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Comariosn of Maceration, Ultrasound- and Microwave-Assisted Extraction Methods for Phenolic Recovery and Antioxidant Activity from Apple and Banana Peels

Hamideh Shafahi[1], Leila Nouri[1], Ali Najafi[*1], Seid Mahdi Jafari[2,3]

1-Department of Food Science and Technology, Da.C., Islamic Azad University, Damghan, Iran.

2- Department of Food Materials and Process, Design Engineering, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran – 3- Halal Research Center of IRI, Iran Food and Drug Administration, Ministry of Health and Medical Education, Tehran, Iran

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*Corresponding Author E-

Ali.Najafi@iau.ac.ir

ABSTRACT

Fruit peels, as by-products of the fruit processing industry, are rich sources of bioactive compounds, particularly phenolic compounds with antioxidant properties, and can be utilized in developing natural ingredients for food systems. The aim of this study was to compare the efficiency of three extraction methods maceration, ultrasound-assisted extraction, and microwave-assisted extraction in recovering phenolic compounds and evaluating the antioxidant activity of extracts from apple and banana peels. After extraction, total phenolic content (TPC) and total flavonoid content (TFC) were measured, and antioxidant activity was evaluated using DPPH radical-scavenging and ferric reducing antioxidant power (FRAP) assays. The results showed that apple peel extracts contained significantly higher levels of phenolic and flavonoid compounds than banana peel extracts ($p < 0.05$). The highest total phenolic content was observed in ultrasound extracts, reaching 48.16 and 45.16 mg GAE/g DW for apple and banana peels, respectively. Similarly, the highest total flavonoid content was found in ultrasound extracts of apple and banana peels, with values of 28.45 and 19.82 mg QE/g DW, respectively. The DPPH assay indicated that ultrasound extracts exhibited the strongest radical-scavenging activity, whereas microwave extraction showed the highest reducing power in the FRAP assay. Maceration resulted in the lowest levels of phenolic and flavonoid compounds and antioxidant activity in both samples. HPLC analysis confirmed the presence of a range of phenolic compounds, particularly flavonoids and flavan-3-ol derivatives, and a positive correlation was observed between total phenolic content, especially flavonoids, and antioxidant activity. Overall, ultrasound extraction was the most efficient method for enhancing phenolic recovery and radical-scavenging activity, whereas microwave extraction showed the greatest effect on reducing power. These findings highlight the potential of apple and banana peels as natural antioxidant sources for food applications and development of bioactive compounds.

1- INTRODUCTION

The utilization of industrial fruit by-products, especially peels, as substantial reservoirs for bioactive constituents like phenolics and flavonoids has gained significant traction. These natural compounds are increasingly viewed as essential building blocks for developing potent antioxidants and functional food ingredients [1-3]. Considering the immense quantities of waste generated during fruit processing and consumption, the systematic recovery of high-value molecules from these matrices is now a strategic priority. This shift not only addresses pressing environmental concerns but also fosters the upcycling of food-grade residues [4-6].

Of the various residues studied, banana and apple peels have emerged as particularly promising candidates for bioactive recovery, owing to their dense phenolic profiles and robust antioxidant potential. The phenolic richness of banana peel, along with the notable radical scavenging activity of its extracts, is well-documented [7, 8]. Additionally, unique chemical markers such as dopamine have been identified within these banana-derived matrices [8]. Apple peels similarly exhibit a complex polyphenolic makeup, characterized by procyanidins, chlorogenic acid, and phloridzin, which reinforces their standing as effective sources for natural antioxidant extraction [9, 10].

The overall success in isolating these bioactive molecules is largely governed by the specific extraction framework employed [4, 5, 11-13]. While maceration remains a staple due to its operational simplicity and low cost, its industrial application is frequently limited by extensive time requirements and high solvent demand [11]. Consequently, "green" intensification technologies, including Ultrasound-Assisted Extraction (UAE) and Microwave-Assisted Extraction (MAE), have risen to prominence for isolating phenolics from botanical and food-side streams. These advanced protocols facilitate improved mass transfer, abbreviated processing intervals, and minimized solvent use [4, 5]. In the case of UAE, the release of intracellular solutes is driven by acoustic cavitation-induced cell wall rupture. Conversely, MAE leverages dielectric heating to accelerate solute migration, thereby boosting the total extraction efficiency [12, 13].

While the literature provides extensive data on plant-based extractions, direct and comprehensive benchmarking of different methodologies for recovering flavonoids and phenolics specifically from fruit peels remains relatively scarce. Few investigations have conducted a side-by-side performance evaluation of maceration, UAE, and MAE regarding bioactive yields from apple and banana peels, particularly in relation to the resulting antioxidant capacity [14, 15]. To fill this gap, the present study aimed to compare these three techniques for optimizing the recovery of phenolic and flavonoid fractions. Furthermore, the antioxidant efficacy of the resulting extracts was systematically assessed. It was hypothesized that the assisted methods (UAE and MAE) would demonstrate a clear superiority in extraction yields over the conventional maceration approach.

2- Materials and Methods

2.1. Materials

Ripe specimens of banana (*Musa acuminata* "Dwarf Cavendish") and apple (*Malus domestica* "Red Delicious") were sourced from a local market in Shiraz, Iran. To ensure the removal of surface contaminants, the samples were thoroughly rinsed with distilled water. The peels were manually isolated from the pulp using a stainless-steel slicer and then dehydrated in a convective laboratory oven at 40°C until weight stability was achieved. Once dried, the materials were pulverized into a fine powder via a high-speed electric grinder and passed through an 800-µm mesh sieve

to maintain a consistent particle size distribution. All analytical-grade chemicals and standards, including Folin–Ciocalteu, DPPH, and various phenolic compounds, were purchased from Merck (Darmstadt, Germany).

