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Extraction technology-driven changes in phenolic selectivity, color quality and chemical composition of black carrot pomace extracts

Siyah havuç posası ekstraktlarında fenolik seçicilik, renk kalitesi ve kimyasal kompozisyonun ekstraksiyon teknolojisine bağlı değişimi

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ABSTRACT

Objective: Black carrot pomace is a valuable agro-industrial byproduct rich in anthocyanins and phenolic compounds that can be utilized as natural colorants and functional food ingredients. This study aimed to compare conventional extraction, ultrasound-assisted extraction (UAE), microwave-assisted extraction (MAE), and pressurized liquid extraction (PLE) for the recovery of anthocyanins and phenolic compounds from black carrot pomace and to determine the effects of extraction technology on phenolic selectivity, color quality, antioxidant activity, and the co-extraction of minerals.

Methods: The effects of solvent type [water, acidified water containing 0.01% (w/v) citric acid, and 50% (v/v) ethanol], solid-to-solvent ratio, extraction time, and microwave power were investigated. Extraction performance was evaluated in terms of total monomeric anthocyanin (TMA) and total phenolic content (TPC), antioxidant activity, color characteristics, phenolic profile determined by LC-HRMS, and mineral composition.

ÖZET

Amaç: Siyah havuç posası, doğal renklendirici ve fonksiyonel gıda bileşeni olarak değerlendirilebilecek antosiyaninler ve fenolik bileşikler bakımından zengin, değerli bir agroendüstriyel yan üründür. Bu çalışmada, siyah havuç posasından antosiyanin ve fenolik bileşiklerin geri kazanılmasında geleneksel ekstraksiyon, ultrases destekli ekstraksiyon (UDE), mikrodalga destekli ekstraksiyon (MDE) ve basınçlı sıvı ekstraksiyonu (BSE) yöntemlerinin karşılaştırılması; ekstraksiyon teknolojisinin fenolik seçicilik, renk kalitesi, antioksidan aktivite ve minerallerin birlikte ekstraksiyonu üzerindeki etkilerinin belirlenmesi amaçlanmıştır.

Yöntem: Çözücü türü [su, %0,01 (w/v) sitrik asit içeren asitlendirilmiş su ve %50 (v/v) etanol], katı/çözücü oranı, ekstraksiyon süresi ve mikrodalga gücünün etkileri incelenmiştir. Ekstraksiyon performansı; toplam monomerik antosiyanin (TMA) ve toplam fenolik bileşik (TFB) miktarları, antioksidan aktivite, renk özellikleri, LC-HRMS ile belirlenen fenolik profil ve mineral bileşimi açısından değerlendirilmiştir.

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Results: Among the extraction systems evaluated, MAE provided the highest TMA [1.74 mg cyanidin-3-glucoside equivalents (C3G)/g dry matter], TPC [7.16 mg gallic acid equivalents (GAE)/g dry matter], and antioxidant activity [60.3 µmol Trolox equivalents (TE)/g dry matter] under the determined optimum conditions [540 W, 1:20 solid-to-solvent ratio, 30 s, and 50% (v/v) ethanol]. UAE demonstrated high extraction efficiency under milder processing conditions and achieved antioxidant activity comparable to that obtained by MAE. In both MAE and UAE systems, 50% (v/v) ethanol was identified as the most effective solvent, whereas acidified water did not improve anthocyanin recovery under ultrasound conditions. LC-HRMS analyses showed that extraction technology had significant effects on the selective recovery of phenolic compounds. MAE extracts exhibited a broader phenolic profile and were richer in chlorogenic acid, ferulic acid, isoquercitrin, vitexin, and isorhamnetin, whereas UAE extracts had a more selective phenolic composition. Color analyses showed that MAE and UAE extracts had higher color intensity and lower L* values than the extracts obtained by conventional extraction and PLE. Extraction technology also affected the transfer of minerals, particularly phosphorus and calcium, into the extract phase.

Conclusion: The findings demonstrated that extraction technology determined not only the recovery of anthocyanins and phenolic compounds but also the compositional selectivity, functional quality, and color characteristics of the extracts. Therefore, the extraction method should be selected by considering not only the maximum extraction yield but also the targeted compositional and functional properties of the final product. MAE and UAE emerged as promising green extraction technologies for producing anthocyanin-rich natural colorants and functional ingredients from black carrot processing byproducts.

Key Words: Black carrot pomace, anthocyanin recovery, microwave-assisted extraction, ultrasound-assisted extraction, green extraction

Bulgular: Değerlendirilen ekstraksiyon sistemleri arasında MDE, belirlenen optimum koşullarda [540 W, 1:20 katı/çözücü oranı, 30 saniye ve %50 (v/v) etanol] en yüksek TMA [1,74 mg siyanidin-3-glukozit eşdeğeri (C3G)/g kuru madde], TFB [7,16 mg gallik asit eşdeğeri (GAE)/g kuru madde] ve antioksidan aktivite [60,3 µmol Trolox eşdeğeri (TE)/g kuru madde] değerlerini sağlamıştır. UDE, daha ılımlı işlem koşullarında yüksek ekstraksiyon etkinliği göstermiş ve MDE ile benzer antioksidan aktivite değerlerine ulaşmıştır. Hem MDE hem de UDE sistemlerinde %50 (v/v) etanol en etkili çözücü olarak belirlenmiş; asitlendirilmiş su ise ultrases koşullarında antosiyanin geri kazanımını artırmamıştır. LC-HRMS analizleri, ekstraksiyon teknolojisinin fenolik bileşiklerin seçici olarak geri kazanılması üzerinde önemli etkileri olduğunu göstermiştir. MDE ekstraktları klorojenik asit, ferulik asit, izokuersitrin, viteksin ve izoramnetin bakımından daha zengin ve daha geniş bir fenolik profil sergilerken UDE ekstraktları daha seçici bir fenolik bileşime sahip olmuştur. Renk analizleri, MDE ve UDE ekstraktlarının geleneksel ekstraksiyon ve BSE ile elde edilen ekstraktlara kıyasla daha yüksek renk yoğunluğuna ve daha düşük L* değerlerine sahip olduğunu göstermiştir. Ayrıca ekstraksiyon teknolojisinin, özellikle fosfor ve kalsiyumun ekstrakt fazına geçişini etkilediği belirlenmiştir.

Sonuç: Bulgular, ekstraksiyon teknolojisinin yalnızca antosiyanin ve fenolik bileşiklerin geri kazanımını değil, aynı zamanda ekstraktların bileşimsel seçiciliğini, fonksiyonel kalitesini ve renk özelliklerini de belirlediğini ortaya koymuştur. Bu nedenle ekstraksiyon yöntemi seçilirken yalnızca maksimum ekstraksiyon verimi değil, hedeflenen ürünün bileşimsel ve fonksiyonel özellikleri de dikkate alınmalıdır. MDE ve UDE, siyah havuç işleme yan ürünlerinden antosiyanin bakımından zengin doğal renklendiricilerin ve fonksiyonel bileşenlerin üretiminde kullanılabilecek umut verici yeşil ekstraksiyon teknolojileri olarak öne çıkmıştır.

Anahtar Kelimeler: Siyah havuç posası, antosiyanin geri kazanımı, mikrodalga destekli ekstraksiyon, ultrases destekli ekstraksiyon, yeşil ekstraksiyon

INTRODUCTION

Black carrot (*Daucus carota ssp. sativus var. atrorubens*) is an important source of color and of bioactive compounds that contain high levels of acetylated anthocyanins and is intensively studied both as a food colorant and for its potential health benefits. It has been demonstrated that black carrot anthocyanins consist primarily of cyanidin derivatives and their acetylated forms with aromatic acids (e.g., sinapic, ferulic, and p-hydroxybenzoic acids); these structural characteristics confer high stability against changes in light, heat, and pH. For this reason, black carrot extracts are used in the food industry as a natural colorant and alternative to synthetic dyes in various food matrices, such as beverages, dairy products, confectionery, and baked goods. Review and research articles published in recent years regarding the extraction, structural properties, health effects, and application potential of black carrot anthocyanins clearly highlight the scientific and industrial importance of this species (1).

