

Hungarian University of Agriculture and Life Sciences

Doctoral School of Life Sciences

Thesis of the PhD dissertation

Ana Radinović

Budapest

2026



Hungarian University of Agriculture and Life Sciences

**Application of various extraction technologies and green- processes for
sustainable valorization of olive pomace- An Environmental and
Waste Management Approach**

Ana Radinović

Budapest

2026

The PhD School

Name: Doctoral School of Agricultural and Food Sciences

Discipline: Food Sciences

Head of the Doctoral School:

Prof. Dr. Melinda Kovács

University Professor, Full Member of the Hungarian Academy of Sciences

Supervisor(s):

Szilvia Bánvölgyi, PhD

Department of Food Process Engineering
Institute of Food Science and Technology
Hungarian University of Agriculture and Life
Sciences

Prof. Dr. Vatai Gyula

Department of Food Process Engineering
Institute of Food Science and Technology
Hungarian University of Agriculture and Life
Sciences

.....
Approval of the Head of Doctoral School

.....
Approval of the Supervisor(s)

1. INTRODUCTION AND OBJECTIVES

The Mediterranean region is strongly associated with olive oil production, with Spain, Italy, and Greece among the leading producers worldwide. Since only 15–20% of the olive fruit is converted into oil, substantial quantities of olive pomace are generated as a major by-product of the olive oil industry (Donner et al., 2022; Galanakis, 2017). Olive pomace consists mainly of skins, pulp, seeds, and stems, and is characterized by high organic load and elevated concentrations of phenolic compounds. Improper disposal of this material may result in soil and water contamination, unpleasant odors, and greenhouse gas emissions. However, olive pomace is also recognized as a valuable secondary raw material rich in bioactive compounds, dietary fibers, residual oils, and phenolics with potential applications in the food, pharmaceutical, cosmetic, and bioenergy sectors (Dermeche et al., 2013; Socas-Rodríguez et al., 2021)

Although conventional valorization approaches such as composting, animal feed, and energy production are already applied, the sustainable utilization of olive pomace remains limited due to technical, environmental, and economic challenges. The high moisture content, acidic pH, lignocellulosic structure, and elevated phenolic concentrations of olive pomace reduce its biodegradability and restrict its direct agricultural application. Furthermore, uncontrolled disposal practices continue to represent a major environmental issue in olive oil-producing regions. Consequently, there is increasing interest in innovative and sustainable technologies capable of converting olive pomace into valuable products while minimizing environmental impacts (Čepo et al., 2018; Fotiadou et al., 2024; Parascanu et al., 2018).

In recent years, green extraction technologies such as ultrasound-assisted extraction (USAE), microwave-assisted extraction (MWAE), enzymatic extraction, and supercritical fluid extraction have emerged as promising alternatives to conventional extraction methods. These technologies offer several advantages, including improved extraction efficiency, shorter processing times, reduced solvent consumption, and lower environmental burden (Chanioti & Tzia, 2018; Zin & Bánvölgyi, 2021). In addition, biological treatment methods, particularly composting assisted by Effective Microorganisms (EM), have shown potential for improving the stabilization and biodegradation of olive pomace residues.

Therefore, the main objective of this research was to develop sustainable valorization strategies for olive pomace through the integration of advanced extraction technologies, EM-assisted composting, and environmental impact assessment within a circular economy framework. The study aimed to compare conventional extraction, MWAE, and USAE in terms of polyphenol recovery, antioxidant activity, and environmental performance using a carbon-footprint-based Life Cycle Assessment (LCA) approach. Additionally, the residual olive pomace after extraction was evaluated to determine the presence of remaining phenolic compounds, while the effectiveness of EM-assisted composting for stabilization of post-extraction residues was investigated.

The research was based on the hypothesis that advanced extraction technologies, particularly USAE and MWAE, would achieve higher polyphenol recovery and antioxidant activity than conventional extraction while simultaneously reducing environmental impacts. Furthermore, it was hypothesized that the application of Effective Microorganisms during composting could accelerate biodegradation and improve the physicochemical properties of olive pomace residues. The integration of extraction and composting processes was expected to contribute to more sustainable olive pomace management and support circular economy principles within the olive oil industry.

2. MATERIALS AND METHODS

This research is structured into three distinct approaches. The first approach (Figure 1) investigates polyphenol extraction from olive pomace using novel techniques, including microwave-assisted (MW) and ultrasound-assisted (US) methods, in comparison with conventional extraction. The main objective is to determine the optimal extraction method and conditions for maximizing polyphenol yield, as well as to quantify residual polyphenols remaining after extraction.

