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Extraction of Bioactive Compounds from Fresh Blueberry Pomace Using Choline Chloride-Based Systems: Influence of Formulation and Treatment Conditions

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ABSTRACT

Cold-press processing of blueberries for juice production generates a wet pomace that retains bioactive compounds of interest. To recover these compounds from the by-product, this study evaluated the extraction performance of five choline chloride-based natural deep eutectic solvents (NADES), formulated with urea, fructose, malic acid, oxalic acid, or lactic acid at a 1:1 molar ratio and containing 15% water, under three extraction conditions: heating with agitation, ultrasound, and microwave treatment. Six independent extracts were prepared for each combination. In each of six independent pressing operations, 250 g of fruit were processed, yielding 175.3 ± 1.6 g of juice and 70.0 ± 1.5 g of fresh pomace. Solvent formulation and extraction technology significantly affected the recovered amounts of phenolics, flavonoids, and monomeric anthocyanins. Heating with agitation using the choline chloride:lactic acid solvent produced the highest total phenolic and flavonoid values, at 667.43 ± 15.33 mg gallic acid equivalents and 256.36 ± 10.37 mg catechin equivalents per 100 g fresh pomace, respectively. The highest monomeric anthocyanin content was obtained with choline chloride:oxalic acid under heating (187.39 ± 7.18 mg cyanidin-3-glucoside equivalents per 100 g). The acidic formulations emerged as promising candidates for further analytical validation, chromatographic characterization, and process optimization.

KEYWORDS: blueberry pomace, phenolic compounds, green extraction, NADES, anthocyanins, emerging technologies.

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1. INTRODUCTION

Blueberry juice production (*Vaccinium corymbosum L.*) generates a pomace composed of skin, seeds, and residual pulp. Because a substantial proportion of phenolic compounds is concentrated in the outer tissues, this stream retains pigments, fiber, and other compounds of interest and can be regarded as a secondary raw material for circular-economy strategies.[1–3]

Conventional recovery of phenolic compounds commonly uses acidified hydroalcoholic mixtures as extraction solvents. The volatility and flammability of these mixtures have encouraged the search for and evaluation of more environmentally friendly extraction media, including natural deep eutectic solvents (NADES). The definition of DES (and therefore NADES) has been revisited in recent years: Martins et al.[4] proposed distinguishing them from conventional eutectic mixtures by their marked negative deviation from ideal behavior; Fajar et al.[5] noted that the term is frequently used without determination of the solid–liquid equilibrium phase diagram or the actual eutectic composition; and Machingal & Gardas[6] reaffirmed the need for a thermodynamic definition based on system nonideality. Nevertheless, given the lack of a universally accepted definition and the broader usage of the term in the applied literature,[7] this study adopts an operational definition of NADES as mixtures of naturally occurring

compounds whose interactions produce a marked melting-point depression, allowing formation of a liquid phase at room temperature, a criterion also used in recent literature.[8]

The properties of NADES depend on the hydrogen-bond acceptor and donor, molar ratio, and water content, among other factors.[9,10] Heating, ultrasound, and microwaves can enhance mass transfer through different mechanisms. In blueberry pomace, responses have been shown to depend on the matrix, solvent, liquid-to-solid ratio, and applied energy.[1,11,12] Recent studies have confirmed the potential of NADES systems combined with ultrasound for the recovery of anthocyanins and polyphenols from blueberry pomace, although using formulations and conditions that are not directly comparable.[13,14]

The aim of this study was to compare the extraction performance of five choline chloride-based NADES and to evaluate the effects of solvent formulation, treatment condition, and their interaction, on the spectrophotometric responses associated with phenolics, flavonoids, and monomeric anthocyanins in extracts prepared from fresh blueberry pomace. Six independent extracts were prepared for each combination. The fruit, pressing mass balance, pomace, and the main physicochemical properties of the evaluated solvents were also characterized.

2. MATERIALS AND METHODS

2.1 Raw material and pomace production

Blueberries (*Vaccinium corymbosum L.*, cv. Elliott) harvested in 2024 by growers from the Andean Region of the 42nd Parallel, El Bolsón, Río Negro, Argentina, were used. The fruit was transported under refrigeration to the Faculty of Food Science and Technology in Villa Regina, Alto Valle of Río Negro, stored at 5 °C until use, and sorted to discard damaged or overripe berries. Processing was carried out in six independent operations. In each operation, 250 g of fruit were processed using a cold-press auger juicer (Peabody by Hurom PE-HSJO2, China) equipped with a continuous juice–pomace separation system. The unit has a nominal power of 150 W, operates at 70 rpm, and uses a low-speed compression extraction mechanism that minimizes temperature rise during processing and reduces air incorporation into the product.

