From olive oil waste to bioactive resources: optimizing the extraction of polyphenols from *Olea europaea* L.

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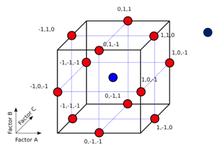
INTRODUCTION

Olive leaves (*Olea europaea* L.) are an abundant, underutilized agro-industrial byproduct generated during pruning and harvesting. They represent a sustainable source of bioactive metabolites (oleuropein, hydroxytyrosol, tyrosol, and other polyphenols) with documented antioxidant, anti-inflammatory, and antimicrobial activities[1], of interest for nutraceutical and pharmaceutical applications within a circular economy perspective[2]. The aim of this study was to optimize the extraction process to maximize phenolic yield and antioxidant activity. To this end, Response Surface Methodology (RSM) based on a Box-Behnken design was employed to investigate the combined effect of time, temperature and solvent composition and to identify optimal conditions[3].

METHODS



- **Experimental design:** Box-Behnken with 3 factors (ehanol 0-80%, temperature 25-80°C, time 30-240 min)[4];
- **Response variables**: total phenolic content (Folin-Ciocalteu), antioxidant activity (DPPH, FRAP), and concentrations of oleuropein, hydroxytyrosol, tyrosol;
- **Instrumental analysis**: LC-HRMS (positive/negative ionization); coumpound identification by analytical standards or MS/MS data;
- **Quantification**: external calibration curves (R^2>0.99); TPC as mg GAE/g, antioxidant activity as mg TE/g;
- **Validation:** optimal conditions experimentally validated (optimized extract, OE).

RESULTS

RSM optimization identified 30% ethanol, 80°C, and 240 min as the optimal conditions to maximize polyphenol yield and antioxidant activity, significantly outperforming literature benchmarks. The model's validity was confirmed by high statistical precision (R^2 up to 0.98) and the identification of 37 additional bioactive compounds via LC-HRMS.

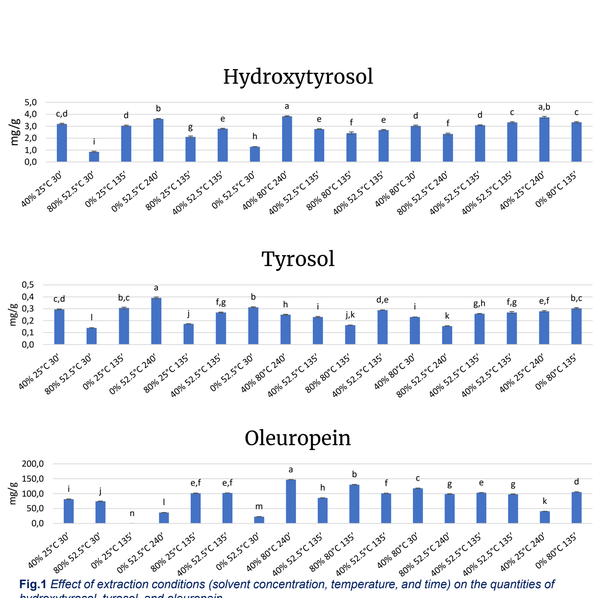


Fig.1 *Effect of extraction conditions (solvent concentration, temperature, and time) on the quantities of hydroxytyrosol, tyrosol, and oleuropein.*

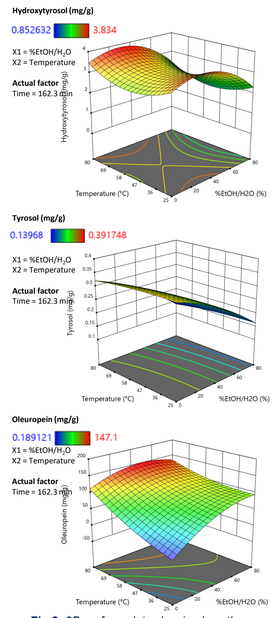


Fig 2. *3D surface plots showing how the independent variables (time, solvent, and temperature) influence the concentrations of hydroxytyrosol, tyrosol, and oleuropein (dependent variables).*

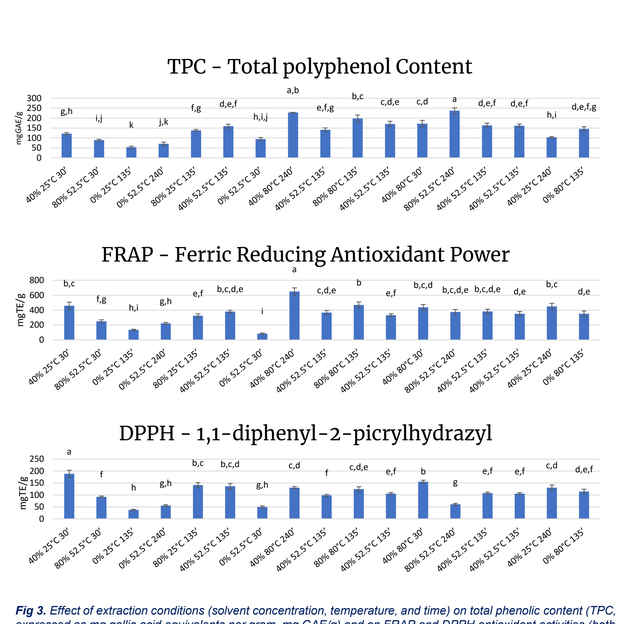


Fig 3. Effect of extraction conditions (solvent concentration, temperature, and time) on total phenolic content (TPC, expressed as mg gallic acid equivalents per gram, mg GAE/g) and on FRAP and DPPH antioxidant activities (both expressed as mg Trolox equivalents per gram, mg TE/g).

