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VALORIZATION OF APPLE POMACE AS A SUSTAINABLE SOURCE OF BIOACTIVE COMPOUNDS: A COMPARATIVE STUDY OF MICROWAVE-ASSISTED AND SOXHLET EXTRACTIONS

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Abstract: This study aims to evaluate and compare the efficiency of microwave-assisted extraction (MAE) and conventional Soxhlet methods for the recovery of bioactive compounds from apple pomace. The total phenolic content (TPC) of extracts obtained under various temperature and time conditions was determined using the Folin-Ciocalteu reagent. The antioxidant activity was evaluated through the 1,1-Diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging assay to calculate IC_{50} values. The highest total phenolic content was obtained with Soxhlet (9.78 ± 0.18 mg GAE/g extract) and MAE-5 (9.7 ± 0.18 mg GAE/g extract) methods (P>0.05). The IC_{50} values of MAE extracts ranged from 17.8 ± 1.2 to 33.3 ± 0.9 µg/mL, with the highest antioxidant activities observed in MAE-4 (17.8 ± 1.2 µg/mL) and MAE-2 (18.8 ± 1.3 µg/mL) samples. Notably, no significant statistical difference was observed between MAE-4 and MAE-2 (P>0.05), indicating that short-duration microwave treatment preserves antioxidant potency effectively across the tested temperature range. The Soxhlet extract (25 ± 1.2 µg/mL) showed significantly lower antioxidant activity compared to most MAE samples P<0.05). Statistical analysis confirmed that MAE is more effective than Soxhlet for phenolic recovery and antioxidant activity (P<0.05). Moderate temperature and short-duration MAE conditions were found to be more advantageous for phenolic stability. Apple pomace is a valuable bioactive resource, and MAE offers a sustainable and efficient alternative for its valorization.

Keywords: Apple pomace, Microwave-assisted extraction, Soxhlet extraction, Total phenolic content, Antioxidant activity

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1. Introduction

The food processing industry generates significant amounts of plant-derived waste, which can pose environmental burdens due to its high organic content and rapid biodegradation. Therefore, the "waste-to-value" paradigm has garnered considerable interest, promoting the conversion of agricultural-industrial by-products into high-value bioactive components, rather than relying on traditional waste management practices (Banerjee et al, 2017). Apple pomace-the main by-product of apple juice and cider production—is among the most abundantly generated fruit-processing residues and represents a valuable source of phenolic compounds, flavonoids, chlorogenic acids, and dietary fibers, making it a promising raw material for nutraceutical, pharmaceutical, and functional food applications (Nawirska and Kwaśniewska, 2005; Kruczek et al., 2023). Phenolic compounds are well-known for their strong antioxidant activity and their ability to mitigate oxidative stress, a significant factor in the pathogenesis of chronic diseases (Shahidi and Ambigaipalan, 2015). Apple pomace is particularly rich in chlorogenic acid, catechin, epicatechin, and proanthocyanidins, bioactive molecules with documented antioxidant and anti-inflammatory activities (Persic et al., 2017). Therefore, developing efficient extraction strategies to recover these compounds has become a growing interest in natural product research and pharmaceutical sciences. The extraction method plays a critical role in determining the yield, composition, and stability of recovered phenolics. Although Soxhlet extraction remains widely used due to its strong solvent penetration, it has limitations such as long processing times, high solvent consumption, and the potential degradation of thermolabile compounds (Mustafa and Turner, 2011). Microwave-assisted extraction (MAE) offers a fast, energy-efficient, and environmentally friendly alternative that enhances mass transfer, reduces solvent usage, and promotes the release of bound phenolics via dielectric heating (Eskilsson and Björklund, 2000; Chemat, 2017). Several studies have shown that microwave irradiation effectively disrupts plant cell matrices, improving the extraction of free and cell-wall-bound phenolic compounds, particularly in lignocellulosic residues such as apple pomace (Zou, 2025). The antioxidant activity of extracts is commonly evaluated using the DPPH radical scavenging assay,

where the IC_{50} value represents the concentration needed to neutralize 50% of the DPPH radical (Brand-Williams, 1995). Lower IC_{50} values indicate stronger antioxidant properties, and numerous studies confirm an inverse relationship between total phenolic content and IC_{50} values (Shahidi and Ambigaipalan, 2015). Valorization of apple pomace through innovative extraction technologies aligns with circular economy principles and offers environmental and economic advantages. As the demand for natural antioxidants continues to increase in pharmaceutical and nutraceutical industries, sustainable and high-efficiency extraction strategies are gaining importance (Garofalo et al., 2025).