2.2. Maceration Extraction (ME) of Apple (AE) and banana (BE) peel extracts

Extraction was carried out via conventional maceration, following the methodology of Ranjha et al. (2020) with slight modifications [13]. To start, the pulverized fruit peel was immersed in a 70% ethanol-water solution at a 1:20 (w/v) solid-to-solvent ratio. We utilized an orbital shaker to provide continuous agitation at 150 rpm for 24 hours, keeping the ambient temperature steady at 25±2°C. After the extraction period, the resulting suspensions were passed through Whatman No. 1 filter paper. To maximize yield, the remaining solid residue underwent a secondary wash with fresh solvent. The combined extracts were then concentrated using a Heidolph rotary evaporator (Germany) at 40°C under reduced pressure. Finally, these fractions were stored in a -18°C freezer for subsequent analysis.

2.3. Ultrasound-assisted extraction (UAE) of AE and BE.

The extraction process for apple and banana peels was conducted via ultrasound-assisted extraction, following the protocol delineated by Shafahi et al. (2025). Briefly, 20 g of each dehydrated peel powder was homogenized with a 50% (v/v) ethanol–water solvent system. The resulting mixtures were then subjected to ultrasonic treatment using an ultrasonic bath (Bandelin Sonorex, Digitech, Germany) operating at a frequency of 20 kHz and a controlled temperature of 40°C for a duration of 30 min. Post-treatment, the extracts were clarified by filtration through Whatman No. 1 filter paper. Solvent removal was subsequently performed using a rotary evaporator (Heidolph, Germany) at 45°C. The obtained concentrated extracts were then stored under cryogenic conditions (−18°C) until further analytical procedures.

2.4. Microwave-assisted extraction (MAE) of AE and BE

The microwave-assisted extraction of bioactive compounds from apple and banana peels was performed following established protocols for fruit by-products with minor modifications [18]. Initially, 1 g of each pulverized sample was suspended in 70% ethanol at a solid-to-solvent ratio of 1:30 (g/mL). The extraction was executed using a microwave system (LG, South Korea), where the irradiation parameters—specifically a power output of 550W for a duration of 165s were optimized based on prior literature regarding phenolic recovery from botanical matrices [18]. Subsequent to the microwave treatment, the crude extracts were centrifuged (Hettich, Germany) at 10,000 × g for 10 min at 4°C to facilitate phase separation. The resulting supernatant was then concentrated under reduced pressure using a rotary evaporator at 45°C. To ensure protection against photo-oxidation, the final extracts were transferred into foil-wrapped glass vials and maintained under freezing conditions (−18°C) until further utilization.

2.5. Determination of Total Phenolic Content (TPC)

The total phenolic content of the obtained extracts was quantified using the Folin–Ciocalteu colorimetric assay, following the methodology described by [16] with slight modifications. Briefly, an aliquot of 30 µL of each extract was homogenized with 150 µL of Folin–Ciocalteu reagent (previously diluted 1:10 v/v with distilled water). Subsequently, 120 µL of 7.5% (w/v) sodium carbonate solution was added to the mixture. To facilitate the development of the chromophore, the reaction mixtures were incubated in total darkness for 45 min at ambient temperature. The absorbance was then recorded at 765 nm using a UV-

Vis spectrophotometer (UV-550; Jasco, Japan) against a distilled water blank. TPC values were extrapolated from a standard calibration curve prepared with gallic acid and expressed as mg gallic acid equivalents (GAE) per gram of dry weight.

2.6. Determination of Total Flavonoid Content (TFC)

The total flavonoid content (TFC) of the AE and BE was quantified using the aluminum chloride colorimetric method, as previously described [13]. Initially, 0.01 g of the extract was solubilized in 5 mL of ethanol. A 1 mL aliquot of this solution was then transferred into a 10 mL volumetric flask containing 4 mL of distilled water, followed by the addition of 0.3 mL of 5% (w/v) $NaNO_2$. After a 5-min incubation period, 0.3 mL of 10% (w/v) $AlCl_3$ was introduced into the mixture. Following an additional 6 min of reaction time, 2 mL of 1 M NaOH was added, and the final volume was adjusted to 10 mL with distilled water. The absorbance of the resulting solution was recorded at 415 nm using a UV-Vis spectrophotometer (UV-550; Jasco, Japan). TFC values were extrapolated from a standard calibration curve of quercetin and expressed as milligrams of quercetin equivalents per gram of dry weight (mg QE/g DW).

2.7. DPPH radical scavenging activity

The antioxidant potential of the extracts was evaluated by assessing their ability to scavenge 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radicals, following the procedure outlined by [17]. Briefly, a 50 µL aliquot of each extract was homogenized with 1.95 mL of a 0.13 mM DPPH methanolic solution. The reaction mixtures were subsequently incubated in the dark at ambient temperature for 30 min to allow for radical stabilization. Following incubation, the absorbance was measured at 517 nm using a UV-Vis spectrophotometer (UV-550; Jasco, Japan). The DPPH radical inhibition percentage (I%) was calculated using the following equation (1):

$$\text{DPPH (\%)} = \frac{Ac - As}{Ac} \times 100 \qquad (1)$$

where Ac represents the absorbance of the control (containing all reagents except the extract) and As denotes the absorbance of the sample.

