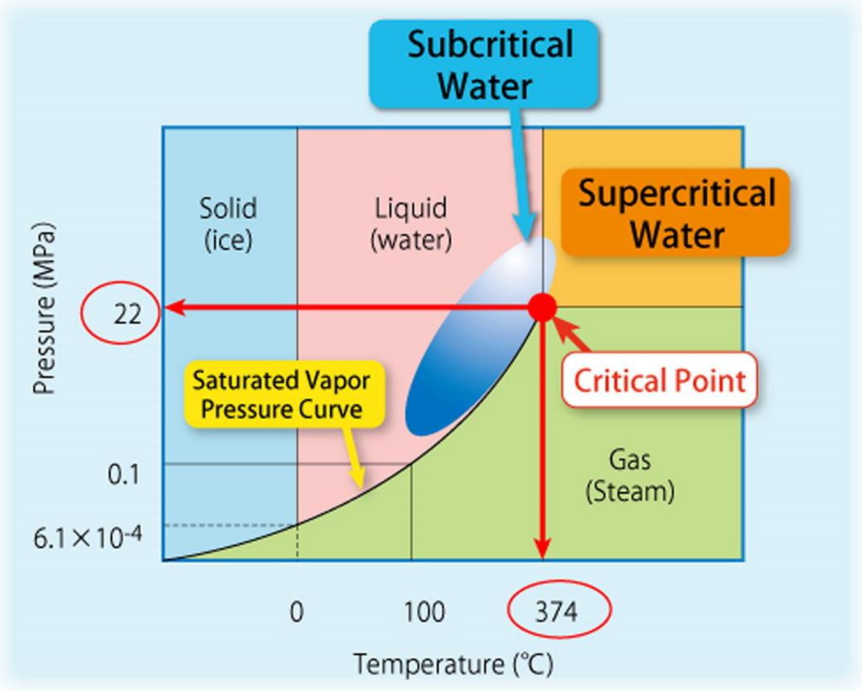
Subcritical Water (subW)

An **alternative technology** to **chemical** and **enzymatic** hydrolysis to release **Phenolic Compounds** from lignocellulosic materials

Subcritical Water (subW)

Water is the **greenest** solvent of all considering the principles of green chemistry

subW is pressurized **water** in its liquid state in the T range from **100 ºC** to **374 ºC.**



Asia Biomass Office. (2014)

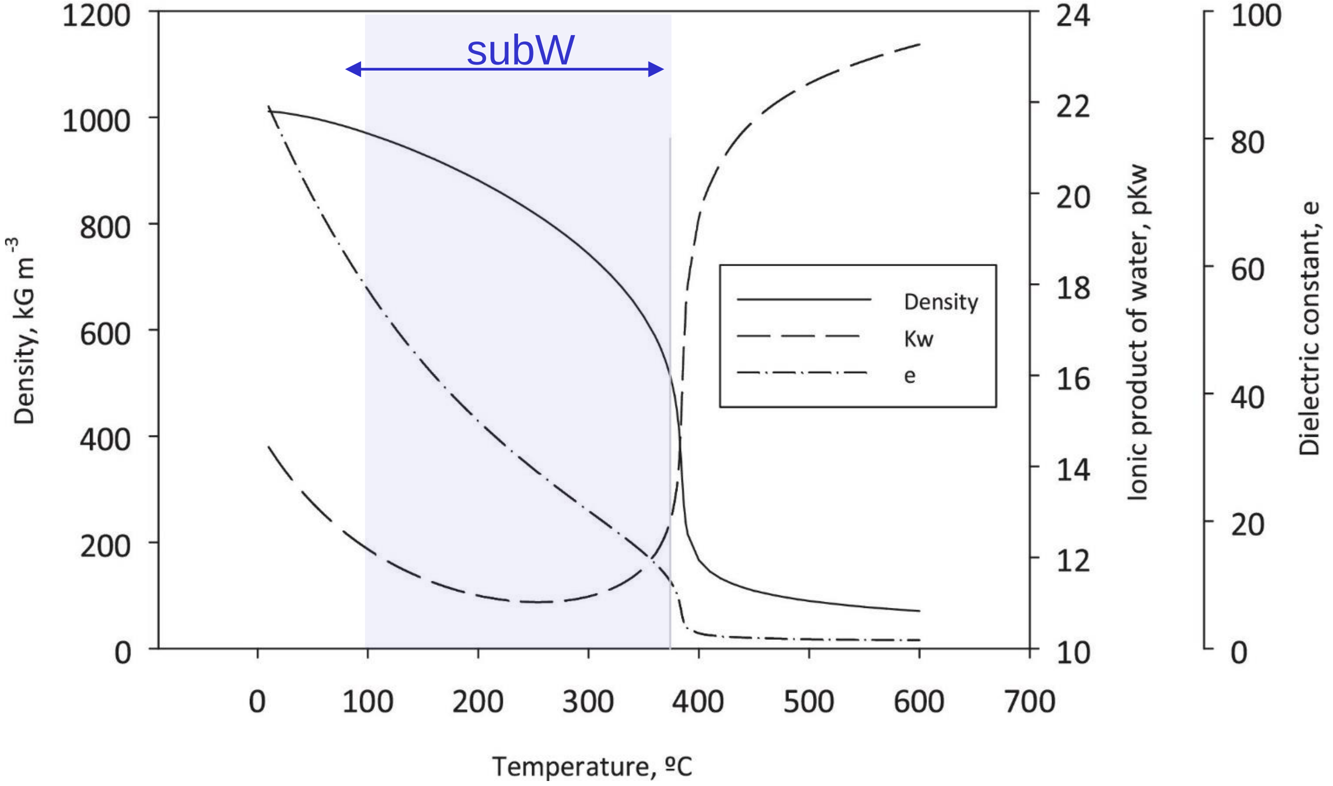
Subcritical Water (subW)

subW presents unique properties

↑ Ionic product

↓ Dielectic constant

↓ Density



Cocero et al. (2018)

↑ Affinity towards non-polar compounds

Subcritical Water (subW)

Tunable properties with **T**

Different selectivity in the release of the bioactive compounds from the biomass

T in subW is a critical parameter

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02 - OBJECTIVES

- ¿Is subW treatment a suitable technology to extract the phenolic compounds from BSG?
- To perform a characterization of the phenolic compounds released to the extraction medium
- To explore the Antioxidant capacity of the subW extracts
- To compare the subW results with the results obtained with another hydrolytic methods

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Preconditioning

BSG generated in a craft beer industry.

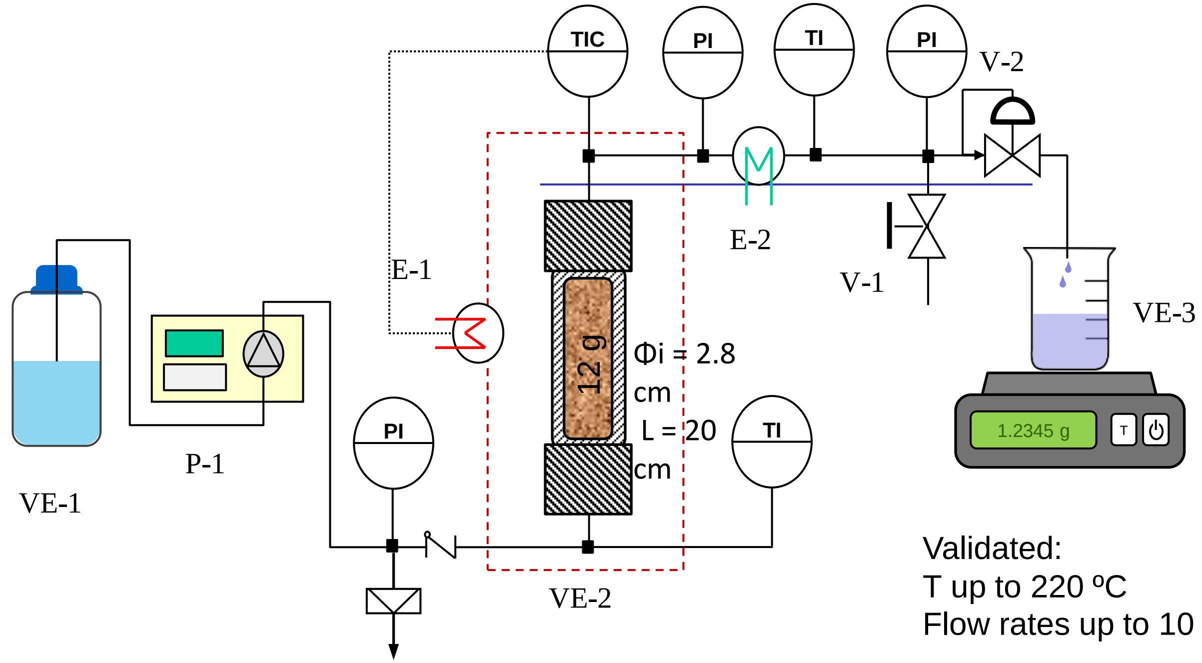
Washing with water

Drying in an air convection oven (45 °C, 3 h)

Humidity< **8 %** (w/w)

Subcritical water (subW) experiments

Semi-continuous fixed-bed reactor



Temperature (125-185 ºC)

P= 50 bar

F= 4 mL/min

Validated:
T up to 220 ºC
Flow rates up to 10 mL/min.

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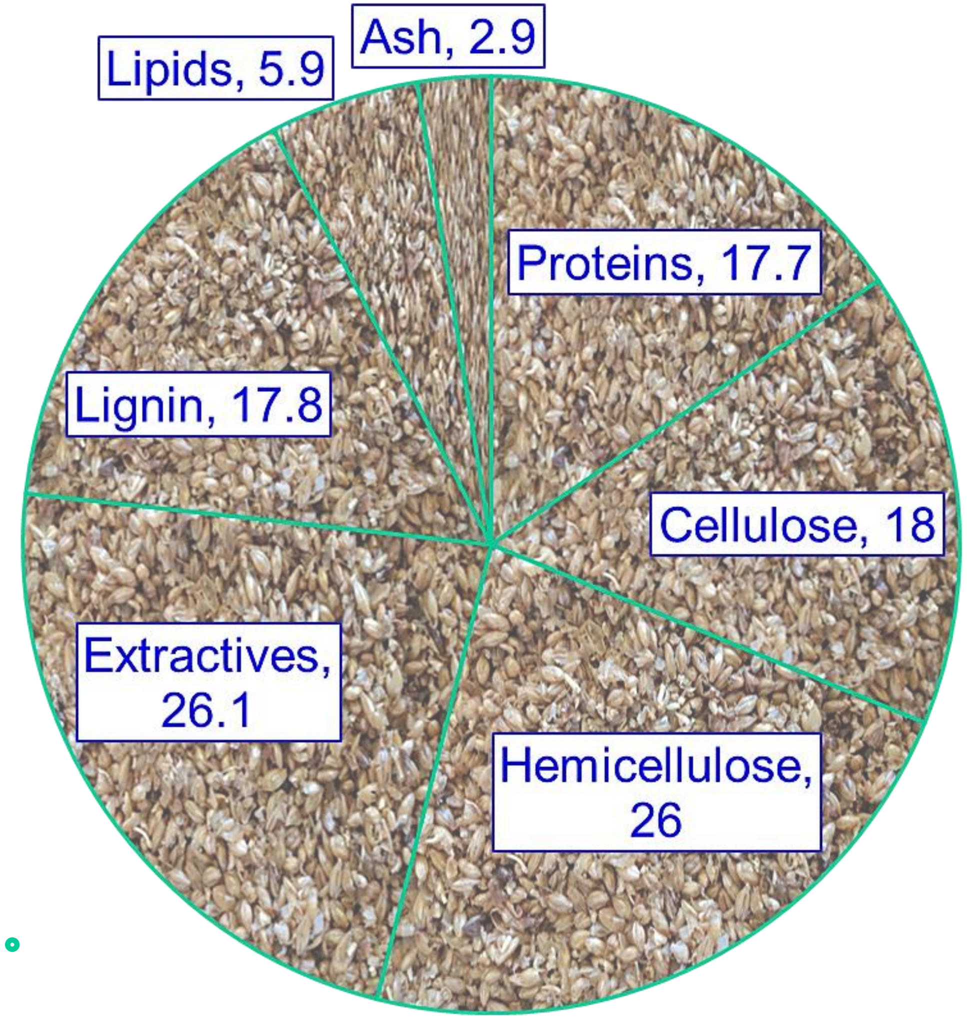
BSG characterization