Comparative studies on microwave-assisted and conventional extraction are common. However, reports optimizing MAE conditions specifically for apple pomace and comparing them directly with Soxhlet extraction regarding antioxidant activity remain limited.

This study aims to comparatively evaluate the total phenolic content and antioxidant activity of apple pomace extracts obtained using microwave-assisted extraction and conventional Soxhlet extraction under different temperature-time conditions.

2. Materials and Methods

2.1. Materials and Plant Material Preparation

The apple pomace used in this study was obtained as an industrial by-product from a local fruit juice processing facility during the apple pressing stage. The freshly collected pomace was transported to the laboratory, manually cleaned to remove foreign materials, and dried in a hot-air oven at 50 °C until it reached a constant weight. The dried material was then ground using a laboratory-scale mill to achieve a homogeneous particle size and stored in airtight containers at room temperature until the extraction method was performed.

2.2. Extraction Methods

2.2.1. MAE

MAE experiments were performed using a closed-vessel microwave extraction system equipped with a temperature control unit and magnetic stirring (Milestone, Italy), following the method described by Ersan et al. (2020) with slight modifications. For each extraction run, 1 g of dried apple pomace was placed into a polytetrafluoroethylene (PTFE)-lined extraction vessel and mixed with 10 mL of ethanol. The vessel was then sealed, and the extraction method was initiated. During extraction, the temperature inside the control vessel was continuously monitored using an integrated temperature sensor, while constant magnetic stirring ensured homogeneous heat distribution throughout the sample. After completion of the extraction, the mixture was cooled to room temperature and filtered to separate the solid residue from the liquid extract. The filtrate was concentrated using a rotary evaporator under reduced pressure to remove ethanol, and the resulting extracts were stored at 4 °C until further analysis. Based on the solvent's boiling point (~78.4 °C for ethanol) and the thermal stability of phenolics (Wang and Weller, 2006; Chan et al., 2011; Plamada et al., 2024), MAE was performed at two temperatures (80 °C and 100 °C) and three extraction times (15, 30, and 60 min) to optimize extraction without causing significant degradation (Lu and Yeap Foo, 2000; Ersan et al., 2020). The experimental conditions were coded according to temperature and time parameters, as commonly reported in the literature (Table 1).

Table 1. Experimental design and parameters for MAE and Soxhlet extraction*

Sample Code	Temperature (°C)	Time (minutes)	Solvent Volume Used (mL)
MAE-1	100	60	10
MAE-2	100	15	10
MAE-3	100	30	10
MAE-4	80	15	10
MAE-5	80	30	10
MAE-6	80	60	10
Soxhlet	78	360	300

*This coding scheme (MAE-1–MAE-6) was used to facilitate comparison among extraction conditions in subsequent analyses.

The selection of MAE temperatures (80–100 °C) and extraction times (15–60 min) was based on established protocols for fruit residues to maximize phenolic yield while preventing thermal degradation of heat-sensitive compounds like quercetin (Wang and Weller, 2006; Chan et al., 2011; Plamada et al., 2024; Zeng et al., 2025). Previous studies on apple pomace have demonstrated that these ranges are effective for optimizing the balance between total phenolic yield and antioxidant integrity (Lu and Yeap Foo, 2000; Carbone et al., 2011; Kruczek et al., 2023).

2.2.2. Soxhlet extraction

A Soxhlet apparatus was used for conventional extraction. Ethanol was preferred as the solvent in the extraction method. For this purpose, 10 g of ground apple pomace was placed in a cellulose extraction cartridge, and 300 mL of ethanol was added to a Soxhlet flask. The system was placed on a heater, and continuous extraction was carried out for 6 hours. After the extraction time was completed, the ethanol extract was evaporated under low pressure using a rotavapor, and a dry extract was obtained. The extracts were stored at +4 °C until analysis.

The sample-to-solvent ratios for MAE (1:10) and Soxhlet (1:30) were specifically selected based on the technical requirements of each apparatus. Soxhlet extraction necessitates a higher solvent volume to ensure a continuous siphoning cycle and to maintain the complete immersion of the sample within the extraction thimble. Conversely, the 1:10 ratio used in MAE was optimized to ensure efficient microwave energy transfer and to prevent excessive pressure buildup within the closed-vessel system, consistent with the principles of operational safety and extraction efficiency.