Before extraction, the berries were sorted, washed, and drained. They were then manually fed into the juicer, simultaneously yielding two fractions: juice and pomace. Both fractions were collected separately in the containers supplied by the manufacturer and weighed immediately after processing to determine juice yield and the proportion of pomace generated. Processing losses were calculated by difference from the initial mass. Each pomace batch was homogenized separately and stored frozen in an ultralow-temperature freezer until analysis.

2.2 Physicochemical characterization of the fruit

Measurements were performed on homogenized fresh-fruit pulp using official AOAC methods.[15] For moisture (AOAC 920.151), 5.00 g of sample were placed in pre-weighed porcelain dishes containing sand and dried in a vacuum oven at 70 °C to constant mass. Soluble solids (AOAC 932.12) were measured in juice separated by filtration through voile fabric using an ATAGO Type 1 Abbe refractometer and expressed as °Brix. For pH and titratable acidity, 5.00 g of pulp were suspended in 100 mL of previously boiled distilled water. pH was determined according to AOAC 981.12 using an Orion EA 940 analyzer with a combined electrode; acidity was determined by titration with 0.1 mol/L NaOH to pH 8.1 (AOAC 942.15) and expressed as grams of anhydrous malic acid per 100 g fresh fruit.

2.3 Proximate composition and dietary fiber

Ash, fat, protein, and total dietary fiber were determined using AOAC methods 940.26, 954.02, 955.04, and 991.43, respectively. Moisture content of the fresh pomace was determined gravimetrically, and dry matter was calculated by difference. Results were expressed on a fresh-weight basis unless otherwise stated.

2.4 Extraction and determination of bioactive compounds in blueberry fresh fruit

For each extract, 2.50 g of fresh-fruit pulp were mixed with 20 mL of ethanol acidified with 1% HCl (v/v) and maintained for 30 min at 37 °C in a thermostated Dubnoff bath with agitation. The suspension was vacuum-filtered, and the residue was extracted three additional times with 20 mL portions of the same solvent. The pooled extracts were brought to 100 mL with distilled water. Three independent extracts were prepared. Total phenolics were determined using Fast Blue BB,[16] total flavonoids by $AlCl_3$ complexation,[17] monomeric anthocyanins by the pH differential method,[18] and DPPH radical-scavenging antioxidant activity, expressed as the antioxidant activity index (AAI).[19]

2.5 Color measurement

The color of the fruit, juice, and pomace was evaluated in the CIELAB color space using a Minolta CR-400 colorimeter (Konica Minolta Sensing Inc., Osaka, Japan), illuminant C and 2° observer. The instrument was calibrated with a white ceramic tile (L* = 95.55; a* = −0.10; b* = 2.69). Ten readings were taken for each matrix, recording L* (lightness), a* (green–red axis), and b* (blue–yellow axis).[20] Total color difference was calculated relative to the mean fruit coordinates: $\Delta E^*ab = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$.

2.6 Preparation of NADES systems

Five extraction solvents were formulated by combining choline chloride (ChCl), as the hydrogen-bond acceptor (HBA), with urea (U), fructose (Fru), malic acid (MA), oxalic acid (OA), or lactic acid (LA), as hydrogen-bond donors (HBD), at a 1:1 molar ratio. The formulations were designated ChCl:U, ChCl:Fru, ChCl:MA, ChCl:OA, and ChCl:LA. Water was added to a final content of 15% (w/w), and the mixtures were heated at 50 °C under magnetic stirring until transparent, homogeneous liquids with no visible crystals were obtained.

2.7 Physicochemical and rheological characterization of the systems

Density was determined gravimetrically from the mass of a known volume. Conductivity was measured using a Jenco 3173 conductivity meter, and pH using an Orion EA 940 analyzer with a combined electrode. Six observations per system were recorded for each property. Rheological behavior was evaluated at 25 and 40 °C using an Anton Paar MCR 302 rheometer (Anton Paar GmbH, Austria), with CC10 concentric-cylinder geometry and C-PTD200 temperature control. An ascending shear-rate ramp from 1 to 500 s^{-1} was applied, with 100 logarithmically spaced points and 2 s per point. Shear stress and apparent viscosity were recorded; for comparison, the value at 97.8 s^{-1}, the experimental point closest to 100 s^{-1}, was reported. One run was performed per system and temperature; therefore, these results were interpreted descriptively.