2.8. Ferric reducing antioxidant power (FRAP) assay

The ferric reducing capacity of the peel extracts was determined according to the method described by [14]. The FRAP working solution was freshly prepared by mixing 300 mM acetate buffer (pH 3.6), 10 mM TPTZ (2,4,6-tripyridyl-s-triazine) in 40 mM HCl, and 20 mM $FeCl_3 \cdot 6H_2O$ in a volumetric ratio of 10:1:1, respectively. Subsequently, a 10 µL aliquot of the extract was integrated with the prepared FRAP reagent. The reaction mixture was then incubated for 30 min at ambient temperature to facilitate the reduction of the ferric-tripyridyltriazine (Fe^{3+} TPTZ) complex to its ferrous (Fe^{2+}) form. The resulting blue-colored solution's absorbance was measured at 593 nm using a UV-Vis spectrophotometer (UV-550; Jasco, Japan). The antioxidant activity was expressed based on the change in absorbance compared to a blank.

2.9. High-performance liquid chromatography (HPLC) analysis

The identification and quantification of phenolic constituents within the extracts were executed using an Agilent 1260 series HPLC system (Waldbronn, Germany). Chromatographic separation was achieved on a reversed-phase Eclipse C18 column (150×4.6150 \times 4.6150×4.6 mm, 1.8 µm particle size). The mobile phase was composed of deionized water (Solvent A) and 0.05% trifluoroacetic acid (TFA) in acetonitrile (Solvent B), delivered at a constant flow rate of 0.9 mL/min. A linear gradient elution profile was implemented as follows: the initial proportion of Solvent A was 82%, which was adjusted to 80% over the first 5 min. This was followed by a further reduction to 60% Solvent A between

5 and 8 min, which was maintained isocratically until 12 min. Subsequently, the concentration of Solvent A was restored to 82% from 12 to 15 min and held constant until 16 min, concluding the run at 20 min. Detection was performed using a multi-wavelength detector (MWD) set at 280 nm. The injection volume was 5 µL, and the column temperature was strictly maintained at 40°C during the analysis [6]. Phenolic compounds were identified by comparing their retention times (t_R) with those of high-purity standards analyzed under identical chromatographic conditions. The relative abundance of each compound was calculated via peak area normalization and expressed as the relative peak area percentage.

2.10. Statistical analysis

All experimental procedures were executed in a completely randomized design (CRD) with three independent replications for each treatment. The collected data were initially subjected to the Shapiro–Wilk test to verify the normality of distribution, while the homogeneity of variances was assessed using Levene's test via SPSS software (version 22.0, IBM Corp., Armonk, NY, USA). To evaluate significant differences between treatment means, a one-way analysis of variance (ANOVA) was performed, followed by Duncan's multiple range test as a post-hoc analysis at a significance level of $p<0.05$. The interrelationships between TPC, TFC, and antioxidant indices (DPPH and FRAP) were determined using Pearson's correlation coefficient. Data are presented as mean ±standard deviation (SD). All graphical illustrations and data visualizations were generated using Microsoft Excel (version 2019).

3-Results and discussion

3.1. Total phenolic content (TPC)

The experimental results indicated that the total phenolic content (TPC) was significantly influenced by both the botanical source and the extraction methodology ($p<0.05$). As illustrated in Figure 1, AE consistently exhibited higher TPC values compared to BE across all extraction techniques. Among the methods evaluated, ultrasound-assisted extraction (UAE) proved to be the most efficient approach for both matrices, yielding TPC values of 45.16 and 48.164 (mg GAE/g DW) for BE and AE, respectively. Microwave-assisted extraction (MAE) also demonstrated substantial recovery of phenolic compounds, with TPC levels reaching 42.95 (mg GAE/g DW) for BE and 46.23 (mg GAE/g DW) for AE. In contrast, the conventional maceration technique resulted in the lowest recovery, with values recorded at 40.06 and 41.39 (mg GAE/g DW) for BE and AE, respectively. These findings underscore the superior efficiency of modern assisted-extraction techniques, particularly UAE and MAE, in enhancing the extractability of polyphenols compared to traditional methods.

The enhanced efficiency of UAE can be primarily attributed to the phenomenon of acoustic cavitation. The rapid formation and violent collapse of micro-bubbles within the solvent generate intense localized pressure and shear forces, leading to the mechanical disruption of plant cell walls. This process increases the permeability of the plant tissue, thereby facilitating mass transfer and the release of sequestered phenolic compounds into the solvent matrix [20, 21]. Similarly, the effectiveness of MAE is likely associated with the rapid and volumetric heating of the plant matrix induced by microwave radiation, which promotes the internal pressure build-up and subsequent exudation of bioactive constituents into the extraction medium [22].

The observed superiority of assisted extraction methods aligns with previous investigations into various plant matrices, including lemon peel, propolis, and apple-based samples, which have highlighted the advantages of UAE in optimizing polyphenol recovery [18, 23–25].

Furthermore, it has been reported that the recovery of phenolics from apple peels is highly dependent on the solvent system and extraction energy, with aqueous ethanol combined with ultrasound showing better performance than maceration [13]. The broad range of TPC values reported for banana peels in the literature can be attributed to variations in cultivar, solvent polarity, solid-to-solvent ratios, and specific extraction parameters [26]. The significant antioxidant potential of these peels is fundamentally driven by their heterogeneous phenolic composition, wherein the collective presence of flavonols, anthocyanins, flavan-3-ols, and phenolic acids plays an integral part in neutralizing oxidative stress [8, 22].

