



Tailoring sodium acetate-based NADES for selective recovery of phenolics from red plum peel: impact of composition, preparation method, and multivariate analysis

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Received: 3 April 2026 / Accepted: 11 June 2026 / Published online: 18 June 2026
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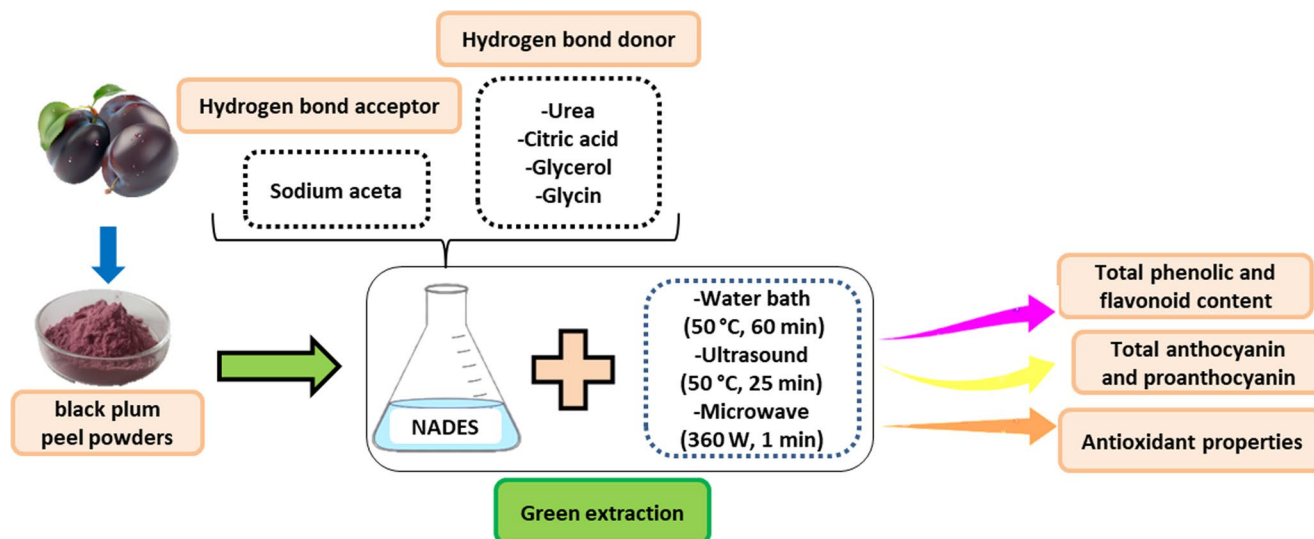
Abstract

The present study systematically investigated sodium acetate-based natural deep eutectic solvents (NADES) formulated with different hydrogen bond donors (urea, citric acid, glycerol, and glycine) and prepared using water bath (WB), ultrasound (US), and microwave (MW) methods, with a specific focus on elucidating the role of solvent composition and preparation strategy in extraction efficiency. These solvents were applied for the recovery of phenolics, flavonoids, monomeric anthocyanins, and proanthocyanidins from red plum (*Prunus domestica* L.) peels, while antioxidant capacity was determined using DPPH radical scavenging, ferric reducing antioxidant power (FRAP), and cupric reducing antioxidant capacity (CUPRAC) assays. Urea-based NADES prepared by MW (N3) exhibited the highest TPC (48.46 g GAE/kg dm), DPPH activity (179.03 g TEAA/kg dm), and FRAP values (189.67 g TEAA/kg dm). The highest TFC was obtained using urea-based NADES prepared by US (N2), reaching 3884.98 mg CE/kg dm. Regarding pigment recovery, glycerol-based NADES prepared by US (N8) achieved the highest TMAC (7103.19 mg C3G/kg dm) and TPAC (34.04 mg cyanidin/kg dm), followed by glycine-based NADES prepared by WB (N10) (7093.63 mg C3G/kg dm; 29.43 mg cyanidin/kg dm). Overall, urea-based systems were more effective for phenolics and antioxidant activity, whereas glycerol- and glycine-based NADES were superior for anthocyanin and tannin extraction. These findings demonstrate, for the first time, that tailoring both NADES composition and preparation methodology enables targeted compound recovery, highlighting the strong potential of sodium acetate-based NADES as sustainable solvents for fruit by-product valorisation.

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Graphical abstract



Highlights

- Sodium acetate-based NADES enabled efficient recovery of plum peel phenolics.
- Preparation method (MW, US, WB) significantly influenced extraction efficiency.
- Urea-based NADES showed superior performance for TPC, TFC, and antioxidant assays.
- Glycine-based systems yielded the lowest recoveries, confirming limited suitability.
- PCA revealed distinct clustering of antioxidant activity and phenolic composition.
- Red plum peel valorisation supports sustainable use of fruit-processing by-products.