In the black carrot processing industry, the generation of large amounts of pulp during juice and concentrate production poses a significant challenge in terms of sustainability and waste management. Black carrot pulp is an agro-industrial byproduct containing high-value components such as anthocyanins, total phenolic compounds, dietary fiber, and pectin; however, it is often considered low-value feed material or waste. Therefore, converting black carrot pulp into products such as bioactive compound-enriched extracts, fiber fractions, or pectin is viewed as a significant opportunity in terms of resource efficiency within the circular economy and the food industry. Indeed, studies comparing microwave, ultrasonic, and conventional heating for pectin extraction from black carrot pulp have demonstrated that this byproduct holds high potential for the recovery of functional components (2) .

For many years, traditional extraction methods based on acidic organic solvents have been used for the extraction of anthocyanins from food by-products; however, these methods have drawbacks such as long processing times, high solvent consumption, and often limited yields. Recently, within the framework of the green extraction approach, innovative techniques aimed at obtaining anthocyanins in a shorter time, with less solvent use, and lower energy consumption have come to the fore. Methods such as ultrasound-assisted extraction (UAE), microwave-assisted extraction (MAE), pressurized liquid extraction (PLE) have been successfully applied for the extraction of anthocyanins and phenolic compounds from food by-products. Comprehensive evaluations of these methods regarding extraction kinetics, yield, product quality, and environmental impacts have been detailed in studies published in recent years (3).

A similar trend is evident in the literature on black carrots and black carrot pulp. Gizir et al. (2008) investigated the extraction of anthocyanins from black carrots using pressurized acidified water and demonstrated the effects of temperature and pressure on anthocyanin degradation and yield (4). Agcam et al. (2017), on the other hand, optimized the application of thermo-sonication for the extraction of anthocyanins from black carrot pulp; they reported a significant increase in yield compared to the traditional method due to the synergy of ultrasound and temperature (5). In more recent studies, approaches combining natural deep eutectic solvents and ultrasound for the extraction of anthocyanins from black carrots have led to the development of systems that reduce solvent consumption and are biodegradable and low in toxicity (6). However, a review of the current literature reveals that while studies focusing on a single innovative method for the anthocyanins extraction from black carrot pulp are predominant, there are only a limited number of studies that systematically and directly compare different “green extraction” techniques on the same matrix. In particular, no study has been found that compares ultrasonic-assisted extraction (UAE), microwave-assisted extraction (MAE), and

pressurized liquid extraction (PLE) methods on black carrot pulp—along with process parameters such as extraction time, solid-to-solvent ratio, and solvent type (water, acidified water, 50% ethanol) under the same experimental design. Furthermore, in many studies, the evaluation criteria are largely limited to total monomeric anthocyanins and, occasionally, total phenolic compound content; and parameters that directly influence product quality and potential applications—such as the phenolic and flavonoid composition of the extracts, color properties (L, a, b, color intensity, polymeric color ratio), radical scavenging or antioxidant activity, and mineral profile—are not comprehensively addressed. However, extracts intended for use as natural colorants or functional components in the food industry must be characterized not only by high yield but also by color stability, antioxidant capacity, and nutritional composition.

In this context, the present study examines black carrot pulp as a model agro-industrial material and systematically compares conventional extraction, pressurized liquid extraction (PLE), ultrasound-assisted extraction (UAE), and microwave-assisted extraction (MAE) for the recovery of anthocyanins and phenolic compounds from this matrix. In the study, the effects of key process parameters—such as extraction time, pulp-to-solvent ratio, and solvent type—on total monomeric anthocyanins (TMA), total phenolic contents (TPC), and extraction yield were investigated for each method, and the phenolic and flavonoid profiles, pH, °Brix, color intensity, polymeric color ratio, antioxidant activity, and mineral composition of the extracts obtained under the conditions yielding the highest extraction yield were determined.

MATERIAL and METHOD

Materials

The black carrot pulp, a byproduct generated during the production of black carrot juice and concentrate, was obtained from the Anadolu Etap Penkon A.Ş. facility. Samples were stored at –18 °C until analysis and were thawed under controlled conditions prior to homogenization.

Solvent Selection

Extraction solvents were selected based on the polarity-pH sensitivity of anthocyanins and the conditions recommended in the literature (7-9). Accordingly, three different solvent systems were used: distilled water, 50% (v/v) ethanol in water, and water acidified with 0.01% (w/v) citric acid.

EXTRACTION PROCEDURES

Conventional Extraction

The conventional solid-liquid extraction method was applied based on standard procedures described in the literature (10). Black carrot pulp was extracted at pulp-to-solvent ratios of 1:10, 1:20, and 1:30 (g/mL) in a water bath at 25 °C for 24 hours. After extraction, the samples were filtered and stored in amber bottles at –18 °C for analysis.

Pressurized Liquid Extraction

Pressurized liquid extraction was performed using the Armfield Rapid Extractor (FT111, Armfield, UK). The device operated at a maximum working pressure of 9 bar, and extraction cycles of 12 minutes were conducted, consisting of a 5-minute dynamic phase and a 7-minute static phase per cycle. Extraction studies were conducted using three different solid-to-solvent ratios (1:10, 1:20, 1:30) and three different extraction durations (36, 60 and 84 minutes). All extractions were carried out using water in accordance with the manufacturer's recommendations. Acidified water and ethanol-containing solvents were not evaluated in the PLE system because the manufacturer recommends water as the extraction medium for safe operation of the equipment under the applied pressure and temperature conditions.

Ultrasound-Assisted Extraction

UAE processes were carried out in a temperature-controlled ultrasonic bath (Bandelin Sonorex RK 100 H, Germany; 35 kHz, 80 W). The extraction temperature was maintained at 50 °C using the built-in temperature control system of the ultrasonic bath throughout the extraction process. Three solvent systems were used, with solid-to-solvent ratios of 1:10, 1:20, and 1:30 and extraction times of 1, 10, 30, and 60 minutes. The temperature was maintained at 50 °C during all extractions.

Microwave-Assisted Extraction

MAE processes were performed at atmospheric pressure by coupling a microwave oven (GMOM 25, General Electric, USA) to a condenser. Experiments were conducted at power levels of 180, 360, and 540 W and at durations ranging from 0.5 to 20 minutes. The solid-to-solvent ratios were kept consistent with those used in UAE and conventional extraction (1:10, 1:20, and 1:30, w/v).

ANALYTICAL METHODS

Moisture Content

Moisture content was measured at 105 °C with an infrared moisture analyzer (HB43-S, Mettler Toledo, USA).

pH and Brix

Soluble solids content was determined using a refractometer (RX5000α, Atago, Japan), while pH values were measured directly with a pH meter (AD8000, Adwa Instruments, Romania).

Calculation of Yield (Relative Anthocyanin Recovery)

To facilitate comparisons among extraction conditions, relative anthocyanin recovery (%) was calculated according to Equation (1). The highest total monomeric anthocyanin (TMA) value obtained among all extraction treatments was considered as 100%, and the anthocyanin contents obtained under the remaining conditions were expressed relative to this value.

$$\text{Relative anthocyanin recovery (\%)} = \frac{\text{TMA under a given condition (mg/gDM)}}{\text{Highest TMA obtained among all treatments (mg/gDM)}} \times 100 \quad (1)$$

where TMA under a given condition represents the total monomeric anthocyanin content measured for a specific extraction treatment, and the highest TMA obtained among all treatments corresponds to the maximum anthocyanin value observed in the study.

Total Monomeric Anthocyanin (TMA)

The total monomeric anthocyanin content was determined by the pH differential method, which exploits the reversible transformation of anthocyanins from the colored flavylium form at pH 1.0 to the colorless carbinol form at pH 4.5. Samples were diluted 1:35 with 0.025 M potassium chloride buffer (pH 1.0) and 0.4 M sodium acetate buffer (pH 4.5). After 15 minutes of equilibration, absorbance was measured at 516 nm and 700 nm using a UNICAM UV2/UV VIS spectrophotometer (11) .

The net absorbance (A) was calculated using formula (2)

$$A = (A_{516} - A_{700})\ \text{at pH 1.0} - (A_{516} - A_{700})\ \text{at pH 4.5} \quad (2)$$

Total monomeric anthocyanin content was expressed as cyanidin-3-glucoside equivalents (C3G/g DM, MW: 449.2 g/mol, ε: 26,900 L/mol·cm).