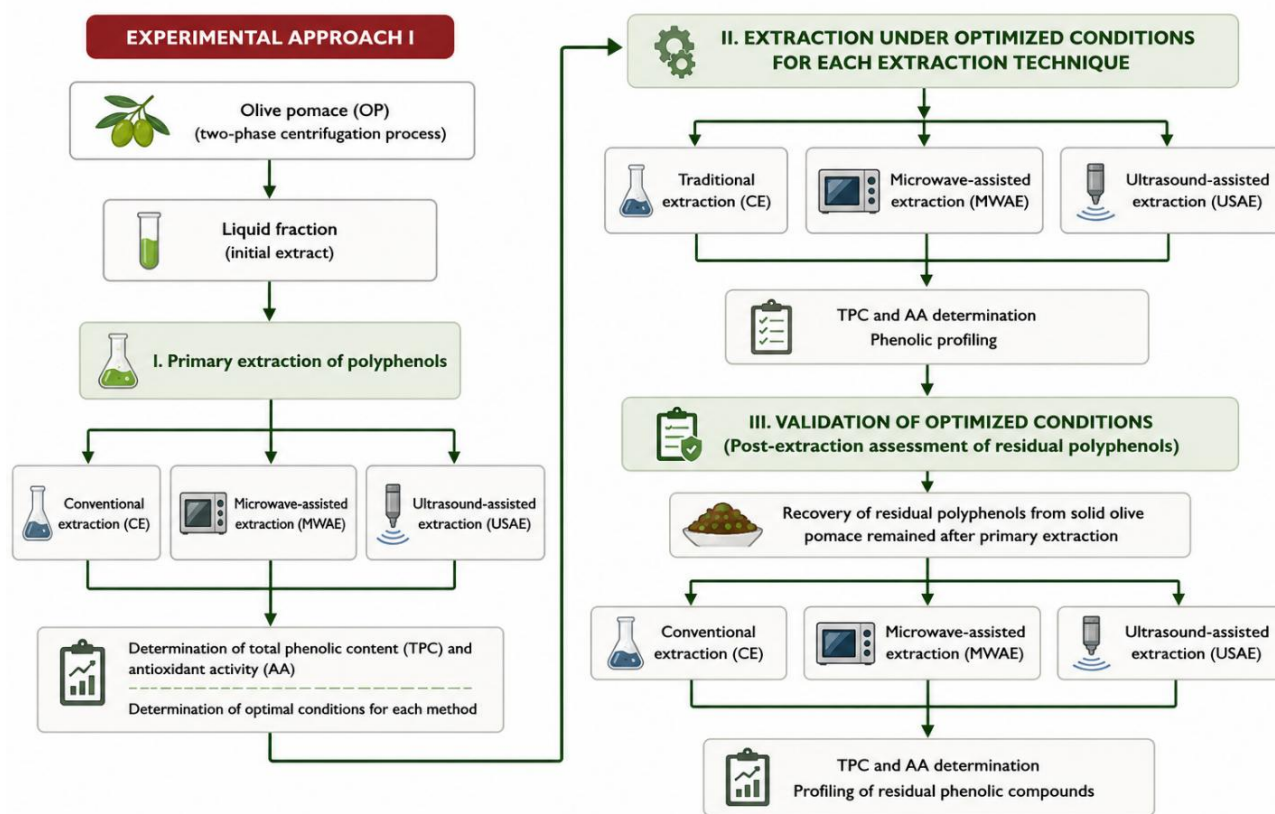


Figure 1. Experimental approach 1: Sequential extraction and post-extraction evaluation of polyphenols from olive pomace.

The second approach (Figure 2) examines the composting of wet olive pomace through the application of effective microorganisms. Considering the well-documented environmental and socio-economic impacts of olive oil production, producing compost for use as fertilizer in olive groves offers a sustainable and cost-effective strategy.

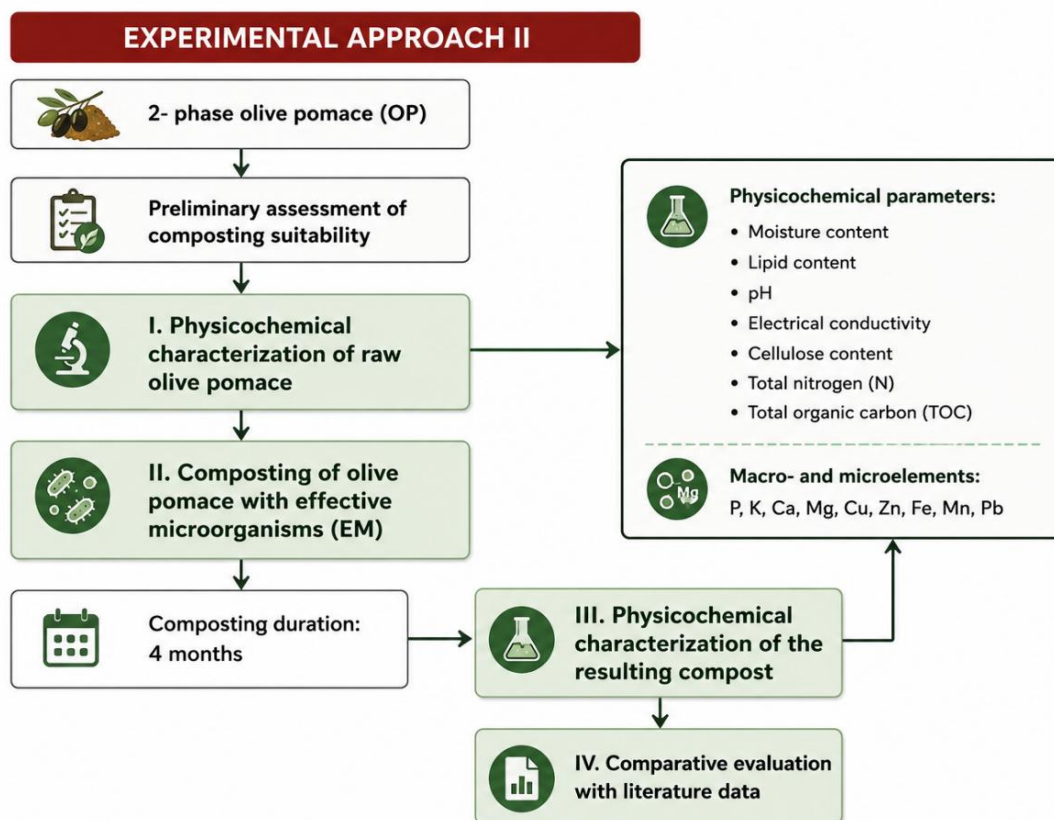


Figure 2. Experimental approach II: Assessment of composting suitability and microbial treatment of two-phase olive pomace.

The third approach (Figure 3) focuses on evaluating the environmental impact of the polyphenol extraction methods (USAE, MWAE, and CE) and comparing their carbon footprints. Taken together, these experiments are designed to determine the extraction technique that maximizes polyphenol recovery while minimizing associated environmental impacts.

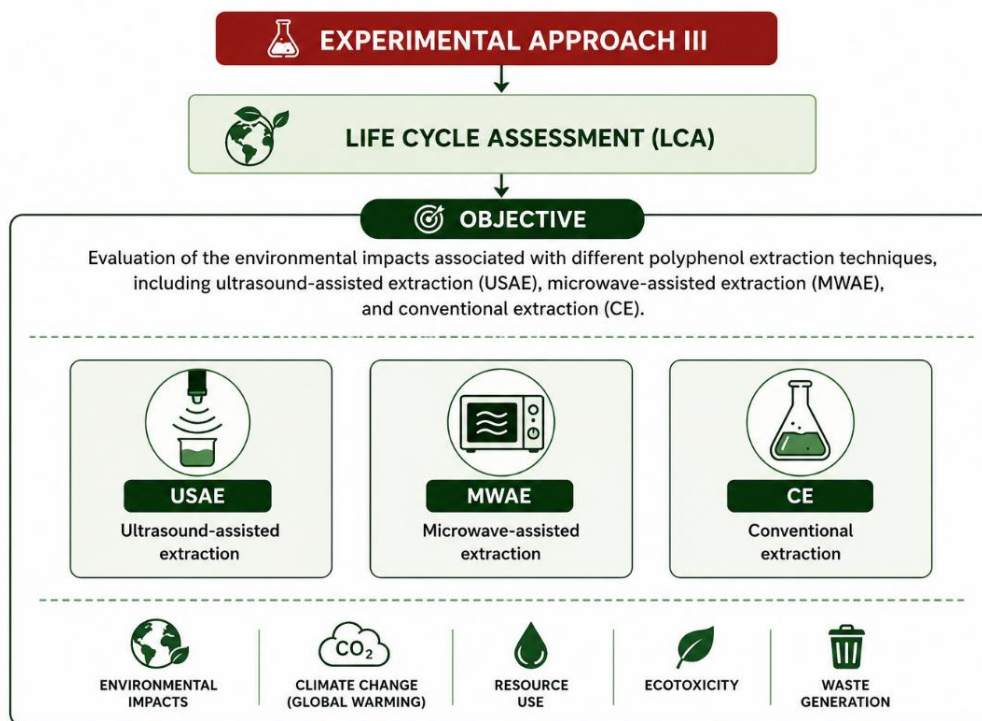


Figure 3. Experimental approach III: Comparative life cycle assessment (LCA) of conventional, ultrasound-assisted, and microwave-assisted polyphenol extraction methods using openLCA software.