Keywords Sodium acetate-based NADES · Green extraction · By-product valorisation · Sustainable processing

Introduction

NADES have emerged as sustainable alternatives to conventional organic solvents due to their composition from natural metabolites, low toxicity, biocompatibility, and adjustable polarity [20]. Acid-based NADES have been effectively used to extract various bioactive compounds, including coumarins, saponins, isoflavones, and phlorotannins [4, 23, 55, 59]. Most formulations employ choline chloride (ChCl) as the hydrogen bond acceptor with donors like ascorbic acid, lactic acid, malonic acid, citric acid, glycerol, or urea [7, 29, 50, 51, 71].

Different hydrogen bond donors play a critical role in determining the physicochemical properties and extraction performance of NADES. Urea-based systems have frequently demonstrated high extraction efficiency for phenolic compounds due to their strong hydrogen-bonding capacity and relatively low viscosity [34, 62]. Glycerol-based NADES are widely recognized for their ability to stabilize anthocyanins and other pigments through extensive hydrogen-bonding networks [9, 57]. Citric acid-based systems

provide acidic environments that may enhance the recovery and preservation of pH-sensitive bioactive compounds [66, 71]. In contrast, amino acid-based NADES, including glycine-containing formulations, have attracted increasing attention because of their biocompatibility and sustainability, although their extraction performance remains highly dependent on solvent composition and processing conditions [37, 48].

In the framework of sustainable process development, the 12 Principles of Green Chemistry emphasize waste minimization (low E-factor), the use of renewable and non-toxic materials, reduced energy consumption, and the preservation of environmental and societal balance [45, 68]. In this context, ionic liquids, defined as salts that remain liquid at or near room temperature, have attracted attention due to their negligible vapor pressure and high thermal stability [18]. However, their application in food-related and bio-based systems remains limited by concerns over toxicity, high production costs, and insufficient biodegradability, which restrict their compliance with green chemistry principles [77].

The preparation technique is another important factor influencing NADES structure and functionality. Conventional heating and water-bath methods are the most frequently employed approaches because they promote stable hydrogen-bond formation and homogeneous solvent generation [20, 40]. However, alternative techniques such as ultrasound-assisted and microwave-assisted synthesis have gained considerable attention in recent years. Ultrasound promotes cavitation, improves mass transfer, and accelerates solvent formation, while microwave irradiation enables rapid and energy-efficient synthesis through volumetric heating [16, 21]. Several studies have demonstrated that these alternative approaches can reduce preparation time, modify solvent microstructure, and improve extraction performance compared with conventional heating methods [30, 61, 65].

The application of NADES to fruit and vegetable by-products has shown high potential. ChCl-based systems outperformed ethanol for flavonoids and cinnamic acids from *Mentha piperita* [43], and yielded higher recovery of polar compounds from *Carthamus tinctorius* L. [20]. Recent studies have indicated enhanced recovery and stability of phenolics and anthocyanins from fruit by-products, including melon wastes [62], grape pomace [58], apple pomace [34], orange peel waste [57], mango peels [46], avocado wastes [32], araza pulp [63] and pomegranate peel [10].

However, despite the increasing interest in NADES-based extraction systems and their successful application to various fruit-processing by-products [10, 32, 34, 46, 57, 58, 62, 63], sodium acetate-based NADES remain largely unexplored for the recovery and stabilization of phenolic compounds from red plum peel. Furthermore, comparative studies evaluating how different preparation methods influence the performance of sodium acetate-based NADES are still scarce. Therefore, the combined effects of solvent composition and preparation strategy on extraction efficiency and bioactive compound recovery have not yet been systematically investigated.

In this study, sodium acetate-based NADES formulated with urea, citric acid, glycerol, and glycine were prepared using three different techniques (water bath heating, ultrasound-assisted, and microwave-assisted methods) and applied for the extraction of bioactive compounds from red plum peel. Ethanol was used as a reference solvent. To the best of our knowledge, this is the first comprehensive and systematic evaluation of sodium acetate-based NADES prepared by multiple methodologies for red plum peel valorisation. The findings provide new mechanistic insights into the structure–function relationships of NADES systems and offer a sustainable and efficient platform for the high-value utilisation of fruit processing wastes.

Material and methods

Materials

Fresh red plums (*Prunus domestica* L.) were obtained from a local market in Konya, Türkiye. Sodium acetate ($\text{CH}_3\text{COONa} \cdot 3 \text{H}_2\text{O}$), citric acid, urea, glycerol, glycine, Folin–Ciocalteu reagent, sodium carbonate (Na_2CO_3), 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ), copper(II) chloride dihydrate ($\text{CuCl}_2 \cdot 2 \text{H}_2\text{O}$), neocuproine, and ammonium acetate were of analytical grade and purchased from Sigma–Aldrich (St. Louis, MO, USA). Ethanol (HPLC grade) was obtained from Merck (Darmstadt, Germany). Deionized water was used throughout the experiments.