Total Phenolic Content (TPC)

The total phenolic content (TPC) was quantified using the Folin–Ciocalteu reagent-based colorimetric assay. The procedure involved mixing 2.5 mL of 0.2 N Folin-Ciocalteu reagent, 5 mL of sodium carbonate solution (75 g/L), and 0.5 mL of the diluted sample. The mixture was incubated for 1 hour at room temperature, after which the absorbance was measured at 720 nm. Results were expressed as mg gallic acid equivalent/g dry matter (12).

Determination of the phenolic profile by Liquid Chromatography-High-Resolution Mass Spectrometry (LC-HRMS)

The phenolic compound profiles of the extracts with the highest anthocyanin content were determined using an LC-HRMS system. UHPLC–HRMS analyses were performed using a DIONEX UltiMate 3000 RS UHPLC system coupled with an Exactive Plus Orbitrap high-resolution mass spectrometer (Thermo Fisher Scientific, USA) fitted with a heated electrospray ionization (HESI) source. Separation was conducted on a 3C18-EB UHPLC column (100 × 2 mm, COSMOSIL) using ultrapure water containing 0.5% (v/v) acetic acid (A) and LC–MS-grade methanol (B) as the mobile phases. The column was maintained at 30 °C, while the flow rate and injection volume were set at 0.3 mL min^{-1} and 20 µL, respectively.

Extracted samples were dissolved in a methanol: acetic acid-in-water (1:1, v/v) mixture prior to analysis, filtered through a 0.22 µm PTFE filter, and injected into the instrument. The identification of phenolic compounds was performed using the retention times and high-resolution mass spectra of standard compounds.

Color measurements

Color analyses were performed by directly reading the L*, a*, and b* values of the extracts using a Konica-Minolta (USA) colorimeter. The average of three readings was recorded. L* represents lightness (brightness). L* can range from a maximum of 100 (white) to a minimum of 0 (black). Positive a* and b* values indicate red and yellow, respectively, while negative a* and b* values indicate green and blue, respectively.

Color intensity and polymeric color (PC)

Color intensity was evaluated because it is an important indicator of the stability and visual quality of anthocyanin-rich extracts. The color intensity of the extract is directly proportional to its anthocyanin content. % PC is also a crucial parameter, as it provides insight into the polymeric compounds present in the extract. The % PC serves as an indicator of anthocyanin degradation in the polyphenolic extract. Color density and polymeric color were analyzed according to the method described by Khandare et al. (2011) (13). The method is based on the principle that natural monomeric anthocyanins react with sodium bisulfite to form colorless compounds; polymeric anthocyanin-tannin complexes and melanoidin pigments retain their color despite the bleaching effect of bisulfites. Color intensity and the percentage of polymeric color were determined by measuring absorbance at the sample's maximum wavelength before bisulfite addition and at 420 nm after bisulfite treatment. These values were calculated based on the difference in absorbance between the two conditions.

Determination of DPPH radical scavenging activity

The method was performed according to the procedure developed by Brand-Williams et al. (1995), which uses DPPH (1,1-diphenyl-2-picrylhydrazine) to determine the radical scavenging activity of samples (14). The method is based on detecting the decrease in color intensity that results from the scavenging of the DPPH radical by antioxidant molecules, measured as absorbance at 515 nm, after treatment with different sample concentrations. For this purpose, a 6×10^{-4} M DPPH stock solution was first prepared. Subsequently, the DPPH stock solution was diluted with methanol to prepare a 6×10^{-5} M DPPH analytical standard. After appropriate dilution, 0.1 mL of each sample was taken, and 3.9 mL of the DPPH analytical standard was added. Absorbance measurements of the samples against methanol at 515 nm were recorded, and the reaction was determined to be complete after 30 minutes. The DPPH radical concentration of the samples was calculated using the equation derived from a calibration curve constructed from absorbance measurements of DPPH solutions at different concentrations at 515 nm. The percentage of DPPH remaining in the solution at the equilibrium point was calculated for all samples using equation 3.

$$Residual\ DPPH(\%) = \frac{DPPH_T}{DPPH_{T=0}} x100 \quad (3)$$

(DPPH)T represents the concentration of the DPPH radical at the equilibrium point (30th minute), while (DPPH)T=0 represents the initial concentration.

TEAC antioxidant capacity determination

The TEAC decolorization method described by Miller et al. (1995) was used to determine total antioxidant capacity (15). The method is based on the comparison between the antioxidant capacity of Trolox (6-hydroxy-2,5,7,8-tetramethylchromen-2-carboxylic acid) and that of the sample. The first step of the method is the preparation of the ABTS radical cation. For this purpose, potassium persulfate ($K_2S_2O_8$) is added to an ABTS solution (2,2'-azinobis-3-ethylbenzothiazol-6-sulfonic acid). The ABTS radical cation is a dark blue solution that exhibits maximum absorbance at a wavelength of 734 nm. When the solution is treated with an antioxidant, its color lightens. The lightening of the sample's color serves as a measure of antioxidant capacity, which is expressed in Trolox equivalents. For the analysis, phosphate buffer (50 mmol/L), ABTS stock solution (7000 µmol/L), ABTS working solution (140 µmol/L), and Trolox stock solution (2.5 mmol/L) were prepared first. The samples were diluted with phosphate buffer to adjust their pH to 7.2–7.4. Two cuvettes were prepared for the measurements: one for the blank and one for the sample. Both cuvettes were filled with 1900 µL of ABTS assay solution; the blank received 100 µL of phosphate buffer solution, and the sample received 100 µL of sample solution. The solutions in the cuvettes were mixed, and the absorbance of the sample was measured against the blank at 6 minutes. Absorbance measurements were performed at a wavelength of 734 nm using a spectrophotometer. Antioxidant capacity was calculated by converting the absorbance values to their Trolox equivalents using the Trolox calibration curve.

Determination of Mineral Composition

The mineral profile of the extracts was determined according to NMKL Method 161. Concentrations of Mg, Na, Ca, K, and P were determined (16). Working solutions prepared from 1000 µg/mL standard stock solutions of the elements were analyzed using the instrument to establish a standard calibration curve with at least three data points. During the sample preparation stage, a closed-system microwave (220 PSI, 180 °C, Ramp Time 15–25 min, Hold Time 10–15 min) was used. The sample, weighed to fall within the calibration curve range, was subjected to a combustion process in the microwave after adding 8 mL of concentrated HNO_3 and 2 mL of H_2O_2. Following the combustion process, the samples were filtered through ash-free filter paper and analyzed by the instrument.

Statistical analysis

Statistical analyses were performed using MINITAB version 15.1.1.0 (Minitab Inc., State College, PA, USA). All extraction experiments were conducted in triplicate (n = 3), and all analytical measurements were performed in duplicate. Results are presented as mean ± standard deviation. Differences among means were evaluated by one-way analysis of variance (ANOVA), and significant differences were determined at the 95% confidence level ($p < 0.05$). Mean comparisons were performed using Tukey's multiple comparison test.

RESULTS

Effect of extraction technology, time, and solid-to-solvent ratio on anthocyanin and phenolic recovery

When the solid-to-solvent ratio and extraction time were evaluated together, the recovery of anthocyanins and total phenolic contents from black carrot pulp exhibited distinct kinetic profiles depending on the extraction technique applied. In the conventional maceration system, increasing the solvent concentration (from 1:10 to 1:30) produced

statistically significant increases in both TMA and TPC; however, these increases were observed only after a 24-hour extraction period (Figure 1).