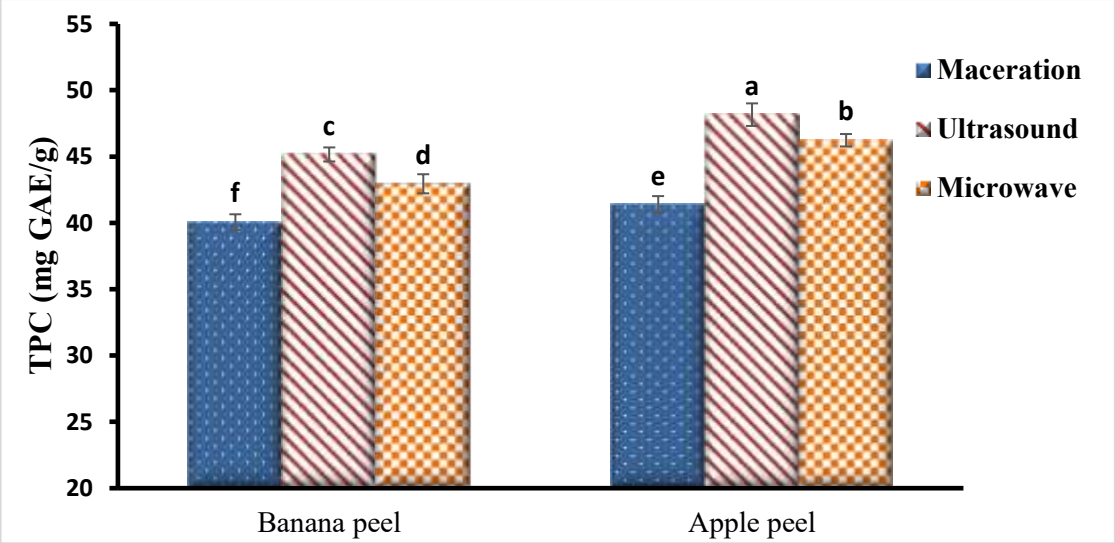

Figure 1. Total Phenolic Content (TPC) of AE and BE obtained by maceration, ultrasound-assisted extraction (UAE) and microwave-assisted extraction (MAE). Different letters indicate statistically significant differences at the 5% level.

3.2. Total flavonoid content (TFC)

Flavonoids represent a critical subclass of polyphenolic metabolites in the plant kingdom, renowned for their multifaceted biological activities, including potent antioxidant, antimicrobial, and anticarcinogenic properties [27]. Analysis of variance (ANOVA) revealed that the TFC was significantly influenced by the botanical source, the extraction technique, and the synergistic interaction between these two factors ($p<0.05$). As depicted in Figure 2, AEs consistently exhibited a significantly higher flavonoid concentration compared to BEs across all methodologies. Interestingly, the efficiency of the extraction methods appeared to be matrix-dependent. For apple peels, UAE achieved the highest recovery (28.45 mg QE/g DW), demonstrating a statistically significant advantage over other methods. Conversely, for BEs, microwave-assisted extraction (MAE) proved slightly more effective (20.39 mg QE/g DW), followed closely by UAE (19.82 mg QE/g DW). In both plant sources, the conventional maceration process yielded the lowest flavonoid concentrations.

The observed disparities in extraction efficiency can be elucidated through the distinct physical mechanisms governing the release of bioactive compounds. In UAE, the phenomenon of acoustic cavitation facilitates the mechanical disruption of cell walls through the implosion of microscopic bubbles, thereby enhancing the mass transfer of flavonoids into the solvent. Furthermore, the relatively lower operating temperatures in UAE may prevent the degradation of thermolabile flavonoids, thus preserving their structural integrity and increasing the final yield [28, 29]. In contrast, the mechanism of MAE relies on instantaneous and volumetric heating, which induces a rapid increase in internal

pressure, leading to the efficient rupture of the plant matrix [12]. It appears that the denser, more resilient fibrous structure of banana peels responds more favorably to the internal pressure buildup generated by microwave radiation, facilitating a more effective liberation of sequestered flavonoids [8].

These findings are in strong agreement with previous literature. For instance, the superiority of UAE for extracting flavonoids from heat-sensitive sources aligns with the observations of Hanula et al. (2020) regarding acai berries [28]. The presence of thermolabile constituents such as hyperoside, isoquercetin, and various catechins in apple peels [22, 30] further supports the rationale behind the higher efficiency of UAE for this specific matrix. Additionally, pre-extraction processing such as drying and comminution can significantly impact flavonoid yield by altering the cellular architecture [37]. In banana peels, which possess a dense lignocellulosic framework, these preparatory steps may play a more decisive role in enhancing solvent accessibility compared to the more porous structure of apple peels [38]. Ultimately, the quantitative variations reported across different studies are likely influenced by a combination of cultivar selection, fruit maturity, environmental conditions, and specific extraction parameters [30].