2.1. Equipment and sample preparation

The experimental work was conducted using laboratory and processing equipment to ensure precise and reproducible results. Conventional extraction was performed using a jacketed extraction vessel connected to a circulating water bath and equipped with an overhead stirrer operating at 220 rpm. Microwave-assisted extraction (MWAE) was carried out using a microwave oven with a power range of 100–800 W, while ultrasound-assisted extraction (USAE) was performed using a Power Ultrasound System operating at 20 kHz and 400 W, equipped with three probes of different ultrasonic intensities. The influence of probe size and ultrasonic intensity on extraction efficiency was evaluated. Identification and quantification of phenolic compounds were performed using LC–MS/MS equipped with an electrospray ionization source. For composting experiments, olive pomace was composted in plastic containers and treated with Effective Microorganisms (EM).

Olive pomace samples were obtained from olive oil mills in Montenegro using a two-phase centrifugation system. Samples originated from the Žutica and Frantoio olive varieties and were stored at $-18\text{ }^{\circ}\text{C}$ prior to analysis. Before extraction, the pomace was dried at $40\text{ }^{\circ}\text{C}$ and $60\text{ }^{\circ}\text{C}$ to evaluate the influence of drying temperature on polyphenol stability. Moisture content was reduced below 5%, after which samples were homogenized using a knife mill.

For composting experiments, three treatment conditions were applied: aerobic composting with EM solution, anaerobic composting with EM solution, and anaerobic composting with EM solution supplemented with Bokashi bran to enhance microbial activity and decomposition efficiency.

2.2. Experimental Designs for extractions

Before the main extraction experiments, water, ethanol, and isopropanol were evaluated as solvents for polyphenol recovery from olive pomace using conventional extraction. The influence of drying temperature ($40\text{ }^{\circ}\text{C}$ and $60\text{ }^{\circ}\text{C}$) on total polyphenol content (TPC) was also investigated. Based on preliminary results, the most effective solvents were selected and further optimized using a central composite design (CCD), considering solvent concentration, extraction temperature, and solid-to-solvent ratio (Alu'datt et al., 2010; Goldsmith et al., 2018; Maldonado Espinel, 2022)

Conventional extraction (CE), ultrasound-assisted extraction (USAE), and microwave-assisted extraction (MWAE) were subsequently optimized using CCD experimental designs. For CE, the effects of temperature, extraction time, and solid-to-solvent ratio were evaluated. USAE optimization included extraction time, solid-to-solvent ratio, and ultrasound probe size, while MWAE experiments investigated the influence of microwave power, extraction time, and solid-to-solvent ratio. Total polyphenol content (TPC) and antioxidant activity (AA) were used as the main response variables in all extraction methods. After extraction, samples were filtered, centrifuged, and stored at $-18\text{ }^{\circ}\text{C}$ prior to analysis.

The second experimental phase focused on EM-assisted composting of two-phase olive pomace. Raw olive pomace was initially characterized by physicochemical parameters including moisture content, pH, electrical conductivity, total organic carbon, total nitrogen, and mineral composition. Composting experiments were conducted for four months under controlled laboratory conditions

to evaluate the influence of Effective Microorganisms (EM) on olive pomace stabilization and biodegradation. Four treatments were investigated: aerobic composting with EM, anaerobic composting with EM, anaerobic composting with EM and Bokashi bran, and untreated olive pomace as the control. The physicochemical properties of the composted material were analyzed after the composting period to assess compost maturity and the effectiveness of EM-assisted treatment for sustainable olive pomace management.

Total polyphenol content (TPC) of olive pomace extracts was determined using the Folin–Ciocalteu colorimetric method following previously reported procedures (Alu'datt et al., 2010; Genwali et al., 2013). Antioxidant activity (AA) was evaluated using the Ferric Reducing Antioxidant Power (FRAP) assay, with results expressed as ascorbic acid equivalents.

Identification and quantification of individual phenolic compounds were performed using LC–MS/MS analysis with electrospray ionization in multiple reaction monitoring (MRM) mode, enabling sensitive detection of phenolic compounds in olive pomace extracts (Petrović et al., 2025). Additionally, physicochemical parameters of olive pomace compost, including moisture content, pH, electrical conductivity, total organic carbon, total nitrogen, lipids, cellulose, and mineral composition, were analyzed using standardized analytical methods.

2.3. Environmental Impact Methodology

Life Cycle Assessment (LCA) was applied to evaluate the environmental performance of conventional extraction (CE), ultrasound-assisted extraction (USAE), and microwave-assisted extraction (MWAE), with particular focus on carbon footprint (CF) expressed as kg CO₂-eq. The assessment followed ISO 14040 and ISO 14044 standards and included raw material handling, solvent production, energy consumption, and post-extraction residue management. The functional unit was defined as 1 kg of wet olive pomace processed.

The life cycle inventory was based on experimentally obtained laboratory-scale data, including solvent consumption, extraction time, energy demand, transport, and polyphenol yield. Environmental impacts of the extraction methods were compared using IDEMAT database data in order to identify the most sustainable extraction technology with the lowest environmental burden.

3. RESULTS AND DISCUSSIONS

3.1. Evaluation of Pure Solvents for Polyphenol Extraction and the Influence of Olive Pomace Drying Temperature

The solvent screening experiment demonstrated that extraction efficiency strongly depended on solvent polarity. Water achieved the highest total polyphenol content (TPC), followed by ethanol, while isopropanol showed the lowest extraction efficiency. Based on these results, water and ethanol were selected for further optimization due to their complementary ability to extract both hydrophilic and moderately polar phenolic compounds. Lower drying temperature (40 °C) significantly improved TPC values compared to 60 °C, indicating reduced thermal degradation of phenolic compounds during sample preparation (Böhmer-Maas et al., 2020; Katsinas et al., 2021).