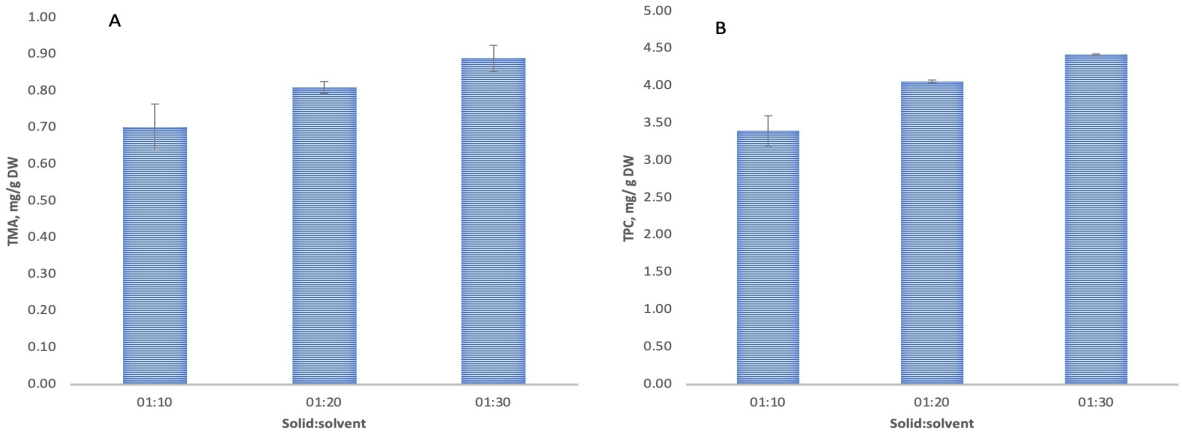


Figure 1. Influence of solid-to-solvent ratio on TMA and TPC obtained by conventional extraction. Data are expressed as mean ± standard deviation

In pressurized liquid extraction (PLE), the highest TMA and TPC values were obtained at a solid-to-solvent ratio of 1:20 and an extraction time of 84 min (Figure 2-A). Although PLE reduced the extraction time substantially compared with conventional extraction, the maximum TMA values obtained by PLE were statistically similar to those achieved by conventional extraction. Ultrasound-assisted extraction (UAE) resulted in rapid anthocyanin and phenolic recovery. At a solid-to-solvent ratio of 1:30, a substantial proportion of both TMA and TPC was recovered within the first 10 min of extraction (Figure 2-B). Similar TMA and TPC levels were also achieved at lower solid-to-solvent ratios (1:10 and 1:20) when longer extraction times were applied. In microwave-assisted extraction (MAE, 360 W), the effects of the solid-to-solvent ratio and extraction time on TMA content were limited (Figure 2-C). Anthocyanins were largely extracted within the first few minutes of the extraction process. In contrast, TPC values increased with increasing solvent loading.

Solvent-dependent extraction selectivity

When comparing solvent types in the UAE system, the highest TMA and TPC values were obtained with a 50% (v/v) ethanol mixture. Under these conditions, TMA and TPC increased by approximately 78% and 48%, respectively, compared with conventional extraction (Table 1). In contrast, the use of acidified water resulted in an average decrease of 15% in both TMA and TPC values.

Solvent type significantly affected extraction performance in microwave-assisted extraction (MAE). When water and acidified water were used as extraction solvents, TMA and TPC contents increased by approximately 35% and 55%, respectively, compared with conventional extraction. In contrast, MAE performed with 50% (v/v) ethanol resulted in approximately 95% and 60% increases in TMA and TPC contents, respectively (Table 1). In addition, substantial anthocyanin recovery was achieved within the first few minutes of extraction when 50% (v/v) ethanol was used. However, the optimal conditions for TMA and TPC recovery did not completely overlap.

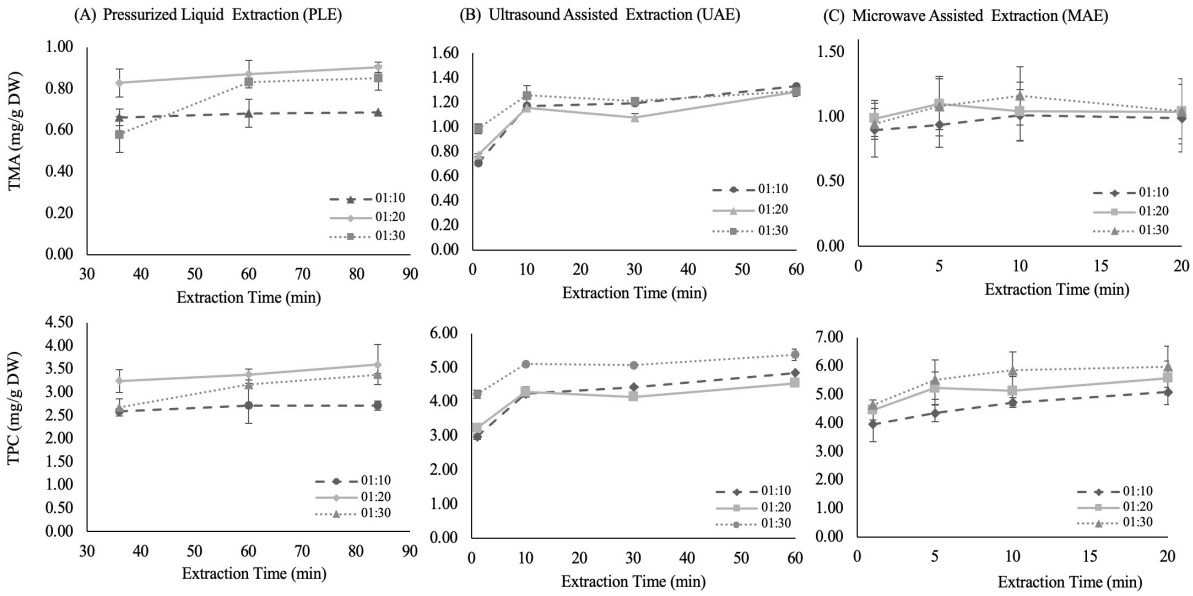


Figure 2. Influence of extraction time and solid-to-solvent ratio on TMA and TPC obtained by, pressurized liquid, ultrasound-assisted, and microwave-assisted extraction using water as a solvent. For MAE, an intermediate microwave power level (360 W) was selected to allow comparative extraction behavior evaluation among extraction techniques. Data are expressed as mean ± standard deviation.

Table 1. Comparison of different solvents and conventional extraction methods that achieve maximum extraction

Extraction type	Solvent type	Solid:solvent ratio	Time	Microwave power	TMA*	TPC**
Conventional	Water	1:30	24 hours	-	0.89±0.04^{dB}	4.42±0.01^{cB}
UAE	Water	1:30	60 min		1.29 ±0.01^{b}	5.38 ± 0.16^{b}
	Acidic water	1:30	10 min		1.08 ±0.03^{c}	4.46 ± 0.05^{c}
	Ethanol (50% (v/v)	1:30	60 min		1.58 ± 0.02^{a}	6.53 ± 0.10 ^{a}
MAE	Water	1:30	10 min	180 W	1.20±0.25^{B}	5.96±0.85^{AB}
	Acidic water	1:30	10 min	540 W	1.40 ± 0.06^{B}	6.13±0.33^{AB}
	Ethanol (50% (v/v)	1:20	30 sec	540 W	1.74 ± 0.03^{A}	7.16 ± 0.12^{A}

Different lowercase letters indicate significant differences among UAE treatments and the conventional extraction ($p < 0.05$).