Drying plum peel

The fruits were washed thoroughly, and the peels were manually separated from the pulp. The collected peels were dried in a hot-air oven (Nüve, FN 500P, Ankara, Turkey) at 50 °C for 72 h until constant weight was achieved. The drying temperature and time were adapted from the method described by Jawad et al., [39]. Dried samples were ground into a fine powder using a laboratory grinder (Delonghi, KG49, Treviso, Italy) and sieved to obtain a particle size of approximately 0.25–0.50 mm. The powders were stored in airtight, light-protected containers at room temperature until further use.

Preparation of NADES

NADES were prepared using sodium acetate as the hydrogen bond acceptor (HBA) and four different hydrogen bond donors (HBDs): urea, citric acid, glycerol, and glycine. Ethanol was selected as the control solvent. The experimental design of the formulations and preparation conditions is outlined in Table 1.

For each NADES, the components were weighed at the specified ratios (Table 1) and subsequently mixed to reach a final volume of 6 mL. In instances where adjustment of the final volume was required, distilled water was added accordingly. In contrast, ethanol was used in its pure form without the addition of water. The component ratios for NADES formulations were selected according to previously reported protocols that ensure stable HBD–acceptor interactions and homogeneous solvent formation [20, 40, 61]. The preparation conditions were not independently optimized in the present study but were selected and adapted from previously published protocols reporting successful NADES formation and extraction performance [20, 61, 64]. Three distinct preparation techniques were applied independently to evaluate their influence on NADES formation and

Table 1 Experimental design of the extraction conditions of various NADES

HBA (acceptor)	HBD (donor)	Sodium acetate (g)	HBD amount (g/mL)	Applied methods
Sodium acetate	Urea	5.00	11.00 g	WB US MW
	Citric acid	1.88	14.12 g	WB US MW
	Glycerol	3.48	12.52 mL	WB US MW
	Glycine	4.27	11.73 g	WB US MW
	Ethanol	-	20.0 mL	WB

*Each DES formulation was prepared by combining the specified amounts of HBA and HBD with 4.0 mL of deionized water, followed by heating or processing as described

*Abbreviations: *HBA* Hydrogen bond acceptor, *HBD* Hydrogen bond donor, *WB* Water bath, *US* Ultrasonic treatment, *MW* Microwave irradiation

subsequent extraction performance. (i) the WB method: The mixtures were then subjected to heating in a WB (Nüve, NB-20, Ankara, Türkiye) at 50 °C for a duration of 60 min, with constant magnetic stirring (IKA C-MAG HS7, Staufen, Germany). This process was continued until transparent liquids were obtained [20]. (ii) the US method: The mixtures were then placed in sealed vials and treated in an ultrasonic bath (Nükleon NUB-10, Türkiye; 240 W, 35 kHz) at 50 °C for 25 min. Cavitation has been demonstrated to facilitate more rapid homogenization and dissolution of components compared to conventional heating methods [64]. (iii) the microwave-assisted method: The mixtures were then irradiated in a microwave oven (LG Solardome, Seoul, Korea) at 360 W for 1 min, followed by brief stirring to ensure the homogeneous distribution of the solvents [61].

All prepared NADES were cooled to room temperature before being stored in airtight glass containers for subsequent use in extraction experiments. The ethanol samples were subjected exclusively to the WB condition, thus serving as the conventional solvent control.

The extraction procedure

The solid-to-solvent ratio employed for all extractions was 1:20 (w/v), a commonly adopted proportion in NADES-based extraction studies to achieve efficient mass transfer and reproducible yields [6, 44]. In each experiment, 1 g of plum peel powder was mixed with 20 mL of the solvent in

screw-capped test tubes. The tubes were placed in a WB at 50 °C and stirred for 60 min to facilitate the transfer of phenolic compounds into the solvent. Subsequently, samples were subjected to a centrifugal process at 6000 rpm for 15 min (Hermle Z326K, Germany). Following this, the samples were filtered through Whatman No. 1 paper, and the clear extract was collected and stored in amber bottles at 4 °C until analysis. The ethanol extractions (control) followed the same procedure.

Determination of total phenolic content (TPC)

TPC was assessed using the Folin–Ciocalteu method with minor modifications based on Eyiz et al., [26]. A 0.4 mL aliquot of extract was combined with 2.4 mL distilled water, 0.4 mL 10% Folin–Ciocalteu reagent, and 1.2 mL 7.5% sodium carbonate. After 2 h of incubation at room temperature, absorbance was read at 765 nm. TPC was expressed as grams of gallic acid equivalents per kilogram of dry matter (g GAE/kg dm).

Antioxidant capacity using the DPPH, FRAP, CUPRAC methods

The antioxidant activity was evaluated using the DPPH radical scavenging method as described by Suárez et al., [72]. Briefly, 0.5 mL of each extract was mixed with 0.95 mL of 0.1 mM DPPH solution prepared in methanol and incubated for 30 min at room temperature. The absorbance was measured at 517 nm using a spectrophotometer (Biochrom, Libra S22, Cambridge, UK). Results were expressed as g Trolox equivalent/kg dry matter (g TEAA/kg dm).