*NREL protocols

BSG composition, g/100g$_{\text{BSG-dry}}$

*National Renewable Energy Laboratory

04 - RESULTS

BSG characterization

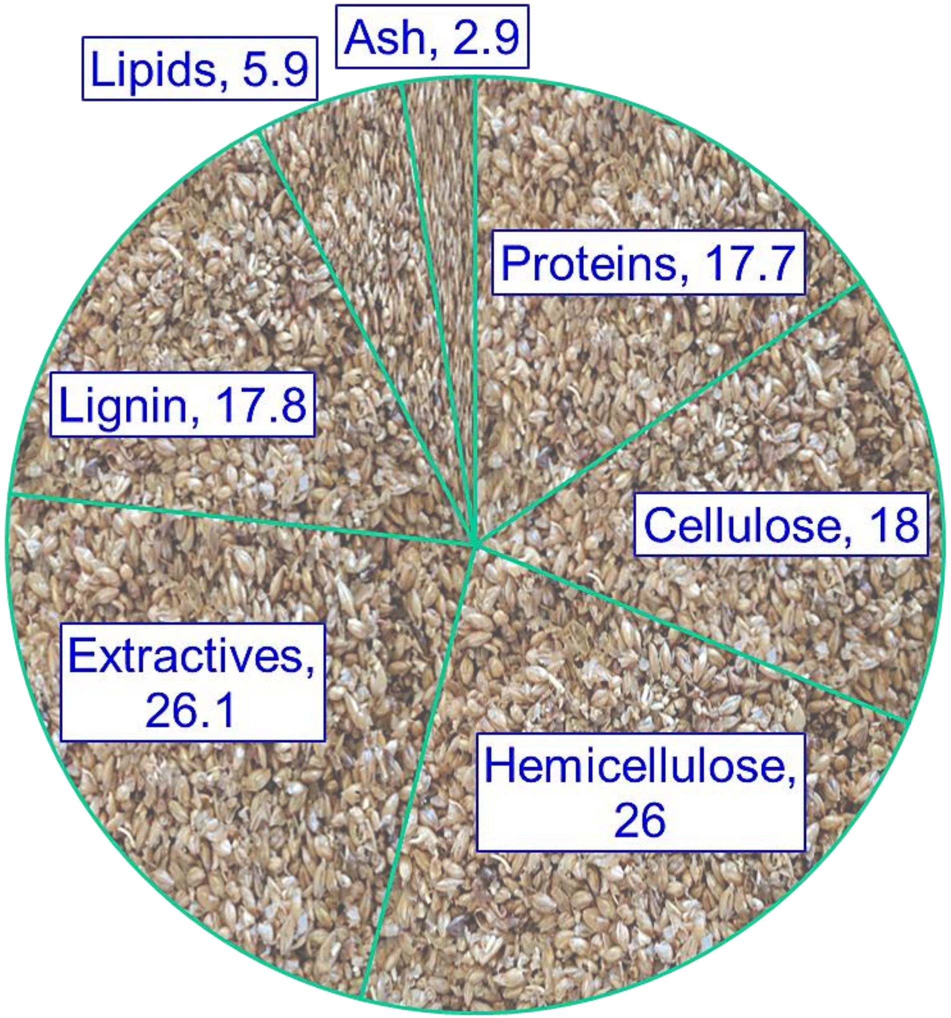
Extractives

Water: 24.3 %
TPC:
2.7 $mg_{GAE}/g_{BSG\text{-}dry}$

Soluble proteins:
5.02 $g/100_{\text{-}BSG\text{-}dry}$

Ethanol: 1.8 %
TPC:
1.6 $mg_{GAE}/g_{BSG\text{-}dry}$

NREL protocols



BSG composition, $g/100g_{BSG\text{-}dry}$

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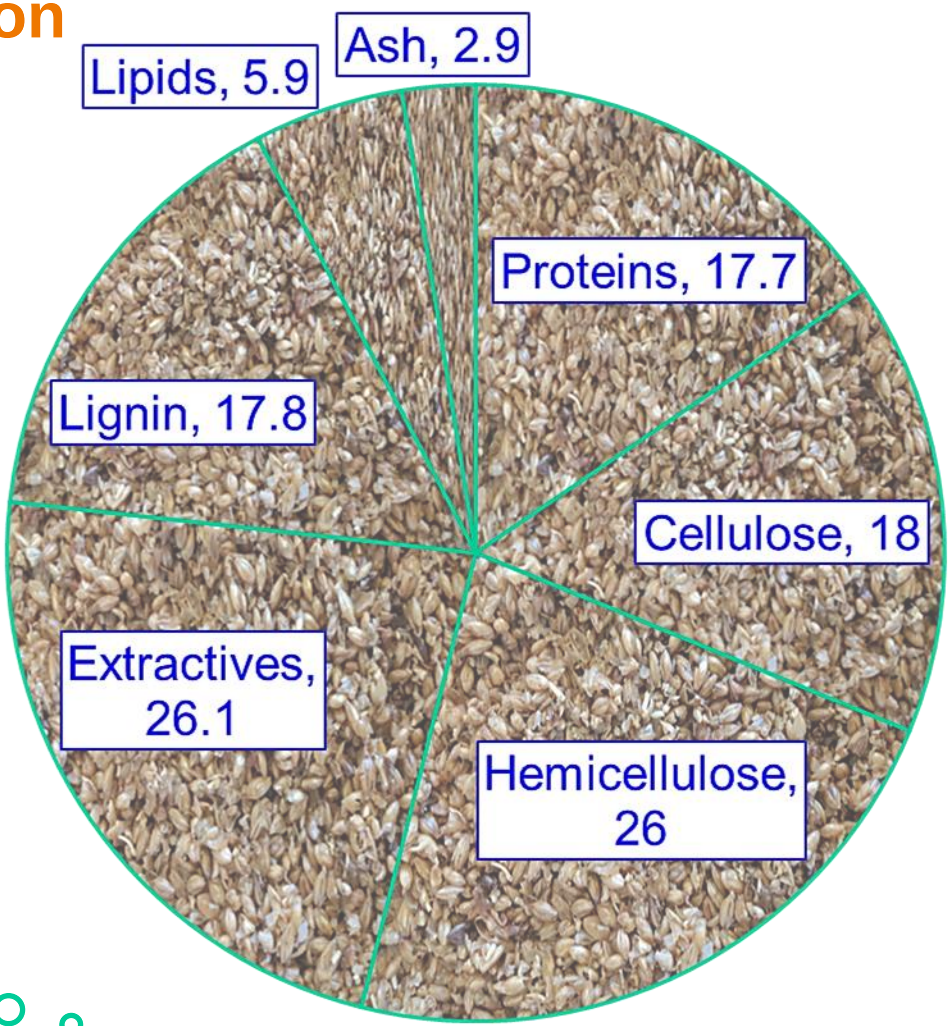
BSG characterization



NREL protocols

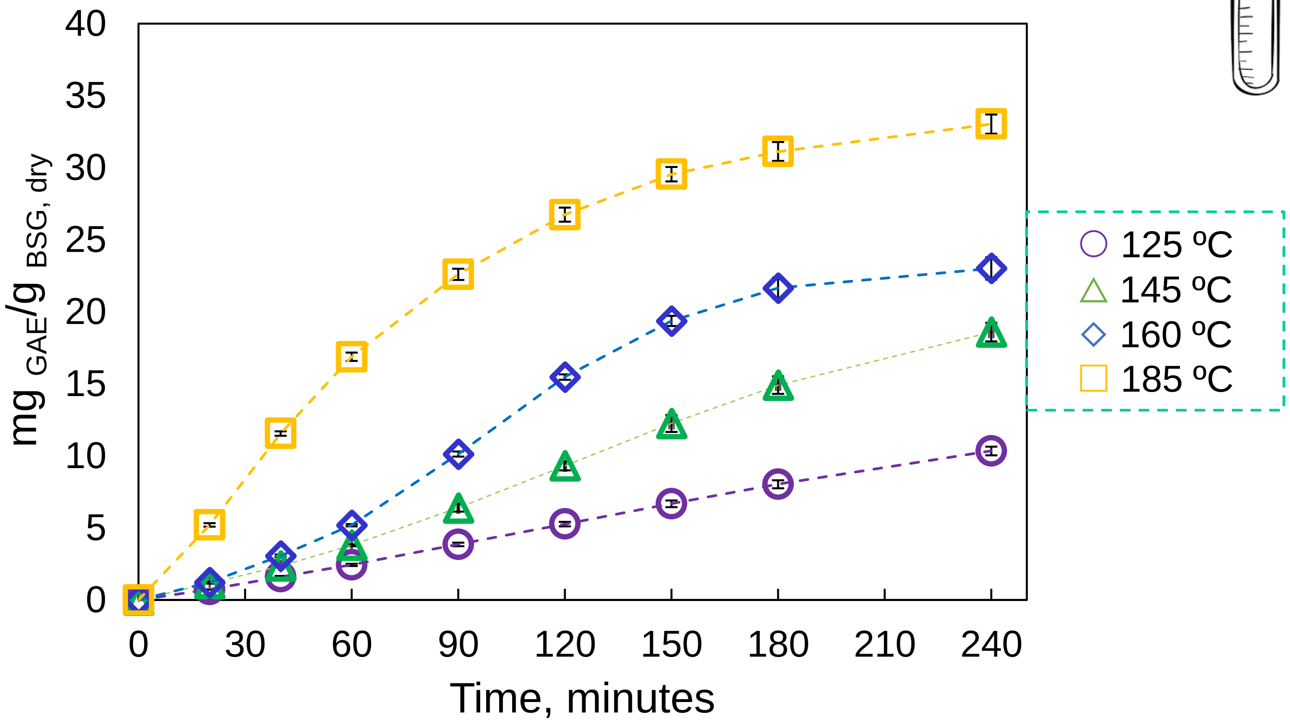
Hemicellulose

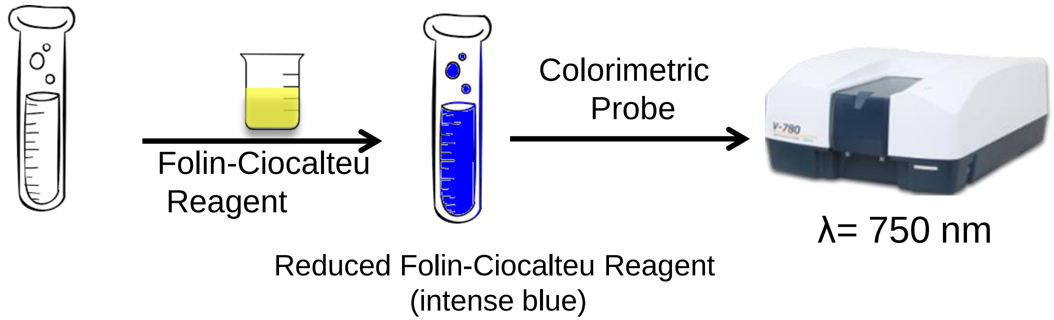
- Xylans: 14.6 %
- Arabinans: 7.9 %
- Acetyl groups: 3.6 %