2.8 Extraction of bioactive compounds from blueberry pomace

Six independent extracts were prepared for each combination (n = 6). Each replicate began by separately weighing 300 mg of fresh pomace into an individual tube and adding 4.00 mL of solvent, corresponding to a liquid-to-solid ratio of 13.3 mL/g. Each suspension was vortex-mixed for 30 s and independently subjected to one of three conditions: heating with agitation (HA), 40 °C for 30 min; ultrasound-assisted extraction (UAE), using a 40 kHz bath at a nominal power of 120 W for 30 min while maintaining the temperature below 40 °C; or microwave-assisted extraction (MAE), at a nominal power of 90 W in three 5-s cycles separated by 5-s pauses. After treatment, samples were centrifuged for 15 min at 4000 rpm (approximately 1800 × g), and the supernatants were recovered. Ethanol acidified with 1% HCl (v/v) was included as a reference for HA and UAE, but not for MAE for safety reasons.

2.9 Total phenolics

Total phenolic content (TPC) was determined using Fast Blue BB. To 2.00 mL of extract, 200 µL of 1% (w/v) Fast Blue BB in methanol and 200 µL of 5% (w/v) NaOH were added. After 60 min at room temperature in the dark, absorbance was measured at 420 nm. Reagent blanks and solvent-specific blanks were prepared, subjected to the same procedure, and their absorbances were subtracted from those of the samples. Quantification was performed using gallic acid, and results were expressed as mg GAE/100 g fresh pomace.

2.10 Total flavonoids

Total flavonoids (TF) were determined by $AlCl_3$ complexation. An aliquot of extract within the calibration range was brought to 5.00 mL with water; 300 µL of 5% $NaNO_2$ were added, followed after 5 min by 300 µL of 10% $AlCl_3$ and, after a further 6 min, by 2.00 mL of 1 mol/L NaOH. The volume was adjusted to 10.00 mL and absorbance was measured at 510 nm. The corresponding blank was processed for each solvent and absorbance was corrected accordingly. Quantification was performed using catechin, and results were expressed as mg CE/100 g fresh pomace.

2.11 Monomeric anthocyanins

Monomeric anthocyanins (ACY) were determined using the pH differential method. Aliquots of 1.00 mL were diluted with 4.00 mL of either 0.025 mol/L KCl, pH 1.0, or 0.4 mol/L sodium acetate, pH 4.5. After 15 min in the dark, absorbance was measured at 520 and 700 nm. Solvent-specific blanks were included in both buffers. Calculations used a molar mass of 449.2 g/mol, ε = 26,900 $L \cdot mol^{-1} \cdot cm^{-1}$, and a 1-cm path length. Results were expressed as mg C3G/100 g fresh pomace.

2.12 Experimental design and statistical analysis

The experimental unit for extraction responses was the independently prepared and processed extract (n = 6 per combination); the six replicates were not repeated technical readings of a single extract. For the five choline chloride-based systems, a completely randomized 5 × 3 factorial ANOVA (solvent × technology) was applied. The comparison including ethanol was restricted to the common 6 × 2 design (HA and UAE). Residuals were examined graphically to assess normality, homogeneity, and influential observations, and α = 0.05 was adopted. When a significant interaction was detected, solvents were compared within each technology using Tukey's HSD test at α = 0.05. Analyses were performed using InfoStat (Grupo InfoStat, FCA, Universidad Nacional de Córdoba, Argentina).[21] Results are presented as mean ± SD of the six independent extracts.

3. RESULTS

3.1 Fruit characterization and color

Fruit composition, pressing mass balance, and fresh pomace characteristics are summarized in Table I.

Table I. Physicochemical characterization of the fruit, pressing mass balance, and fresh pomace composition

Block	Parameter	Result
Fruit	Moisture (%)	83.20 ± 1.20
Fruit	Soluble solids (°Brix)	12.75 ± 0.27
Fruit	pH	2.85 ± 0.04
Fruit	Titratable acidity (g malic acid/100 g)	1.62 ± 0.01
Fruit	Ash (%)	1.44 ± 0.13
Fruit	Protein (%)	0.88 ± 0.02
Fruit	Total dietary fiber (g/100 g)	3.72 ± 0.10
Fruit	Fat (g/100 g)	0.31 ± 0.01
Fruit	Total phenolics (mg GAE/100 g)	506.00 ± 29.00
Fruit	Flavonoids (mg CE/100 g)	204.02 ± 8.99
Fruit	Anthocyanins (mg C3G/100 g)	150.00 ± 4.01
Fruit	AAI	0.78 ± 0.04
Processing	Juice (g/250 g fruit)	175.3 ± 1.6
Processing	Fresh pomace (g/250 g fruit)	70.0 ± 1.5
Processing	Juice yield (%)	70.1 ± 0.7
Processing	Pomace yield (%)	28.0 ± 0.6
Processing	Processing losses (%)	1.87 ± 0.06
Pomace	Moisture (%)	55.5 ± 0.7
Pomace	Dry matter (%)	44.5 ± 0.7

Note. Results are expressed as mean ± standard deviation (SD). The mass balance per 250 g fruit, yields, processing losses, and pomace composition correspond to six independent operations (n = 6). GAE, gallic acid equivalents; CE, catechin equivalents; C3G, cyanidin-3-glucoside equivalents; AAI, antioxidant activity index. Contents are expressed on a fresh-weight basis.