The FRAP (Ferric Reducing Antioxidant Power) assay was performed according to the procedure by Chen et al., [17]. The FRAP reagent was freshly prepared by mixing 300 mM acetate buffer (pH 3.6), 10 mM TPTZ in 40 mM HCl, and 20 mM ferric chloride in a 10:1:1 (v/v/v) ratio. A 0.5 mL sample was added to 4.5 mL of FRAP reagent and incubated for 30 min. Absorbance was recorded at 593 nm, and results were expressed as g TEAA/kg dm.

The CUPRAC (Cupric Reducing Antioxidant Capacity) assay was performed according to Apak et al., [2]. The reaction mixture consisted of equal volumes (1:1:1, v/v/v) of 10 mM CuCl₂ solution, 1 M ammonium acetate buffer, and 7.5 mM neocuproine solution prepared in ethanol. Subsequently, 0.5 mL of plum peel extract (diluted 50-fold) and 0.6 mL of distilled water were added to 3.0 mL of the reaction mixture. The samples were incubated at room temperature for 30 min, and the absorbance was measured at 450 nm using a spectrophotometer (Biochrom, Libra S22, Cambridge, UK). Results were expressed as g TEAA/kg dm [2].

Determination of total flavonoid content (TFC)

TFC was measured following Chang et al., [15], with slight modifications. Rutin was used as the standard. In brief, 1 mL of each extract was mixed with 0.4 mL of 5% NaNO₂ in a 10 mL volumetric flask. After 6 min, 0.4 mL of 10% Al(NO₃)₃ was added. Following another 6-min reaction, 4 mL of 5% NaOH was added, and the mixture was left for 15 min. Absorbance was recorded at 510 nm using a spectrophotometer. The TFC was expressed as g CE/kg dm.

Determination of total monomeric anthocyanin content (TMAC) of extracts

TMAC was quantified using the pH differential method as described by Tontul and Topuz [76]. The extracts were diluted with buffer solutions at pH 1.0 and pH 4.5 and incubated in the dark for 20 min. Absorbance readings were then taken at 510 nm and 700 nm using distilled water as the blank. TMAC values were calculated according to Eq. (1) and expressed as mg cyanidin-3-glucoside per kg dry matter (mg C3G/kg dm).

$$TMAC \left(\frac{mg}{kg} \right) = \frac{[(518 - 700)]_{pH 1.0} - [(518 - 700)]_{pH 4.5} \times MW \times Df \times 1000}{\epsilon \times l} \quad (1)$$

MW: molecular weight of C3G (449.2 g/mol),

Df: dilution factor,

ϵ : molar extinction coefficient of cyanidin-3-glucoside (26,900 L/(mol.cm)),

l: path length of spectrophotometer (1 cm).

Determination of total proanthocyanin content (TPAC) of extracts

TPAC was performed according to the method proposed by Eyiz and Acar [25]. A solution of FeSO₄·7 H₂O (154 ppm) was prepared using a 2:3 (v/v) mixture of HCl and n-butanol. In sealed glass tubes, 2.5 mL of the prepared reagent was added to 0.25 mL of the extract. The tubes were then incubated in a WB at 95 °C for 15 min. Following incubation, the absorbance was measured at 540 nm. Results were expressed as mg cyanidin per kg dm (mg C3G / kg dm).

Statistical analysis

All experiments were conducted in triplicate, and the results were expressed as mean ± standard deviation. Statistical analyses, including Pearson's correlation, were carried out using SPSS version 21.0 (SPSS Inc., Chicago, IL, USA). Differences between groups were assessed using one-way ANOVA, with statistical significance set at $P < 0.05$.

Greenness assessment in this study was qualitatively evaluated based on solvent safety, volatility, energy demand, and waste generation, in line with sustainable chemistry principles.

Results and discussion

Total phenolic and flavonoid content of NADES-based plum peel extracts

Most previous studies on NADES-based extraction primarily report extraction yields and antioxidant capacities, with limited mechanistic interpretation of how solvent composition and preparation strategies govern solvent structure, solute–solvent interactions, and compound stability. In contrast, the present study provides a comprehensive evaluation of how different sodium acetate-based NADES formulations and preparation methods modulate hydrogen bonding networks, viscosity, polarity, and mass transfer behavior, thereby directly affecting phenolic solubilization and antioxidant performance. This integrative approach enables a deeper understanding of structure–function relationships in NADES systems and advances the rational design of green extraction solvents.

