

Efficient Extraction of Bioactive Compounds from Black Truffles: Comparing Supercritical Fluid and Pressurized Liquid Extraction Techniques

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INTRODUCTION & AIM

The black truffle (*Tuber melanosporum*) is a rich source of bioactive compounds, particularly polyphenols and antioxidants, which have been recognized for their potential health benefits. The high amount of by-products derived from truffle processing presents a valuable opportunity for obtaining bioactive compounds. This study focuses on the extraction of these bioactive compounds from black truffles using non-conventional extraction techniques, including supercritical fluid extraction (SFE) with supercritical CO₂ and pressurized liquid extraction (PLE). Moreover, the cytotoxicity of the obtained extracts is assessed.

METHOD

The SFE parameters used in the present study were: 30 MPa, 40°C, 30 min of extraction, absolute flow of 16 ml/min with 10% of modifying co-solvent (i.e., 14.4 ml/min scCO₂ and 1.6 ml/min EtOH). Regarding PLE, the parameters used were: 120°C, 15 min extraction time (3 static cycle x 5 min) and 101.3 bar pressure. Absolute ethanol was used as solvent. Chemical characterization was performed through Total Phenolic Content (TPC), Trolox Equivalent Antioxidant Capacity (TEAC), and Oxygen Radical Antioxidant Capacity (ORAC) assays. Finally, PLE extract cytotoxicity was studied using human keratinocytes (HaCaT cells) through an MTT test at 24 h of exposure, in a concentration range from 0.0625% up to 1% (v/v) in medium (Figure 1).

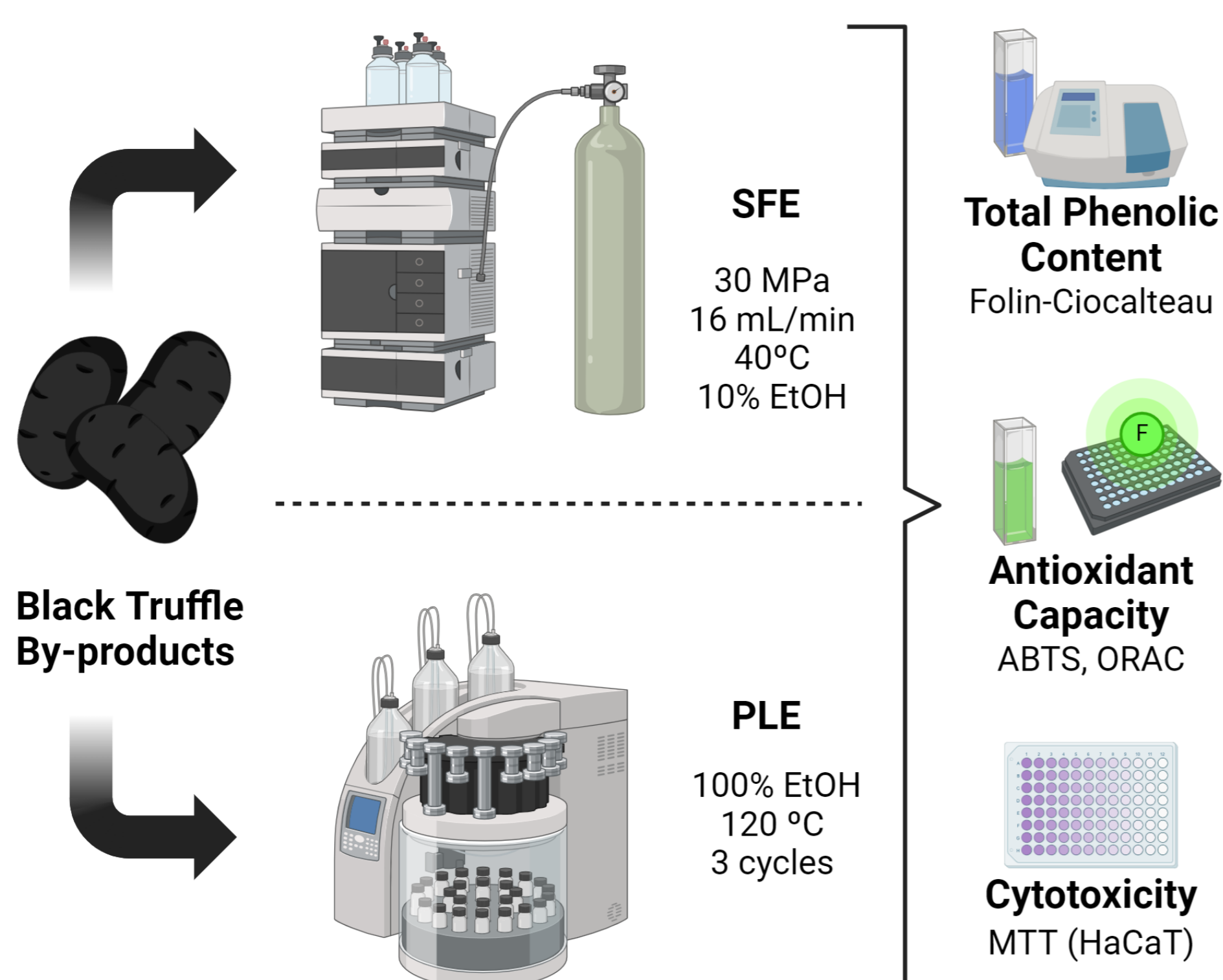


Figure 1. Representative scheme of the process followed to obtain and analyse extracts from black truffle by-products.

