Modification of brewer's spent grain after sc-CO_2 extraction: improvement of sugar and phenolic compounds release

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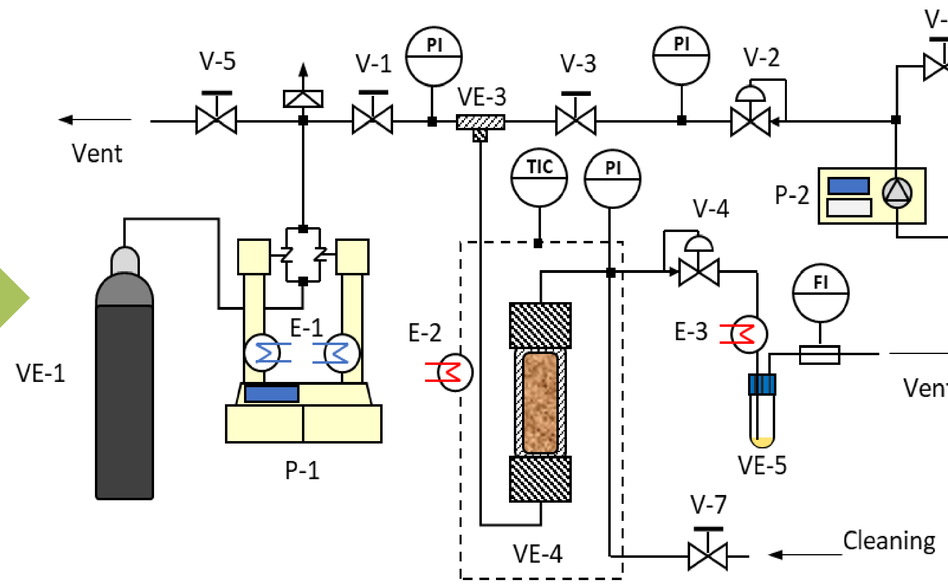
BSG is the most abundant brewing industry by-product (85%), generated after the mashing and wort filtration process.

Brewer´s spent grain (BSG)
Milled (<0.5 mm)

BSG presents a valuable chemical composition:

- ✓ **Protein (**10-30%)
- ✓ **Carbohydrates** (>50 %)
- ✓ **Lipids (**~ 6%)
- ✓ **Phenolic compounds.**



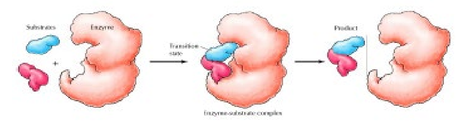
Supercritical CO_2 extraction 80 °C, 40 MPa

Extraction yield: 5.70g /100 g_{BSG}

BSG oil
(linoleic acid)

Raffinate

Enzymatic hydrolysis by cellulase



1,4-(1,3:1,4)-β-D-Glucan 4-glucanohydrolase, EC 3.2.1.4 from *Aspergillus niger* (Sigma-Aldrich)

Cellulase activity: 1.18 U/mg

Operating conditions

T= 50 °C
pH= 5 (acetate buffer)
5% dry BSG (% *w/v*)
% Cellulase = 0.25 % -1 %, enzyme:BSG ratio (*w/w*)

Carbohydrate composition of the BSG and the sc-CO_2 raffinate in a dry and free-fat basis (g/100 g_{BSG}) .

Carbohydrate	BSG (%)	Raffinate (%)
Glucans	42 ± 2	43 ± 1
Xylans	15 ± 1	15.2 ± 0.1
Arabinans	8 ± 1	7.5 ± 0.5