2.3. Preparation of Extracts for Analysis

All extracts obtained from the MAE and Soxhlet methods were dissolved in methanol at appropriate concentrations prior to analysis. While ethanol was selected for the extraction stage due to its optimal dielectric constant for the selective recovery of polar and semi-polar phenolic fractions and its status as a sustainable 'green' solvent, methanol was strictly utilized for the analytical preparations (TPC and DPPH assays). Methanol ensures superior solubility and maximum chemical stability for phenolic compounds during spectrophotometric measurements, providing higher reproducibility compared to other solvents. Furthermore, the use of methanol aligns with standardized international protocols, ensuring methodological consistency and allowing for a direct comparison with the existing literature. Since all samples were analyzed under identical conditions, this dual-solvent approach ensures both high extraction efficiency and precise quantification without introducing bias into the comparative interpretation (Brand-Williams, 1995; Chemat et al., 2012).

2.4. Determination of Total Phenolic Content (TPC)

The TPC of the apple pomace extracts was quantified using the Folin–Ciocalteu colorimetric method with slight modifications adapted from previously published procedures (Eroğlu et al., 2025). A series of gallic acid standard solutions (25–800 mg/L) was prepared to construct the calibration curve. For each analysis, 500 µL of the sample solution (5 mg/mL) was mixed with 1 mL of Folin–Ciocalteu reagent, followed by the addition of 750 µL of 2% (w/v) sodium carbonate solution. The reaction mixtures were incubated in the dark for 30 minutes at room temperature. Absorbance readings were subsequently recorded at 714 nm using a UV–Vis spectrophotometer. TPC values were calculated and expressed as milligrams of gallic acid equivalents per gram of dry extract (mg GAE/g extract). All measurements were carried out in triplicate, and results are presented as mean ± standard deviation (SD).

2.5. DPPH Radical Scavenging Assay

The antioxidant capacities of the extracts were determined using the DPPH free radical scavenging assay according to the procedure described by Eroğlu et al., (2023) with minor adjustments. A stock solution of each extract (1 mg/mL) was prepared, and working solutions at various concentrations (1–1000 µg/mL) were prepared by mixing an equal volume (100 µL) of 0.15 mM DPPH solution. After incubating the mixtures for 30 minutes in the dark at room temperature, the decrease in absorbance was measured at 517 nm using a microplate reader. The radical scavenging activity was calculated using equation 1:

$$\% \text{ DPPH scavenging activity} = [(A_0 - A_1) / A_0] \times 100 \quad (1)$$

where A_0 represents the absorbance of the DPPH control, and A_1 corresponds to the absorbance of the sample–DPPH mixture. All experiments were conducted in triplicate. Butylated hydroxytoluene (BHT) was used as the reference antioxidant. The IC_{50} values, representing the extract concentration required to scavenge 50% of the DPPH radicals, were determined using linear interpolation between the two concentrations that yielded inhibition percentages immediately above and below 50% (equation 1).

$$IC_{50} = [((50 - y_1) * (x_2 - x_1)) / (y_2 - y_1)] + x_1 \quad (2)$$

where y_1 and y_2 are the inhibition percentages (%), and x_1 and x_2 are the corresponding extract concentrations (µg/mL). All measurements were performed in triplicate, and results were expressed as mean ± standard deviation (SD).

2. 6. Statistical Analysis

All experimental analyses were performed in triplicate, and the results were expressed as mean ± standard deviation (SD). Data were subjected to one-way analysis of variance (ANOVA). Significant differences between the means of extraction methods were determined using Tukey's post-hoc test at a significance level of $P<0.05$.

3. Results and Discussion

This study comprehensively evaluates the changes in total phenolic content and antioxidant activity of apple pomace extracts obtained by MAE at different temperature-time combinations, comparing them with conventional Soxhlet extraction. Apple pomace, a significant byproduct of the fruit juice industry, is known to be particularly rich in bioactive phenolic compounds such as chlorogenic acid, catechins, floridzin, quercetin derivatives, and various phenolic acids. These compounds significantly contribute to the antioxidant, functional, and nutritional potential of apple pomace, making it an attractive raw material for value-added applications (Lu and Yeap Foo, 2000; Kruczek et al., 2023). In conclusion, the extraction method and processing conditions play a decisive role in determining both the yield and biological efficacy of the obtained phenolics.