BSG composition, g/100g$_{\text{BSG-dry}}$

Total Phenolic Content (TPC)

TPC. Folin-Ciocalteu method





Effect of temperature on TPC release in subW extracts

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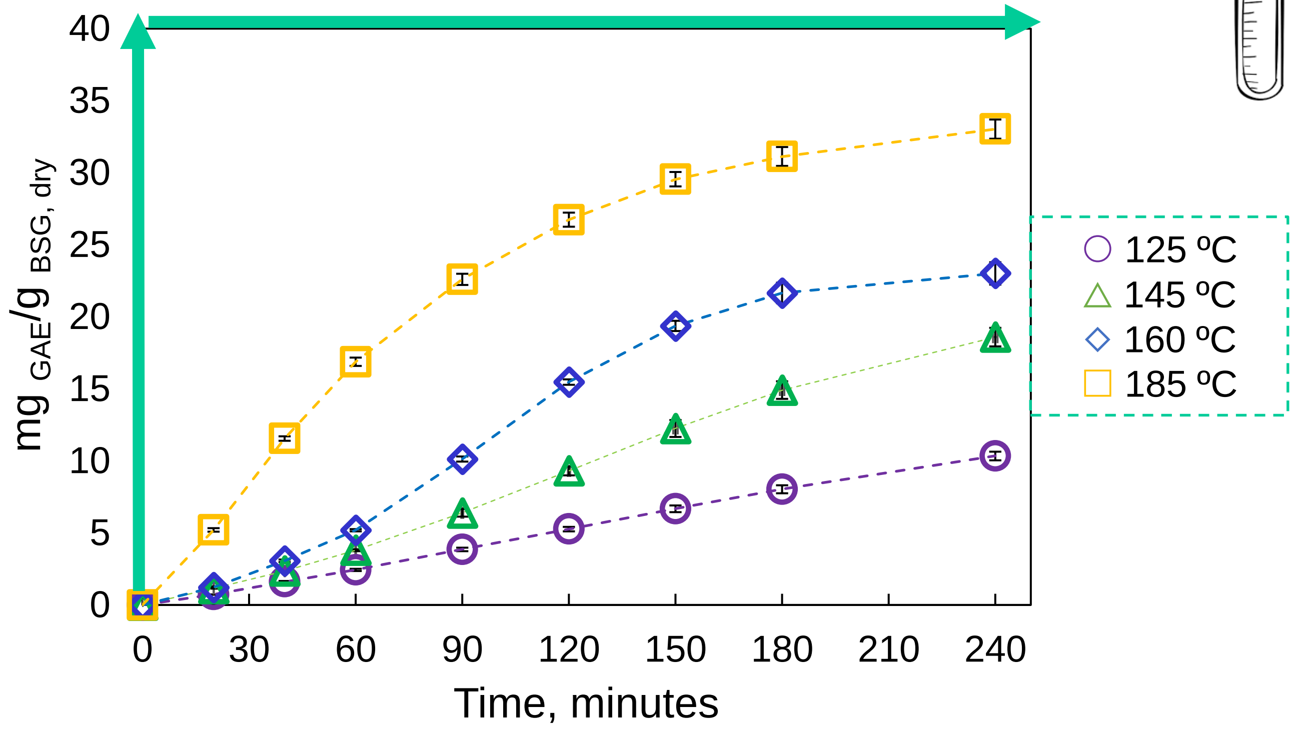
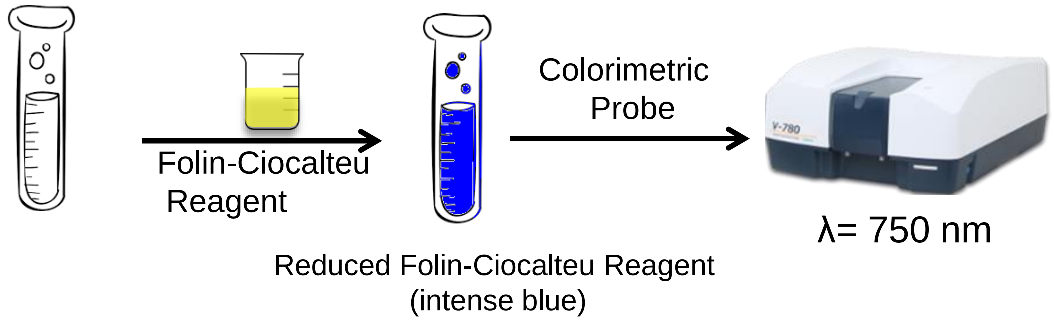
Total Phenolic Content (TPC)

TPC. Folin-Ciocalteu method





An increase in the operating **temperature** led to an **increase in TPC** in the extraction medium

Effect of temperature on TPC release in subW extracts

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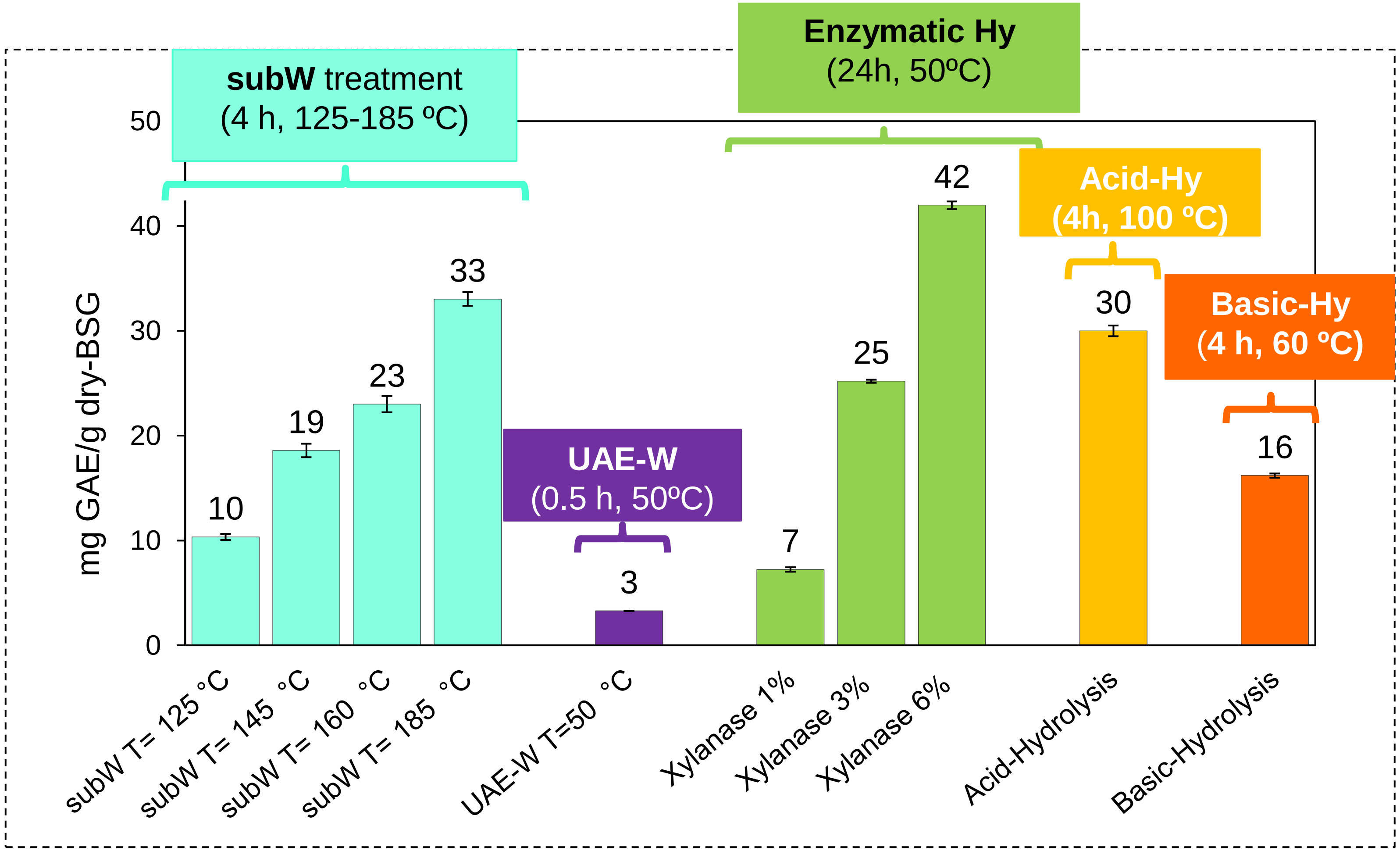
Total Phenolic Content (TPC)

Comparison between the different hydrolytic methods

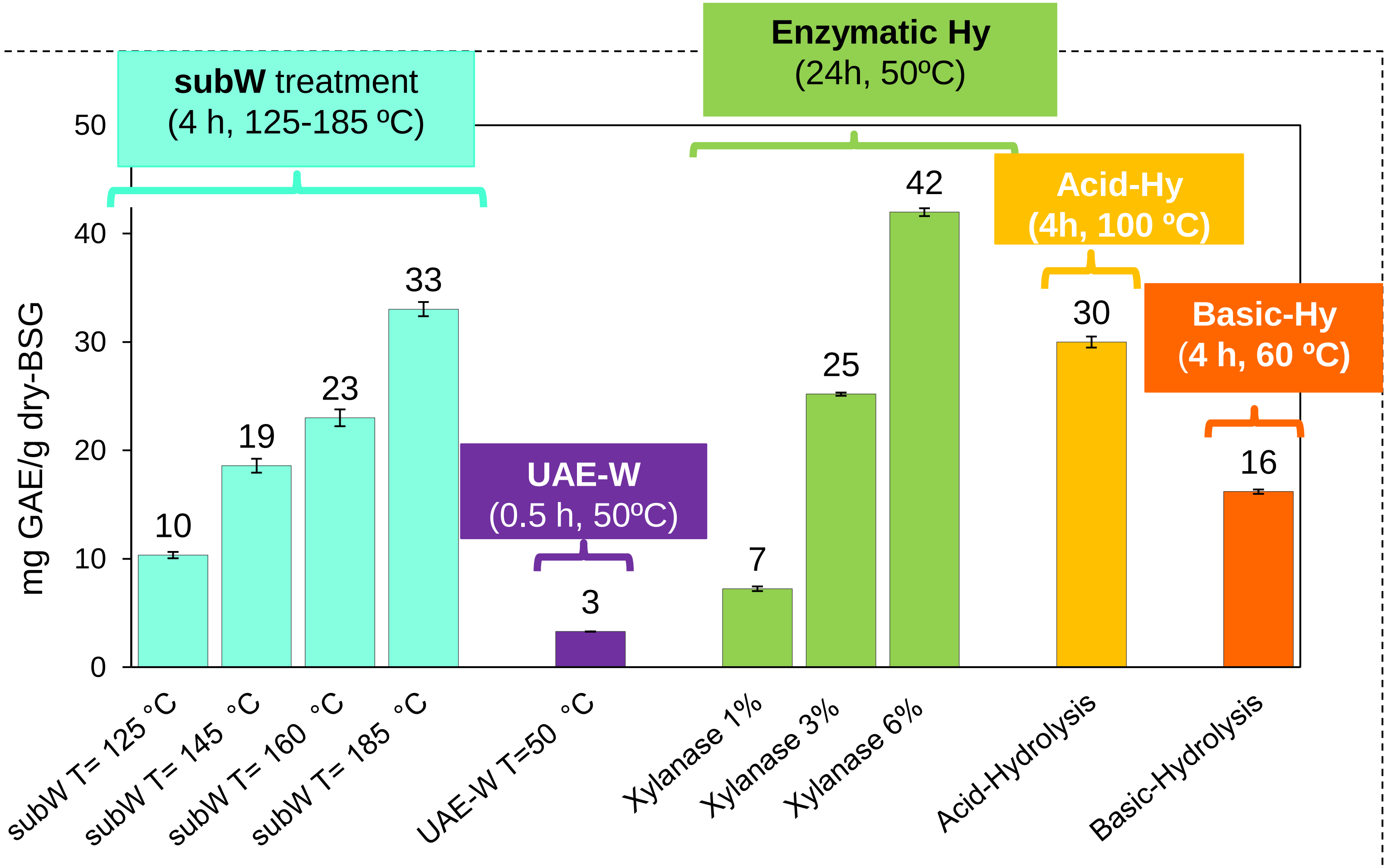


TPC determined by Folin-Cioculteau in different extracts. UAE-W= Ultrasonic Assisted Extraction with water

