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PRODUCTION OF VALUE ADDED FOOD DERIVED SUB-PRODUCTS VIA SSF USING *Rhizopus* sp.

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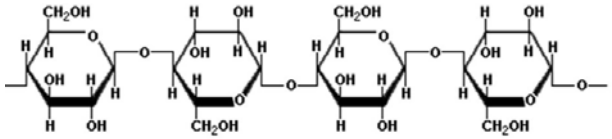
Brewer's spent grain (BSG)

- **85 % of total by-products** generated during beer production > 7 million tones of residues in Europe breweries annually
- Rich in fiber (70 %) and protein (20 %)



Value

enzymes

Microbial fermentation

Value

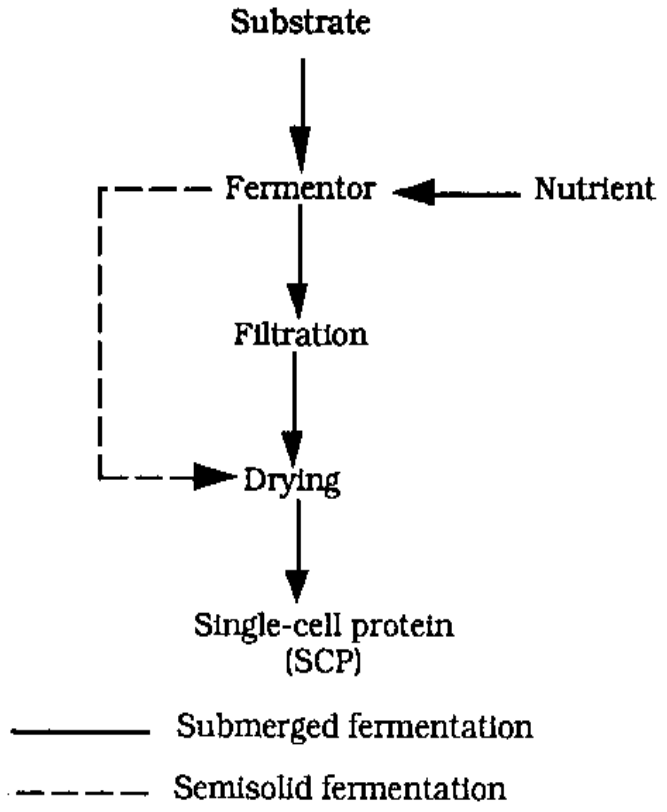
Antioxidant peptides ✓
Antihypertensive peptides ✓
Phenolic compounds ✓

High economical cost ✗

SSF as valorization strategy

- **Fungi** main microorganisms used in SSF
  - Known to produce enzymes which degrade plant cell walls
  - Improve biochemical composition and bioactivity of the employed substrates

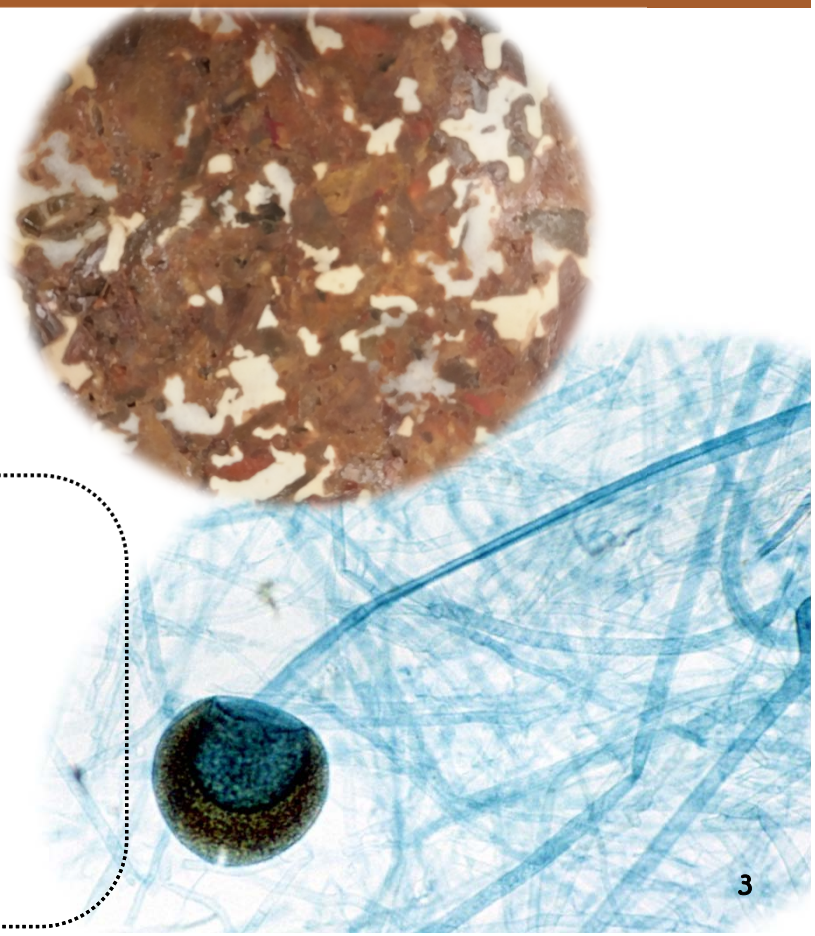
- Microorganism for SCP production should have…
  - High affinity for the substrate
  - Low nutritional requirements
  - Ability to use complex substrates
  - Ability to develop high cell density
  - Good tolerance to temperature and pH
  - Balanced protein and lipid composition
  - Low nucleic acid content
  - Good digestibility and non-toxic