As presented in Table 2, the TPC of red plum peel extracts ranged from 20.85 to 48.46 g GAE/kg dm. A clear extraction trend was observed, with MW extraction being the most effective method, followed by US extraction and WB extraction in that order, for all NADES formulations. The MW-assisted urea NADES (N3: 48.46 g GAE/kg dm) produced the highest yield, followed by the US-assisted urea NADES (N2: 47.86 g GAE/kg dm), whereas the WB condition (N1: 42.45 g GAE/kg dm) yielded a lower recovery. Conversely, the ethanol control (N13: 20.85 g GAE/kg dm) demonstrated the minimal activity. Glycine-based systems (N10–N12: 21.21–26.36 g GAE/kg dm) also demonstrated consistently lower performance in comparison to the other NADES formulations ($P \leq 0.05$). The enhanced performance of urea-based NADES can be attributed to their potent hydrogen-bonding capacity, which enables interactions with hydroxyl-rich phenolic compounds. Rico et al., [62] on melon peel sodium acetate–urea NADES were found to demonstrate significantly superior performance in comparison to ethanol. Furthermore, MW and US intensification have been demonstrated to enhance mass transfer and reduce solvent viscosity, thereby accelerating extraction efficiency in urea-based systems [61]. These findings are consistent with the remarkable yields obtained in the present study. Glycerol-based NADES produced intermediate TPC values (38.01–42.97 g GAE/kg dm). The performance of the system is associated with the multiple

Table 2 Total phenolic, flavonoid, anthocyanin, and proanthocyanidin contents of NADES-based red plum peel extracts

Sample	TPC (g GAE/kg dm)	TFC (mg CE/kg dm)	TMAC (mg C3G/kg dm)	TPAC (mg cyanidin/km dm)
N1	42.45±0.21* ^{b†}	2020.02±26.93 _i	4006.53±79.72 g	28.28±1.15bc
N2	47.86±1.41a	3884.98±25.58a	4898.12±81.24f	28.06±2.04bc
N3	48.46±0.67a	3608.06±30.38b	3805.88±99.91 h	27.91±2.92bc
N4	34.41±1.44d	2911.12±13.00f	6183.98±110.46d	24.30±0.52d
N5	30.53±0.64e	2500.40±10.31 g	3523.94±76.92 _i	20.23±0.42e
N6	39.01±0.44c	3389.58±15.56d	6691.01±78.69c	26.14±1.46 cd
N7	42.97±1.93b	3461.04±15.91c	6679.34±42.47c	27.10±2.40bc
N8	40.65±2.11bc	3344.47±61.77e	7103.19±128.33a	34.04±1.98a
N9	38.01±0.79c	3363.19±15.43e	6794.97±87.09b	27.18±0.63bc
N10	26.36±0.50f	2050.59±14.63 h	7093.63±90.23a	29.43±2.27b
N11	21.21±4.75 g	1950.92±13.24j	3816.69±95.70 h	14.29±1.14 g
N12	24.91±6.42f	1912.48±17.36k	5297.30±78.19e	17.61±0.26f
N13	20.85±0.23 g	2057.65±20.38 h	4814.45±44.13f	12.89±0.35 g

*Abbreviations: N1 Urea+WB, N2 Urea+US, N3 Urea+MW, N4 Citric acid+WB, N5 Citric acid+US, N6 Citric acid+MW, N7 Glycerol+WB, N8 Glycerol+US, N9 Glycerol+MW, N10 Glycine+WB, N11 Glycine+US, N12 Glycine+MW, N13 Control (Ethanol)

*Mean±standard deviation ($n=3$)

†Statistical differences in the same column are shown by different lower case letters ($P<0.05$). Statistical results obtained from Duncan methods

hydroxyl groups of glycerol, which improve solubility. However, extraction efficiency is restricted by the high viscosity of these solvents. The findings of this study are consistent with those of previous research on banana peel [14] and orange peel [57], where the combination of glycerol NADES with US was found to be more effective than conventional solvents. Citric acid-based NADES achieved moderate results (30.53–39.01 g GAE/kg dm). The acidity and chelating ability of citric acid have been demonstrated to support phenolic release. However, it is evident that viscosity once again limited extraction efficiency, particularly under WB conditions. Comparable outcomes have been reported in extractions from apple pomace [31], mango peel [66], and peach/apricot by-products [71], where US or MW assistance improved recovery. Glycine-based NADES consistently yielded the lowest phenolic concentrations (21.21–26.36 g GAE/kg dm). Although amino acid-based NADES are regarded as biocompatible and sustainable [48], their comparatively weaker hydrogen-bonding networks result in reduced solubilization efficiency. However, US extraction yielded higher values than WB extraction, which is consistent with findings reported in the study on blueberry leaves [65]. Amino acid-based natural deep eutectic solvents also still require substantial optimisation to achieve comparable efficiency to urea- or glycerol-based formulations. In one study, amino acid-based systems (glycerol–L-proline, glycerol–L-alanine, glycerol–glycine, glycerol–L-histidine, glycerol–L-threonine, glycerol–L-lysine, and glycerol–L-arginine) were functional but generally less efficient than choline chloride–glycerol, highlighting the need for further optimisation [37].