Total Phenolic Content (TPC)

subW treatment at **185** °C led to **higher** amount of **TPC than acid** and **basic** hydrolysis and **UAE-W**

Comparison between the different hydrolytic methods



A **maximum TPC** release was found with **6%** of **xylanase** for **24 h**

TPC determined by Folin-Cioculteau in different extracts. UAE-W= Ultrasonic Assisted Extraction with water

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Total Phenolic Content (TPC)

Comparison between the different hydrolytic methods

TPC Productivity (P)*

$$P = \frac{mg_{GAE}}{g_{BSG,\ dry} \cdot \Delta t}$$

Method	P $mg_{GAE}/(g_{BSG\ dry} \cdot min)$
subW T= 160 ºC	0.100 ± 0.001
subW T= 185 ºC	0.28 ± 0.01
UAE T= 50 ºC	0.109 ± 0.002
Basic Hydrolysis	0.55 ± 0.04
Xylanase 6%	0.087 ± 0.001

Productivity by enzymatic hydrolysis was lower than for subW at 160 ºC and 185 ºC.

*Evaluated from the initial linear extraction curve.

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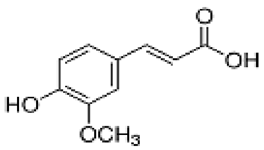
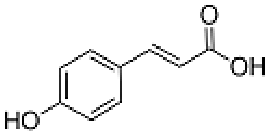
Individual Phenolic Compounds

Hydroxycinnamic acids

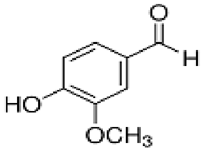


◇◆ **Ferulic acid**

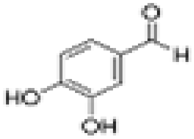


△▲ **p-Coumaric acid**

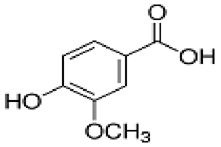
Phenolic aldehydes



□ ■ **Vanillin**

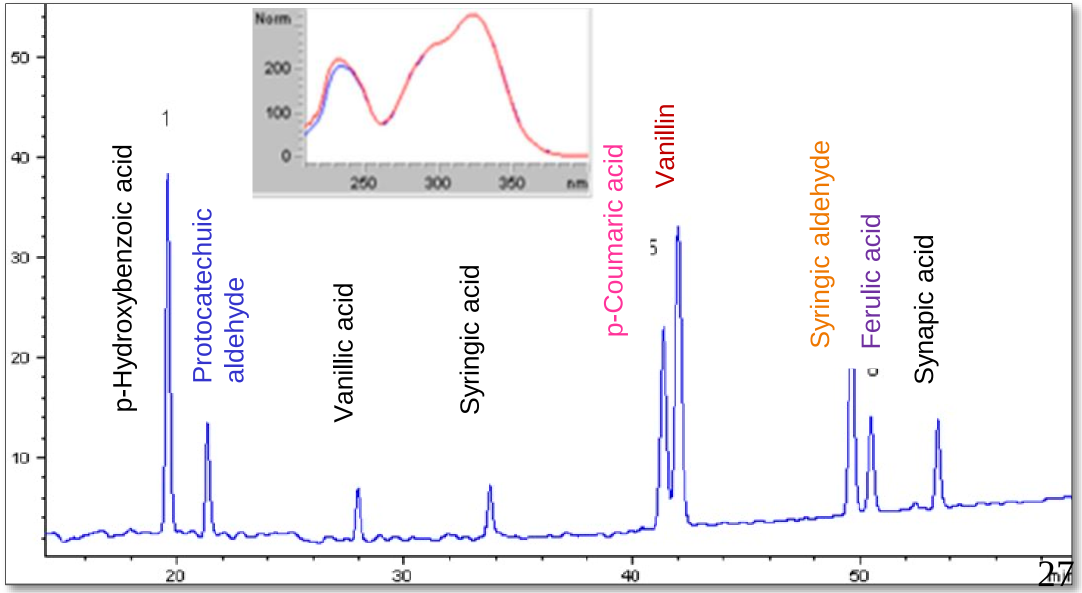


◇◆ **Protocatechuic aldehyde**



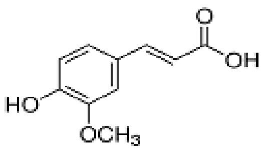
○ ● **Syringic aldehyde**

HPLC-DAD

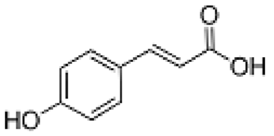


Individual Phenolic Compounds

Hydroxycinnamic acids

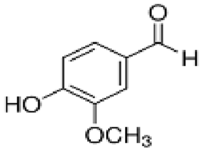


◇◆ Ferulic acid

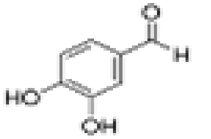


△▲ p-Coumaric acid

Aldehydes



□■ Vanillin



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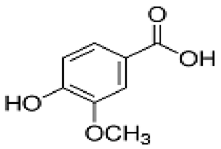
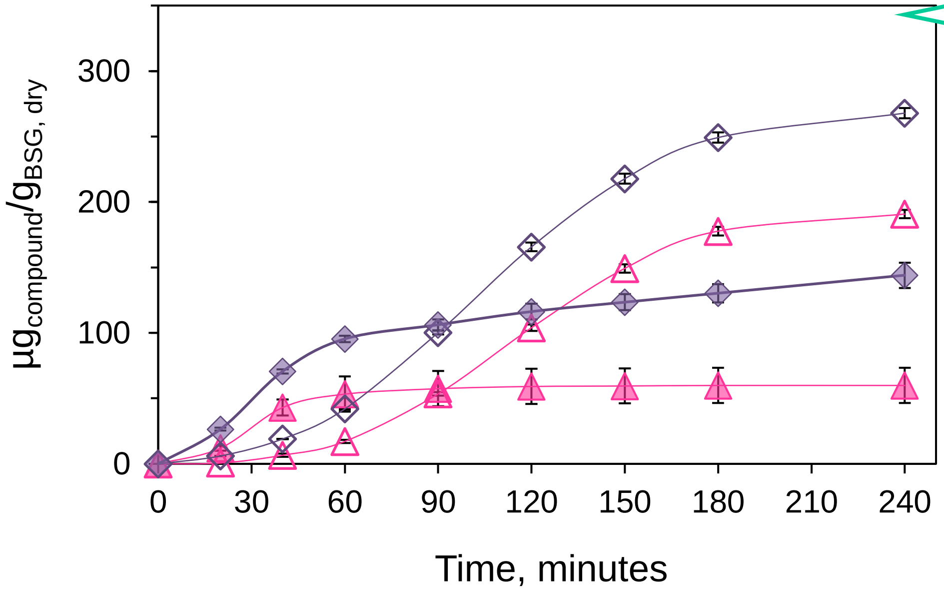
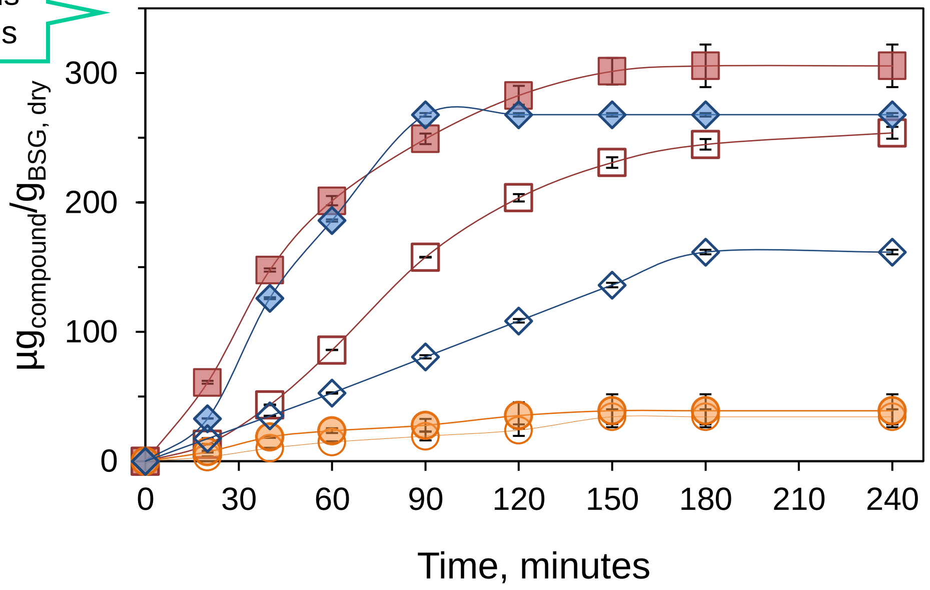


○● Syringic aldehyde

160 ºC: open symbols
185 ºC: filled symbols

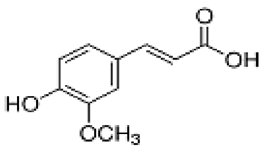




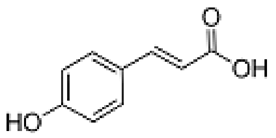
Accumulative release of the identified individual phenolic compounds in the subW extracts

Individual Phenolic Compounds

Hydroxycinnamic acids

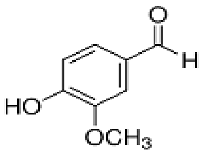


◇◆ **Ferulic acid**

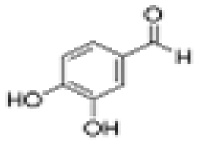


△▲ **p-Coumaric acid**

Aldehydes



□ ■ **Vanillin**



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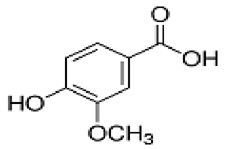