The fruit contained 83.20% moisture and 12.75 °Brix, with a pH of 2.85 and titratable acidity of 1.62 g malic acid/100 g. In each operation, 250 g of fruit were processed, yielding 175.3 ± 1.6 g of juice and 70.0 ± 1.5 g of fresh pomace, corresponding to yields of 70.1 ± 0.7% and 28.0 ± 0.6%, respectively. Processing losses were 1.87 ± 0.06%, equivalent to approximately 4.7 g per operation. The pomace contained 55.5 ± 0.7% moisture and 44.5 ± 0.7% dry matter.

In addition to fruit characterization, the immediate effect of pressing on the color of the three resulting matrices was examined. Table II presents the CIELAB coordinates and total color difference calculated relative to the whole fruit.

Table II. Colorimetric parameters of blueberry fruit, juice, and pomace

Matrix	L*	a*	b*	ΔE*ab
Whole fruit	36.46 ± 2.85 a	0.28 ± 0.25 c	−5.99 ± 0.93 c	2.51 ± 1.44 b
Juice	28.50 ± 2.50 b	5.80 ± 1.20 a	−0.80 ± 1.00 b	11.18 ± 1.99 a
Pomace	27.20 ± 1.80 b	3.90 ± 0.80 b	0.40 ± 0.50 a	11.89 ± 1.49 a

Note. Values are expressed as mean ± SD of ten readings (n = 10). L, lightness; a*, green–red coordinate; b*, blue–yellow coordinate; ΔE*ab, total color difference relative to the mean fruit coordinates. For whole fruit, ΔE*ab represents the distance of each individual reading from the fruit's own chromatic centroid; therefore, its mean is not zero. Within each column, means with different letters differ according to Tukey's HSD test ($p < 0.05$).*

Pressing decreased lightness: L* declined from 36.46 in the fruit to 28.50 in the juice and 27.20 in the pomace, with no significant difference between the latter two matrices. At the same time, a* increased from 0.28 to 5.80 in the juice and 3.90 in the pomace, indicating a greater contribution of red hues after disruption of the bluish waxy fruit surface. The b* coordinate also shifted from negative blue values (−5.99) toward −0.80 in the juice and 0.40 in the pomace. Overall differences relative to the fruit were large and similar for juice and pomace (ΔE*ab = 11.18 and 11.89, respectively). These changes describe the optical redistribution caused by tissue disruption and phase separation.

3.2 Characterization of choline chloride-based systems

Before evaluating extraction performance, the five NADES were characterized visually, physicochemically, and rheologically. Instrumental characterization included density, conductivity, apparent pH, and apparent viscosity at 25 and 40 °C. These measurements describe properties relevant to handling and mass transfer during the extraction of compounds with bioactive potential. Results are summarized in Table III.

Table III. Physicochemical properties and apparent viscosity of choline chloride-based systems

System	Density (g/mL)	Conductivity (mS/cm)	Apparent pH	Appearance	η at 25 °C (mPa·s)	η at 40 °C (mPa·s)
ChCl:urea	1.18 ± 0.04	17.76 ± 0.32	6.00 ± 0.50	Colorless	17.3	10.4
ChCl:fructose	1.25 ± 0.02	16.05 ± 0.15	5.50 ± 0.71	Light amber	387.0	88.7
ChCl:malic acid	1.23 ± 0.09	19.78 ± 0.25	1.97 ± 0.16	Slightly yellow	125.0	54.4
ChCl:oxalic acid	1.30 ± 0.05	15.32 ± 0.14	1.36 ± 0.19	Colorless	148.0	66.3
ChCl:lactic acid	1.08 ± 0.03	12.98 ± 0.05	2.45 ± 0.47	Colorless	447.0	139.0

Note. Density, conductivity, and apparent pH: mean ± SD of six determinations (n = 6). Density, g/mL; conductivity, mS/cm; η, apparent viscosity in mPa·s measured at 97.8 s^{-1}. Viscosity values were obtained from one run per system and temperature and are interpreted descriptively. ChCl, choline chloride.