Different uppercase letters indicate significant differences among MAE treatments and the conventional extraction ($p < 0.05$).*mg cyanidin-3-glucoside/g DM, **mg gallic acid/g DM

Effect of microwave power

In MAE performed with water, increasing microwave power at solid-to-solvent ratios of 1:10, 1:20, and 1:30 did not produce statistically significant changes in TMA or TPC levels ($p > 0.05$). In MAE performed with acidified water, the effect of microwave power varied depending on the solid-to-solvent ratio and extraction time. While increasing microwave power did not significantly affect TMA and TPC levels at a 1:10 solid-to-solvent ratio, a positive effect of power was observed at shorter extraction times when 1:20 and 1:30 ratios were applied. At a 1:20 solid-to-solvent ratio, increasing microwave power from 180 W to 540 W increased TMA content by approximately 28% and 19% at the 1st and 5th min of extraction, respectively ($p < 0.05$) (Figure 3-A). However, no significant effect of power on TMA content was observed at longer extraction times. Similarly, at a 1:30 solid-to-solvent ratio, increasing microwave power from 180 W to 540 W increased TMA content by approximately 29% (Figure 3-B) and TPC content by approximately 32% (Figure 3-D), but only during the first minute of extraction. Similarly, in 50% (v/v) ethanol, increasing microwave power at a 1:10 solid-to-solvent ratio did not affect TMA and TPC extraction ($p > 0.05$). In contrast, at a 1:20 solid-to-solvent ratio, increasing the microwave power from 180 W to 540 W during the first 30 s of extraction resulted in an approximately 60% increase in TMA content (Figure 3-C). However, the effect of microwave power on TMA became insignificant as extraction time increased. At a 1:30 solid-to-solvent ratio, increasing microwave power did not affect TMA content (data not shown), whereas TPC content increased by approximately 29% during the 30-s extraction period (Figure3-D). Considering all process variables simultaneously, the highest TMA value in 50% (v/v) ethanol was obtained at a solid-to-solvent ratio of 1:20, a microwave power of 540 W, and an extraction time of 30 s, reaching 1.74 ± 0.03 mg cyanidin-3-glucoside/g DM. In contrast, the highest TPC value (7.70 ± 0.29 mg GAE/g DM) was achieved at a solid-to-solvent ratio of 1:30, a microwave power of 180 W, and an extraction time of 5 min.

Quality attributes of optimal extracts: pH, Brix, antioxidant activity

When evaluating the quality attributes of extracts containing maximal anthocyanin content, it was observed that the extraction technology significantly affected not only the recovery of TMA and TPC but also the functional properties of the extracts (Table 2). In particular, extracts obtained via UAE and MAE exhibited higher values of TMA, TPC, and antioxidant activity than those obtained from conventional and pressure-assisted extraction systems. The MAE extracts exhibited the highest values for TMA (1.74 mg cyanidin-3-glucoside/g DM), TPC (7.16 mg GAE/g DM), and TEAC antioxidant activity (60.3 µmol TEAC/g DM). The UAE extract similarly exhibited high TMA (1.58 mg cyanidin-3-glucoside/g DM), TPC (6.53 mg GAE/g DM), and antioxidant activity (53.5 µmol TEAC/g DM).

Using the maximum TMA and TPC values obtained by MAE as benchmarks, conventional and pressure-assisted extraction achieved approximately 50% of the anthocyanin recovery obtained by MAE, whereas UAE reached nearly 90% of the maximum anthocyanin recovery. Similarly, when the highest TPC value was used as the reference, UAE achieved approximately 91% phenolic recovery. The pH values of extracts exhibiting maximum anthocyanin content ranged between 4.3 and 5.2. UAE and MAE extracts showed higher pH values than those obtained by conventional extraction. The °Brix values of UAE and MAE extracts were 16.9%, which was significantly higher than those obtained by conventional and pressure-assisted extraction methods. DPPH radical scavenging activity and TEAC antioxidant activity results showed that UAE and MAE extracts exhibited higher antioxidant capacities than extracts obtained by conventional and pressure-assisted extraction methods. In particular, MAE extracts showed the lowest residual DPPH percentage and the highest TEAC value among all extraction methods (Table 2).

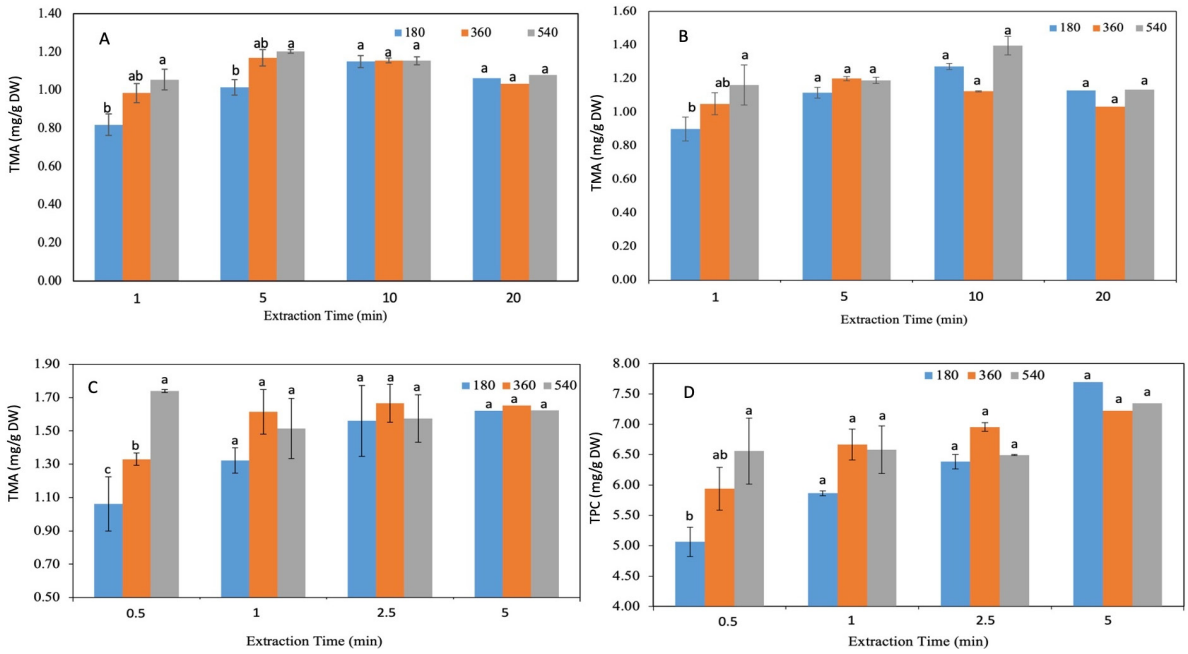


Figure 3. Effect of microwave power on TMA and TPC recovery from black carrot pomace under different solvent systems and solid-to-solvent ratios. (A) TMA obtained by microwave-assisted extraction with acidified water at a 1:20 solid-to-solvent ratio. (B) TMA obtained by microwave-assisted extraction with acidified water at a 1:30 solid-to-solvent ratio. (C) TMA obtained by microwave-assisted extraction with 50% (v/v) ethanol at a 1:20 solid-to-solvent ratio. (D) TPC obtained by microwave-assisted extraction with 50% (v/v) ethanol at a 1:30 solid-to-solvent ratio. Data are expressed as mean ± standard deviation. Different letters indicate statistically significant differences between microwave power levels within the same extraction time ($p < 0.05$).

Table 2. pH, Brix, TMA, TPC, Radical Scavenging, and Antioxidant Activity values of extracts with the highest anthocyanin content

Conditions	TMA*	TPC **	pH	Brix ***	Radical Scavenging Activity ****	Antioxidant Activity *****
Conventional	0.89 ±0.04^{b}	4.42 ±0.01^{b}	4.4 ±0.19^{b}	0.13 ± 0.01^{b}	69.3^{a}	34.7 ±0.0 ^{b}
PLE (1:20 solid-to-solvent ratio, 84 minutes)	0.90 ±0.03^{b}	3.60 ±0.43^{b}	4.3 ±0.16^{b}	0.15 ± 0.03^{b}	64.2^{b}	33.2 ±0.0 ^{b}
UAE (1:30 solid-to-solvent ratio, 50% (v/v) ethanol, 60 min.)	1.58 ±0.02^{a}	6.53 ±0.10^{a}	4.9 ±0.04^{a}	16.92 ±0.06^{a}	59.8^{b}	53.5 ±0.04^{a}
MAE (1:20 solid:solvent, 540 W, 50% (v/v) ethanol, 0.5 min.)	1.74 ±0.03^{a}	7.16 ±0.12^{a}	5.2 ±0.05^{a}	16.87 ±0.01^{a}	50.4^{b}	60.3 ±0.02^{a}

* mg cyanidin-3-glucoside/g DM, ** mg gallic acid equivalent/g DM, *** %, **** % residual DPPH, ***** µmol TEAC/g DM,

a, b Differences between values denoted by different letters in the same column are statistically significant.

