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# **Brewers' Spent Grain Flour: Antioxidant Properties and Applications**

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# **INTRODUCTION & AIM**

Beer is a widely consumed carbonated beverage made from natural ingredients, including malted cereal, hops, yeast and water [1]. During the brewing process, large quantities of Brewers' Spent Grain (BSG), derived from barley malt, represent the residue left after wort extraction and before fermentation. Traditionally regarded as waste, BSG has primarily been used for applications such as animal feed, organic fertiliser, or brick production [2]. This study aims to evaluate the development of flour from BSG and assess its antioxidant properties to determine its functional benefits as a strategy to valorise this by-product, with potential applications in the food and nutraceutical industries.

### **RESULTS & DISCUSSION**

Data are expressed in table 1 as the mean ± SD of three independent measures.

Table 1: Results of antioxidant capacity by DPPH and FRAP assays, total phenolic and total flavonoid contents of BSG flour.

Antioxidant capacity Total Phenols Total Flavonoids	t capacity Total Phe	enols Total Flavonoids
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# METHOD

BSG samples were collected from the brewery Crafters (Sintra, Portugal) after the brewing process.

The collected BSG was refrigerated and then dried in an oven with forced air circulation at 50°C, milled into flour, and sieved through a 400  $\mu$ m mesh. The flour was subsequently packed, sealed in polyethylene bags to prevent exposure to oxygen, and stored at -20°C, away from light, until analysis.



Figure 1: BGS sample and flour used in the tests.

#### 1. Total Phenolic Content (TPC) and Total Flavonoid Content (TFC)

The Total Phenolic Content (TPC) was determined according to the modified Folin-Ciocolteau colorimetric method [3]. The absorbance of the blue coloration formed was read at 725 nm. Total phenolics were calculated with respect to Gallic Acid standard curve (concentration range of 0.001-0.200 mg/mL). Results are expressed in mg of Gallic Acid equivalent (GAE)/g of BSG.

The Total Flavonoid Content was measured using the aluminium chloride colorimetric method [4]. The absorbance was read at 490 nm. The Total Flavonoid Content (TFC) was quantified based on a Quercetin standard curve (concentration range of 0.004-4 mg/mL). The results are expressed in mg of Quercetin equivalent (QE)/g of BSG.

Sample	DPPH (µmol TE/g BSG)	FRAP (µmol TE/g BSG)	mg GAE/g BSG	mg QE/g BSG
BSG	$1.69 \pm 0.16$	2.12 ± 0.37	1.27 ± 0.03	3.05 ± 0.54



Figure 2: Spectrophotometric equipment and sample preparation for the DPPH method

- The BSG showed significant levels of total phenolic content of 1.27 ± 0.03 mg GAE/g BSG flour and total flavonoid content of 3.05 ± 0.54 mg QE/g BSG flour.
- BSG showed a promising antioxidant capacity of 2.12  $\pm$  0.37 µmol TE/g by the Frap method and 1.69  $\pm$  0.16 µmol TE/g by the DPPH assay.
- These results highlight the potential of BSG flour as a valuable source of natural polyphenolic compounds and its potential use as a food additive to increase bioactive content.
- Future work will investigate the phenolic compound profile using high performance liquid chromatography (HPLC) to clarify the phenolic bioactive capacity of BSG flour.

#### 2. Antioxidant Capacity

#### A) DPPH Method

Radical scavenging capacity was determined by the DPPH assay described by Pereira *et al.* 2023 [3]. To prepare the samples, 150  $\mu$ L of BSG and 2850  $\mu$ L of the DPPH standard solution were measured. The absorbance was read at 580 nm. The antioxidant activity was determined by constructing a calibration curve with Trolox (concentration range of 25-800  $\mu$ mol/L). Results are expressed as  $\mu$ mol trolox equivalent (TE)/g of BSG.

#### B) FRAP Method

The FRAP assay was carried out according to the procedure described by Cartas *et al* 2024 [5]. The principle of this method is based on the ability of substances to reduce Fe(III)- 2,4,6-Tri(2-pyridyl)-s-triazine (TPTZ). The absorbance was read at 593. A calibration curve of iron (II) sulfate heptahydrate (concentration range of 10-1000  $\mu$ mol/L) was used, and results are expressed as  $\mu$ mol TE/g of BSG.

# CONCLUSION

The results highlight the potential of BSG as a valuable source of natural polyphenolic compounds. The developed flour could be used as an eco-friendly antioxidant ingredient with applications in the food and nutraceutical industries, improving product preservation and functional properties.



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