SSF as valorization strategy

- *Rhizopus* sp. one of the most promising fungal genus
  - Consume a great range of carbon sources
  - Enzyme and organic acid production
  - By-product valorization

- Advantages for feed application
  - Generally recognized as safe (GRAS) by the FDA
  - Inoculum for human food production (tempeh, ragi…)
  - Significantly reduced the amount of testing required
  - High content of protein (35-60 %)
  - Low levels of nucleic acids
  - Pleasant taste and smell of the fungal biomass



Analytical methods

Food-derived *Rhizopus* strain isolated and characterized in our laboratory (ROR004)[1] and a strain derived from ROR004 after mutagenesis experiments (ROR00G), UV, Ethidium bromide and MNNG .

- **Dry weight:** drying at 60 °C, until constant weight
- **Protein and total nitrogen content:** Kjeldahl method
- **Total Amino acids**: hydrolysis with 6N HCl and HPLC
- **Essential Amino Acid Index:** (2)

$$EAAI = \sqrt[n]{\sum n \frac{mg\ of\ EAA\ in\ 1\ g\ of\ tested\ protein}{mg\ of\ EAA\ in\ 1\ g\ of\ reference\ protein}} \quad (2)$$

- **Antioxidant activity:** DPPH radical scavenging activity (TEAC, Trolox equivalent capacity)
- **Total phenolic content (TPC):** Folin–Ciocalteu method (GAE, Gallic acid equivalent)
- **Reducing Sugar**: Dinitrosalicylic acid (DNS) method
- **Soluble protein** : BSA method
- **Degree of hydrolysis** (DH): Phthaldialdehyde (OPA) method
- **Antibacterial activity:** agar diffusion method *Salmonella enterica* (CECT 4156) and *Escherichia coli* (CECT 516)

(1) Ibarruri, J., Hernández, I.: *Rhizopus oryzae* as fermentation agent in food derived subproducts. Waste and Biomass Valorization (2017). doi:10.1007/s12649-017-0017-8

(2) Concentration and characterization of microalgae proteins from Chlorella pyrenoidosa Ashish G. Waghmare, Manoj K. Salve, Jean Guy LeBlanc and Shalini S. Arya. Bioresources and bioprocessing (2016) 3:16

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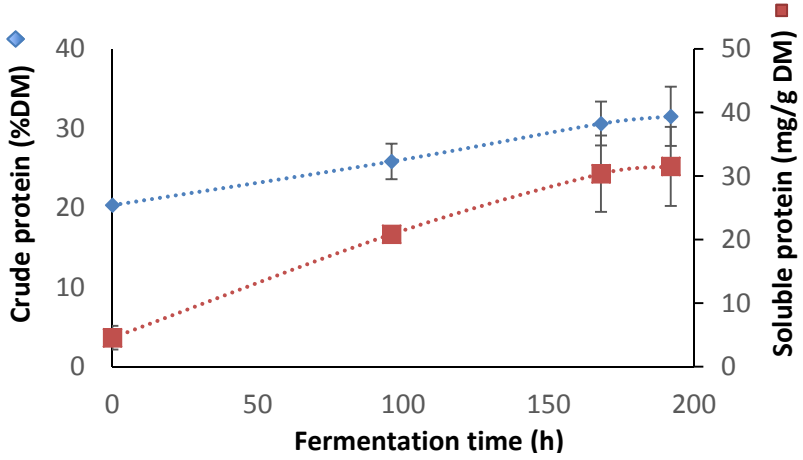
BSG