Densities ranged from 1.08 to 1.30 g/mL. Systems containing organic acids showed apparent pH values between 1.36 and 2.45, compared with 5.50–6.00 for ChCl:fructose and ChCl:urea. Conductivity was highest for ChCl:malic acid (19.78 mS/cm) and lowest for ChCl:lactic acid (12.98 mS/cm), with no simple relationship with pH. Viscosity was strongly composition-dependent: at 25 °C it ranged from 17.3 mPa·s for ChCl:urea to 447 mPa·s for ChCl:lactic acid. Increasing temperature reduced the viscosity of all systems by 39–77%, suggesting lower transport resistance during thermal treatment. Because only one rheological run was performed per condition, these differences were used descriptively and were not subjected to statistical inference.

3.3 Effect of solvent and technology

The effects of formulation and extraction-assistance technology were evaluated by factorial ANOVA for the five choline chloride-based NADES (Table IV). The comparison with ethanol was performed separately within the common HA–UAE domain. The distribution of responses among the 17 tested combinations is shown in Figure 1.

Table IV. Factorial analysis of variance for the effects of solvent and extraction technology

Response	Source	df	F	P
Total phenolics	Solvent	4	286.19	<0.001
Total phenolics	Technology	2	93.90	<0.001
Total phenolics	Solvent × technology	8	3.69	0.0011
Total flavonoids	Solvent	4	335.48	<0.001
Total flavonoids	Technology	2	78.46	<0.001
Total flavonoids	Solvent × technology	8	2.47	0.0196
Monomeric anthocyanins	Solvent	4	1241.06	<0.001

Monomeric anthocyanins	Technology	2	24.88	<0.001
Monomeric anthocyanins	Solvent × technology	8	2.08	0.0486

Note. 5 × 3 factorial ANOVA with six independent extracts per combination (n = 6). df, degrees of freedom; F, Fisher's F statistic; p, associated probability. Effects were considered significant at p < 0.05.

Solvent, technology, and their interaction significantly affected all three responses. The solvent × technology interaction was significant for total phenolics ($F_{8,75} = 3.69$; $p = 0.0011$), total flavonoids ($F_{8,75} = 2.47$; $p = 0.0196$), and monomeric anthocyanins ($F_{8,75} = 2.08$; $p = 0.0486$). Therefore, effects were interpreted from the specific solvent–technology combinations rather than from marginal means alone.