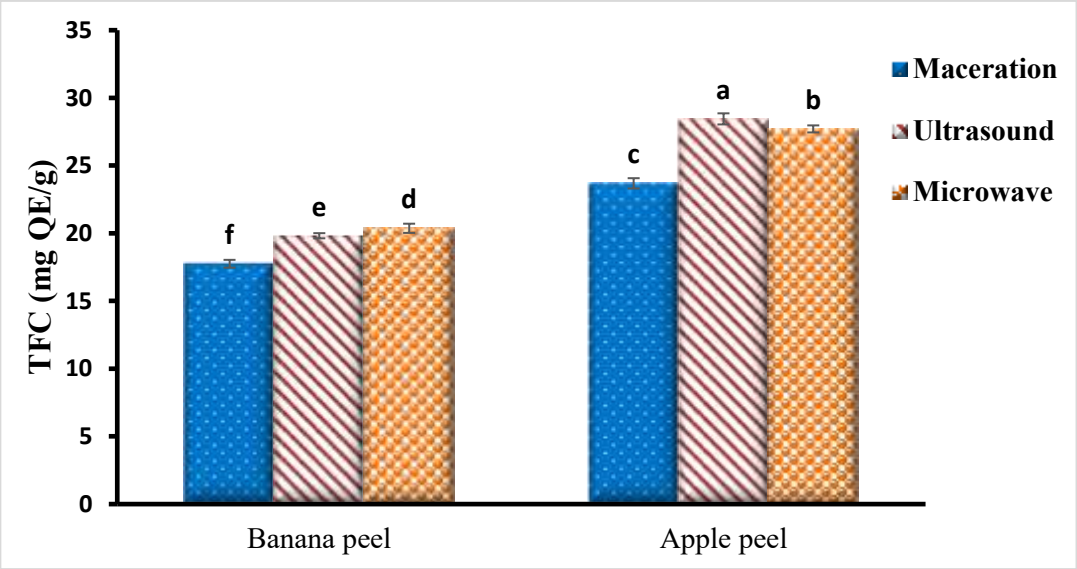

Figure 2. Total flavonoid content (TFC) of AE and BE obtained by maceration, UAE and MAE. Different letters indicate statistically significant differences at the 5% level.

3.3. Antioxidant activity of the extracts

In the present study, the antioxidant potential of AE and BE was rigorously evaluated through two complementary assays: DPPH and FRAP. Statistical analysis via ANOVA confirmed that the antioxidant capacity was significantly modulated by the botanical source, the extraction technique, and the synergistic interaction between these variables ($p<0.05$).

The DPPH assay is fundamentally based on the capability of antioxidant molecules to quench the stable DPPH radical (•DPPH). The mean values for the DPPH radical scavenging percentage across various extraction treatments for both BE and AE are illustrated in Figure 3. The results demonstrated that apple peel extracts possessed a significantly higher radical scavenging capacity than their banana peel counterparts ($p<0.05$). Among the extraction methodologies investigated, ultrasound-assisted extraction (UAE) yielded the most potent antioxidant activity for both matrices, with inhibition percentages reaching 76.59% and 88.47% for BE and AE, respectively. Notably, according to Duncan's multiple range test, no statistically significant difference was observed between the UAE and MAE

(74.24%) treatments for BE ($p>0.0$), suggesting a comparable efficiency of these assisted methods in recovering antioxidant constituents from this specific matrix. Conversely, the lowest radical scavenging efficiency was recorded for the extracts obtained via conventional maceration, which exhibited inhibition values of 68.83% and 74.35% for BE and AE, respectively.

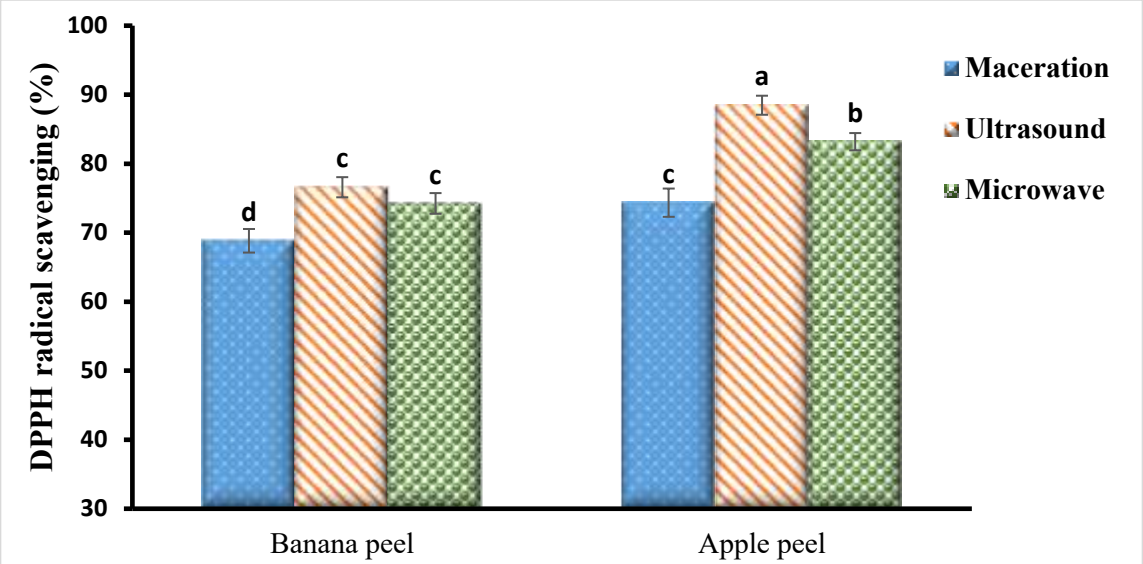

Figure 3. DPPH radical scavenging values of BE and AE obtained by maceration, UAE and MAE. Different letters indicate statistically significant differences at the 5% level.

A comparative analysis of the Ferric Reducing Antioxidant Power (FRAP) for BE and AE obtained through different methodologies is presented in Figure 4. Generally, the FRAP values for AE were significantly higher than those recorded for BE ($p<0.05$). Regarding the extraction techniques for BE, the highest reducing capacity was observed in extracts produced via microwave-assisted extraction (MAE) (%32.69), while the lowest was associated with conventional maceration (%27.89). Similarly, for AE, the MAE treatment yielded the maximal FRAP value (%35.81); however, this result was not statistically different from the values obtained via ultrasound-assisted extraction (UAE) (^35.54) ($p>0.05$). The minimum ferric reducing potential among AEs was again observed in the macerated samples (33.28). These findings are in strong alignment with the elevated TPC and TFC values previously reported for AEs, further validating the instrumental role of phenolic and flavonoid constituents in the overall antioxidant efficacy. The superior antioxidant performance can be ascribed to both the quantitative abundance of these bioactive metabolites and their specific chemical structures, particularly the arrangement of functional groups that facilitate radical scavenging through hydrogen atom donation or electron transfer, as well as the chelation of metal ions [31, 32].