As demonstrated in Table 2, the TFC of red plum peel extracts ranged between 1912.48 and 3884.98 mg CE/kg dm. The highest recovery was obtained with urea and US (N2: 3884.98 mg CE/kg dm), followed by urea and MW (N3: 3608.06 mg CE/kg dm). The lowest recorded value was observed in the glycine + MW group (N12: 1912.48 mg CE/kg dm). The ethanol (N13: 2057.65 mg CE/kg dm) yielded significantly lower values than the most effective NADES systems ($P \leq 0.05$). The overall extraction trend was consistent with the observations for TPC, where urea-based NADES demonstrated the greatest efficiency, particularly under US and MW assistance. These findings are in agreement with recent reports highlighting the superior performance of urea-containing systems for flavonoid recovery from agri-food by-products [53, 75]. Conversely, glycerol- and citric-acid-based NADES exhibited moderate recovery rates, while glycine systems consistently yielded the lowest yields, thereby confirming their limited suitability for flavonoid extraction. However, the employment of US assistance enhanced the efficiency of glycerol and glycine formulations in comparison with WB extraction, a phenomenon that has also been observed in the context of blueberry leaves [65] and other fruit residues [87].

Total anthocyanin (TMAC) and proanthocyanin (TPAC) content of NADES-based plum peel extracts

According to Table 2, TMAC values ranged from 3805.88 to 7103.19 mg C3G/kg dm. The highest anthocyanin contents were observed in N8 (Glycerol + US, 7103.19 mg C3G/kg) and N10 (Glycine + WB, 7093.63 mg C3G/kg) ($P \leq 0.05$).

These findings indicate that both glycerol- and glycine-based NADES, under the applied extraction conditions, are highly effective in recovering anthocyanins from plum peel. Glycerol combined with US benefits from reduced viscosity and enhanced mass transfer, while glycine under WB conditions may provide a stabilizing microenvironment that protects anthocyanins from thermal degradation [78, 83]. Moreover, glycerol-based NADES, especially when combined with US, proved to be highly efficient, which is consistent with previous reports where choline chloride–glycerol NADES were successfully applied for anthocyanin extraction from bilberry [42]. In contrast, the lowest TMAC values were found in N11 (Glycine + US, 3816.69 mg C3G/kg) and N3 (Urea + MW, 3805.88 mg C3G/kg). The suboptimal performance of glycine + US may be attributable to the instability of amino acid-based NADES under cavitation conditions. Conversely, the low efficiency of urea + MW indicates that anthocyanins are more susceptible to degradation under microwave heating, despite the high efficacy of urea NADES in terms of total phenolics. The ethanol (N13: 4814.45 mg/kg) exhibited a lower level of performance than N11 and N3, yet its yield remained significantly lower than that of most NADES systems. This finding indicates that while ethanol can extract anthocyanins to a moderate extent, NADES formulations consistently outperform ethanol, particularly when combined with US or WB methods [84]. Notably, this is the first report demonstrating that sodium acetate-based NADES can provide superior anthocyanin and proanthocyanidin recovery from red plum peel, thereby expanding their applicability beyond conventional choline chloride-based systems.

As illustrated in Table 2, TPAC values ranged from 12.89 to 34.04 mg cyanidin/kg dm ($P \leq 0.05$). The highest content was found in N8 (Glycerol + US, 34.04 mg C3G/kg dm), followed by N10 (Glycine + WB, 29.43 mg C3G/kg dm). In contrast, the lowest TPAC levels were recorded for N11 (Glycine + US, 14.29 mg C3G/kg dm) and the ethanol (N13: 12.89 mg C3G/kg dm). It is noteworthy that this distribution closely mirrors the TMAC results, wherein N8 and N10 also achieved the highest recoveries, while N11 and N13 consistently produced the lowest values. This parallel trend suggests that the NADES systems that facilitate anthocyanin recovery are equally effective in enhancing proanthocyanidin extraction. The superior performance of glycerol in combination with US highlights the synergistic effect of polyol-based NADES and cavitation. The application of US has been demonstrated to reduce viscosity and promote solvent penetration, thereby facilitating the release of condensed tannins. Comparable results were reported by Wang et al., [80], who achieved efficient recovery of procyanidins from grape residue using ultrasound-assisted extraction. Zannou et al., [83] also demonstrated that

choline chloride–glycerol NADES under US can recover high amounts of anthocyanins and related compounds from blackberry. The substantial outcome observed for glycine + WB (N10) indicates that amino acid-based NADES have the capacity to act as a stabilising medium for condensed tannins when subjected to mild heating conditions. This finding is consistent with the results reported by Grisales-Mejía et al., [32], who demonstrated the efficacy of amino acid NADES in combination with UAE/PLE for tannin-rich avocado residues. Conversely, glycine + US (N11) resulted in a markedly diminished TPAC, presumably due to the destabilisation of solvent–pigment interactions caused by cavitation. This explanation is supported by recent reports showing that ultrasound cavitation can disrupt hydrogen bonding networks and generate localised hotspots, leading to degradation or destabilisation of polyphenolic compounds [22, 78]. The ethanol (N13) also exhibited substandard performance in comparison to the vast majority of NADES formulations, thereby substantiating the limited efficacy of ethanol in terms of tannin recovery. This finding is consistent with the recent reports in [84], which demonstrated that ethanol consistently yielded lower levels of anthocyanin and proanthocyanidin when compared to NADES under US/MW intensification conditions.