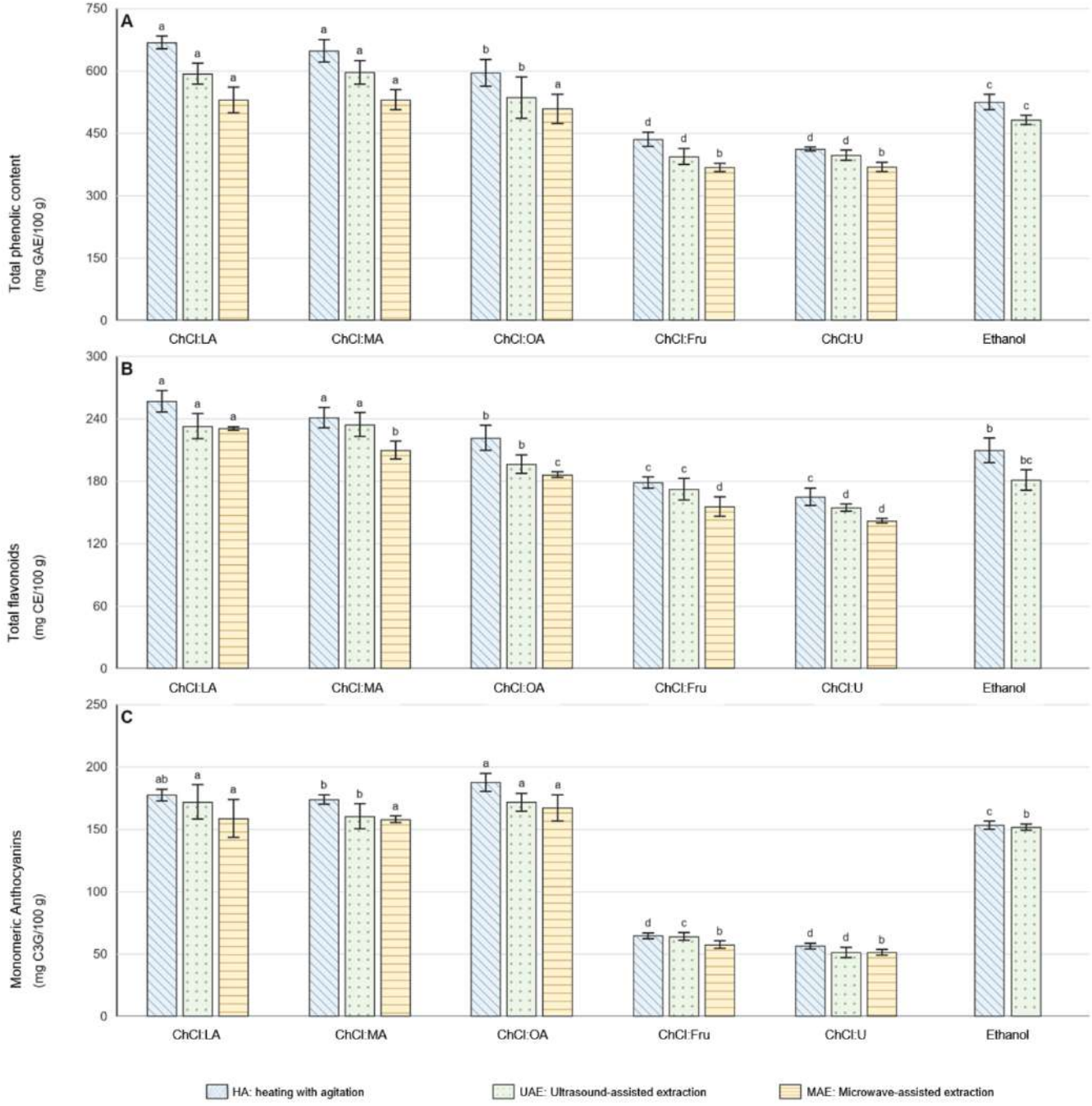

Figure 1. Total phenolics content (A), total flavonoids (B), and monomeric anthocyanins (C) in fresh blueberry pomace extracts. Bars represent mean ± SD of six independent extracts (n = 6). For each panel and within the same technology, bars sharing at least one letter do not differ according to Tukey's HSD test ($p \geq 0.05$); letters should not be compared across technologies or panels. HA, heating with agitation; UAE, ultrasound-assisted extraction; MAE, microwave-assisted extraction. Ethanol was not evaluated by MAE.

ChCl:lactic acid with HA produced the highest total phenolic (667.43 ± 15.33 mg GAE/100 g) and flavonoid (256.36 ± 10.37 mg CE/100 g) values. Compared with acidified ethanol under HA, the differences were 143.40 mg GAE/100 g (27.4%) and 47.20 mg CE/100 g (22.6%), respectively; in both cases, the means belonged to different Tukey groups ($p < 0.05$). The significant interaction indicates that the magnitude of the solvent effect depended on the applied technology.

For anthocyanins, the maximum was obtained with ChCl:oxalic acid and HA (187.39 ± 7.18 mg C3G/100 g), 34.42 mg C3G/100 g above acidified ethanol under HA (22.5%); the two means belonged to different Tukey groups ($p < 0.05$). Although HA produced the highest absolute values, the treatments were not matched for time or specific energy; therefore, the data do not support conclusions regarding energy superiority, industrial productivity, or technological equivalence.

4. DISCUSSION

4.1 Raw material characteristics and effect of pressing on color

Soluble solids (12.75 °Brix) and titratable acidity (1.62 g malic acid/100 g) were within the ranges reported for different blueberry cultivars.[22] The mass balance showed that pomace represented 28.0% of the processed fruit and had a dry matter content of 44.5%. Given its yield and composition, this by-product constitutes a secondary stream of interest for obtaining ingredients rich in bioactive compounds.

The total phenolic content of the fruit (506 mg GAE/100 g) was higher than the mean value of 179 mg GAE/100 g reported for a broad set of highbush blueberry genotypes.[23] This comparison should be considered indicative because cultivar, extraction conditions, and analytical method differed among studies. In particular, Fast Blue BB has a different selectivity from the Folin–Ciocalteu reagent.[16] The monomeric anthocyanin content of the fruit (150 mg C3G/100 g FW) was within the broad range reported for ripe blueberries. Kim et al.[22] reported total anthocyanin contents ranging from 167.6 to 677.8 mg C3G/100 g FW among 45 cultivars, with Elliott reaching 552.7 mg C3G/100 g FW. In contrast, Hwang et al.[24] reported 70.89 mg C3G/100 g FW for ripe Elliot fruit.. The antioxidant activity index (AAI) was 0.78, corresponding to moderate antioxidant activity according to the classification of Scherer & Godoy[19].