RESULTS & DISCUSSION

The PLE extract obtained higher values than the SFE extract in the TPC assay: $517.3 \pm 170.8 \mu\text{g GAE/mL}$ vs. $69.5 \pm 24.0 \mu\text{g GAE/mL}$, respectively (Figure 2a). Moreover, a higher antioxidant activity was detected for the PLE extract in comparison to the SFE extract: $402.80 \pm 84.85 \mu\text{M TE}$ vs. $31.67 \pm 15.16 \mu\text{M}$ for TEAC assay and $3531.36 \pm 906.84 \mu\text{M TE}$ vs. $1875.72 \pm 906.84 \mu\text{M TE}$ for ORAC assay, respectively (Figure 2b and 2c). Finally, the cytotoxicity study revealed that none of the tested concentrations decreased cell viability by more than 25% compared to the control (Figure 2d).

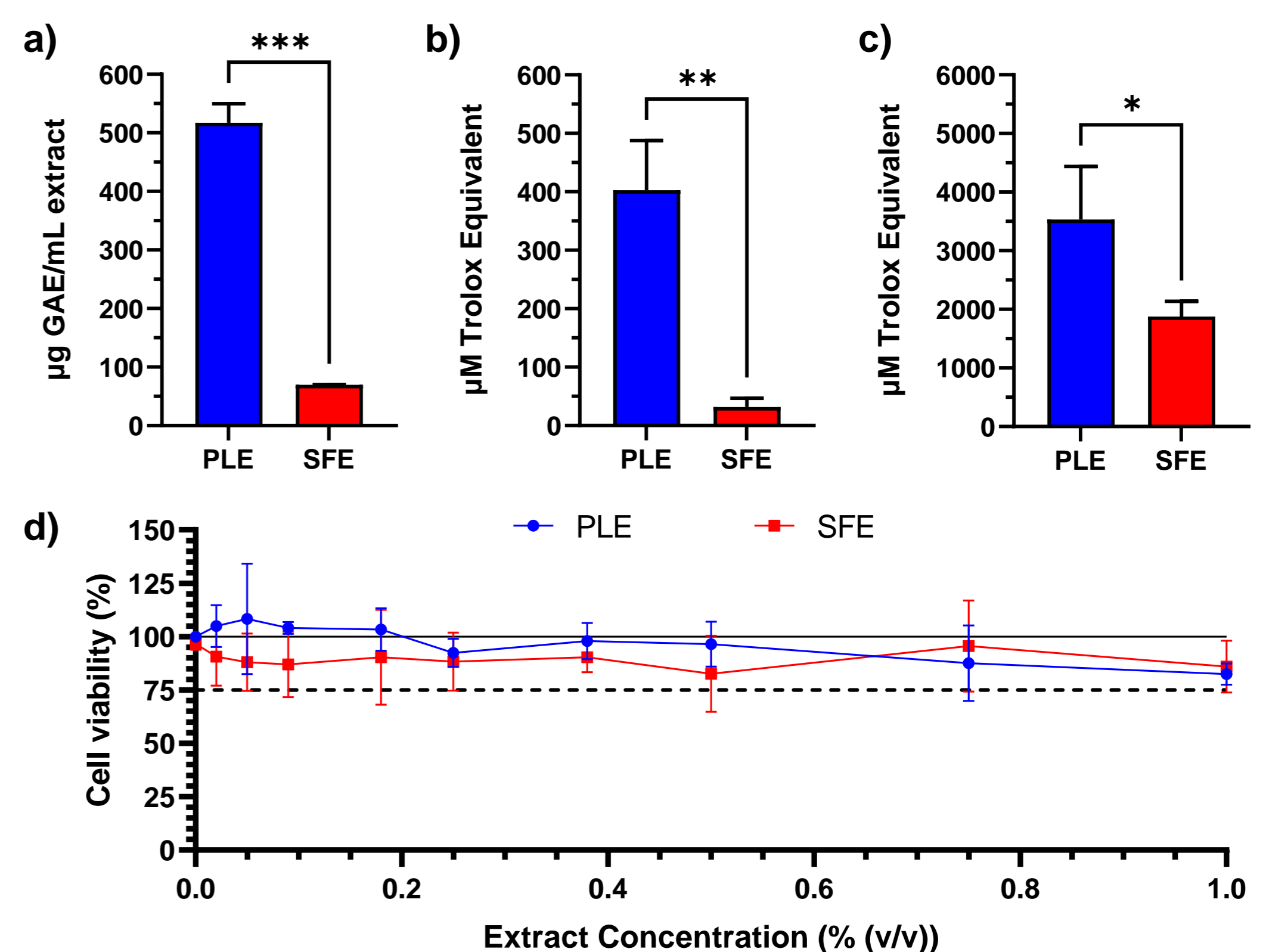


Figure 2. Total phenolic content (a), antioxidant capacity measured by ABTS (b) and ORAC (c) tests and cytotoxicity evaluation on HaCaT cells by MTT assay (d) of PLE and SFE extracts from black truffle by-products.

CONCLUSION

PLE is more effective than SFE in obtaining bioactive compounds from black truffles, providing higher antioxidant activity and phenolic recovery.

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