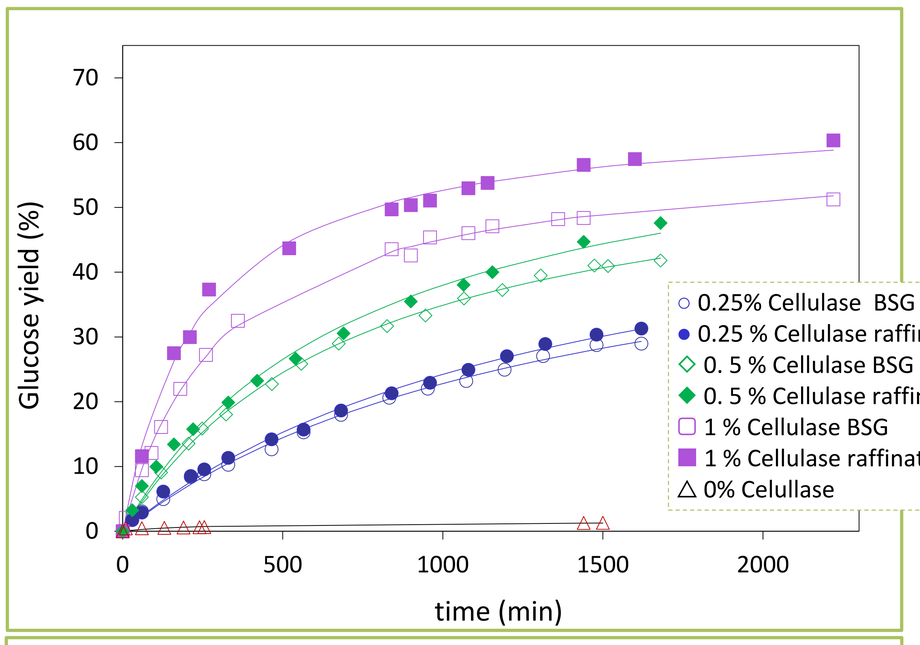
CH´s composition were not significantly different after sc-CO_2 treatment.

Double effect of **sc-CO_2** in a **biorefinery** context to include the BSG into a **circular economy** concept:

- ✓ Green solvent for **oil recovery**
- ✓ **Pretreatment** agent for further improvement of the **enzymatic hydrolysis** yield of the sc-CO_2 treated BSG

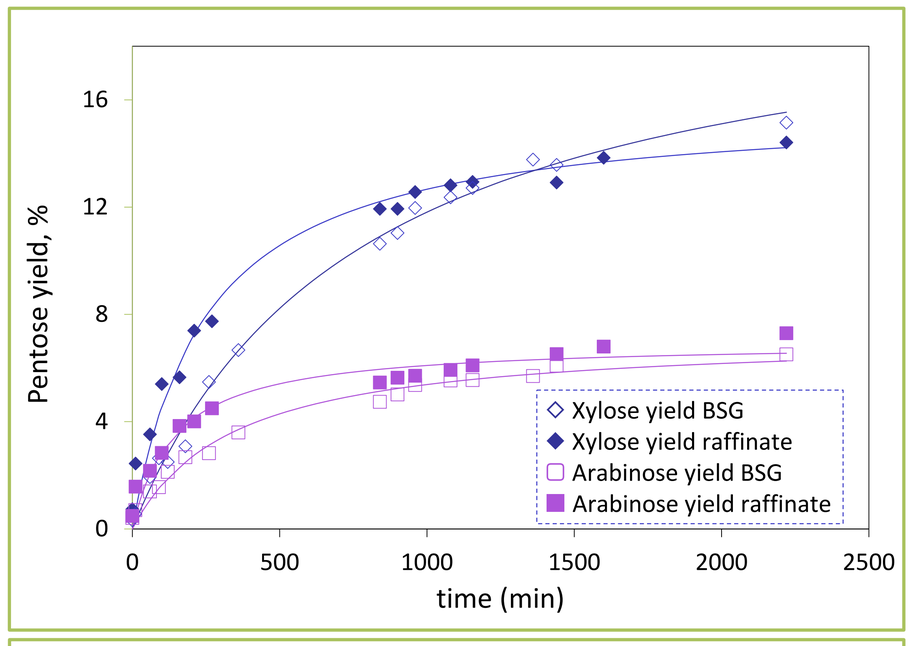
sc-CO_2 treatment enhanced **glucose yields** for all the enzyme concentrations assayed