Pressing significantly modified all three CIELAB coordinates. Lightness (L*) decreased from 36.46 in whole fruit to 28.50 in juice and 27.20 in pomace, with no significant difference between the latter two matrices. This decrease may be related to disruption of the fruit surface structure during pressing. The intact epidermis and epicuticular wax bloom contribute to light scattering, whereas tissue disruption produces wet, heterogeneous surfaces with different optical properties.[24] In pomace, the presence of skin fragments, seeds, and pulp also generates simultaneous absorption, scattering, and reflection phenomena. Thus, the decrease in L* does not by itself demonstrate pigment degradation and may also reflect physical changes caused by processing.

The a* coordinate increased from 0.28 in whole fruit to 5.80 in juice and 3.90 in pomace, with significant differences among all three matrices. This shift toward positive values indicates a greater contribution of red hues after tissue disruption. The higher a* value in juice could be associated with a more homogeneous distribution of pigments in the liquid phase and less interference from seeds and insoluble particles. However, this interpretation is exclusively optical and does not establish that juice contains a higher anthocyanin concentration than pomace, because a* also depends on pH, turbidity, copigmentation, and the presence of polymerized pigments.[24,25]

The b* coordinate shifted from −5.99 in whole fruit to −0.80 in juice and 0.40 in pomace. The decrease in the blue component and the shift toward values close to zero indicate that pressing altered the visual balance among bluish, reddish, and yellowish hues. The blue color of whole blueberries depends not only on anthocyanin composition but also on the wax bloom and the interaction of light with the intact epidermis.[24] After pressing, disruption of this surface and exposure of internal tissues reduce the visual blue contribution. The slightly positive b* value in pomace could also be related to the presence of seeds, less-pigmented tissues, or early oxidative reactions; these possible contributions were not evaluated separately.

Total color differences relative to the fruit were $\Delta Eab = 11.18$ for juice and $\Delta Eab = 11.89$ for pomace, indicating a marked chromatic change after pressing.[20] Although ΔEab values were similar, juice and pomace showed different chromatic profiles, as reflected by their a* and b* values. Blueberry CIELAB coordinates can vary with cultivar and ripening stage and should therefore be interpreted in light of the physiological and structural characteristics of the sample.[24] Likewise, subsequent operations such as drying and milling can modify L*, a*, and b* through changes in solids concentration, particle size, and anthocyanin stability.[25] Consequently, CIELAB parameters describe macroscopic changes caused by processing but do not replace chemical identification and quantification of pigments.

4.2 System properties and their relationship with extraction

NADES formulated with organic acids as HBDs showed apparent pH values between 1.36 and 2.45 and produced the highest responses in specific combinations. ChCl:oxalic acid with HA yielded the highest monomeric anthocyanin value, whereas ChCl:lactic acid with HA yielded the highest phenolic and flavonoid values. The significant interaction confirms that the effect of formulation depended on the technology, although it does not by itself establish the underlying mechanism. The observed behavior

may be related to the combined properties of each system, including polarity, hydrogen-bonding capacity, water activity, ionization state, and stability of the extracted compounds.[26,27] Solvent-specific blanks corrected background absorbance, although they do not replace formal validation of potential matrix effects in each assay.

The addition of 15% water improved fluidity and facilitated preparation, homogenization, and handling of the systems. This water content maintained media concentrated in their constituent components while reducing the resistance associated with high viscosity. Water modifies the hydrogen-bond network of eutectic solvents and may enhance mass transfer; the extent of this effect depends on its proportion and on the composition of each formulation.[28] Under the conditions studied, water addition produced homogeneous, transparent systems suitable for direct use in the extraction treatments.

Viscosity provided complementary information for interpreting the formulations. ChCl:lactic acid showed the highest apparent viscosity at both 25 and 40 °C and nevertheless produced high phenolic and flavonoid responses. When temperature increased from 25 to 40 °C, its viscosity decreased from 447 to 139 mPa·s, a change consistent with lower transport resistance during heating. However, rheological characterization was based on one run per system and temperature, and no quantitative relationship between viscosity and extraction was established; this mechanism should therefore be regarded as a plausible interpretation.

4.3 Influence of technology and comparison with previous studies

The significant interaction between formulation and technology indicates that both variables jointly affected extraction responses. HA and UAE were applied for 30 min, whereas MAE involved only 15 s of effective irradiation. Despite this markedly shorter treatment time, MAE yielded quantifiable responses for all formulations. Because the treatments differed in processing time and were not standardized in terms of absorbed energy or final temperature, the comparison reflects extraction performance under the specific operating conditions evaluated rather than relative process efficiency under equivalent conditions. In this context, HA can be identified as the condition that produced the highest responses under the conditions evaluated, while MAE emerges as a promising alternative for further optimization.