LC-HRMS fingerprinting of optimal UAE and MAE extracts

LC-HRMS analysis of the UAE and MAE extracts exhibiting the highest anthocyanin yields revealed that both extracts were characterized by a phenolic profile dominated by hydroxycinnamic acids and flavonol derivatives. Chlorogenic acid, quinic acid, quercetin, and isoquercitrin were identified as common compounds in both extracts (Table 3). Although the two extracts shared a similar phenolic core, the MAE extract displayed a broader and more intense phenolic fingerprint. Chlorogenic acid was the predominant compound in both extracts, reaching 3859.6µg/g extract in UAE and 7940.6 µg/g extract in MAE. UAE exhibited a narrower phenolic profile than MAE. Major compounds such as chlorogenic acid and quercetin were detected in UAE extracts; however, ferulic acid, vitexin, and isorhamnetin, which were identified in MAE extracts, were not detected. In contrast, luteolin-7-rutinoside was detected exclusively in the UAE extract. In addition, quercetin and isoquercitrin concentrations were higher in MAE than in UAE (Table 3).

Table 3. Phenolic compound profile and calculated concentrations of optimal UAE and MAE extracts determined by LC-HRMS

Compound	Class	UAE (µg/g extract)	MAE (µg/g extract)
Ethyl ester of protocatechuic acid	Hydroxybenzoic acid derivative	29.06	51.33
Ferulic acid	Hydroxycinnamic acid	N/A	3.26
Chlorogenic acid	Hydroxycinnamic acid/ caffeoylquinic acid	3859.58	7940.61
Quinic acid	Organic acid / chlorogenic acid-related structure	4803.13	1621.56
Vitexin	Flavone C-glucoside	N/A	5.06
Luteolin-7-rutinoside	Flavone glycoside	27.56	N/A
Quercetin	Flavonol	500.87	635.80
Isoquercitrin	Quercetin glycoside	204.60	351.80
Isorhamnetin	Methoxylated flavonol	N/A	15.49
Vitamin C	Antioxidant vitamin	4.07	3.16

Color quality and polymeric color

Extraction technology affected not only the recovery of TMA but also the color characteristics of the extracts, including brightness and color intensity (Table 4). UAE and MAE extracts exhibited lower L* values and higher color intensity than extracts obtained by conventional and pressure-assisted extraction methods. A significant decrease in L* values was observed in UAE and, particularly, MAE extracts compared with conventional and pressure-assisted extraction methods (Table 4). Among the color parameters, the lowest a* (redness) value was observed in MAE extracts.

Mineral co-extraction profile

Extraction technology influenced not only the recovery of phenolic compounds but also the transfer of inorganic components into the extract phase (Table 5). Na content was lower in all alternative extraction

techniques than in conventional extraction (Table 5). No significant differences were observed in Mg and K concentrations among the extraction methods ($p > 0.05$). In contrast, significant differences were detected for Ca and P. Ca concentrations were 17.0 and 17.3 ppm in conventional and PLE extracts, respectively, whereas they increased to 20.7 and 19.0 ppm in UAE and MAE extracts. The most pronounced difference among the mineral elements was observed for phosphorus (P) (Table 5). The P content of the UAE extract was approximately 7.7-fold higher than that of the conventional extract.

Table 4. Color values of extracts with maximum anthocyanin content

Conditions	L*	a*	b*	Color intensity	Polymeric color ratio (%)
Conventional	27.23±0.07^{a}	10.89±0.08^{a}	2.29 ± 0.05 ^{a}	0.36 ± 0.03 ^{d}	23^{b}
PLE (1:20 solid-to-solvent ratio. 84 minutes)	26.2 ± 0.63 ^{a}	11.6 ± 0.43 ^{ab}	2.9 ± 0.97^{a}	0.69 ± 0.04 ^{c}	23^{b}
UAE (1:30 solid-to-solvent ratio, 50% (v/v) ethanol, 60 min.)	23.0 ± 0.1 ^{b}	10.6 ± 0.2 ^{b}	2.6 ± 0.0 ^{a}	1.20±0.00^{b}	40^{a}
MAE (1:20 solid:solvent, 540 W, 50% (v/v) ethanol, 0.5 min.)	21.0 ± 0.014 ^{c}	7.4 ± 0.028 ^{c}	2.9 ± 0.035 ^{a}	1.59 ± 0.04 ^{a}	35^{a}

Different letters within the same column indicate statistically significant differences among values ($p < 0.05$).

Table 5. Mineral profile of extracts with the highest anthocyanin content

Conditions	Na (ppm)	Mg (ppb)	P (ppb)	K (ppm)	Ca (ppm)
Conventional	42.4 ±4.0^{a}	3759 ±319^{a}	749.5 ±11.5^{c}	61.0 ±8.2^{a}	17.0 ±0.5^{b}
PLE (1:20 solid-to-solvent ratio, 84 minutes)	20.7 ± 2.1 ^{b}	3199 ±1081^{a}	2606.3 ±15.7^{b}	68.9 ±12.5^{a}	17.3 ± 0.0 ^{b}
UAE (1:30 solid-to-solvent ratio, 50% (v/v) ethanol, 60 min.)	26.9 ±2.1^{b}	3180 ±11.7^{a}	5748.2 ±243^{a}	53.5 ±0.1^{a}	20.7 ±0.4^{a}
MAE (1:20 solid:solvent, 540 W, 50% (v/v) ethanol, 0.5 min.)	30.2 ±0.2^{b}	2366 ±537.7^{a}	3493.2 ±380.7^{b}	55.2 ±3.2^{a}	19.0 ±0.9^{a}

Different letters within the same column indicate statistically significant differences among values ($p < 0.05$).

DISCUSSION

Extraction efficiency

The observed dependence of anthocyanin recovery on solvent loading suggests that mass transfer during conventional extraction is largely governed by diffusion processes. Under these conditions, increasing solvent availability may enhance the concentration gradient between the plant matrix and the extraction medium, thereby facilitating anthocyanin release. Similar observations have been reported for black carrot and other phenolic-rich plant by-products, where improved extraction efficiency often requires higher solvent volumes and extended extraction times (17). The results suggest that pressure-assisted extraction may enhance solvent penetration into the plant matrix, allowing efficient recovery of anthocyanins and phenolic compounds at lower solvent volumes. However, despite the accelerated extraction process, the comparable TMA values observed between PLE and conventional extraction indicate that pressure mainly improves extraction behaviour rather than the ultimate extraction yield. Similar findings have been reported in previous studies, which showed that pressure can increase diffusion rates and mass transfer efficiency without necessarily leading to higher maximum recoveries (3). The rapid recovery observed during the initial stage of UAE may be attributed to acoustic cavitation, which enhances mass transfer by disrupting plant cell structures and facilitating the release of soluble phenolic compounds into the extraction medium. The ability to obtain comparable TMA and TPC levels at lower solvent loadings by extending extraction time suggests a compensatory relationship between solvent availability and extraction duration. Similar observations have been reported by Tao et al. (2014), who demonstrated that the majority of anthocyanins and phenolic compounds are recovered during the first 10 min of ultrasound-assisted extraction, after which the extraction rate gradually decreases. These findings indicate that UAE can achieve high extraction efficiency without requiring excessive solvent volumes or prolonged processing times (8). The rapid recovery of anthocyanins observed during the initial stage of extraction suggests that microwave energy promotes rapid and homogeneous heating of the plant matrix, facilitating the release of soluble anthocyanin fractions. The different responses of TMA and TPC to extraction conditions further indicate that the optimal extraction parameters for anthocyanins and other phenolic compounds may not fully overlap in MAE. Similar observations have been reported in the literature, where short-duration microwave treatments were found to be sufficient for anthocyanin recovery, whereas longer extraction times promoted the extraction of a broader phenolic profile (18). In general, the extraction techniques exhibited fundamentally different recovery dynamics. While UAE provided rapid and efficient recovery through the fragmentation of plant tissues via cavitation, MAE facilitated the rapid release of anthocyanins owing to volumetric heating and enhanced solvent penetration. These findings indicate that the extraction performance of black carrot pulp cannot be interpreted solely based on maximum recovery values; rather, extraction kinetics, solvent efficiency, and process-specific selectivity must also be considered.