○ ● **Syringic aldehyde**

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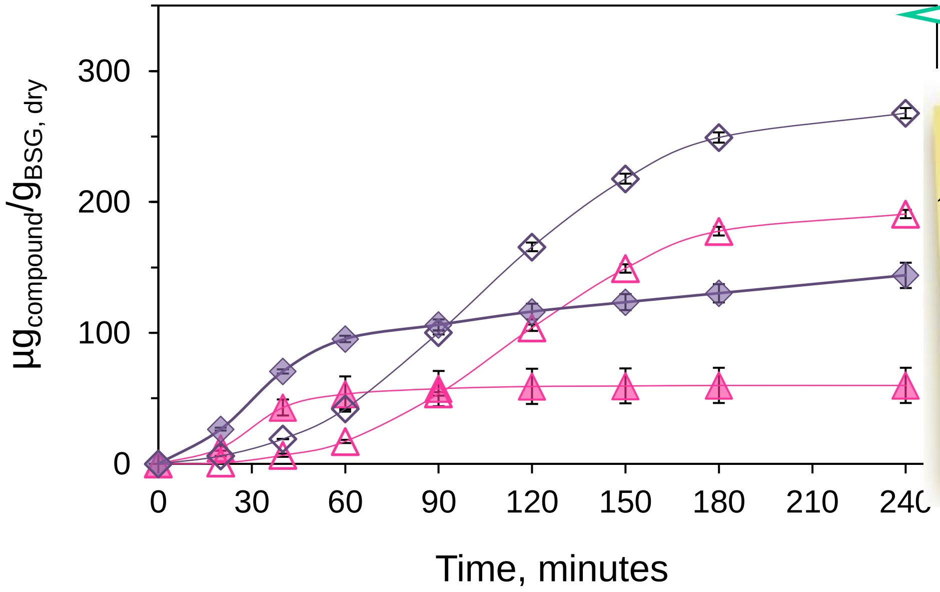
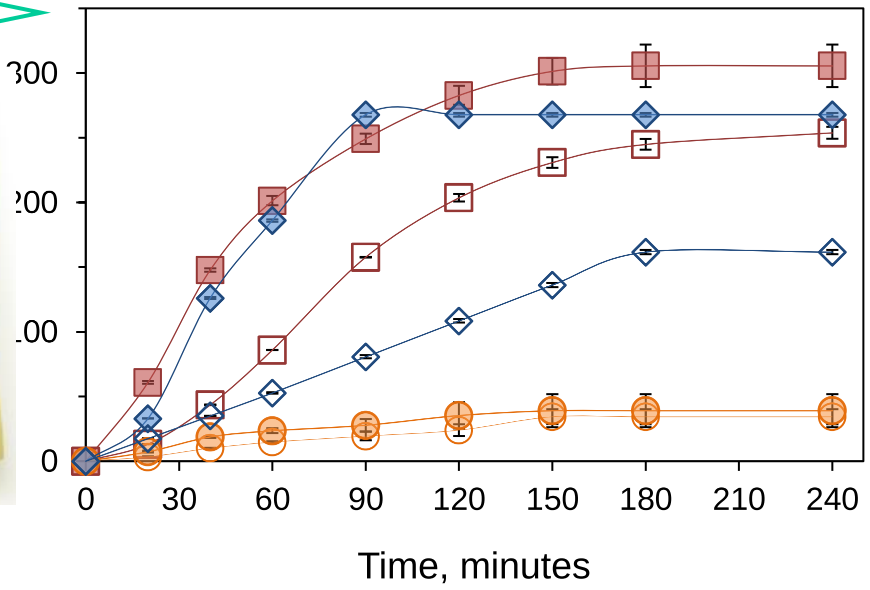
185 ºC: filled symbols



↑ Hydroxycinnamic ac.
160 ºC
↑ Phenolic aldehydes
185 ºC



Accumulative release of the identified individual phenolic compounds in the subW extracts

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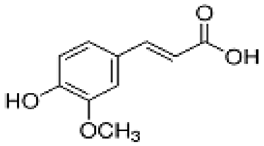
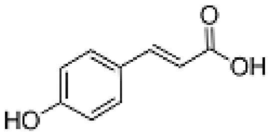
Individual Phenolic Compounds

Hydroxycinnamic acids

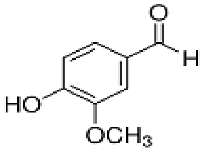


◇◆ Ferulic acid

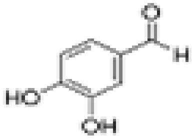


△▲ p-Coumaric acid

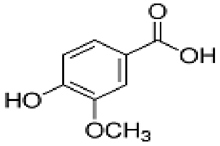
Aldehydes



□ ■ Vanillin



◇◆ Protocatechuic aldehyde



○ ● Syringic aldehyde

160 ºC: open symbols
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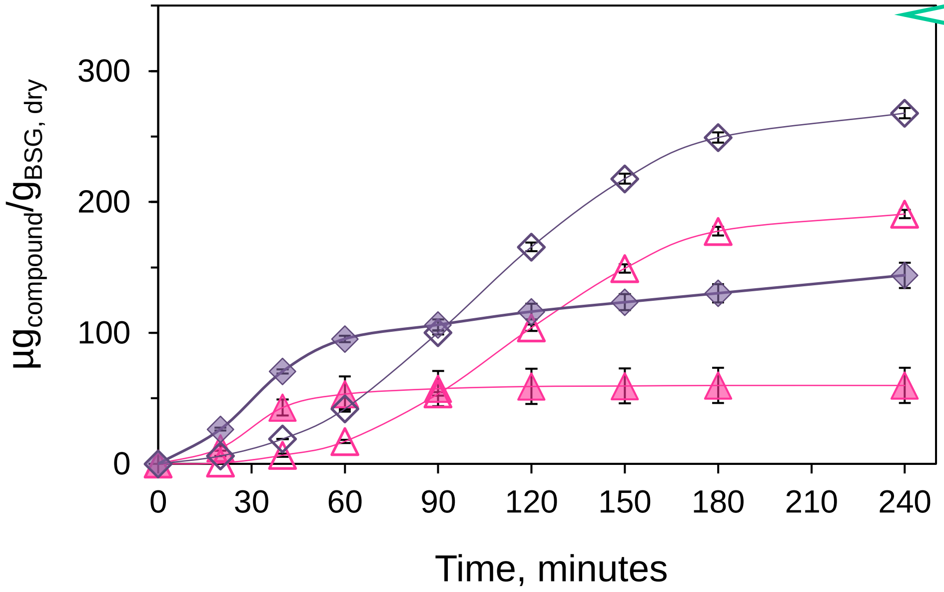
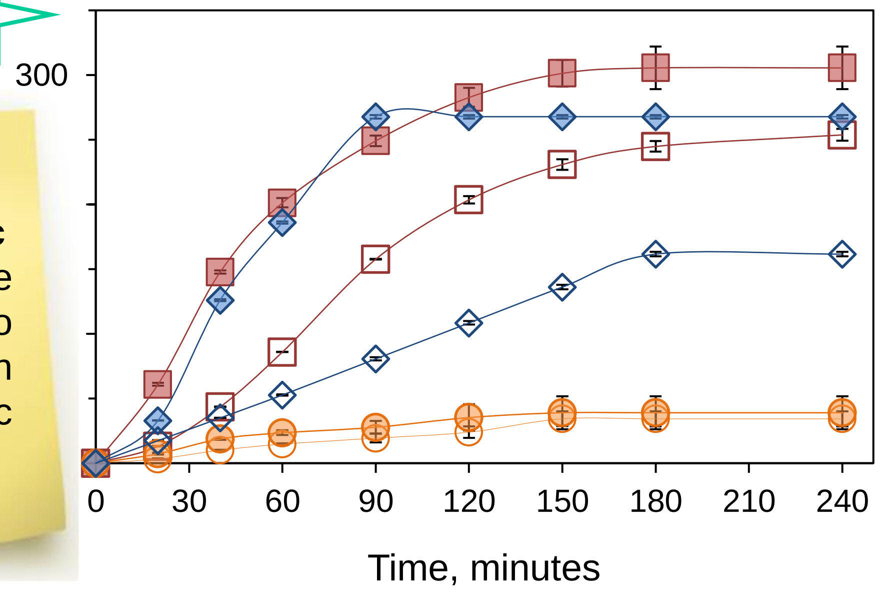


Hydroxycinnamic acids were more **sensitive** to temperature than **aldehyde** phenolic compounds.



Accumulative release of the identified individual phenolic compounds in the subW extracts

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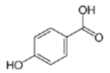
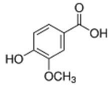
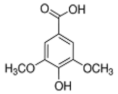
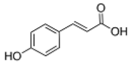
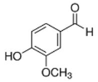
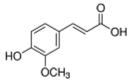
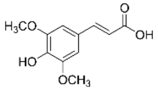
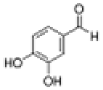
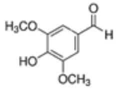
Individual Phenolic Compounds

Comparison between the different hydrolytic methods

Phenolic compounds identified by HPLC-DAD (μg $_{compound}$/$g_{BSG,dry}$)

Compound	Formula	SubW T= 160 ºC	SubW T= 185 ºC	UAE-W T= 50 ºC	Acid-Hy	Alkaline-Hy	Xylanase 6%
p-Hydroxibenzoic acid		n.d.	n.d.	10.0 ± 0.5	n.d.	59 ± 2	n.d.
Vanillic acid		17.9 ± 0.4	n.d.	n.d.	n.d.	49 ± 2	61 ± 3
Syringic acid		n.d.	n.d.	n.d.	n.d.	106.1 ± 5.7	n.d.
p-Coumaric acid		191 ± 3	60 ± 1	n.d.	n.d.	538 ± 4	5.3 ± 0.4
Vanillin		254 ± 5	306 ± 10	n.d.	n.d.	217 ± 1	203 ± 10
Ferulic acid		250 ± 3	144 ± 7	10.7 ± 0.3	54.4 ± 0.3	1305.7 ± 0.5	292 ± 3
Synapic acid		n.d.	n.d.	2.8 ± 0.2	31.1 ± 0.5	27 ± 1	15 ± 1
Protocatechuic aldehyde		162 ± 2	268 ± 1	n.d.	n.d.	n.d.	n.d.
Syringic aldehyde		34 ± 5	39 ± 2	n.d.	n.d.	n.d.	n.d.

n.d. not detected

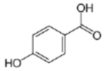
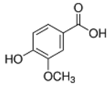
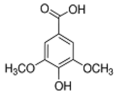
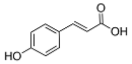
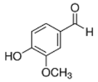
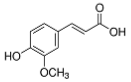
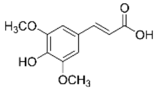
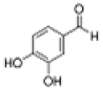
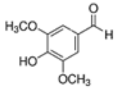
UBU

04 - RESULTS

Individual Phenolic Compounds

Comparison between the different hydrolytic methods

Phenolic compounds identified by HPLC-DAD (µg $_{compound}$/g$_{BSG,dry}$)

Compound	Formula	SubW T= 160 ºC	SubW T= 185 ºC	UAE-W T= 50 ºC	Acid-Hy	Alkaline-Hy	Xylanase 6%
p-Hydroxibenzoic acid		n.d.	n.d.	10.0 ± 0.5	n.d.	59 ± 2	n.d.
Vanillic acid		17.9 ± 0.4	n.d.	n.d.	n.d.	49 ± 2	61 ± 3
Syringic acid		n.d.	n.d.	n.d.	n.d.	106.1 ± 5.7	n.d.
p-Coumaric acid		191 ± 3	60 ± 1	n.d.	n.d.	538 ± 4	5.3 ± 0.4
Vanillin		254 ± 5	**306 ± 10**	n.d.	n.d.	217 ± 1	203 ± 10
Ferulic acid		250 ± 3	144 ± 7	10.7 ± 0.3	54.4 ± 0.3	1305.7 ± 0.5	292 ± 3
Synapic acid		n.d.	n.d.	2.8 ± 0.2	31.1 ± 0.5	27 ± 1	15 ± 1
Protocatechuic aldehyde		162 ± 2	268 ± 1	n.d.	n.d.	n.d.	n.d.
Syringic aldehyde		34 ± 5	39 ± 2	n.d.	n.d.	n.d.	n.d.

