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Sustainable Antioxidants: Exploring Beer By-products for Cosmetic and Pharmaceutical 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. It is rich in nutrients and contains carbohydrates, minerals, vitamins, amino acids and polyphenols [1]. The brewing process, however, generates a significant amount of solid waste, including hot trub, a slurry of entrained wort, hop particles and mainly unstable high molecular weight colloidal proteins that coagulate during the boiling of the wort [2]. Given the environmental impact of agro-industrial waste, finding sustainable methods to reuse these wastes by transforming them into bio-products is crucial. The aim of this study was to evaluate the potential of transforming these by-products into biologically active extracts, suitable for use as functional ingredients in cosmetic and pharmacological formulations.

METHOD

Hot trub was supplied by a brewery named Musa, Lisbon, Portugal.

The material was subjected to the drying process at 45ºC in lab, until dry and stored at room temperature.

Figure 1. Hot trub after drying process.

1. Preparation of the Hot trub extracts

Three different extractions were performed: Two extractions using the Soxhlet method – Hydroalcoholic, EH (ethanol/water 70:30 (v/v)), and Alcoholic, EA (99.9% ethanol (v/v)) during 90 min at high temperature; and one more Hydroalcoholic Extraction, EM (ethanol/water 70:30 (v/v)) by maceration in agitation (900 rpm) at room temperature for 24 h. All the extracts were evaporated in a rotary evaporator and the resulting extracts were stored in Eppendorf tubes and frozen.

Figure 2. Soxhlet Extraction Equipment.

Figure 3. Maceration Extraction Method.

Figure 4. Rotary Evaporator Equipment.

2. 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: 0.001-0.200 mg/mL). Results are expressed in mg of Gallic Acid equivalent (GAE)/g of hot trub extract. 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: 0.004-4 mg/mL). The results are expressed in mg of Quercetin equivalent (QE)/g of hot trub extract.

3. Antioxidant Activity

A) DPPH Method

Radical scavenging capacity was determined by the DPPH assay described by Pereira *et al*. 2023 [3]. To prepare the samples, 150 µL of each extract and 2850 µL 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: 25-800 µmol/L). Results are expressed as µmol trolox equivalent (TE)/g of hot trub extract.

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 (10-1000 µmol/L) was used and results are expressed as mmol $FeSO_4.7H_2O$/g of hot trub extract.

RESULTS & DISCUSSION

Data are expressed in figures 5, 6, 7 and 8 as the mean ± SD of three independent measures. The statistical analysis was done by one-way ANOVA using the Scheffé test and Statistica® software, v. 8.0. (StatSoft, Tulsa, OK, USA). Different letters between columns represent significant differences ($p < 0.05$).

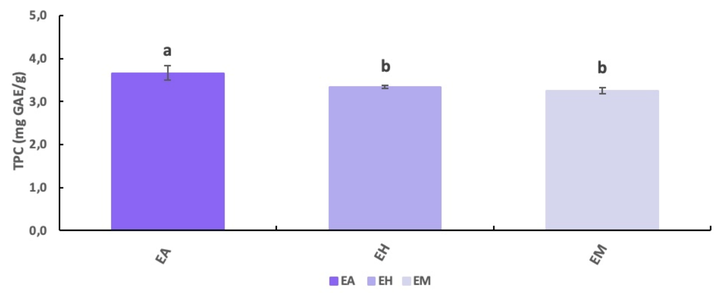

Figure 5. Total phenolic content (TPC) in hot trub extracts (mg GAE/g of extract). EA - Alcoholic Extract Soxhlet; EH - Hydroalcoholic Extract Soxhlet; EM - Hydroalcoholic Extract Maceration.

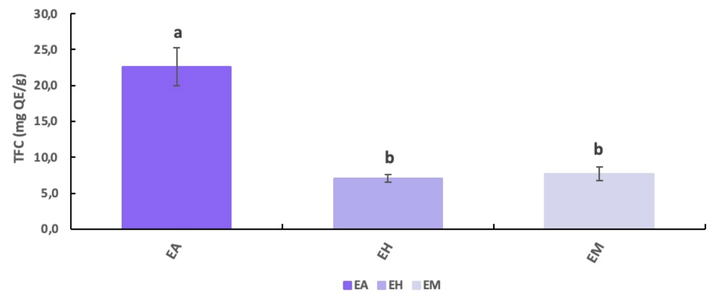

Figure 6. Total flavonoid content (TFC) in hot trub extracts (mg QE/g of extract). EA - Alcoholic Extract Soxhlet; EH - Hydroalcoholic Extract Soxhlet; EM - Hydroalcoholic Extract Maceration.