Optimization experiments using response surface methodology showed that TPC and antioxidant activity (AA) were significantly influenced by extraction temperature and solid-to-solvent ratio, while solvent concentration had a less pronounced effect. The highest TPC and AA values were obtained using hydroethanolic mixtures under moderate ethanol concentration, elevated temperature, and high solid loading. Statistical analysis confirmed that the developed quadratic models adequately described the extraction process and enabled prediction of optimal extraction conditions.

The optimized extraction parameters for simultaneous maximization of TPC and AA were identified at approximately 52.7% ethanol, elevated extraction temperature, and high solid-to-solvent ratio. These findings are consistent with previous studies reporting improved polyphenol recovery using hydroethanolic solvents and elevated temperatures for olive pomace extraction (Alu'datt et al., 2010; Antónia Nunes et al., 2018; Lafka et al., 2011). Based on these results, the optimized ethanol–water mixture was selected for all subsequent extraction experiments.

3.2. Conventional Extraction

The conventional extraction experiments demonstrated that extraction conditions strongly influenced both total polyphenol content (TPC) and antioxidant activity (AA). TPC values ranged from 0.34 to 8.56 mg GA/gdw, while AA varied between 1.42 and 22.57 mg AS/gdw. A strong positive correlation between TPC and AA ($r = 0.73$) confirmed the important contribution of phenolic compounds to antioxidant capacity, consistent with previous findings for olive-derived matrices (Gülçin, 2012).

Response surface analysis showed that the solid-to-solvent ratio was the most influential factor affecting extraction efficiency, while temperature and extraction time had less pronounced effects. Lower solid-to-solvent ratios generally resulted in higher TPC and AA values due to improved mass transfer and extraction efficiency. Statistical analysis confirmed that the developed quadratic models adequately described the extraction behavior and could successfully predict optimal extraction conditions.

The observed trends were consistent with previous studies reporting the strong influence of solid loading on phenolic recovery from olive pomace (Böhmer-Maas et al., 2020) (Katsinas, Bento Da Silva, et al., 2021). Numerical optimization identified the optimal conventional extraction conditions at elevated temperature, shorter extraction time, and low solid-to-solvent ratio, resulting in the highest predicted TPC and antioxidant activity values.

3.3. Ultrasound extraction

The ultrasound-assisted extraction (USAE) experiments confirmed that this technique is effective for recovering phenolic compounds from olive pomace, with total polyphenol content (TPC) ranging from 3.87 to 19.50 mg GA/gdw and antioxidant activity (AA) between 3.31 and 11.15 mg AS/gdw. A strong positive correlation between TPC and AA ($r = 0.85$) indicated that phenolic compounds significantly contributed to the antioxidant potential of the extracts, consistent with previous studies on olive by-products (Cabrera et al., 2024; Gortzi et al., 2008; Gülçin, 2012).

Statistical analysis showed that the solid-to-solvent ratio and ultrasound probe size were the most influential factors affecting extraction efficiency, while extraction time had a less pronounced effect. Lower solid-to-solvent ratios generally resulted in higher TPC and AA values due to improved mass transfer. The response surface models demonstrated good predictive capability and confirmed the suitability of USAE for polyphenol recovery from olive pomace.

Optimization results identified low solid-to-solvent ratio and moderate extraction time as the most favorable conditions for maximizing both TPC and AA. Although the medium probe achieved the highest individual TPC value, the large probe demonstrated more stable and reproducible extraction performance across the experimental domain. The improved efficiency of the large probe was attributed to more uniform ultrasonic energy distribution and stable cavitation, minimizing localized overheating and degradation effects. Similar observations regarding the importance of ultrasonic intensity and cavitation behavior have been reported in previous studies (Dujmić et al., 2020; Natolino & Celotti, 2022; Sancho-Galán et al., 2019).

3.4. Microwave extraction

Microwave-assisted extraction (MWAE) demonstrated effective recovery of phenolic compounds from olive pomace, with total polyphenol content (TPC) ranging from 6.86 to 15.30 mg GA/gdw and antioxidant activity (AA) between 5.54 and 10.00 mg AS/gdw (Mardokić et al., 2023). A strong positive correlation between TPC and AA ($r = 0.812$) confirmed that higher phenolic content contributed to increased antioxidant activity. The obtained results were comparable to previously reported values for microwave-assisted extraction of olive by-products (Chanoti et al., 2021).

Statistical analysis revealed that microwave power and solid-to-liquid ratio were the most significant factors influencing both TPC and AA, whereas extraction time had a limited effect. Higher microwave power enhanced extraction efficiency due to intensified heating and improved mass transfer, while lower solid-to-liquid ratios promoted better solvent penetration and phenolic recovery. Similar trends have been reported in previous studies on microwave-assisted extraction of olive pomace (Macedo et al., 2021; Tapia-Quirós et al., 2020).

Optimization results identified the most favorable extraction conditions at high microwave power, longer extraction time, and low solid-to-liquid ratio, resulting in the highest predicted TPC and AA values. However, prolonged microwave treatment may increase solvent evaporation and energy consumption, highlighting the importance of balancing extraction efficiency with process sustainability and operational feasibility.

3.5. Comparative evaluation of the Extraction Techniques of Polyphenols

The three extraction techniques investigated—conventional extraction (CE), ultrasound-assisted extraction (USAE), and microwave-assisted extraction (MWAE)—showed clear differences in extraction efficiency and sensitivity to operational parameters. Overall, MWAE achieved the highest and most consistent total polyphenol content (TPC) and antioxidant activity (AA), while USAE also provided high extraction efficiency under relatively mild conditions. Conventional extraction showed the lowest and most variable performance due to its diffusion-controlled mechanism and longer processing time.

The results confirmed that low solid-to-solvent ratios improved extraction efficiency across all methods by enhancing mass transfer and solvent accessibility. In MWAE, microwave power was the dominant factor affecting extraction performance, whereas in USAE, probe size and cavitation intensity played key roles. Although MWAE achieved the highest extraction efficiencies, practical limitations such as solvent evaporation at high power levels required optimization of operating conditions.

Based on experimental results and numerical optimization, specific operating conditions were selected for further comparative analyses. For MWAE, slightly lower microwave power was chosen to reduce solvent evaporation while maintaining high extraction efficiency. For USAE, the large probe and low solid-to-solvent ratio provided the most stable and reproducible extraction performance. Overall, the intensified extraction techniques demonstrated clear advantages over conventional extraction in terms of efficiency, processing time, and sustainability.