Cellulose dose (% w/w)	Increase in glucose yield (%)
0.25	8
0.5	14
1	18

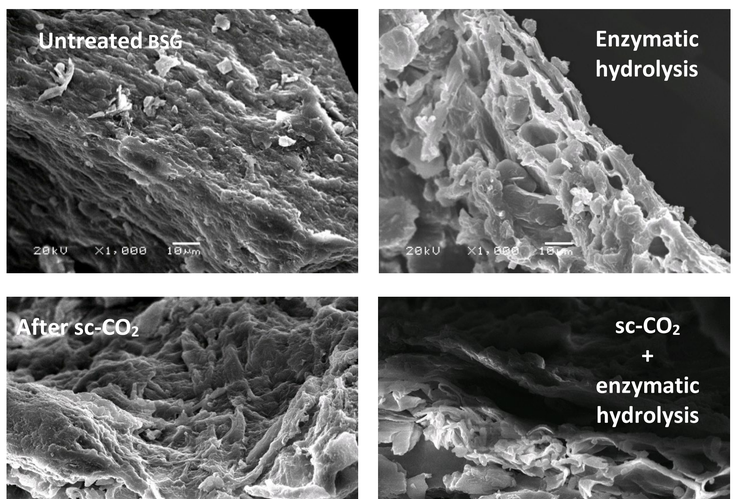


Glucose monomer yield by enzymatic hydrolysis at 50 °C and different cellulase dose. Continuous lines represent the Holtzapple model.

The initial **hydrolysis rate for pentoses** were significant higher for the sc-CO_2 treated BSG than for the untreated samples



Pentose monomer yield by enzymatic hydrolysis at 50 °C and 1 % of cellulase dose. Continuous lines represent the Holtzapple model.



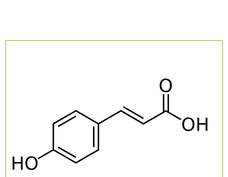
SEM micrographs (1000 x magnifications) of the different BSG samples: untreated BSG, sc-CO_2 treated BSG and after enzymatic hydrolysis

- ➢ **Untreated BSG**: more rigid and continuous surface
- ➢ **sc-CO_2 treated BSG**: irregular porosity and lamellar structure.

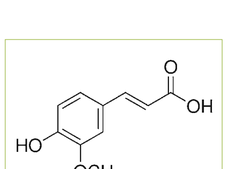
The **improvement** in enzymatic hydrolysis rate and yield after sc-CO_2 treatment could be attributed to:

- ➢ the **removal** of the **lipid** fraction.
- ➢ **surface morphology modification.**

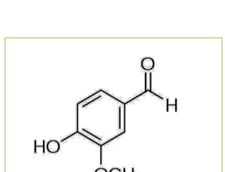
Improvement of phenolic compounds release after sc-CO_2 treatment



p-coumaric acid



ferulic acid



vanillin

- ✓ **p-Coumaric acid** concentration increased 30%
- ✓ **Ferulic acid** concentration increased 25%
- ✓ The concentration of **vanillin** was similar in both hydrolysates.

Phenolic compounds release yield by different treatments

Treatment	Cumaric acid, µg/g_{BSG}	Vanillin, µg/g_{BSG}	Ferulic acid, µg/g_{BSG}
Celullase, 1 %	3.0 ± 0.3	20 ± 1	274 ± 4
sc-CO_2 + Celullase, 1 %	3.9 ± 0.3	21 ± 2	341 ± 6
Xilanase, 1 %	6 ± 1	111 ± 3	52.4 ± 0.9
Alakaline hydrolysis	538 ± 4	217 ± 1	1305.7 ± 0.5
Subcritical water 185 °C	60 ± 8	330 ± 11	144 ± 10

The release **of ferulic acid** by CO_2 + Celullase, 1 % was noticeable higher than those obtained by other hydrolytic methods with exception than alkaline hydrolysis

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