- Provided by Boga Cooperative (an artisanal brewery situated in Mungia, Spain)
- Sterilized at 110 °C, 15 min
- Inoculation (10^6 cfu/g)
- Density was maintained at 0.09 g/cm^2

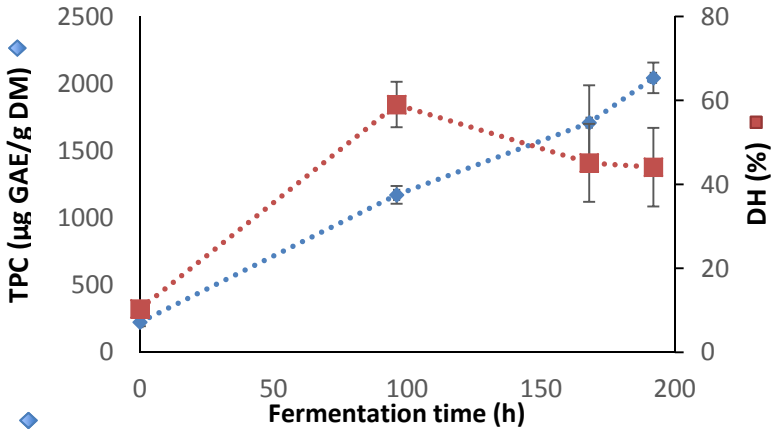
SSF

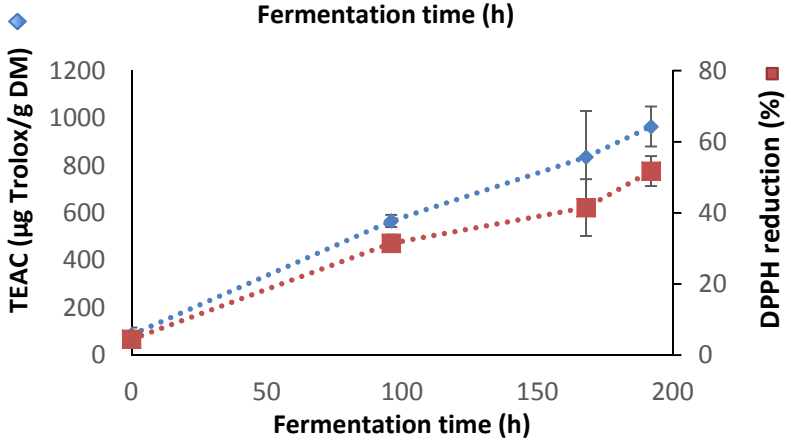
- Sterile Petri plate (surface area 143.1 cm^2), 30 °C, from T0 to T192 h to determine the variables that change along the SSF
- Factorial design (2^3) (8 runs) and central points (4 runs) was used for optimal conditions determination

Factor	Low(-)	0	High (+)
A: Time (h)	48	144	216
B: Temperature (°C)	20	25	30
C: Strain	1 (Mutant)		2 (ROR004)



- Antibacterial activity was not detected in the extracts obtained from fermented BSG
- Not differences in reducing sugars





SSF EVOLUTION

Time 0 h → Time 72 h → Time 120 h →

Time 144 h → Time 168 h → Time 192 h

Ideal substrate for SSF

- **composition**
- **particle size**

Small particles with rigid internal structure allow a complete fungal growth and a complete use of the inter-particle space

Homogenous fermented material and avoid the presence of under-fermented areas

Model	P-value	R^2
Protein	**0.0297***	65.4
Soluble protein	**0.0000***	96.8
DH	**0.0196***	77.7
TEAC	**0.0177***	78.4
DPPH	**0.0080***	82.9
TPC	**0.0005***	92.6