3.1. Total Phenolic Content

The total phenolic content of the apple pomace extracts, expressed as gallic acid equivalents (GAE), is presented in Figure 1. In the present study, total phenolic contents were expressed as mg GAE/g extract by normalizing the experimental measurements to the extract concentration used in the assays. This normalization allows for a more

meaningful comparison of extraction efficiencies under different conditions and facilitates direct comparison with previously published studies. The results clearly demonstrate that MAE efficiency is highly dependent on both extraction temperature and time, consistent with previous reports (Eskilsson and Björklund, 2000; Wang and Weller, 2006; Ersan et al., 2020). Among the MAE conditions tested, the highest phenolic recovery was achieved at 80 °C for 30 minutes (MAE-5; 9.7 ± 0.18 mg GAE/g extract, assigned to Group a), which showed no significant difference from the traditional Soxhlet extraction (9.78 ± 0.18 mg GAE/g extract, also in Group a) ($P>0.05$). This statistical grouping indicates that MAE can reach the maximum extraction capacity of the Soxhlet method in a significantly shorter duration, confirming the efficiency of the microwave-assisted process. The high phenolic recovery achieved in our study (9.7 mg GAE/g) aligns with the framework proposed by Lyu et al. (2020), who categorized apple pomace as a prime candidate for the recovery of value-added compounds like dihydrochalcones and flavanols. While Lyu et al. (2020) highlighted that the complex lignocellulosic structure of the pomace matrix often acts as a physical barrier to phenolic release, our findings demonstrate that the application of optimized MAE effectively overcomes these constraints by disrupting the cell-wall lignification. Consequently, the high-purity extracts obtained in our work establish MAE as a critical technology for industrial-scale valorization, as envisioned in current literature. Although extraction at 80 °C for 15 minutes (MAE-4) also yielded a high phenolic content (9.3 ± 0.16 mg GAE/g extract), the additional 15 minutes in MAE-5 further optimized the release of phenolic compounds from the plant matrix. These findings demonstrate that combining moderate temperatures with optimized extraction times is sufficient to disrupt the plant cellular matrix while minimizing thermal degradation. Similar trends have been reported for MAE of phenolics from various plant matrices (Wang and Weller, 2006; Chan et al., 2011). In contrast, MAE processes performed at higher temperatures or excessive durations, such as MAE-6 (80 °C, 60 min), did not improve recovery and potentially promoted oxidation or structural degradation of heat-sensitive compounds. These observations are consistent with studies highlighting the negative effects of excessive heat input on phenolic stability (Oracz et al., 2023; Zeng et al., 2025). Consequently, while Soxhlet extraction produces high phenolic yields, its inherent long extraction time raises concerns about reduced biological activity compared to the more efficient and rapid MAE process.

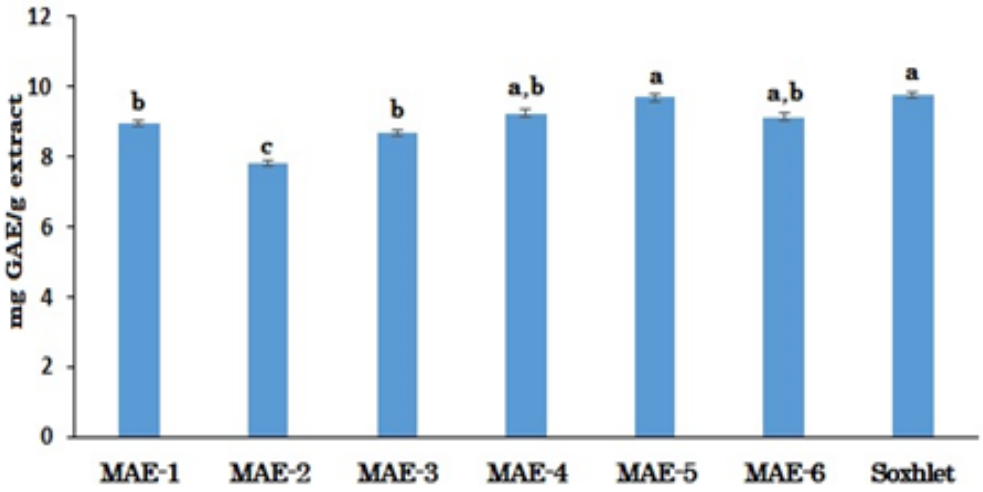

Figure 1. Total phenolic content of apple pomace extracts obtained by MAE under different temperature–time conditions and by conventional Soxhlet extraction, expressed as mg gallic acid equivalents (GAE) per gram of extract. Values are mean ± standard deviation (n = 3). Different lowercase letters (a–c) above the columns indicate statistically significant differences between extraction methods ($P<0.05$); means sharing the same letter are not significantly different.