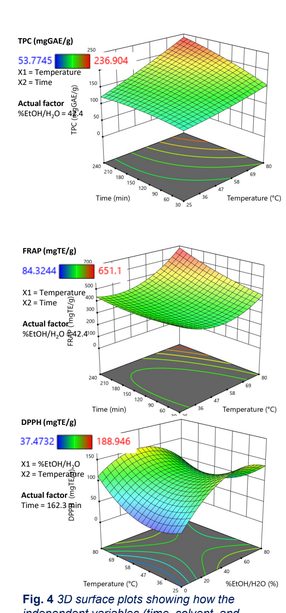


Fig. 4 *3D surface plots showing how the independent variables (time, solvent, and temperature) influence total phenolic content (TPC), and the antioxidant activities DPPH and FRAP (dependent variables)*

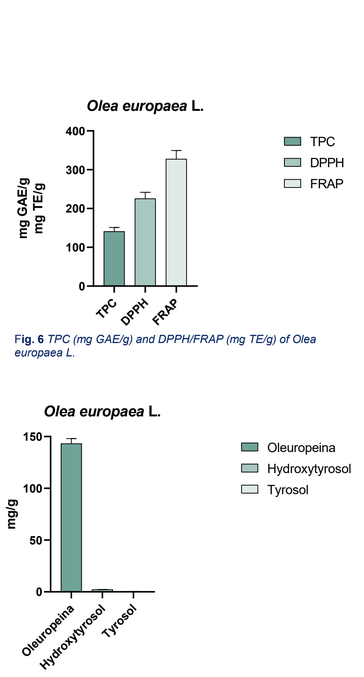


Fig. 6 *TPC (mg GAE/g) and DPPH/FRAP (mg TE/g) of Olea europaea L.*

Fig. 7 *Concentration mg/g of oleuropein, hydroxytyrosol, tyrosol.*

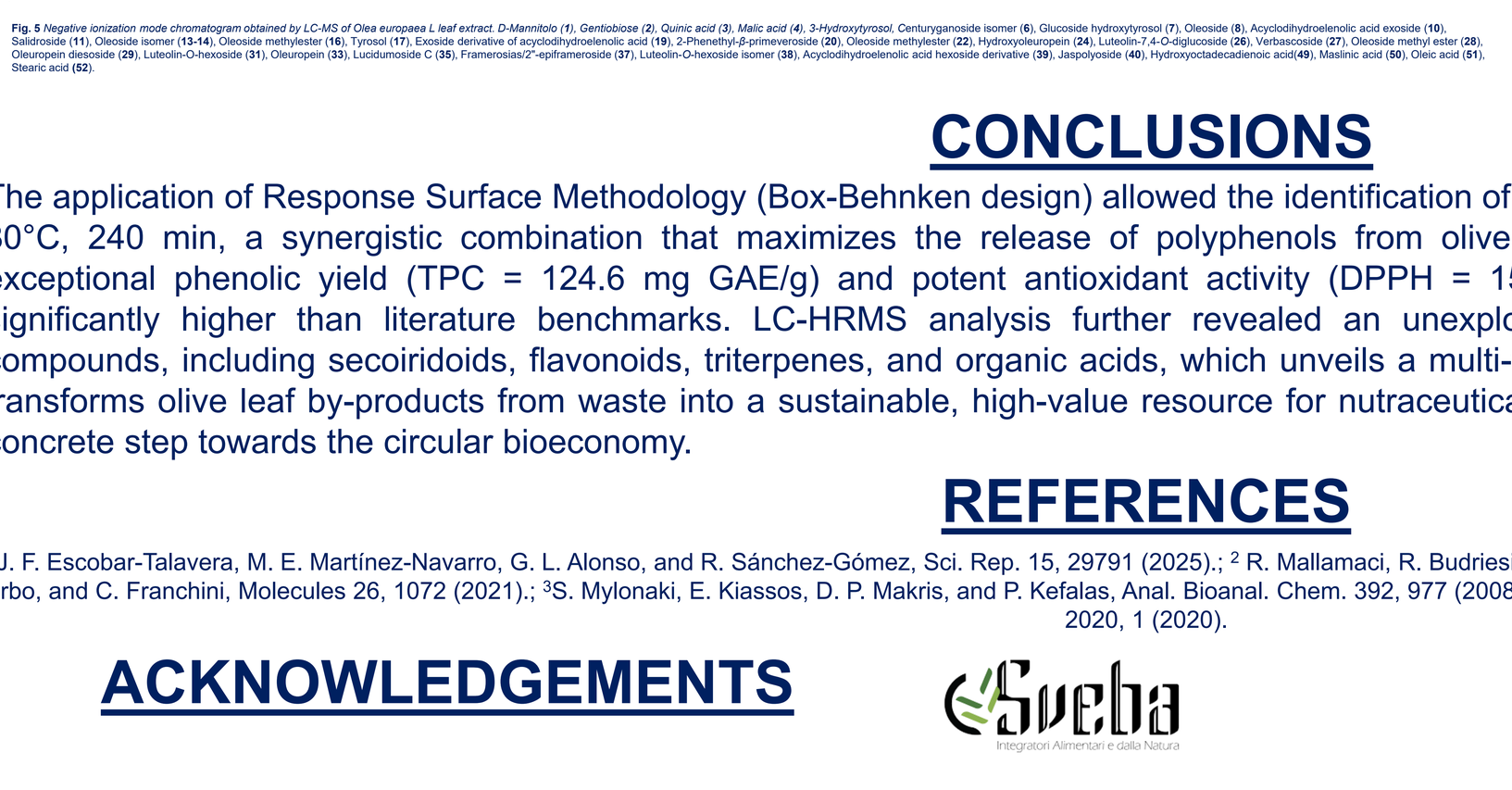


Fig. 5 *Negative ionization mode chromatogram obtained by LC-MS of Olea europaea L leaf extract. D-Mannitolo (1), Gentiobiose (2), Quinic acid (3), Malic acid (4), 3-Hydroxytyrosol,* Centuryganoside isomer (**6**), Glucoside hydroxytyrosol (**7**), Oleoside (**8**), Acyclodihydroelenolic acid exoside (**10**), Salidroside (**11**), Oleoside isomer (**13-14**), Oleoside methylester (**16**), Tyrosol (**17**), Exoside derivate of acyclodihydroelenolic acid (**19**), 2-Phenethyl-*β*-primeveroside (**20**), Oleoside methylester (**22**), Hydroxyoleuropein (**24**), Luteolin-7,4-*O*-diglucoside (**26**), Verbascoside (**27**), Oleoside methyl ester (**28**), Oleuropein diesoside (**29**), Luteolin-*O*-hexoside (**31**), Oleuropein (**33**), Lucidumoside C (**35**), Framerosias/2″-epiframeroside (**37**), Luteolin-*O*-hexoside isomer (**38**), Acyclodihydroelenolic acid hexoside derivative (**39**), Jaspolyoside (**40**), Hydroxyoctadecadienoic acid(**49**), Maslinic acid (**50**), Oleic acid (**51**), Stearic acid (**52**).

VARIABLE	95 % TI*
TPC	123.196 - 311.338
FRAP	220.569 - 547. 116
DPPH	20.872 - 279.026
Hydroxytyrosol	1.819 - 6.853
Tyrosol	0.119 - 0.478