	Adjusted	Observed
Protein (% DM)	31.1	31.7 ± 7.6
Soluble protein (mg/g DM)	48.6	47.4 ± 3.8
DH (%)	29.9	27.4 ± 7.0
TEAC (µg TE/g DM)	746	795 ± 102
DPPH reduction (%)	58.5	63.8 ± 1.9
TPC (µg GAE/g DM)	2736	2747 ± 112

Overall strategy for BSG valorization is to optimize all responses at a time

- 30 °C, 216 h of fermentation time

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Amino Acid Profile

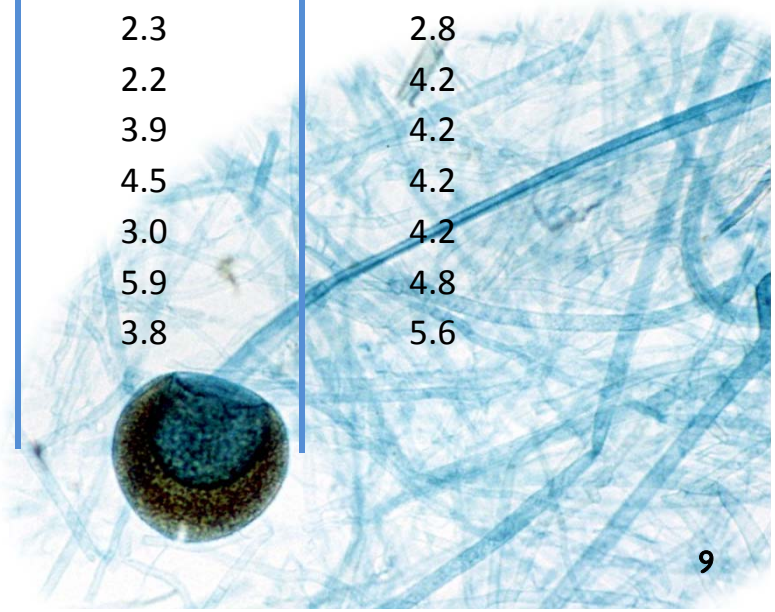
[3] FAO/WHO: Protein and amino acid requirements in human nutrition. Expert Consultation, vol. 935. WHO Technical Report Series, pp. 1-7. World Health Organization, Geneva, (2007).
[4] FAO (WHO) (1974) Protein advisory group guidelines no. 15 on the nutritional and safety aspects of novel sources of protein for animal feeding. United Nations, Rome

	EAA profile (%)		EAA content (% DM BSG)	
	Unfermented BSG	Fermented BSG	Unfermented BSG	Fermented BSG
His	2.9 ± 0.1^{a}	2.7 ± 0.5^{a}	0.6 ± 0.0^{a}	0.8 ± 0.1^{b}
Ile	3.7 ± 0.2^{a}	3.7 ± 0.2^{a}	0.7 ± 0.0^{a}	1.2 ± 0.1^{b}
Leu	8.2 ± 0.1^{a}	7.6 ± 0.3^{a}	1.7 ± 0.0^{a}	2.4 ± 0.1^{b}
Lys	6.6 ± 0.2^{a}	6.8 ± 0.4^{a}	1.3 ± 0.0^{a}	2.1 ± 0.1^{b}
Met	1.1 ± 0.0^{a}	1.6 ± 0.2^{b}	0.2 ± 0.0^{a}	0.5 ± 0.1^{b}
Cys	0.9 ± 0.2^{a}	1.3 ± 0.3^{a}	0.2 ± 0.1^{a}	0.4 ± 0.1^{b}
Phe	5.5 ± 0.0^{a}	4.7 ± 0.4^{a}	1.1 ± 0.0^{a}	1.5 ± 0.1^{b}
Tyr	3.7 ± 0.2^{a}	4.4 ± 1.1^{a}	0.7 ± 0.0^{a}	1.4 ± 0.3^{b}
Thr	4.0 ± 0.3^{a}	4.6 ± 0.3^{a}	0.8 ± 0.1^{a}	1.4 ± 0.1^{b}
Val	5.8 ± 0.2^{a}	5.9 ± 0.5^{a}	1.2 ± 0.0^{a}	1.8 ± 0.1^{b}
Total essential	42.4 ± 0.2^{a}	43.1 ± 2.3^{a}	**8.6 ± 0.0^{a}**	**13.6 ± 0.7^{b}**