3.6.Evaluation of results in extracts and post- extracts generated using optimized parameters

LC–MS/MS analysis confirmed the reliability and selectivity of the analytical method, with no detectable peaks observed in blank samples and excellent calibration linearity for all analyzed phenolic compounds. Optimized extraction conditions were subsequently applied to two olive pomace cultivars (Frantoio and Žutica), enabling comparison of extraction methods and evaluation of residual phenolic content after post-extraction treatment.

The results showed that ultrasound-assisted extraction (USAE) generally produced the highest total polyphenol content (TPC) and antioxidant activity (AA), particularly for the Žutica cultivar, while microwave-assisted extraction (MWAE) and conventional extraction achieved moderate but comparable performance. Statistical analysis confirmed that extraction method significantly influenced TPC and AA, whereas cultivar effects were less pronounced. The superior performance of USAE was mainly attributed to cavitation-induced matrix disruption, which enhanced solvent penetration and mass transfer (Chemat et al., 2011; Kumar et al., 2021).

Post-extraction analysis demonstrated that only low residual levels of phenolic compounds remained in the exhausted olive pomace, confirming the high efficiency of the optimized extraction procedures. Residual antioxidant activity was minimal in most samples, indicating that the majority of bioactive compounds had been removed during the primary extraction step. From a waste management perspective, this reduction in phenolic content is particularly important because it decreases phytotoxicity and improves the suitability of olive pomace for further valorization pathways such as composting or bioenergy production (Paraskeva & Diamadopoulos, 2006; Roig et al., 2006a).

LC–MS/MS profiling identified a wide range of phenolic compounds belonging to flavonoids, phenolic acids, phenylethanoids, and secoiridoids. Secoiridoids and phenylethanoids, particularly secologanoside, hydroxytyrosol, oleacein, and verbascoside, represented the dominant phenolic groups, consistent with previous reports for olive-derived residues (Servili et al., 2004; Cioffi et al., 2010). Considerable differences between cultivars were observed for several individual compounds, confirming cultivar-dependent variability in phenolic composition.

Comparison of primary extracts and post-extraction residues showed a substantial reduction in both phenolic concentration and diversity after extraction. Several compounds, including ferulic acid, oleuropein, apigenin, and gallic acid, were detected only in primary extracts, whereas more stable glycosylated flavonoids and selected secoiridoids remained detectable in residual material.

3.6.1. EM-Assisted Composting of Olive Pomace

The raw olive pomace exhibited characteristics typical of two-phase olive mill residues, including high moisture and organic matter content, acidic pH, and notable concentrations of macro- and micronutrients. Its high total organic carbon (TOC) and mineral content confirmed its suitability for composting and further biological treatment (Aparicio & Harwood, 2013; Mekersi et al., 2022).

After four months of composting, all treatments showed substantial physicochemical changes, although the extent of these changes depended on oxygen availability and EM application. The most pronounced effects were observed in the EM-treated samples, particularly under anaerobic and Bokashi conditions, where significant reductions in TOC indicated enhanced organic matter degradation and microbial activity. Similar trends have been reported in previous composting studies of olive mill residues (Iglesias Jim & Garcia, 1992; Khater, 2015).

Moisture content remained relatively high in all treatments, while EM-treated samples also maintained acidic pH values due to fermentative microbial activity. Therefore, additional aeration or curing would be required to achieve fully mature compost suitable for agricultural application. Nevertheless, electrical conductivity values remained below phytotoxic thresholds, and heavy metal concentrations were very low, indicating no significant environmental risk (Bernal et al., 2009; Tiquia et al., 1997).

The composting process also influenced lipid degradation, cellulose transformation, and nutrient dynamics. EM-treated samples showed lower residual fat content and more efficient lignocellulosic degradation compared to the untreated control, suggesting improved microbial decomposition. Macro- and micronutrients remained within expected ranges for olive-pomace-based composts, supporting their potential agricultural value.

The results demonstrated that EM-assisted composting, particularly under anaerobic conditions, can enhance stabilization and degradation of olive pomace. However, further optimization, including improved aeration, use of bulking agents, and extended curing phases, would be necessary to obtain fully mature and stable compost.

3.7. Environmental impact of the extraction methods

The carbon footprint (CF) assessment revealed clear differences in environmental performance among the investigated extraction methods. Microwave-assisted extraction (MWAE) exhibited the lowest total CF (24.61 kg CO₂-eq/FU), followed by ultrasound-assisted extraction (USAE) with 29.35 kg CO₂-eq/FU, while conventional extraction (CE) showed the highest impact (59.26 kg CO₂-eq/FU). Across all methods, electricity consumption represented the dominant contributor to total emissions, particularly in conventional extraction due to prolonged heating requirements. Ethanol use was the second most important contributor, whereas water use, transport, wastewater treatment, and spent pomace had negligible impacts.

When normalized to polyphenol yield, MWAE and USAE demonstrated substantially lower environmental burdens compared to conventional extraction. The CF per gram of recovered gallic acid equivalents was 1.61 kg CO₂-eq for MWAE, 1.51 kg CO₂-eq for USAE, and 6.92 kg CO₂-eq for CE, indicating that the higher extraction efficiency of intensified technologies compensates for their energy demand. Similar trends have been reported in previous studies highlighting the environmental advantages of green extraction technologies based on improved process efficiency and reduced processing time.

The results identified electricity consumption and solvent use as the main environmental hotspots in olive pomace extraction processes. Under the investigated laboratory-scale conditions, MWAE showed the most favorable environmental performance, followed closely by USAE, while conventional extraction exhibited the highest climate-change impact due to significantly higher energy consumption. Although the assessment was limited to carbon footprint analysis, the findings emphasize the importance of energy efficiency and solvent management in developing sustainable olive pomace valorization strategies

4. CONCLUSIONS & RECOMMENDATIONS

This research investigated the sustainable valorization of olive pomace through the integration of advanced extraction technologies, biological treatment, and environmental assessment within a circular economy framework. The results demonstrated that ultrasound-assisted extraction (USAE) and microwave-assisted extraction (MWAE) significantly improved polyphenol recovery and antioxidant activity compared to conventional extraction. Under optimized conditions, USAE achieved the highest polyphenol yields, while MWAE provided rapid extraction with lower processing time and favorable environmental performance. In contrast, conventional extraction showed lower extraction efficiency and higher energy demand.