Interestingly, the relative superiority of UAE in the DPPH assay versus the strong performance of MAE in the FRAP assay suggests a differential recovery of specific bioactive fractions by each method. This discrepancy likely stems from the fundamental mechanistic differences between the two assays: while the DPPH assay primarily evaluates the capacity of antioxidants to neutralize free radicals, the FRAP assay specifically measures the reducing power through a single electron transfer (SET) mechanism. Consequently, the varied extraction energies—acoustic cavitation in UAE versus volumetric heating in MAE may preferentially facilitate the liberation of different phenolic subclasses with varying degrees of

hydrogen-donating or electron-reducing potential.

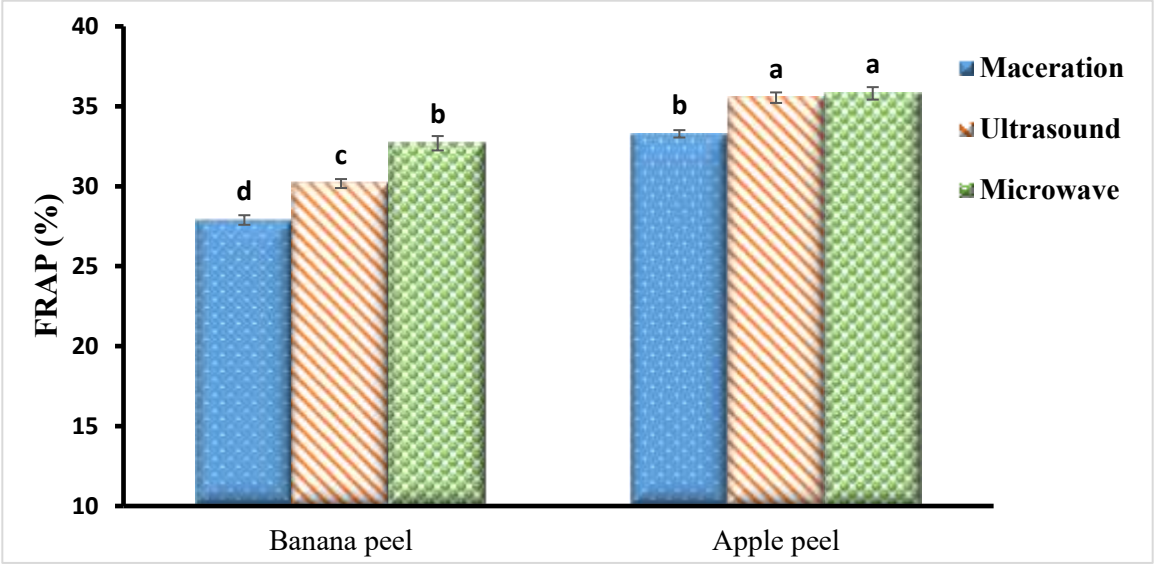

Figure 4. FRAP values of BE and AE obtained by maceration, UAE and MAE. Different letters indicate statistically significant differences at the 5% level

The results obtained in this study are in full consonance with earlier investigations that highlighted the substantial antioxidant potential inherent in BE and AE [8, 9, 26, 34]. Also, the literature corroborates that ultrasound-assisted extracts from various plant matrices such as lemon peel and acai berries often exhibit superior radical scavenging activity due to their enriched phenolic profiles. In contrast, microwave-assisted extracts have been reported in several instances to demonstrate enhanced reducing power [18, 28]. Such findings underscore the promising potential of these extracts to serve as potent natural antioxidants and functional ingredients within food formulations. In this context, previous research has established that plant derived extracts not only provide robust antioxidant protection but also significantly extend the shelf life of diverse food systems, including fresh fruits and meat products, while effectively bolstering the oxidative stability of complex matrices like whipped cream [6, 19, 35, 36].

3.4. Phenolic Compounds Profile and their Correlation with Antioxidant Activity

The qualitative and quantitative profile of phenolic compounds in the ultrasound-assisted extracts was characterized utilizing HPLC analysis to identify the primary bioactive constituents responsible for the documented antioxidant efficacy. The identified phytochemicals, along with their respective retention times (t_R) and relative percentages for both AE and BE, are comprehensively compiled in Table 1. The chromatographic fingerprints revealed that AE possessed a highly diverse polyphenolic constitution, including chlorogenic acid, catechin, epicatechin, procyanidins, rutin, quercetin, and phloridzin. These compounds represent the predominant phenolic classes typically recovered from apple pomace, and they are widely acknowledged for their potent radical scavenging capabilities [39].

According to the chromatographic data (Table 1), flavonoid compounds constituted the dominant fraction of the identified phenolics in AE. Specifically, quercetin represented the most abundant constituent (%44.35), followed by myricetin (%10.22), kaempferol (%3.46), epicatechin (%2.45), and rutin (%1.97). Among the phenolic acids, chlorogenic acid was identified as a major representative, accounting for %9.35 of the total profile. In addition, the substantial presence of procyanidins (%11.25) highlights the critical

contribution of oligomeric flavan-3-ols to the overall phenolic architecture of apple peels. Flavan-3-ols, such as monomeric catechin, epicatechin, and their oligomeric configurations (procyanidins), possess multiple hydroxyl moieties on their aromatic rings. This structural feature enables them to actively neutralize free radicals through both single-electron transfer (SET) and hydrogen atom transfer (HAT) pathways [39]. Consequently, the high relative abundance of these highly active molecules strongly correlates with the elevated TPC, TFC, and superior antioxidant performance observed for AE in both DPPH and FRAP assays.