Amino Acid Profile

[3] FAO/WHO: Protein and amino acid requirements in human nutrition. Expert Consultation, vol. 935. WHO Technical Report Series, pp. 1-7. World Health Organization, Geneva, (2007).
[4] FAO (WHO) (1974) Protein advisory group guidelines no. 15 on the nutritional and safety aspects of novel sources of protein for animal feeding. United Nations, Rome

	Fermented BSG	Soya protein	Casein	FAO Reference	FAO Reference
His	2.7 ± 0.5	1.2	3.0	1.5	
Thr	4.6 ± 0.3	1.9	4.3	2.3	2.8
Cys+Met	2.9 ± 0.3	0.7	2.9	2.2	4.2
Val	5.9 ± 0.5	2.4	5.9	3.9	4.2
Lys	6.8 ± 0.4	3.4	8.2	4.5	4.2
Ile	3.7 ± 0.2	2.1	4.9	3.0	4.2
Leu	7.6 ± 0.3	3.5	9.7	5.9	4.8
Phe+tyrosine	9.1 ± 1.4	2.4	10.7	3.8	5.6
EAAI FAO [3]	1.58 ± 0.08	0.63	1.78		
EAAI FAO [4]	1.28 ± 0.05	0.50	1.44		

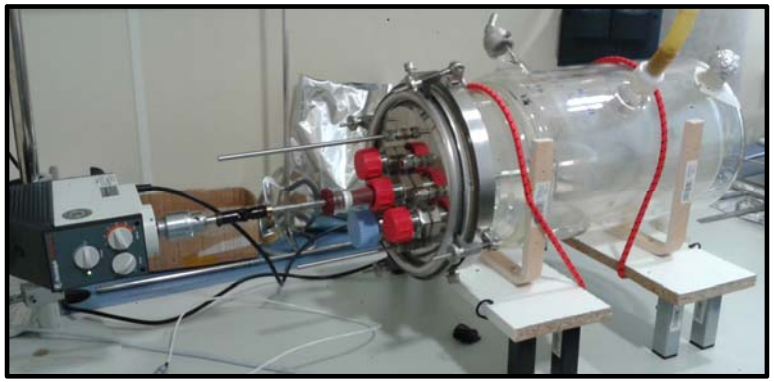


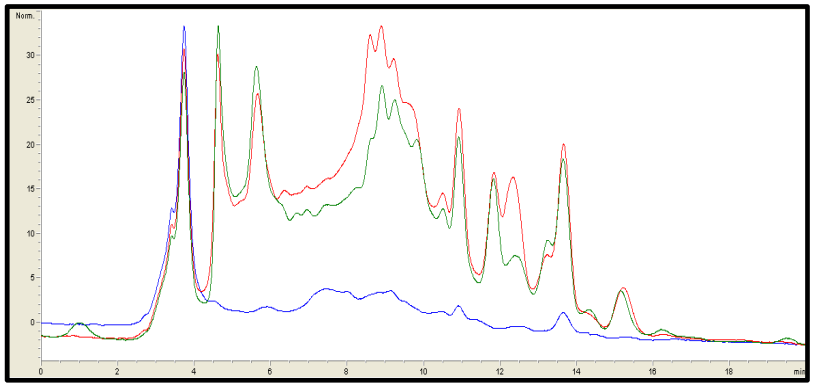
- Using filamentous fungi as bio-fermentation of BSG leads to a **protein rich substrate** with higher **essential amino** acid content and **hydrolysis degree** which could improve protein digestibility
- SSF also increases the **antioxidant activity** of the fermented BSG related to the release of **phenolic compounds**
- SSF is a **promising alternative** to revalorize this agro-industrial by-product as **ingredient for feed and food applications**

- BSG is also known for containing peptides related to **antihypertensive activity** and for **specific phenolic compounds** (ferulic, caffeic and p-coumaric acid) related to **anti-cancer, anti-atherogenic and anti-inflammatory effects**
- Further research is needed to evaluate the effect of SSF by *Rhizopus* sp. in the liberation of those specific peptides and phenolic compounds

Ongoing research

- Scaling up in drum bioreactor
- SSF effect of antihypertensive peptide liberation (positive preliminary results)
- Obtained peptides profile
- Anti-inflammatory and immunostimulatory effects of the fermented product
- Enzyme production
- Economical, nutritional, safety and environmental viability of the process





Unfermented BSG Fermenetd BSG (30 ºC, 9 days)

Thank you for your attention

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