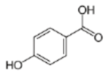
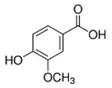
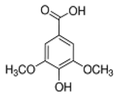
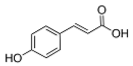
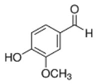
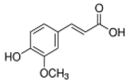
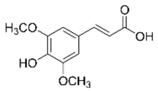
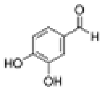
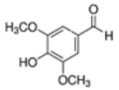
n.d. not detected

subW 185 ºC → Vanillin

Individual Phenolic Compounds

Comparison between the different hydrolytic methods

Phenolic compounds identified by HPLC-DAD (μg $_{compound}$/g$_{BSG,dry}$)

Compound	Formula	SubW T= 160 ºC	SubW T= 185 ºC	UAE-W T= 50 ºC	Acid-Hy	Alkaline-Hy	Xylanase 6%
p-Hydroxibenzoic acid		n.d.	n.d.	10.0 ± 0.5	n.d.	59 ± 2	n.d.
Vanillic acid		17.9 ± 0.4	n.d.	n.d.	n.d.	49 ± 2	61 ± 3
Syringic acid		n.d.	n.d.	n.d.	n.d.	106.1 ± 5.7	n.d.
p-Coumaric acid		191 ± 3	60 ± 1	n.d.	n.d.	538 ± 4	5.3 ± 0.4
Vanillin		254 ± 5	**306 ± 10**	n.d.	n.d.	217 ± 1	203 ± 10
Ferulic acid		250 ± 3	144 ± 7	10.7 ± 0.3	54.4 ± 0.3	1305.7 ± 0.5	292 ± 3
Synapic acid		n.d.	n.d.	2.8 ± 0.2	31.1 ± 0.5	27 ± 1	15 ± 1
Protocatechuic aldehyde		162 ± 2	268 ± 1	n.d.	n.d.	n.d.	n.d.
Syringic aldehyde		34 ± 5	39 ± 2	n.d.	n.d.	n.d.	n.d.

n.d. not detected

subW 185 ºC → Vanillin

Alkaline hydrolysis → Hydroxycinnamic acids

Individual Phenolic Compounds

Comparison between the different hydrolytic methods

Phenolic compounds identified by HPLC-DAD (μg $_{compound}$/g$_{BSG,dry}$)

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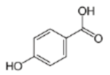
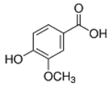
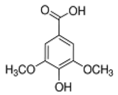
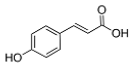
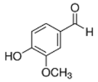
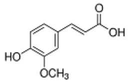
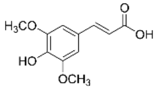
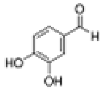
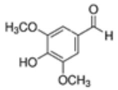
p-Hydroxibenzoic acid		n.d.	n.d.	10.0 ± 0.5	n.d.	59 ± 2	n.d.
Vanillic acid		17.9 ± 0.4	n.d.	n.d.	n.d.	49 ± 2	61 ± 3
Syringic acid		n.d.	n.d.	n.d.	n.d.	106.1 ± 5.7	n.d.
p-Coumaric acid		191 ± 3	60 ± 1	n.d.	n.d.	538 ± 4	5.3 ± 0.4
Vanillin		254 ± 5	**306 ± 10**	n.d.	n.d.	217 ± 1	203 ± 10
Ferulic acid		250 ± 3	144 ± 7	10.7 ± 0.3	54.4 ± 0.3	1305.7 ± 0.5	292 ± 3
Synapic acid		n.d.	n.d.	2.8 ± 0.2	31.1 ± 0.5	27 ± 1	15 ± 1
Protocatechuic aldehyde		162 ± 2	268 ± 1	n.d.	n.d.	n.d.	n.d.
Syringic aldehyde		34 ± 5	39 ± 2	n.d.	n.d.	n.d.	n.d.

n.d. not detected

subW 185 ºC → Vanillin

Alkaline hydrolysis → Hydroxycinnamic acids

Xylanase 6% vs **subW:**

↑ Ferulic acid

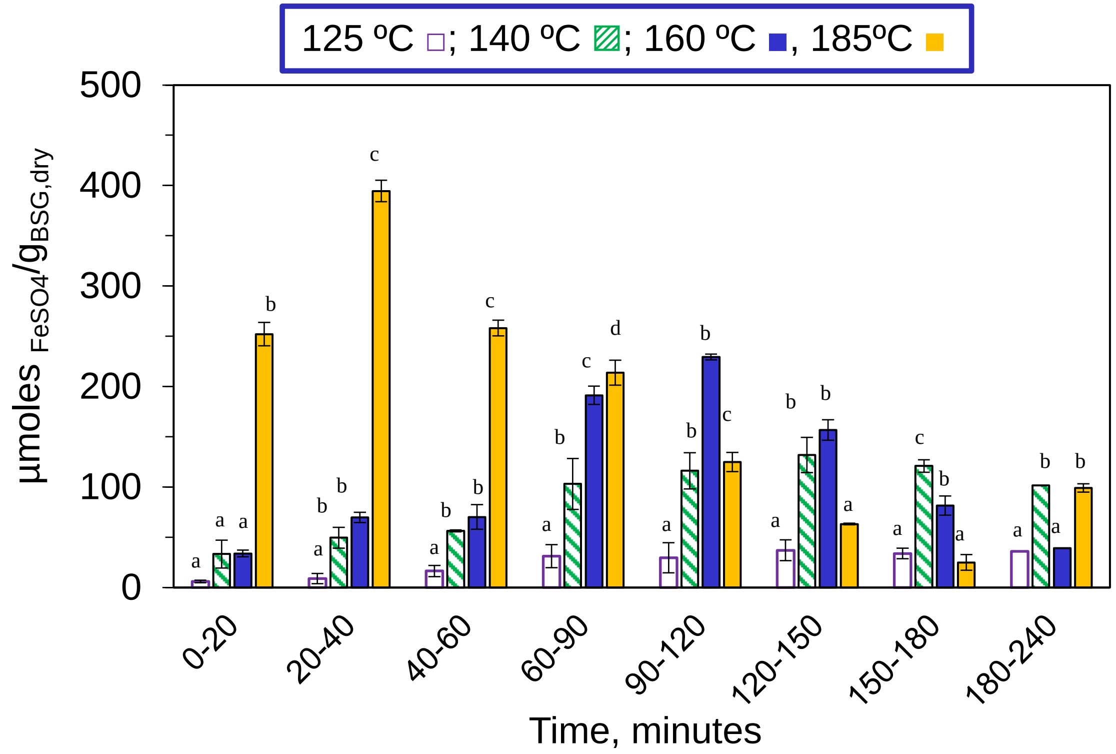
↓p-Coumaric acid

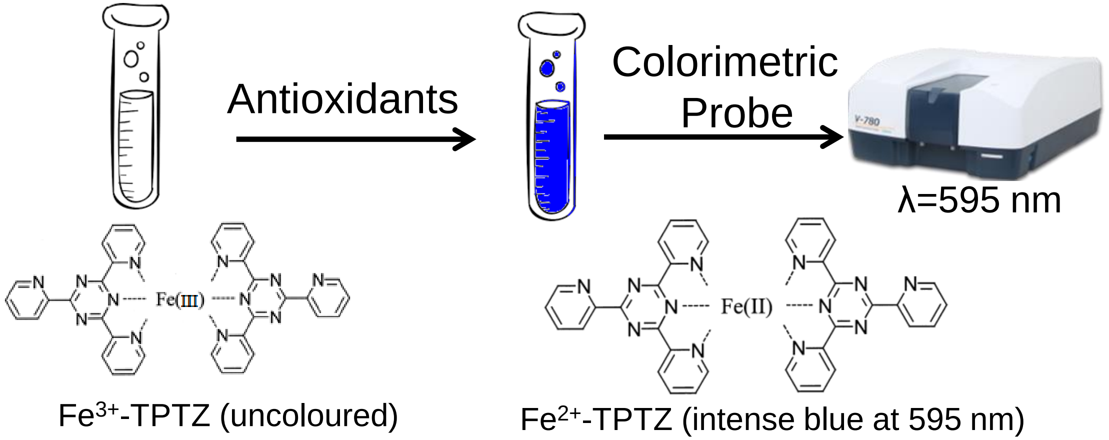
↓Aldehydes

UBU

Antioxidant activity

FRAP ***(Ferric Reducing Antioxidant Power)***





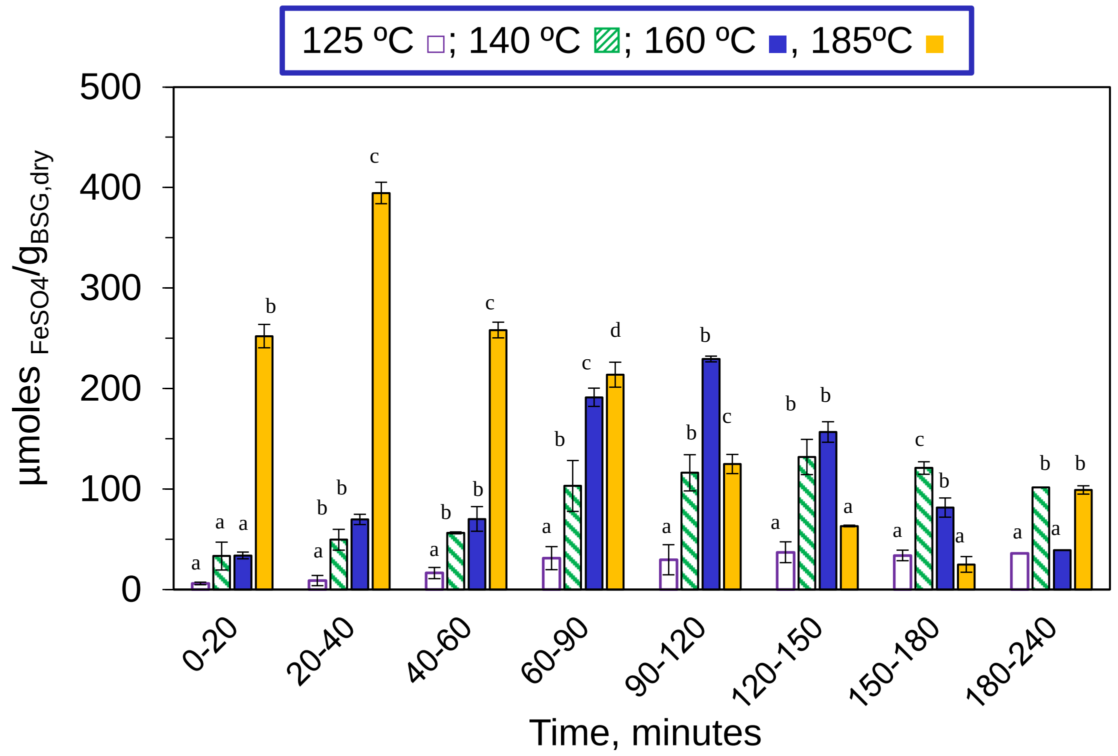
Antioxidant activity of subW extracts at different time intervals

*Values with different letters at each interval time are significantly different when applying the Fisher's least significant differences (LSD) method at p-value ≤ 0.05.