Effect of solvent composition

The superior performance of 50% (v/v) ethanol suggests that solvent polarity and solubility characteristics play a critical role in the recovery of anthocyanins and phenolic compounds during UAE. Similar findings have been reported for grape pomace, apple pomace, and citrus by-products, where ethanol-containing solvent systems enhanced phenolic recovery by 6–35% (19). Conversely, the lower recoveries observed in acidified water may be associated with the highly acylated structure of black carrot anthocyanins and the potential formation of free radicals during ultrasonication under acidic conditions. Previous studies have shown that organic

acids can accelerate free radical formation during sonication, thereby promoting oxidative degradation reactions (20). Likewise, Machado et al. (2017) reported lower anthocyanin and phenolic recoveries in acid-assisted UAE systems compared with ethanol-containing solvents (21). Collectively, these findings suggest that acidification does not provide an advantage for anthocyanin extraction and may adversely affect pigment stability in anthocyanidin-rich matrices such as black carrot pomace. The superior performance of 50% (v/v) ethanol indicates that solvent properties play a crucial role in MAE efficiency. Ethanol–water mixtures may not only improve the solubility of phenolic compounds but also enhance microwave energy absorption and volumetric heating within the extraction matrix. Similar observations have been reported for various plant materials, including tea leaves, Herba Epimedii, and sugar beet molasses, where ethanol–water mixtures containing 50–70% ethanol were identified as highly effective extraction media. Routray and Orsat (2012) further emphasized that solvent dielectric properties and dipole rotation behavior directly influence energy transfer efficiency during microwave-assisted extraction. The rapid anthocyanin recovery observed during the initial extraction period supports the process-intensification effect of microwave energy. Furthermore, the different optimal conditions obtained for TMA and TPC suggest that anthocyanins and other phenolic compounds exhibit distinct extraction behaviors under microwave treatment. Therefore, solvent composition and process parameters appear to influence not only overall recovery but also extraction selectivity (22). When all findings are considered collectively, solvent composition can be regarded as a key determinant of anthocyanin and total phenolic compound recovery in both UAE and MAE. Nevertheless, the effectiveness of a given solvent depends strongly on the extraction mechanism, emphasizing that solvent selection and process optimization should be performed in an integrated manner. Under the conditions investigated, a 50% (v/v) ethanol yielded the highest recovery of anthocyanins and total phenolic compounds in both extraction systems. Overall, the results demonstrate that maximizing extraction efficiency requires consideration of the synergistic relationship between solvent properties and the mode of energy transfer employed during extraction.

Microwave power and extraction selectivity

Microwave power is one of the fundamental process parameters governing energy transfer, heating rate, and matrix disruption during microwave-assisted extraction. However, the present findings indicate that its effect should not be considered independently, but rather in combination with extraction time, solid-to-solvent ratio, and solvent composition. The absence of significant changes in TMA and TPC levels with increasing microwave power in aqueous systems suggests that anthocyanin and phenolic compound recovery may be constrained not only by energy input but also by mass-transfer limitations and solvent–matrix interactions. Therefore, the benefits of microwave irradiation may only be fully realized when combined with a solvent system capable of efficiently solubilizing the target compounds. These findings further emphasize the importance of solvent selection for maximizing the effectiveness of microwave-assisted extraction. The greater effectiveness of microwave power during the initial stages of extraction suggests that increased energy input primarily enhances mass-transfer rates before equilibrium conditions are reached. As extraction time increases, the contribution of higher microwave power appears to diminish, indicating that the extraction system gradually approaches equilibrium and becomes less responsive to additional energy input. The substantial increase in TMA observed during the initial extraction period at a 1:20 solid-to-solvent ratio indicates that high microwave power can enhance anthocyanin release within a short processing time when ethanol is used as the extraction medium. However, the diminishing effect of microwave power with increasing extraction

time suggests that the advantage of high-power treatment is primarily limited to the early stages of extraction. Furthermore, the different responses of TMA and TPC to microwave power indicate that anthocyanins and other phenolic compounds may exhibit distinct extraction behaviors, suggesting that different phenolic classes are recovered within different power–time windows. The different optimal conditions observed for TMA and TPC indicate that anthocyanins and other phenolic compounds exhibit distinct extraction behaviors during microwave-assisted extraction. Short-duration, high-power treatments appear to favor anthocyanin recovery, whereas the extraction of a broader range of phenolic compounds may benefit from longer extraction times and higher solvent availability. These findings suggest that the optimal MAE conditions depend on the target compound group and that process parameters should be selected according to the desired extraction outcome. Similar observations have been reported in previous studies. Recent MAE investigations have demonstrated that optimal extraction performance is achieved not only through high microwave power but also through an appropriate balance between power, extraction time, and solvent composition (22). Likewise, Aliaño-González et al. (2020) reported that anthocyanins and total phenolic compounds may respond differently to the same extraction parameters during MAE of açai fruit (23). Similarly, Álvarez et al. (2017) showed that microwave treatment enhances the recovery of polyphenols, particularly anthocyanins, from grape pomace, although the process must be optimized in relation to both temperature and extraction time (24).

Extraction-dependent changes in physicochemical characteristics and antioxidant capacity

Higher recoveries of TMA and TPC obtained with microwave-assisted extraction, compared with other methods, can be attributed to the rapid volumetric heating of the solvent and plant matrix by electromagnetic energy, which leads to more effective disruption of cellular structures. In particular, the rapid increase in temperature during microwave application may have facilitated the transfer of phenolic compounds into the solvent phase by increasing cell wall permeability. The decrease in solvent viscosity and the increase in diffusion rate at high temperatures are also considered important factors supporting extraction efficiency (25). Routray and Orsat (2014), on the other hand, noted that the application of microwaves in blueberries particularly increased the recovery of certain phenolic compounds and that this might be related to the more effective release of phenolics bound to the cell membrane (10). Findings demonstrate that ultrasound-assisted extraction can provide anthocyanin and phenolic recoveries comparable to those obtained by microwave-assisted extraction. Consistent with the present results, Tao et al. (2014) reported high anthocyanin and phenolic recoveries from wine pomace using ultrasound-assisted extraction, while subsequent studies have highlighted the suitability of UAE for anthocyanin-rich matrices because of its ability to enhance extraction efficiency under relatively mild temperature conditions (Tao et al., 2014). Nevertheless, the ability of MAE to achieve similarly high recoveries within substantially shorter extraction times represents an important advantage from the perspectives of process intensification and industrial applicability (8). Black carrot anthocyanins are generally considered relatively stable within the pH range observed in the present study. Assous et al. (2014) reported that approximately 19% degradation of black carrot anthocyanins occurred at pH 5, whereas degradation increased markedly at higher pH values. The high degree of acylation characteristic of black carrot anthocyanins may further contribute to pigment stability under moderately acidic conditions. Therefore, the higher pH values observed in UAE and MAE extracts are unlikely to adversely affect anthocyanin stability to a significant extent (26). The higher soluble solids content observed in UAE and MAE extracts may be associated with the more effective

disruption of plant cell structures by ultrasound and microwave treatments. Enhanced cell wall disruption can facilitate the release of soluble constituents into the extraction medium, including compounds associated with pectin, cellulose, and hemicellulose fractions. Previous studies have demonstrated that polyphenols can interact selectively with plant cell wall polysaccharides (27) while anthocyanins in black carrot have been shown to interact specifically with pectin (28). Consequently, the greater disruption of cell wall architecture achieved by UAE and MAE may have promoted both anthocyanin release and the transfer of dissolved solids into the extract phase. The superior antioxidant capacity of UAE and especially MAE extracts is likely associated with their higher anthocyanin and phenolic contents. Previous studies have reported a strong correlation between anthocyanin concentration and antioxidant activity (29). Likewise, Machado et al. (2017) demonstrated a close relationship between total phenolic content and antioxidant activity in ultrasound-assisted extracts obtained from blueberry and blackberry pomace (21). Such mechanisms may contribute to the enhanced antioxidant performance observed in MAE extracts. Collectively, these observations indicate that extraction technology influences not only anthocyanin and phenolic recovery but also the functional quality characteristics of the resulting extracts.