3.2. DPPH Radical-Scavenging Activity

Given the well-established role of phenolic compounds as major contributors to antioxidant activity, the radical-scavenging activity of the apple pomace extracts was further evaluated using the DPPH assay. DPPH radical scavenging assay is widely used as a rapid and reliable method for preliminary evaluation of antioxidant activity in plant extracts, particularly in studies focusing on matrices rich in phenolic compounds (Özay and Mammadov, 2019). The DPPH radical-scavenging activity of the extracts obtained under different MAE conditions and by Soxhlet extraction is presented in Figure 2.

The data reveal a general trend in which samples with higher total phenolic content exhibit stronger DPPH radical-scavenging activity, supporting the view that phenolic compounds are primary contributors to antioxidant activity in plant extracts (Köroğlu et al., 2021). For instance, MAE-4 (80 °C, 15 min) displayed both a high phenolic content and the most potent antioxidant activity with the lowest IC_{50} value (17.8 ± 1.2

µg/mL), placing it in the superior statistical group (Group b). It is noteworthy that while Soxhlet extraction achieved the highest numerical phenolic yield (Group an in TPC), it fell into a significantly lower antioxidant activity group (Group d) in the DPPH assay. This discrepancy is statistically supported by the different letters assigned to these groups ($P<0.05$), proving that the rapid extraction time of MAE-4 (Group b) is more effective in preserving the bioactivity of the molecules than the prolonged Soxhlet process. Notably, MAE-2 (100 °C, 15 min) also exhibited strong antioxidant activity, showing no significant statistical difference from MAE-4 ($P>0.05$). This indicates that at short extraction durations (15 min), the biological potency is well-preserved even at higher temperatures, as the rapid microwave heating minimizes the exposure time to thermal stress. However, the relationship between total phenolic content and antioxidant activity was not perfectly linear. Sample MAE-1 (60 min at 100 °C) exhibited a higher IC_{50} value (20.1 ± 1.3 µg/mL), despite its relatively high phenolic yield. This discrepancy, further evidenced by the significantly lower activity of MAE-6 (Group e; 33.3 ± 1.3 µg/mL), can be attributed to thermal degradation or structural modification under prolonged microwave exposure. High-potential antioxidants like quercetin derivatives are susceptible to heat, while more stable compounds with weaker radical-scavenging activity, such as phloridzin, remain intact. Consequently, TPC analysis (Folin-Ciocalteu) may still detect these degraded structures, but structural changes—such as the loss of hydroxyl groups—significantly limit their biological efficacy. This demonstrates that high phenolic yield does not always equate to high biological activity, as the qualitative composition significantly influences antioxidant behavior (Plamada et al., 2024).

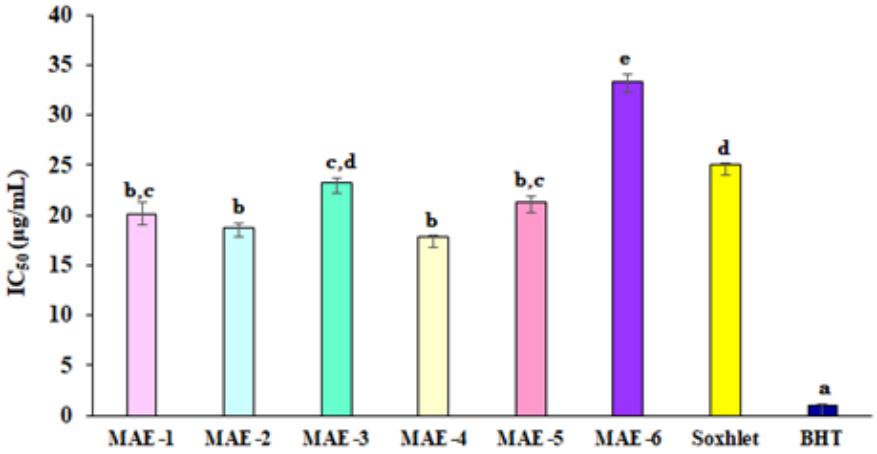

Figure 2. DPPH radical-scavenging activity of apple pomace extracts obtained by MAE under different temperature–time combinations and by conventional Soxhlet extraction, ex pressed as IC_{50} values (µg/mL). Values are mean ± standard deviation (n = 3). Different letters (a–e) represent significant statistical differences ($P<0.05$) in IC50 values. BHT was used as a positive control.