UBU

Antioxidant activity

FRAP ***(Ferric Reducing Antioxidant Power)***



Antioxidant activity of subW extracts at different time intervals

↑ Temperature ↑ Antioxidant activity

$p < 0.05$

CORRELATION

Antioxidant activity ⟷ **R^2 = 0.9511** ⟷ **TPC**

Pearson's correlation coefficient

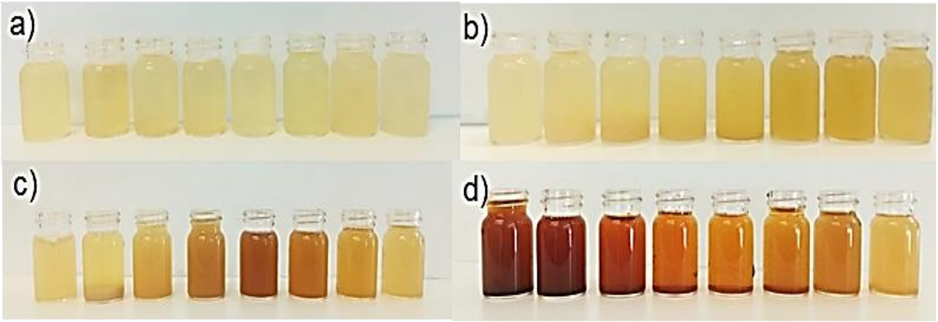
*Values with different letters at each interval time are significantly different when applying the Fisher's least significant differences (LSD) method at p-value ≤ 0.05.

TPC-Antioxidant activity

↑ TPC ↑ Antioxidant activity

↑ Temperature ↑ Browning

Formation of compounds of **Maillard** reactions



a) 125 ºC
b) 140 ºC
c) 160 ºC
d) 185ºC

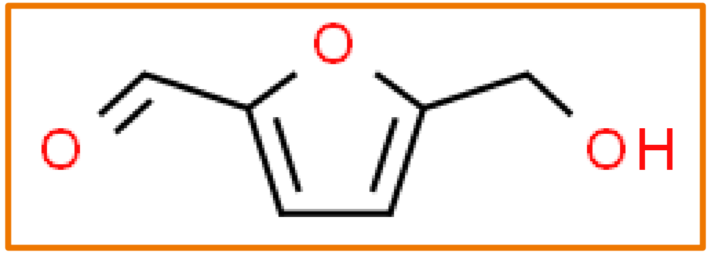
The extracts become darker by ↑ T

The colour change would be due to the formation of **hydrolysis** and **decomposition** products such as HMF and Furfural

UBU

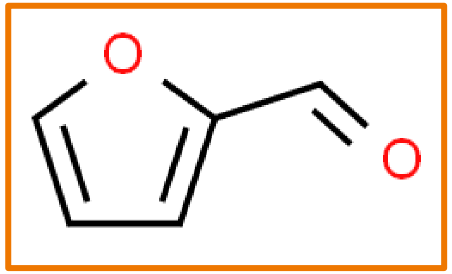
Hmf and Furfural

Concentration of HMF and furfural in subW extracts

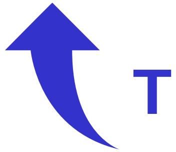


5-Hydroxymethyl furfural (HMF)

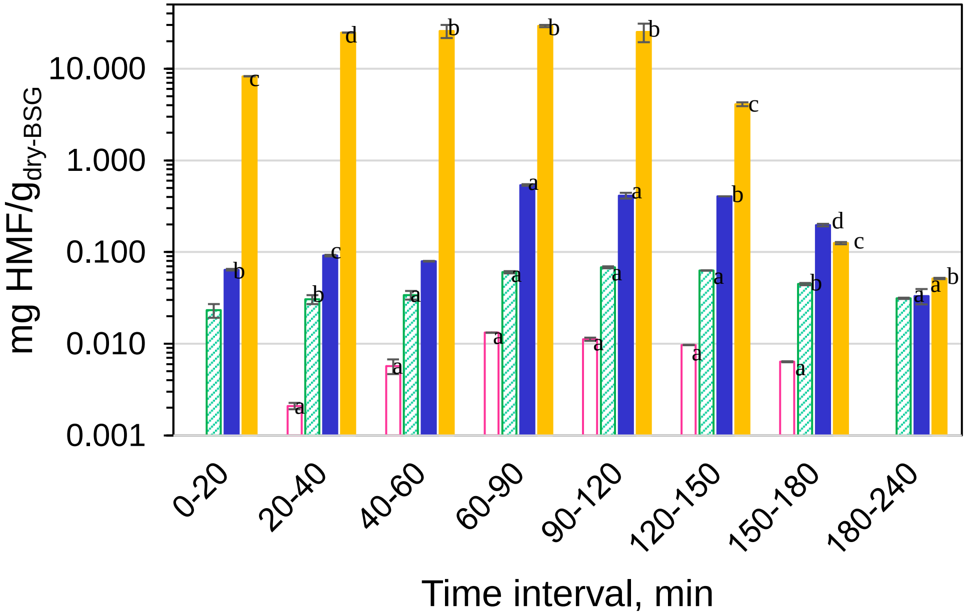
Take part in reactions leading to the formation of melanoidins and other polymers and aromatic substances

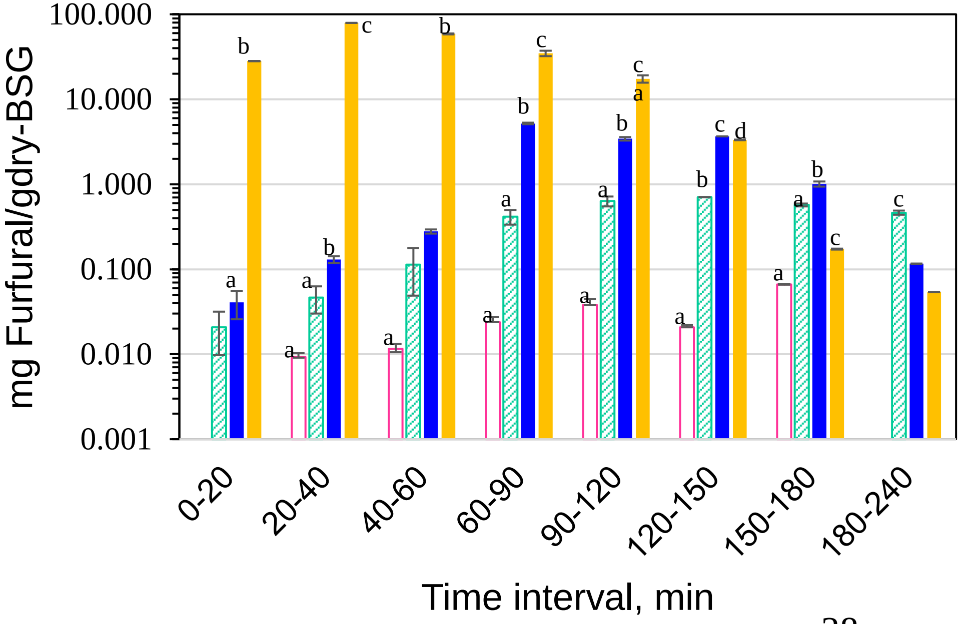


Furfural



125 ºC
140 ºC
160 ºC
185 ºC



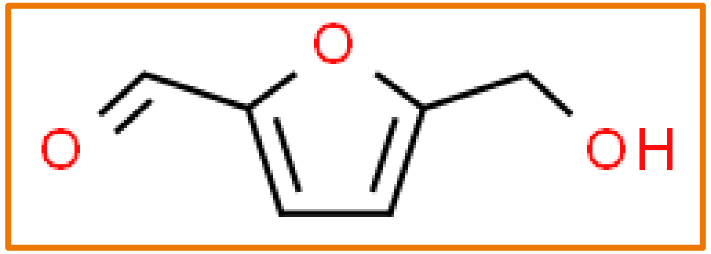


*Values with different letters at each interval time are significantly different when applying the Fisher's least significant differences (LSD) method at p-value ≤ 0.05.

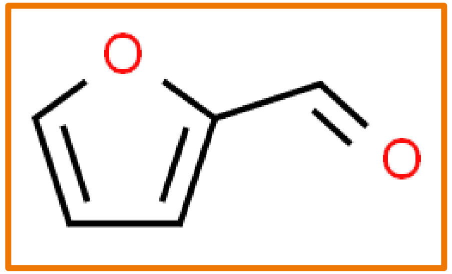
UBU

Hmf and Furfural

Concentration of HMF and furfural in subW extracts



5-Hydroxymethyl furfural (HMF)



Furfural

CORRELATION

Pearsons correlation coefficients

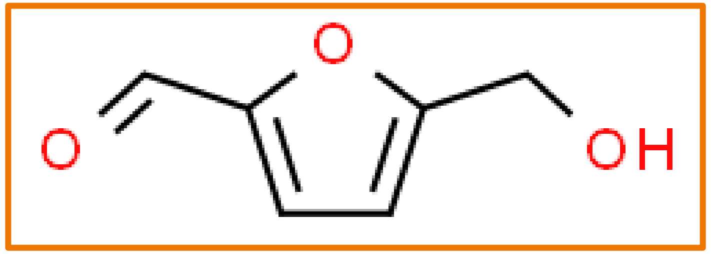
Antioxidant activity ⟷ R^2 = **0.6887** ⟷ **HMF**

Antioxidant activity ⟷ R^2 = **0.8091** ⟷ **Furfural**

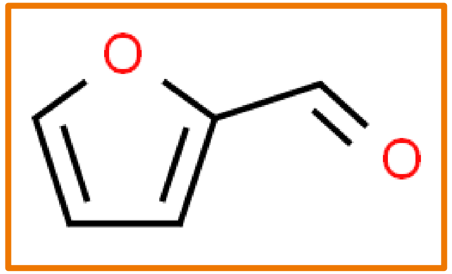
UBU

Hmf and Furfural

Concentration of HMF and furfural in subW extracts

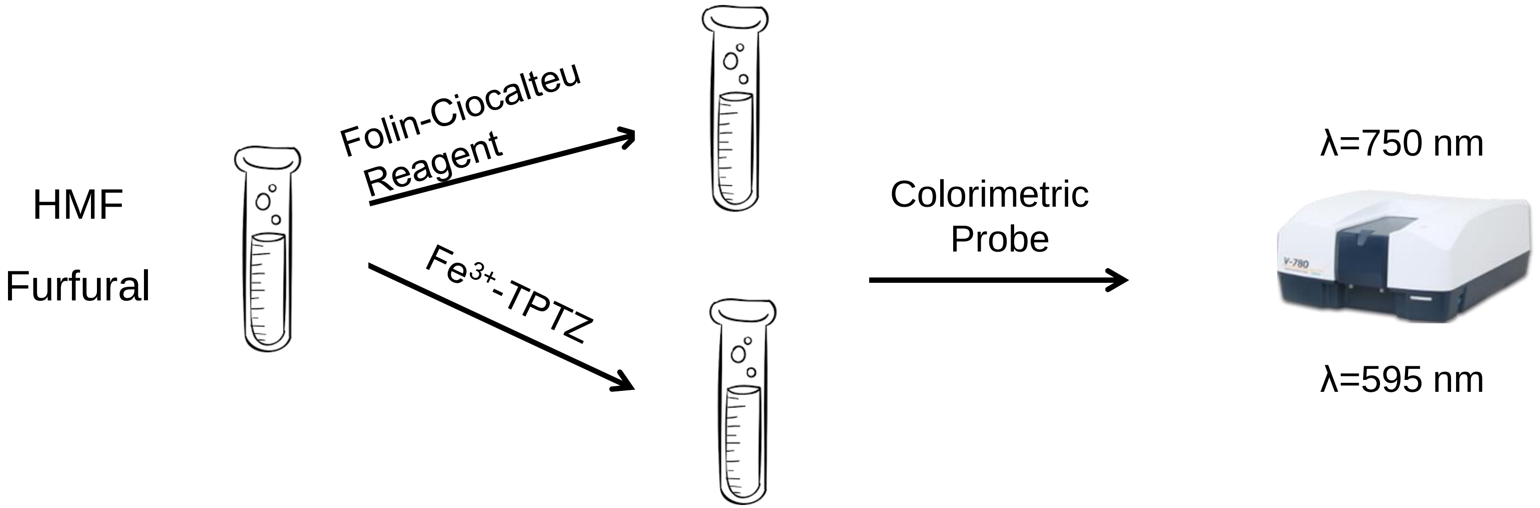


5-Hydroxymethyl furfural (HMF)