Influence of extraction technology on phenolic composition and selectivity

Hydroxycinnamic acids and flavonol derivatives are recognized as major phenolic constituents of black carrot, together with anthocyanins. Previous studies have shown that chlorogenic acid, caffeic acid, ferulic acid, and related hydroxycinnamic acids constitute important components of the black carrot phenolic fraction (30) . The markedly higher chlorogenic acid concentration observed in the MAE extract suggests that microwave-assisted extraction may be more effective in recovering hydroxycinnamic acid derivatives than UAE. This observation is consistent with the broader and more intense phenolic fingerprint obtained by MAE. Given that chlorogenic acid is one of the major contributors to antioxidant activity in black carrot, the enhanced recovery of this compound may also partially explain the superior antioxidant capacity observed in MAE extracts (30). The high concentrations of chlorogenic acid detected in the MAE extracts are consistent with the elevated total phenolic content and antioxidant activity observed in this study. Notably, the MAE extract exhibited the highest TPC and TEAC values among all extraction methods. These findings suggest that the high antioxidant capacity of the MAE extract may be attributed not only to anthocyanins but also to the presence of non-anthocyanin phenolic compounds, including chlorogenic acid, quercetin, isoquercitrin, and ferulic acid. Previous studies have emphasized that phenolic compounds occur in different forms, including glycosides, aglycones, and matrix-bound structures, and that the extraction technique plays a crucial role in determining the resulting phenolic profile (31).

The presence of minor compounds such as ferulic acid, vitexin, and isorhamnetin in the MAE extract—which were not detected in the UAE—suggests that the microwave application can release a broader phenolic fraction. In particular, the detection of ferulic acid exclusively in the MAE extract is quite significant. A significant portion of black carrot anthocyanins consists of cyanidin derivatives acetylated with hydroxycinnamic acids such as ferulic, sinapic, and p-coumaric acids. This acetylated structure contributes to the greater stability of black carrot anthocyanins against changes in heat, light, and pH (32). Therefore, the detection of free ferulic acid in MAE suggests that microwave energy may have exerted a stronger liberating effect on cell wall structures and/or ester-bound phenolic fractions. This interpretation is consistent with the literature reporting that rapid volumetric heating and increased intracellular pressure during MAE may allow for more effective separation of phenolic compounds from the

matrix (33). The differences in phenolic composition between UAE and MAE indicate that extraction technology influences not only the quantity but also the selectivity of phenolic recovery. The detection of luteolin-7-rutinoside exclusively in UAE may suggest that ultrasound-assisted extraction provides a milder extraction environment that favors the preservation of certain glycosylated flavonoids. In contrast, the broader phenolic profile observed in MAE supports the hypothesis that microwave treatment is more effective in releasing matrix-bound or less accessible phenolic fractions. The higher recovery of quercetin and isoquercitrin in MAE further supports its enhanced ability to extract flavonols and flavonol glycosides, which may have contributed to the greater antioxidant capacity observed in MAE extracts.

Color characteristics and pigment stability

The lower L* values and higher color intensity observed in UAE and MAE extracts indicate the production of more concentrated pigment-rich extracts. Enhanced recovery of anthocyanins and other color-contributing compounds through these extraction techniques may explain the greater color intensity obtained compared with conventional and pressure-assisted extraction methods. Lower L* values are generally associated with darker and more intensely colored extracts. The reduced L* values observed in UAE and MAE may be related to the enhanced recovery of pigments and other color-contributing compounds achieved by these extraction techniques. Similar observations have been reported by Stintzing et al. (2002), who found that increased recovery of anthocyanins and proanthocyanidins contributes to the development of more intense color characteristics in plant extracts (34). The lower a* value observed in MAE extracts, together with their high color intensity and polymeric color ratio, suggests that microwave treatment may promote pigment transformation reactions in addition to enhancing pigment extraction. Anthocyanin polymerization can lead to the formation of darker pigment structures with lower redness values, which may explain the simultaneous occurrence of low a* values and high color intensity in MAE extracts. Such behavior indicates the development of a more complex pigment profile through the combined effects of pigment release, co-pigmentation, and polymerization reactions. Polymeric color formation is widely recognized as an important indicator of anthocyanin transformation during processing and has been associated with pigment stability under thermal conditions (35). Previous studies have shown that anthocyanins may undergo structural changes through mechanisms such as chalcone formation, browning reactions, and phenolic–anthocyanin polymerization in response to temperature exposure (36). The formation of high-molecular-weight pigments through interactions among anthocyanins or between anthocyanins and other phenolic compounds may therefore contribute to reduced redness and increased polymeric color development.

Mineral co-extraction and extract composition

The influence of extraction technology on both organic and inorganic constituents supports the concept of holistic extract characterization, which has received increasing attention in recent green extraction studies. According to this approach, extract quality should be evaluated not only on the basis of phenolic composition and antioxidant activity but also by considering the accompanying inorganic fraction. Previous studies have similarly reported that ultrasound-assisted extraction may affect mineral transfer in addition to phenolic recovery, emphasizing the importance of assessing organic and inorganic components together when characterizing extract quality (37). The lower Na concentrations observed in alternative extraction techniques indicate that mineral transfer does not occur uniformly for all elements and may depend on the binding form of each element within the plant matrix. Previous studies have shown that mineral extraction is influenced not only by the applied

extraction energy but also by matrix interactions, solvent characteristics, and the formation of complexes with organic constituents. Kowaluk et al. (2025), for example, reported that stable matrix-associated complexes can limit the release of macro- and microelements during ultrasound-assisted extraction (38). The absence of significant differences in Mg and K concentrations suggests that these elements may occur in more readily soluble forms or in fractions less affected by extraction conditions. Conversely, the higher Ca concentrations observed in UAE and MAE extracts may be associated with enhanced disruption of cell wall structures during extraction. Because calcium is frequently associated with pectin-rich regions and structural components of plant cell walls, greater matrix disruption may facilitate its release into the extract phase. The markedly higher phosphorus concentration observed in the UAE extract suggests that ultrasound treatment enhanced the release of phosphorus-containing compounds from the plant matrix. Acoustic cavitation generates microjets, localized pressure fluctuations, and shear forces that can disrupt cell walls and membrane structures, thereby facilitating the release of matrix-bound mineral fractions. Previous studies have demonstrated that extraction media influence not only the recovery of phenolic compounds and antioxidant activity but also the release of mineral constituents, thereby affecting the overall chemical composition of plant extracts (39). Recent studies on fruit by-product valorization have highlighted that these materials represent important sources of both phenolic compounds and minerals such as K, Ca, and Mg (40). Likewise, ultrasonic treatment has been reported to promote the transfer of selected mineral elements into the extract phase through cavitation-induced disruption of cellular structures (37). The differential responses of mineral and phenolic fractions indicate that extraction technologies exhibit selective effects on specific chemical constituents. Whereas UAE enhanced the transfer of phosphorus and calcium, MAE was more effective in the recovery of phenolic compounds and anthocyanins. These findings highlight the importance of adopting a holistic approach to extract characterization, considering both organic and inorganic fractions when evaluating extraction efficiency and extract quality.

In conclusion; this study demonstrates that extraction technology acts not only as a recovery tool but also as a determinant of the chemical and functional identity of black carrot pomace extracts. Beyond differences in anthocyanin and phenolic yields, the applied extraction approach significantly influenced phenolic selectivity, antioxidant activity, color quality, polymeric color formation, and mineral co-extraction behavior. The results revealed that extraction technologies generate extracts with distinct compositional and functional profiles, even when targeting the same raw material. The findings further indicate that extraction process selection should not be based solely on maximizing extraction yield. Instead, the desired characteristics of the final product—including phenolic composition, color attributes, antioxidant potential, and overall extract functionality—should be considered when designing extraction strategies. In this context, MAE favored the production of extracts with higher anthocyanin recovery, broader phenolic diversity, and enhanced antioxidant activity, whereas UAE provided a more selective phenolic profile under comparatively mild processing conditions. Overall, black carrot pomace was confirmed as a valuable source of anthocyanins and phenolic compounds that can be efficiently valorized through green extraction technologies. The study highlights the importance of aligning extraction technology with the intended application of the extract and provides a basis for the development of natural colorants and functional ingredients from black carrot processing by-products.

ETHICS COMMITTEE APPROVAL

* This study does not require Ethics Committee Approval.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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