A key outcome of this study is the superior performance of MAE compared to conventional Soxhlet extraction in terms of antioxidant activity. Although Soxhlet yielded the highest phenolic content (Group a), its antioxidant activity was statistically lower (Group d; 25.00 ± 1.30 µg/mL) than several MAE samples. This is consistent with reports indicating that prolonged heating during Soxhlet extraction results in the degradation of sensitive phenolic compounds (Cacace and Mazza, 2003; Mandal et al., 2007; Sun et al., 2025). In contrast, MAE benefits from rapid and selective heating, enhanced solvent penetration, and improved mass transfer, leading to more efficient cell wall disruption and phenolic release (Routray and Orsat, 2012). Biorecovery of antioxidants from apple pulp has gained new momentum in recent years with the development of advanced extraction technologies. The high yields obtained with MAE in our study parallel the findings of Ferrentino et al. (2018), who examined the effectiveness of advanced techniques such as supercritical fluid extraction (SFE) on apple pulp. The researchers reported that such advanced methods release bioactive compounds in the raw material matrix in a much cleaner and more protective manner compared to conventional methods. Temperature emerged as a critical parameter in the extraction method. Increasing the extraction temperature from 80 °C to 100 °C did not consistently enhance phenolic recovery or antioxidant activity. Instead, shorter extraction times at moderate temperatures proved more effective than prolonged treatments at higher temperatures. Similar trends have been reported for grape pomace, olive leaves, and other fruit residues, where excessive thermal input reduced phenolic stability and antioxidant activity (Karastergiou et al., 2024; Zeng et al., 2025). Prolonged extraction may also facilitate oxidation, hydrolysis, or re-adsorption of phenolic compounds onto the plant matrix, thereby limiting effective recovery (Ersan et al., 2020). The phenolic contents and IC_{50} values obtained in this work fall well within the ranges reported for apple pomace. Total phenolic contents have been reported between 5

and 70 mg GAE/g extract depending on cultivar and extraction system (Lu and Yeap Foo, 2000; Kruczek et al., 2023), while IC_{50} values typically range from 10 to 70 µg/mL (Tsao et al., 2005; Carbone et al., 2011). This consistency further supports the reliability of the present findings. From an industrial and sustainability perspective, apple pomace represents a readily available and underutilized biomass. These results demonstrate that MAE offers clear advantages over Soxhlet extraction by reducing energy and solvent consumption while improving antioxidant activity. In particular, the MAE-4 condition (80 °C for 15 minutes) appears optimal under the tested parameters, providing the most favorable balance between phenolic yield and biological capacity. These findings support the potential of MAE as an efficient strategy for valorizing apple pomace into high-value antioxidant extracts suitable for food, nutraceutical, and functional ingredient applications.

4. Conclusion

This study demonstrates that MAE is a more efficient and sustainable alternative to conventional Soxhlet extraction for the recovery of bioactive compounds from apple pomace. While Soxhlet and MAE-5 (80 °C, 30 min) yielded the highest total phenolic content (9.7 ± 0.2 mg GAE/g extract) with no significant statistical difference between them ($P>0.05$), MAE-4 (80 °C, 15 min) emerged as the most effective condition by providing statistically superior antioxidant activity (IC_{50}: 17.8 ± 1.2 µg/mL; $P<0.05$). Notably, MAE-2 (100 °C, 15 min) also demonstrated comparable antioxidant potency (IC_{50}: 18.8 ± 1.3 µg/mL), showing no significant difference from MAE-4 ($P>0.05$). This indicates that a short processing time of 15 minutes is a more critical factor for maintaining biological efficacy than the extraction temperature within the 80–100 °C range. The fact that MAE achieved comparable phenolic yields to Soxhlet in a fraction of the time—reducing the process from 6 hours to just 15–30 minutes—represents a significant industrial advantage. From an applied perspective, this drastic reduction in extraction time translates into lower energy consumption and increased daily processing capacity in waste valorization facilities. These findings suggest that high-activity MAE extracts can be utilized as cost-effective, natural preservatives in the food and nutraceutical industries, offering a green and commercially viable strategy for transforming apple processing waste into high-value ingredients.

Author Contributions

The percentages of the author' contributions are presented below. The author reviewed and approved the final version of the manuscript.

	P.E.
C	100
D	100
S	100
DCP	100
DAI	100
L	100
W	100
CR	100
SR	100

C= concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision.

Conflict of Interest

The authors of this article declare that they have no conflict of interest

Ethical Consideration

Ethics committee approval was not required for this study because there was no study on animals or humans.

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