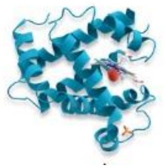


Furfural

TPC AND ANTIOXIDANT ACTIVITY ASSAYS



Antioxidant activity- Proteins



CORRELATION

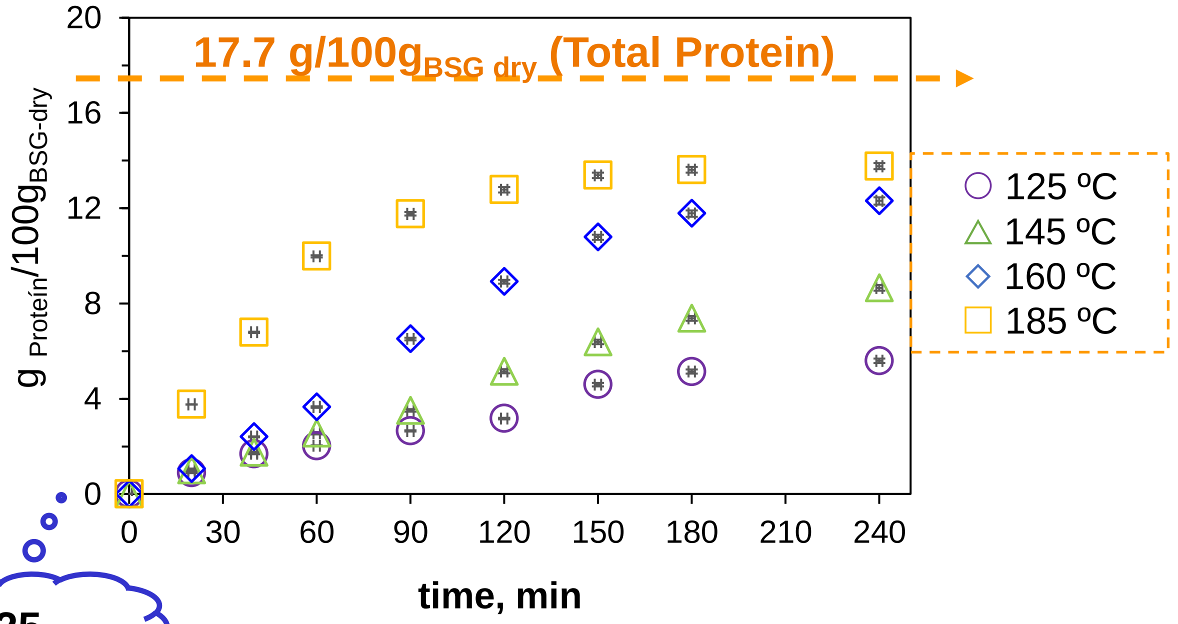
Pearsons correlation coefficients

Antioxidant activity ⟷ R^2 = **0.7629** ⟷ **Proteins**

$$\%\,Yield = \frac{Protein\ in\ the\ subW\ extract}{Protein\ in\ dry\ BSG} \cdot 100$$

T, ºC	Yield, %
185	**77.7**
160	69.5
145	52.6
125	31.6

Accumulative total protein fraction in the subW extracts.

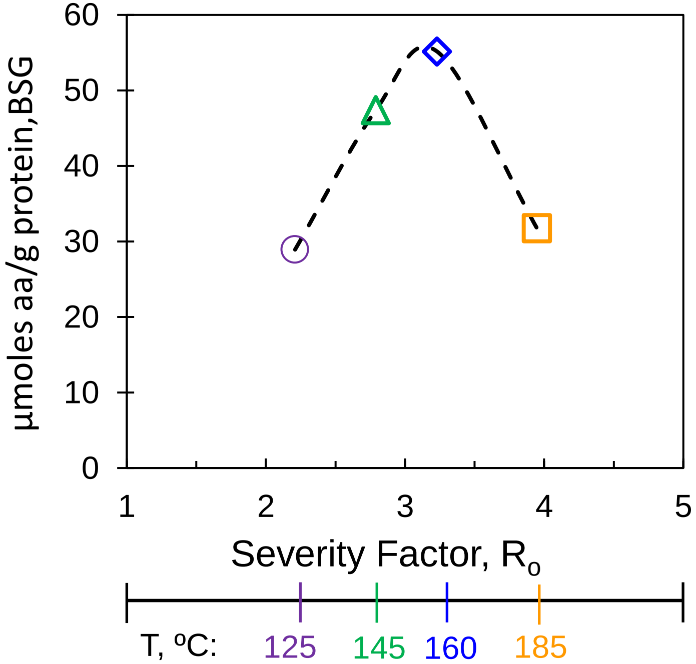


Antioxidant activity- Free amino acids

CORRELATION

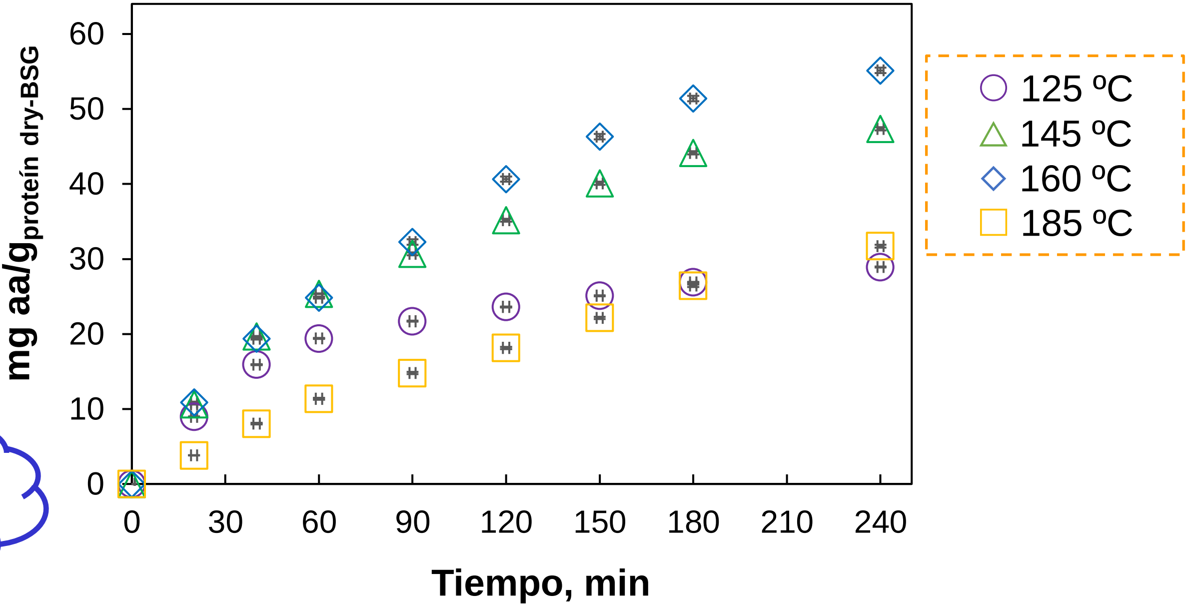
Pearsons correlation coefficients

Antioxidant activity ⇔ **R^2 = 0.1189** ⇔ **Free amino acids**



Sum of aa by GC

Accumulative profile in the subW extracts of **free amino acids**



Highest level at **160 ºC**
aa degradation for
Ro> 3.2

High residence time: **τ ≈ 29 min**

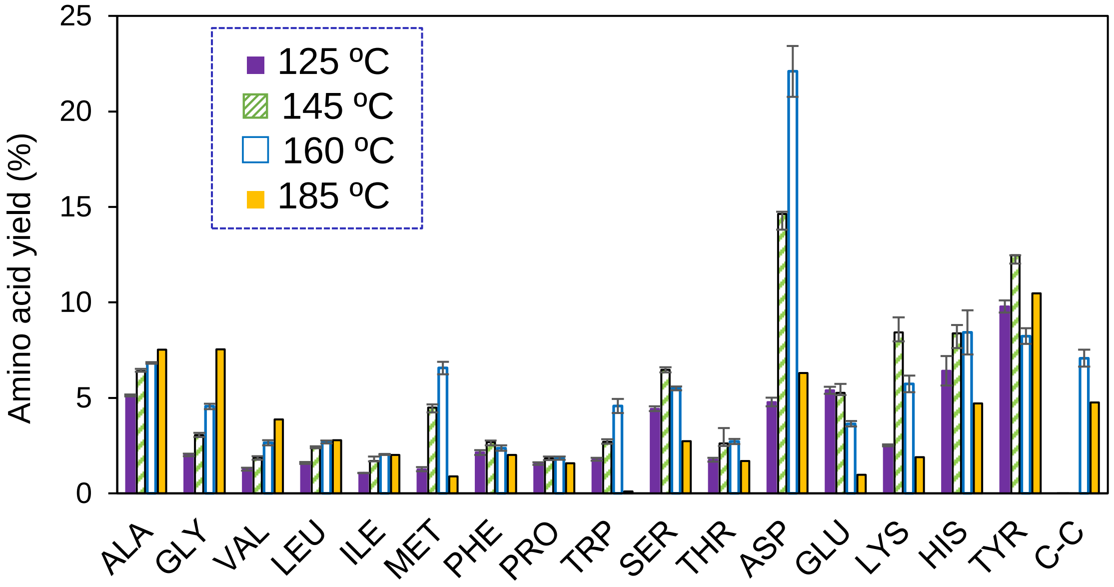
Free amino acids

Amino acid profile of BSG and subW extracts ($mg_{aa}/g_{protein\text{-}dry\text{-}BSG}$)

Amino acid	BSG	125 ºC	145 ºC	160 ºC	185 ºC
ALA	48.1	2.46	3.08	3.29	3.62
GLY	41.9	0.84	1.28	1.91	3.16
VAL	122.6	1.56	2.30	3.25	4.76
LEU	87.1	1.39	2.13	2.35	2.43
ILE	69.3	0.74	1.18	1.42	1.40
THR	41.1	0.73	1.07	1.12	0.70
SER	44.1	1.96	2.86	2.43	1.21
PRO	123.2	1.93	2.25	2.28	1.94
ASP	69.5	3.32	10.17	15.35	4.38
MET	19.0	0.24	0.85	1.25	0.17
HYP	4.3	0.00	0.14	0.10	0.02
GLU	116.8	6.30	6.15	4.26	1.15
PHE	68.0	1.46	1.83	1.61	1.37
LYS	82.7	2.08	6.98	4.74	1.57
HIS	22.8	1.47	1.92	1.93	1.08
HYL	0.0	0.00	0.00	5.03	0.17
TYR	22.5	2.20	2.80	1.85	2.35
TRP	14.7	0.26	0.40	0.67	0.02
C-C	4.7	0.00	0.00	0.33	0.22
TAA	1002.4	28.9	47.4	55.2	31.7

UBU

$$\text{Yield (\%)} = \frac{\text{mg } aa_T}{\text{mg } aa_{BSG}} \cdot 100$$



Final yield of individual amino acids as a function of temperature

↑Aspartic Acid at 160 ºC

TABLE OF CONTENTS

- SubW treatment was confirmed as an efficient extraction/hydrolysis method to extract phenolic compounds from BSG.
- Extraction of phenolic compounds was maximized at the highest temperature studied in this work, 185 °C.
- Hydroxycinnamic acids were more sensitive to temperature than aldehyde phenolic compounds.
- SubW extracts showed a high antioxidant activity that increased with temperature.
- A high protein yield extraction of 78 % was achieved at 185 ºC.

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