Conversely, the polyphenolic profile of BE was characterized by a distinct distribution, marked by a higher proportion of specific phenolic acids and procyanidins. As illustrated in Table 1, procyanidins represented the primary constituent in BE, accounting for %40.75 of the total detected compounds. Within the phenolic acid fraction, protocatechuic acid (%4.95), ferulic acid (%4.66), gallic acid (%2.21), and chlorogenic acid (%1.42) were successfully quantified. Despite the presence of these bioactive acids, the cumulative flavonoid content in BE (%16.24) was significantly lower than that of AE (%63.27). This pronounced divergence in the phytochemical fingerprint, particularly the scarcity of highly active flavonoids, serves as a primary factor explaining the lower antioxidant performance of BE in both DPPH radical scavenging and ferric reducing power.

Moreover, key molecules such as chlorogenic acid, quercetin, and epicatechin, which were recovered in highly substantial quantities in AE, are equipped with vicinal dihydroxy substituents (catechol structures) on the B-ring. This specific configuration dramatically facilitates electron donation and radical stabilization, imparting exceptional reducing power to the molecule [39]. The rich abundance of these electron-donating structures provides a solid mechanistic explanation for the superior reducing capacity of AE demonstrated in the FRAP assay. Collectively, the HPLC findings indicate that the specific composition and relative abundance of phenolic subclasses most notably the overwhelming dominance of flavonoids in AE, exert a decisive role in governing the overall antioxidant activity of the investigated extracts.

Table 1. Phenolic profile of the extracts identified by HPLC analysis and their relative percentages (AE and BE).

Phenolic category	Compounds	Retention time (min)	AE (%)	BE (%)
Phenolic acids				
	Protocatechuic acid	6.19	1.38	4.95
	p-Hydroxybenzoic acid	8.35	0.00	2.70
	Gallic acid	18.62	0.15	2.21
	Chlorogenic acid	15.23	9.35	1.42
	Vanillic acid	20.28	0.11	0.74
	Syringic acid	17.46	0.28	0.00
	Ferulic acid	27.36	0.63	4.66
	Ellagic acid	17.5	0.92	0.67
	Rosmarinic acid	24.45	0.00	3.48
	Subtotal		12.82	20.83
Flavonoids				
	Catechin	11.25	0.82	4.45
	Epicatechin	30.44	2.45	6.87
	Rutin	32.57	1.97	1.88
	Kaempferol	36.08	3.46	0.00
	Quercetin	40.88	44.35	2.07
	Myricetin	43.56	10.22	0.97

	Subtotal		63.27	16.24
Proanthocyanidins				
	Procyanidin	31.99	11.25	40.75
	Subtotal		11.25	40.75
Other phenolics				
	Phloridzin	47.29	2.11	4.85
	Quinic acid	14.07	2.60	7.24
	Homovanillic acid	23.19	0.05	0.20
	Unknown		7.35	8.25
	Subtotal		12.11	20.54
Total Phenolics			**99.45**	**98.36**

3.5. Correlation Analysis between Bioactive Compounds and Antioxidant Efficacy

Pearson's correlation coefficients between total phenolic content (TPC), total flavonoid content (TFC), and the antioxidant activities (DPPH and FRAP) of BE and AE are presented in Table 2. The results demonstrated a significant positive correlation between TPC and DPPH radical scavenging activity ($r=0.939$, $p<0.01$). A significant positive correlation was also observed between TPC and FRAP reducing capacity ($r=0.682$, $p<0.01$), which highlights the primary role of phenolic compounds in the antioxidant activity of the extracts.

Total flavonoid content (TFC) exhibited a significant positive correlation with antioxidant activities, with correlation coefficients for DPPH and FRAP recorded as 0.989 and 0.932 ($p<0.01$), respectively. These results indicate that flavonoids contribute significantly to the antioxidant activity of the extracts. Consistent with the phenolic profile obtained via HPLC, the presence of flavonoids such as rutin, quercetin, and their derivatives can largely explain this strong correlation between TFC and antioxidant indices.

A significant positive correlation was identified between the results of the DPPH and FRAP assays ($r=0.818$, $p<0.01$), demonstrating that both assays effectively assess the antioxidant potential of the extracts, although they may exhibit different sensitivities toward various types of antioxidant compounds. This difference in sensitivity may be related to the distinct reaction mechanisms of these assays and the type of dominant phenolic compounds present in the extracts, for instance, some phenolic and flavonoid compounds identified in the HPLC analysis may play a more effective role in electron transfer reactions.

The relatively high correlation between phenolic and flavonoid compounds and antioxidant indexes indicates that these components are among the most important determinants of antioxidant activity in the extracts. These findings are in agreement with previous reports regarding the role of phenolic and flavonoid compounds in neutralizing free radicals and increasing the reducing power of plant extracts [13, 18, 22, 26]. The integration of correlation data and HPLC results clearly illustrates the variations in the type and abundance of phenolic and flavonoid compounds identified within the extracts. These chemical disparities serve as the primary factor underlying the observed differences in the antioxidant activity of the extracts.

Table 2. Pearson correlation coefficients (r) between TPC, TFC, DPPH radical scavenging activity and FRAP values of AE and BE.

	TPC	TFC	DPPH	FRAP
TPC	1			
TFC	0.754**	1		
DPPH	0.939**	0.989**	1	
FRAP	0.682**	0.932**	0.818**	1

**Correlation is significant at the 0.01 level

4- Conclusion

This research demonstrates that apple and banana peels, as fruit processing by-products, serve as significant sources of phenolic and flavonoid compounds with substantial antioxidant activity. The extraction efficiency of these compounds is significantly influenced by the method employed. Among the techniques evaluated, ultrasound-assisted extraction (UAE) proved most effective for recovering phenolic and flavonoid constituents, whereas microwave-assisted extraction (MAE) yielded the highest reducing power in the FRAP assay. AE exhibited higher bioactive content and superior antioxidant capacity compared to BE. HPLC analysis further revealed that AE contains a greater diversity and concentration of phenolic compounds, particularly flavan-3-ols and flavonoids such as catechin, epicatechin, procyanidins, rutin, and quercetin. In contrast, the profile of BE is predominantly composed of phenolic acids. This variation in phenolic composition largely explains the enhanced antioxidant activity observed in AE.