Results expressed on a fresh-weight basis demonstrate that the pomace retained appreciable amounts of bioactive compounds after pressing. Determination of its moisture content (55.5%) allowed more precise characterization of the matrix and enables conversion of results to a dry-weight basis for comparison with other studies. Differences in cultivar, ripening stage, moisture content, liquid-to-solid ratio, solvent composition, and analytical methodology account for part of the variability among published values. Therefore, the most informative comparisons are those that simultaneously consider the basis of expression and the experimental conditions used.

The present results are consistent with previous studies specifically addressing blueberry pomace, although quantitative comparisons require caution. Fu et al.[29] selected ChCl:oxalic acid and combined it with pulsed ultrasonication for freeze-dried pomace; in addition to optimizing yield, they identified anthocyanins by UPLC-MS and observed greater extract stability than with acidified ethanol. This finding is consistent with the maximum anthocyanin response observed here for ChCl:oxalic acid, although the studies differ in expression basis, matrix moisture, liquid-to-solid ratio, temperature, and ultrasound regime. Zhang et al.[14], in turn, selected ChCl:1,4-butanediol (1:3), 29% water, and 63 °C within a different experimental space and obtained high anthocyanin and polyphenol yields. Taken together, these studies show that there is no universally optimal formulation: performance arises from the interaction among composition, water content, matrix, and mass-transfer conditions.

The most recent evidence further supports this context dependence. Chen et al.[13] optimized a ChCl:lactic acid (1:2) system containing 40% water combined with ultrasound for blueberry pomace and attributed its performance to the combination of structural disruption and solvent–solute affinity; however, their conditions were more intensive and their primary target was anthocyanins. Ouyang et al.[30] found a different optimum—betaine:urea with 60% water—for flavonoids from wild blueberry, further supporting the influence of acceptor identity, donor identity, and water content on selectivity. More recently, Jaouhari et al.[31] compared five betaine-based NADES for blueberry by-products and demonstrated formulation-dependent phenolic profiles by UHPLC-DAD-MS/MS, as well as the potential for direct application of the extract without solvent removal.

The contribution of this study lies in comparing, within a single experimental design, five formulations, three technologies, and three spectrophotometric responses using characterized fresh pomace. The highest values were obtained with ChCl:lactic acid–HA for phenolics and flavonoids and with ChCl:oxalic acid–HA for monomeric anthocyanins. This divergence among responses is compatible with differential extraction or with different analytical responses to the matrix, but it does not demonstrate chemical selectivity, which remains to be established through identification and quantification of individual compounds.

5. CONCLUSION

The results of this study, expressed on a fresh-weight basis, demonstrate that blueberry pomace retains appreciable amounts of bioactive compounds after pressing. The successful recovery of phenolics, flavonoids, and anthocyanins directly from fresh pomace shows that effective extraction can be achieved without prior drying, supporting its potential as an immediately processable feedstock for valorization.

Solvent formulation, extraction technology, and their interaction significantly affected the spectrophotometric responses. ChCl acid combined with heating produced the highest total phenolic and flavonoid values, whereas ChCl acid with heating produced the highest monomeric anthocyanin content. This divergence suggests that formulation choice may be tailored to the targeted class of bioactive compounds rather than to a universally optimal solvent, although chemical selectivity remains to be established by chromatographic characterization.

Acidic NADES (ChCl acid, ChCl acid, and ChCl acid) consistently occupied the upper response range across HA, UAE, and MAE, whereas ChCl and ChCl generally remained in the lower range. Thus, although extraction technology affected response magnitude and interacted with solvent formulation, solvent identity emerged as a major determinant of extraction performance. Moreover, performance was not inversely related to solvent viscosity, as illustrated by the high responses obtained with the comparatively viscous ChCl acid systems.

Overall, these findings identify acidic choline chloride-based formulations as promising candidates for direct valorization of fresh blueberry pomace and indicate that further optimization should emphasize solvent formulation and target-compound composition. The conditions identified are promising rather than optimized and do not yet demonstrate chemical selectivity.

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7. CONFLICTS OF INTEREST

The authors declare no conflicts of interest related to this work.

8. FUNDING AND SUPPORT

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9. AUTHOR CONTRIBUTIONS (CREDIT)

F. C. Namor and C. A. Paulino: conceptualization, methodology, investigation, formal analysis, funding acquisition, and writing—review and editing.

10. DATA AVAILABILITY

The processed data supporting the results are available from the corresponding author upon reasonable request.

11. DECLARATION OF GENERATIVE AI USE IN MANUSCRIPT PREPARATION

During the preparation of this manuscript, generative artificial intelligence (ChatGPT, OpenAI) was used solely for language editing and to improve the clarity and readability of the English text, as the authors are non-native English speakers. The authors reviewed and edited all AI-assisted content and take full responsibility for the final version of the manuscript.

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