Antioxidant properties of NADES-based plum peel extracts

DPPH radical scavenging activity

The DPPH values ranged extensively from 75.06 to 179.03 g TEAA/kg dm (Fig. 1 and Table A.1). T11694_2026_4624_ArticleES prepared by MW (N3: 179.03 g TEAA/kg dm), followed closely by the US-assisted urea system (N2: 177.16 g TEAA/kg dm). In contrast, the lowest value was observed in glycine + US (N11: 75.06 g TEAA/kg dm), while the ethanol (N13: 101.12 g TEAA/kg dm) remained almost 1.8 times lower than the best-performing NADES. Statistical analysis confirmed that both HBD type and preparation method significantly affected the radical scavenging potential (Table A.1). The enhanced performance of MW- and US-assisted NADES is consistent with recent findings that MAE and UAE facilitate mass transfer by improving cell wall disruption and solvent penetration, thereby yielding higher antioxidant activity compared to conventional heating [16, 61]. A secondary critical factor pertains to the composition of NADES. For example, Lazović et al., [47] showed that choline chloride: urea and choline chloride: glycerol NADES provided significantly greater DPPH radical scavenging activity than ethanol. Similarly, Hu et al., [36] demonstrated that optimized NADES formulations yielded markedly higher DPPH and FRAP activities compared to

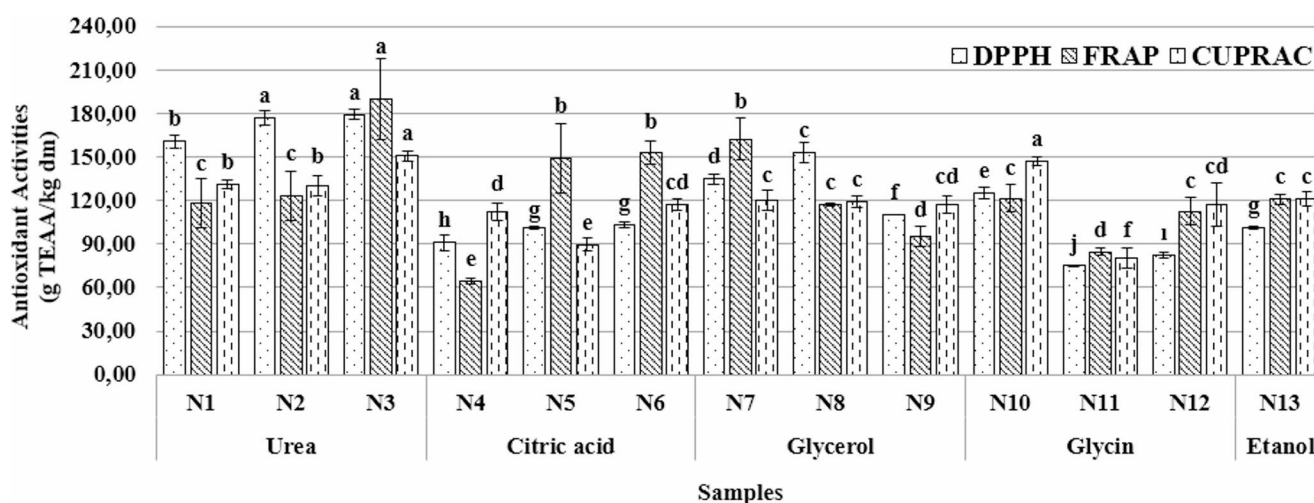


Fig. 1 Antioxidant activities of NADES-based red plum peel extracts (DPPH, FRAP, CUPRAC). *Results are expressed as g Trolox equivalent antioxidant activity (TEAA) per kg dry matter (mean±SD, $n=3$). Different lowercase letters within the same column indicate statistically significant differences among means ($P \leq 0.05$) according to Dun-

can's multiple range test. *Sample codes: N1 = Urea + WB; N2 = Urea + US; N3 = Urea + MW; N4 = Citric acid + WB; N5 = Citric acid + US; N6 = Citric acid + MW; N7 = Glycerol + WB; N8 = Glycerol + US; N9 = Glycerol + MW; N10 = Glycine + WB; N11 = Glycine + US; N12 = Glycine + MW; N13 = Control (Ethanol)