Carbon-footprint assessment revealed that electricity consumption was the main contributor to environmental impacts across all extraction methods. MWAE exhibited the lowest overall carbon footprint, followed by USAE, whereas conventional extraction showed the highest environmental burden. When normalized to polyphenol yield, both assisted extraction techniques demonstrated substantially lower impacts per unit of recovered bioactive compounds, highlighting the importance of extraction efficiency in sustainable process evaluation.

The study also addressed the management of post-extraction olive pomace through EM-assisted composting. Composting promoted biodegradation and stabilization of the material, although additional curing or aeration would be necessary to obtain fully mature compost suitable for agricultural application. The reduced phenolic content in post-extraction residues further supports the suitability of exhausted olive pomace for downstream valorization pathways such as composting or soil amendment. Statistical analysis confirmed that extraction performance was mainly influenced by the applied extraction technique, while cultivar effects were less pronounced. LC–MS/MS profiling revealed a diverse phenolic composition dominated by secoiridoids, phenylethanoids, and flavonoids, confirming the high valorization potential of olive pomace. Although the study was performed at laboratory scale and focused primarily on carbon footprint analysis, the results provide important insight into sustainable olive pomace management. Future research should focus on process scale-up, solvent and energy optimization, broader environmental assessment, and the development of integrated biorefinery systems combining extraction, composting, and energy recovery for improved resource utilization.

5. NEW SCIENTIFIC FINDINGS

The present research generated several original scientific findings that advance the sustainable valorization of olive pomace, particularly in the fields of green extraction technologies, environmental sustainability, and residue management.

1. Comparative performance of extraction technologies

This study provides a direct, experimentally controlled comparison of conventional, ultrasound-assisted (USAE), and microwave-assisted extraction (MWAE) methods for polyphenol recovery from olive pomace under harmonized conditions. The results demonstrate that USAE achieves the highest polyphenol yields under optimized conditions, while MWAE enables rapid extraction with competitive efficiency. In contrast, conventional extraction showed lower recovery efficiency and higher cumulative energy demand, indicating the technical advantages of assisted extraction technologies for olive pomace valorization under the investigated conditions.

2. Influence of olive cultivar on phenolic composition and extraction performance

Statistical analysis showed that olive cultivar influenced the absolute polyphenol content and the composition of individual phenolic compounds for the two cultivars investigated in this study. Qualitative profiling confirmed the presence of a diverse range of phenolic compounds, including hydroxytyrosol, tyrosol, secoiridoid derivatives, flavonoids, and phenolic acids, with variations in their relative abundance between cultivars.

However, under the investigated conditions, no statistically significant interaction was observed between cultivar and extraction method, indicating that the relative ranking of extraction methods was primarily governed by process conditions rather than cultivar-specific responses. These results suggest that, while raw material characteristics influence the absolute phenolic profile and compound distribution, they do not substantially alter the comparative performance of extraction technologies.

3. Role of ultrasonic intensity in extraction efficiency

The study advances understanding of ultrasound-assisted extraction by demonstrating that extraction performance is influenced by effective ultrasonic intensity delivered to the sample, rather than probe size alone. While probe size affects nominal power distribution, the actual

extraction efficiency is governed by the resulting power density and cavitation conditions within the medium. Uniform energy distribution and stable cavitation were associated with improved polyphenol recovery, while minimizing localized overheating.

A discrepancy was observed between the experimentally measured maximum TPC and the model-predicted optimum conditions, suggesting that local variations in cavitation intensity and experimental variability may influence extraction outcomes beyond the predictive capability of the model. These findings support the concept of a cavitation threshold, beyond which increasing power input does not lead to proportional gains in extraction efficiency. These insights are relevant for the optimization and scale-up of ultrasound-based extraction systems.

4. Link between extraction efficiency and environmental performance

By integrating a carbon-footprint-based assessment within an LCA framework with experimentally determined extraction yields, this work shows that environmental performance is strongly influenced by extraction efficiency and functional unit selection. When impacts were normalized to the amount of extracted polyphenols, both USAE and MWAE exhibited substantially lower carbon footprints per unit of bioactive compounds than conventional extraction.

It should be noted that the environmental assessment focused on the carbon footprint (climate change impact category), and that inclusion of additional LCA impact categories would be required for a comprehensive environmental evaluation. This finding highlights the importance of yield-based normalization in sustainability assessment and provides a functionally relevant framework for comparing extraction technologies.

5. Evaluation of the role of Effective Microorganisms (EM) in the treatment of pure olive pomace

This study shows that Effective Microorganisms (EM) can promote organic matter degradation in pure two-phase olive pomace without the use of bulking agents—an experimental condition rarely examined in existing literature. Under the investigated conditions, anaerobic EM treatments were associated with greater reductions in total organic carbon and cellulose compared to other treatments.

These results indicate that EM-assisted treatment under laboratory conditions promotes degradation processes, as reflected by TOC reduction and changes in physicochemical parameters. However, the acidic pH of EM-treated samples indicates that the material cannot be considered fully mature compost and would require additional curing or aeration before agricultural application. Further optimization and validation are therefore required.

The presented findings contribute to an integrated understanding of olive pomace valorization, linking extraction performance, carbon-footprint-based environmental assessment, and residue management. These insights provide guidance for the development of more sustainable and resource-efficient strategies in olive oil producing regions.

6. PUBLICATIONS

Journal Articles:

1. **Mardokić, A.**, Maldonado, A.E., Klosz, K., Molnár, M.A., Vatai, G. and Bánvölgyi, S., 2023. Optimization of conditions for microwave-assisted extraction of polyphenols from olive pomace of Žutica variety: waste valorization approach. *Antioxidants*, 12(6), p.1175.
 - a. DOI: <https://doi.org/10.3390/antiox12061175>
2. **Mardokić, A.**, Jónás, G., Albert, K., Molnár, M.A., Rédey, Á., Vatai, G. and Bánvölgyi, S., 2026. Ultrasound-assisted extraction of bioactive compounds from olive pomace: Polyphenol content and antioxidant activity. *Progress in Agricultural Engineering Sciences*, pp.446-2026.
 - a. DOI: <https://doi.org/10.1556/446.2026.00261>
3. Rédey, Á., Zsom-Muha, V., Stefaniga, T., Gáspár, I., **Mardokić, A.** and Bánvölgyi, S., 2025. Recovery of valuable components from carrot peel as vegetable waste using microwave-assisted extraction. *Progress in Agricultural Engineering Sciences*, 21(1), pp.269-286.
 - a. DOI: <https://doi.org/10.1556/446.2025.00225>
4. Alsobh, A., Zin, M.M., **Mardokić, A.**, Vatai, G. and Bánvölgyi, S., 2024. Heat, ultrasound, and microwave assisted extraction methods for recovering bioactive components from hawthorn fruit (*Crataegus monogyna* Jacq.). *Progress in Agricultural Engineering Sciences*, 20(1), pp.49-63.
 - a. DOI: <https://doi.org/10.1556/446.2024.00103>