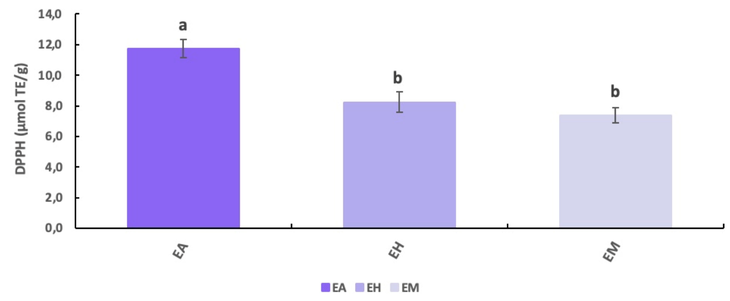

Figure 7. The antioxidant activity in hot trub extracts with DPPH assay (µmol TE/g of extract). EA - Alcoholic Extract Soxhlet; EH - Hydroalcoholic Extract Soxhlet; EM - Hydroalcoholic Extract Maceration.

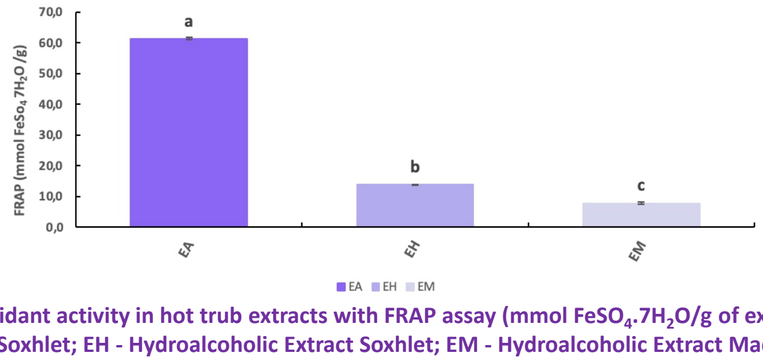

Figure 8. The antioxidant activity in hot trub extracts with FRAP assay (mmol $FeSO_4.7H_2O$/g of extract). EA - Alcoholic Extract Soxhlet; EH - Hydroalcoholic Extract Soxhlet; EM - Hydroalcoholic Extract Maceration.

The results demonstrated the antioxidant activity exhibited by the different extracts, with the alcoholic extract showing the best performance in both methods, obtaining a value of 61.31±0.39 mmol $FeSO_4.7H_2O$/g extract for the FRAP assay, and a value of 11.74±0.57 µmol TE/g extract for the DPPH assay. Consistent with the antioxidant activity finding, the alcoholic extract also exhibited the highest total levels of total phenolic content (TPC = 3.66±0.17 mg GAE/g extract) and total flavonoids content (TFC = 22.61±2.68 mg QE/g extract).

CONCLUSION

The results suggest that beer waste could serve as a promising source of natural polyphenolic compounds, offering potential as an eco-friendly antioxidant ingredient. Such compounds could be incorporated into nutraceutical formulations or applied in pharmaceutical and cosmetic, contributing to waste valorisation and sustainability.

REFERENCES

[1] Ferreira Costa, F., Roquete Amparo, T., Brandão Seibert, J., Silveira, B. M., Gomes da Silva, R., Inocêncio Pereira, D., ... & Bianco de Souza, G. H. (2021). Reuse of hot trub as an active ingredient with antioxidant and antimicrobial potential. *Waste and Biomass Valorization*, *12*, 2037-2047.

[2] dos Santos Mathias, T. R., de Mello, P. P. M., & Sérvulo, E. F. C. (2014). Solid wastes in brewing process: A review. *J. Brew. Distill*, *5*(1), 1-19.

[3] Pereira, N. *et al.* Valorisation of Wasted Immature Tomato to Innovative Fermented Functional Foods. *Foods* 12, (2023).

[4] Shraim, A. M., Ahmed, T. A., Rahman, M. M. & Hijji, Y. M. Determination of total flavonoid content by aluminum chloride assay: A critical evaluation. Lwt 150, (2021).

[5] Cartas, J. *et al.* Influence of geographical origin in the physical and bioactive parameters of single origin dark chocolate. *Eur. Food Res. Technol.* 250, 2569–2580 (2024).