Correlation analysis indicated that total phenolic and, more notably, total flavonoid contents are significantly and positively correlated with antioxidant indices (DPPH and FRAP). These findings underscore the decisive role of these metabolites in the antioxidant efficacy of the extracts. Such results align with the identified phenolic profiles, suggesting that the presence of catechins, procyanidins, and quercetin-derived flavonoids contributes substantially to the observed antioxidant performance.

Overall, the findings highlight the significant potential of AE and BE as valuable natural sources of antioxidants. These fruit wastes represent promising candidates for the recovery of bioactive compounds. The high efficiency of ultrasound-assisted extraction in retrieving phenolic compounds suggests its potential as a rapid, effective, and relatively sustainable approach for extracting bioactives from fruit peel waste for food and pharmaceutical applications. However, this study was limited to two fruit types and a specific set of antioxidant assays. Future research should focus on the precise identification of bioactive constituents, their stability and bioavailability, and their performance within real food matrices or natural preservative and active packaging systems to facilitate the industrial application of these natural resources.

Data Availability

They will be available upon request

Funding Statement

The author declares that no funding was received for this study.

Conflict of Interest

The author confirms that there are no financial conflicts of interest or competing interests in this study.

Author Contributions

All aspects of the study were carried out solely by the author.

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مقاله علمی_پژوهشی

مقایسه‌ی روش‌های استخراج به‌کمک فراصوت، مایکروویو و ماسراسیون بر میزان ترکیبات فنولی و فعالیت آنتی‌اکسیدانی پوست سیب و موز

حمیده شفاهی[۱]، لیلا نوری[۱]، علی نجفی*[۱]، سیدمهدی جعفری[۲و۳]

۱-گروه علوم و مهندسی صنایع غذایی، واحد دامغان، دانشگاه آزاد اسلامی، دامغان، ایران

۲-گروه مهندسی مواد غذایی و طراحی فرآیند، دانشگاه علوم کشاورزی و منابع طبیعی گرگان، گرگان، ایران

۳-مرکز تحقیقات حلال جمهوری اسلامی ایران، اداره کل غذا و دارو، وزارت بهداشت، درمان و آموزش پزشکی، تهران، ایران

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* مسئول مکاتبات:

Ali.Najafi@iau.ac.ir

چکیده

پوست میوه‌ها به‌عنوان یکی از محصولات جانبی صنایع فرآوری میوه، منبعی از ترکیبات زیست‌فعال به‌ویژه فنول‌های دارای خواص آنتی‌اکسیدانی هستند و می‌توانند در توسعه ترکیبات طبیعی مورد استفاده در سیستم‌های غذایی کاربرد داشته‌باشند. هدف این پژوهش، مقایسه کارایی سه روش استخراج شامل ماسراسیون، فراصوت و مایکروویو در بازیابی ترکیبات فنولی و ارزیابی فعالیت آنتی‌اکسیدانی عصاره‌های پوست سیب و موز بود. پس از استخراج، محتوای فنول کل و فلاونوئید کل اندازه‌گیری و فعالیت آنتی‌اکسیدانی با استفاده از آزمون‌های DPPH و FRAP ارزیابی شد. نتایج نشان داد عصاره‌های پوست سیب نسبت به پوست موز به‌طور معنی‌داری($p<۰/۰۵$) دارای مقادیر بالاتری از ترکیبات فنولی و فلاونوئیدی بودند. بیشترین مقدار فنول کل در عصاره‌های استخراج‌شده به روش فراصوت مشاهده شد که برای پوست سیب و موز به‌ترتیب ۴۸/۱۶ و ۴۵/۱۶ (mg GAE/g DW) بودد. همچنین بالاترین مقدار فلاونوئید کل در عصاره‌های فراصوتی پوست سیب و موز به‌ترتیب ۲۸/۴۵ و ۱۹/۸۲ (mg QE/g DW) مشاهده شد. نتایج آزمون DPPH نشان داد که عصاره‌های فراصوتی بیشترین فعالیت مهارکنندگی رادیکال آزاد را ایجادکردند. در حالی که در آزمون FRAP، روش مایکروویو بالاترین قدرت احیاکنندگی را داشت، روش ماسراسیون در هر دو نمونه کمترین میزان ترکیبات فنولی، فلاونوئیدی و فعالیت آنتی‌اکسیدانی را نشان داد. آنالیز HPLC حضور طیفی از ترکیبات فنولی، به‌ویژه فلاونوئیدها و مشتقات فلاوان-۳-اول را تأیید کرد و بین محتوای فنول کل، به‌ویژه فلاونوئید کل با فعالیت آنتی‌اکسیدانی همبستگی مثبت مشاهده شد. به‌طورکلی، فراصوت به‌عنوان کارآمدترین روش برای افزایش بازیابی ترکیبات فنولی و تقویت فعالیت مهارکنندگی رادیکال‌های آزاد بود، در حالی‌که مایکروویو بیشترین اثر را بر قدرت احیاکنندگی داشت. این یافته‌ها بیانگر پتانسیل قابل‌توجه پوست سیب و موز به‌عنوان منابع طبیعی آنتی‌اکسیدان برای کاربرد در سیستم‌های غذایی و توسعه ترکیبات زیست‌فعال است.