Conference Abstracts:

1. Angela Maldonado, Ana Marđokic, Gyula Vatai, Szilvia Bánvölgyi: Polyphenol extraction from Olive pomace of Montenegrin Olive variety Žutica as the initial step in waste-valorization strategy, In: *Fourth International Conference on Food Science and Technology*, June 2022, Budapest, Hungary; ISBN 978-615-01-5422-0
2. Marđokić Ana, Maldonado Angela, Vatai Gyula, Bánvölgyi Szilvia: Microwave Extraction of Polyphenols from Olive pomace of Montenegrin olive variety Žutica as an initial step in valorization process, In: *International Conference Green Extraction of natural products (GNEP 2022)*, 26-28th of October 2022, Poreč, Croatia; ISBN: 978-953-6893-16-4
3. Szilvia Bánvölgyi, Angela Maldonado, Ana Marđokić, Máté Molnár, Katalin Klosz, Gyula Vatai: Extraction of Polyphenols from Olive Pomace of Žutica variety, In: *2nd International Conference on Advanced Production and Processing (ICAPP 2022)*, 20th – 22nd October 2022, Novi Sad, Serbia; ISBN 978-86-6253-160-5
4. Marđokić Ana, Berisha Kaltrina, Koris Andras, Vatai Gyula, Bánvölgyi Szilvia: Ultrasound-Assisted Extraction As A Novel Technology For The Extraction Of Bioactive Compounds From Olive Pomace, In: *21st Wellmann International Scientific Conference*, 18th of April 2024, Hódmezővásárhely, Hungary; ISBN 978-963-306-980-6
5. Marđokić Ana, Vatai Gyula, Bánvölgyi Szilvia: Comparative Analysis of Polyphenol Extraction from Olive Pomace: A Study on Microwave-Assisted and Traditional Methods, In: *International Conference on Science, Technology, Engineering And Economy (ICOSTEE 2024)*, 31st of May 2024, Szeged, Hungary; ISBN: 978-963-306-986-8
6. Marđokić Ana, Vatai Gyula, Bánvölgyi Szilvia: Exploring the Potential of EM-Treated Olive Pomace as a Soil Amendment for Sustainable Agriculture, In: *First International Student Conference on Agriculture, Food Science and Veterinary Medicine*, 12th of July 2024, Prishtina, Kosovo

7. Marđokić Ana, Máté Molnár, Bánvölgyi Szilvia: Microwave-Assisted vs. Traditional Extraction of Polyphenols from Olive Pomace: A Comparative Study, In: *6th International Conference on Biosystems and Food Engineering (BiosysFoodEng 2025)*, 6th of June, 2025, Budapest, Hungary; ISBN 978-615-02-3494-6
8. Ana Marđokić: Kvalitet namirnica sa aspekta sadržaja aditiva, s posebnim osvrtom na sadržaj vještačkih zaslađivača i konzervanasa, *National Conference on Applied Science on Food, Sci-Food*, Faculty of Food Technology, Food Safety and Ecology, april 2017, Podgorica, Montenegro

REFERENCES

- Alu'datt, M. H., Alli, I., Ereifej, K., Alhamad, M., Al-Tawaha, A. R., & Rababah, T. (2010). Optimisation, characterisation and quantification of phenolic compounds in olive cake. *Food Chemistry*, 123(1), 117–122. <https://doi.org/10.1016/j.foodchem.2010.04.011>
- Antónia Nunes, M., Costa, A. S. G., Bessada, S., Santos, J., Puga, H., Alves, R. C., Freitas, V., & Oliveira, M. B. P. P. (2018). Olive pomace as a valuable source of bioactive compounds: A study regarding its lipid- and water-soluble components. *Science of the Total Environment*, 644, 229–236. <https://doi.org/10.1016/j.scitotenv.2018.06.350>
- Aparicio, R., & Harwood, J. (2013). *Handbook of Olive Oil Analysis and Properties* (R. Aparicio & J. Harwood, Eds.; Second Edition). Springer. <https://doi.org/10.1007/978-1-4614-7777-8>
- Bernal, M. P., Albuquerque, J. A., & Moral, R. (2009). Composting of animal manures and chemical criteria for compost maturity assessment. A review. *Bioresource Technology*, 100(22), 5444–5453. <https://doi.org/10.1016/j.biortech.2008.11.027>
- Böhmer-Maas, B. W., Otero, D. M., Zambiasi, R. C., & Aranha, B. C. (2020). Optimization of the extraction of phenolic compounds from olive pomace using response surface methodology. *Revista Ceres*, 67(3), 181–190. <https://doi.org/10.1590/0034-737X202067030003>
- Cabrera, L., Xavier, L., & Zecchi, B. (2024). Extraction of phenolic compounds with antioxidant activity from olive pomace using natural deep eutectic solvents: modelling and optimization by response surface methodology. *Discover Food*, 4(1). <https://doi.org/10.1007/s44187-024-00100-z>
- Čepo, D. V., Radić, K., Jurmanović, S., Jug, M., Rajković, M. G., Pedisić, S., Moslavac, T., & Albahari, P. (2018). Valorization of olive pomace-based nutraceuticals as antioxidants in chemical, food, and biological models. *Molecules*, 23(8). <https://doi.org/10.3390/molecules23082070>
- Chanioti, S., Katsouli, M., & Tzia, C. (2021). Novel processes for the extraction of phenolic compounds from olive pomace and their protection by encapsulation. *Molecules*, 26(6). <https://doi.org/10.3390/molecules26061781>