Oleuropein	92.504 - 202.757

*Tolerance index; mgGAE\g - milligrams of gallic acid equivalent per gram; mgTE\g - milligrams of trolox equivalent per gram

CONCLUSIONS

The application of Response Surface Methodology (Box-Behnken design) allowed the identification of the optimal extraction conditions, 30% v/v ethanol, 80°C, 240 min, a synergistic combination that maximizes the release of polyphenols from olive leaves. The optimized extract (OE) showed an exceptional phenolic yield (TPC = 124.6 mg GAE/g) and potent antioxidant activity (DPPH = 151.6 mg TE/g; FRAP = 472.9 mg TE/g), values significantly higher than literature benchmarks. LC-HRMS analysis further revealed an unexplored chemical diversity, identifying 40 additional compounds, including secoiridoids, flavonoids, triterpenes, and organic acids, which unveils a multi-target bioactive reservoiir. In conclusion, this work transforms olive leaf by-products from waste into a sustainable, high-value resource for nutraceutical and pharmaceutical applications, representing a concrete step towards the circular bioeconomy.

REFERENCES

[1]J. F. Escobar-Talavera, M. E. Martínez-Navarro, G. L. Alonso, and R. Sánchez-Gómez, Sci. Rep. 15, 29791 (2025).; [2] R. Mallamaci, R. Budriesi, M. L. Clodoveo, G. Biotti, M. Micucci, A. Ragusa, F. Curci, M. Muraglia, F. Corbo, and C. Franchini, Molecules 26, 1072 (2021).; [3]S. Mylonaki, E. Kiassos, D. P. Makris, and P. Kefalas, Anal. Bioanal. Chem. 392, 977 (2008); [4]W.-Y. Cho, D.-H. Kim, H.-J. Lee, S.-J. Yeon, and C.-H. Lee, J. Food Qual. 2020, 1 (2020).

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Integratori Alimentari e dalla Natura