- Chanioti, S., & Tzia, C. (2018). Extraction of phenolic compounds from olive pomace by using natural deep eutectic solvents and innovative extraction techniques. *Innovative Food Science and Emerging Technologies*, 48, 228–239. <https://doi.org/10.1016/j.ifset.2018.07.001>
- Dermeche, S., Nadour, M., Larroche, C., Moulti-Mati, F., & Michaud, P. (2013). Olive mill wastes: Biochemical characterizations and valorization strategies. *Process Biochemistry*, 48(10), 1532–1552. <https://doi.org/10.1016/j.procbio.2013.07.010>
- Donner, M., Radic, I., Erraach, Y., & El Hadad-Gauthier, F. (2022). *Implementation of Circular Business Models for Olive Oil*. 1–18.
- Dujmić, F., Ganić, K. K., Čurić, D., Karlović, S., Bosiljkov, T., Ježek, D., Vidrih, R., Hribar, J., Zlatić, E., Prusina, T., Khubber, S., Barba, F. J., & Brnčić, M. (2020). Non-thermal ultrasonic extraction of polyphenolic compounds from red wine lees. *Foods*, 9(4). <https://doi.org/10.3390/foods9040472>
- Fotiadou, R., Fragkaki, I., Pettas, K., & Stamatis, H. (2024). Valorization of Olive Pomace Using Ultrasound-Assisted Extraction for Application in Active Packaging Films. *International Journal of Molecular Sciences*, 25(12). <https://doi.org/10.3390/ijms25126541>
- Galanakis, C. M. (2017). *Olive Mill Waste- Recent Advances for Sustainable Management*. Elsevier.
- Genwali, G. R., Acharya, P. P., & Rajbhandari, M. (2013). Isolation of Gallic Acid and Estimation of Total Phenolic Content in Some Medicinal Plants and Their Antioxidant Activity. *Nepal Journal of Science and Technology*, 14(1), 95–102.
- Goldsmith, C. D., Vuong, Q. V., Stathopoulos, C. E., Roach, P. D., & Scarlett, C. J. (2018). Ultrasound increases the aqueous extraction of phenolic compounds with high antioxidant activity from olive pomace. *LWT*, 89, 284–290. <https://doi.org/10.1016/j.lwt.2017.10.065>
- Gortzi, O., Lalas, S., Chatzilazarou, A., Katsoyannos, E., Papaconstandinou, S., & Dourtoglou, E. (2008). Recovery of natural antioxidants from olive mill wastewater using Genapol-X080. *JAACS, Journal of the American Oil Chemists' Society*, 85(2), 133–140. <https://doi.org/10.1007/s11746-007-1180-z>

- Gülçin, I. (2012). Antioxidant activity of food constituents: An overview. In *Archives of Toxicology* (Vol. 86, Number 3, pp. 345–391). <https://doi.org/10.1007/s00204-011-0774-2>
- Iglesias Jim, E., & Garcia, rez. (1992). *Determination of maturity indices for city refuse composts*.
- Katsinas, N., Rodríguez-Rajo, S., & Enríquez-De-salamanca, A. (2021). Olive pomace phenolic compounds and extracts can inhibit inflammatory-and oxidative-related diseases of human ocular surface epithelium. *Antioxidants*, 10(7). <https://doi.org/10.3390/antiox10071150>
- Khater, E. S. G. (2015). Some Physical and Chemical Properties of Compost. *International Journal of Waste Resources*, 05(01). <https://doi.org/10.4172/2252-5211.1000172>
- Lafka, T. I., Lazou, A. E., Sinanoglou, V. J., & Lazos, E. S. (2011). Phenolic and antioxidant potential of olive oil mill wastes. *Food Chemistry*, 125(1), 92–98. <https://doi.org/10.1016/j.foodchem.2010.08.041>
- Macedo, G. A., Santana, Á. L., Crawford, L. M., Wang, S. C., Dias, F. F. G., & de Mour Bell, J. M. L. N. (2021). Integrated microwave- and enzyme-assisted extraction of phenolic compounds from olive pomace. *LWT*, 138. <https://doi.org/10.1016/j.lwt.2020.110621>
- Maldonado Espinel, A. E. (2022). *Polyphenol extraction from Olive pomace of Montenegrin Olive variety Žutica as the initial step in waste-valorization strategy- MSc Thesis* [Institute of Food Science and Technology]. Hungarian University of Agriculture and Life Sciences.
- Mekersi, N., Kadi, K., Casini, S., Addad, D., Amari, A., & Lekmine, S. (2022). Evaluation of the effects of short-term amendment with olive mill pomace on some soil properties. *Soil Science Annual*, 73(2). <https://doi.org/10.37501/SOILSA/150493>
- Natolino, A., & Celotti, E. (2022). Ultrasound treatment of red wine: Effect on polyphenols, mathematical modeling, and scale-up considerations. *LWT*, 154. <https://doi.org/10.1016/j.lwt.2021.112843>
- Parascanu, M. M., Sánchez, P., Soreanu, G., Valverde, J. L., & Sanchez-Silva, L. (2018). Environmental assessment of olive pomace valorization through two different thermochemical processes for energy production. *Journal of Cleaner Production*, 186, 771–781. <https://doi.org/10.1016/j.jclepro.2018.03.169>

- Petrović, E., Vrandečić, K., Albrecht, A., Gruntar, I., Major, N., Ćosić, J., Užila, Z., Goreta Ban, S., & Godena, S. (2025). Integrated Analysis of Olive Mill Wastewaters: Physicochemical Profiling, Antifungal Activity, and Biocontrol Potential Against Botryosphaeriaceae. *Horticulturae*, 11(7). <https://doi.org/10.3390/horticulturae11070819>
- Sancho-Galán, P., Amores-Arrocha, A., Jiménez-Cantizano, A., Ferreira-González, M., Palacios, V., & Barbero, G. F. (2019). Ultrasound-assisted extraction of anthocyanins and total phenolic compounds in *Vitis vinifera* L. “Tempranillo” winemaking lees. *Vitis - Journal of Grapevine Research*, 58, 39–47. <https://doi.org/10.5073/vitis.2019.58.special-issue.39-47>
- Socas-Rodríguez, B., Álvarez-Rivera, G., Valdés, A., Ibáñez, E., & Cifuentes, A. (2021). Food by-products and food wastes: are they safe enough for their valorization? *Trends in Food Science and Technology*, 114, 133–147. <https://doi.org/10.1016/j.tifs.2021.05.002>
- Tapia-Quirós, P., Montenegro-Landívar, M. F., Reig, M., Vecino, X., Alvarino, T., Cortina, J. L., Saurina, J., & Granados, M. (2020). Olive mill and winery wastes as viable sources of bioactive compounds: A study on polyphenols recover. *Antioxidants*, 9(11).
- Tiquia, S. M., Tam, N. F. Y., & Hodgkiss, I. J. (1997). Effects of turning frequency on composting of spent pig-manure sawdust litter. *Bioresource Technology*, 62(1–2), 37–42. [https://doi.org/10.1016/S0960-8524\(97\)00080-1](https://doi.org/10.1016/S0960-8524(97)00080-1)
- Zin, M. M., & Bánvölgyi, S. (2021). Emerging technology approach for extractability and stability of betalains from the peel of beetroot (*Beta vulgaris* L.). *Biomass Conversion and Biorefinery*. <https://doi.org/10.1007/s13399-021-01975-z>