conventional solvents. In addition, Sik et al., [70] reported that glucose–glycerol NADES efficiently extracted phenolics and exhibited strong radical scavenging from Cornelian cherry fruit residues. Moreover, L-proline: urea NADES demonstrated remarkable performance for flavonoid recovery and DPPH activity. In accordance with the mentioned reports, the present study corroborated the sequence urea > glycerol/citric acid/glycine when assessed under intensified preparation conditions. Previous studies have also demonstrated the effectiveness of sodium acetate: urea NADES in the valorisation of fruit by-products, such as melon peel [62]. Our findings extend this evidence to red plum peel, a matrix that has received little attention to date, thus confirming the potential of acetate–urea systems. Furthermore, some NADES may have intrinsic antioxidant properties and act synergistically with phenolic compounds, which could account for their superior performance compared to ethanol [56]. These results confirm that the synergistic interaction between sodium acetate-based NADES composition and intensified preparation techniques enables enhanced stabilization and activity of antioxidant compounds, representing a significant advancement beyond conventional solvent extraction strategies.

Ferric reducing antioxidant power (FRAP)

The range of FRAP values observed in this study was from 64.27 to 189.67 g TEAA/kg dm (Fig. 1 and Table A.1). The urea-based NADES prepared using a MW (N3: 189.67) demonstrated peak reducing capacity, which was approximately 3 times higher than that of the lowest performer (citric acid + WB, N4: 64.27). The ethanol (N13: ~120.7)

exhibited a significantly lower level of activity compared to the urea-MW extract ($P \leq 0.05$, Table A.1). This outcome is consistent with recent findings that MW- or US-assisted NADES significantly increase FRAP values compared to traditional solvents in grape pomace and blueberry by-products [53]. The high polarity and robust hydrogen bonding interactions intrinsic to urea-based NADES are considered pivotal in enhancing the solubilisation and stabilisation of phenolic compounds across diverse matrices [41, 74]. The strong agreement between TPC and FRAP results suggests that phenolic compounds were major contributors to the reducing capacity of the extracts. Similar relationships between phenolic content and ferric reducing power have been reported for NADES-based extracts obtained from fruit processing by-products [36, 53].

Cupric reducing antioxidant capacity (CUPRAC)

As demonstrated in Fig. 1 and Table A.1, the CUPRAC results ranged from 80.20 to 150.61 g TEAA/kg dm. Once more, urea and MW (N3) yielded the highest result, while glycine and US (N11: ~80.20) yielded the lowest. The ethanol (N13: ~121.2) is situated beneath the upper limit of the NADES result ($P \leq 0.05$). Even though the range of urea-based NADES is comparatively restricted when juxtaposed with that of FRAP, the merits of NADES derived from urea are unmistakable. The extant literature confirms that NADES, particularly those containing urea, enhance CUPRAC outcomes in the extraction of fruit and vegetable residues [24, 43, 74]. It is also noted that the ROS mechanisms underlying CUPRAC are particularly sensitive to phenolic compounds. It has been demonstrated that

MW- and US-assisted NADES protocols intensify interactions between the solvent and the cell matrix, thus raising CUPRAC readings relative to conventional heating [75]. Furthermore, some NADES exhibit intrinsic reducing capacity, which reinforces the apparent antioxidant outcomes through synergy with the extracted phenolic compounds [41, 56]. The consistency observed between CUPRAC, FRAP, and TPC results further indicates that the extraction efficiency of sodium acetate-based NADES is closely associated with their ability to selectively recover redox-active phenolic compounds. Similar trends have been reported for NADES-assisted extraction systems applied to fruit and vegetable residues [43, 75].

Impact of HBD on extraction efficiency

To facilitate the interpretation of the effect of hydrogen bond donor type, the mean values of all analytical parameters were calculated for each HBD irrespective of the preparation method and are summarized in Table 3. The urea-based NADES demonstrated the highest efficiency across the majority of analyses, particularly in terms of TPC and antioxidant assays (DPPH, FRAP, and CUPRAC). This enhanced performance can be attributed to urea's dual hydrogen-bonding functionality, with carbonyl and amino groups enabling both donor and acceptor interactions, thereby enhancing the solubilisation of phenolics, flavonoids, and anthocyanins [34, 62, 81]. The relatively low viscosity of urea-based systems has been demonstrated to promote diffusion and mass transfer further, thus explaining their consistent superiority [47]. Recent studies have consistently demonstrated that urea-containing NADES provide the highest polyphenol recoveries from apple pomace [11] and NADES formulations have been found to exhibit comparable extraction efficiencies to ethanol in other plant matrices [35, 38, 52].

Citric acid-based NADES have been shown to generally exhibit moderate extraction yields. The presence of three

carboxyl and one hydroxyl group provides strong proton-donating capacity and metal-chelating ability, facilitating not only the release of phenolics but also flavonoids and anthocyanins from plant matrices [66]. It has been established through previous studies that citric acid NADES typically display low pH and high viscosity. These parameters have been demonstrated to influence not only the solubility of phenolic compounds, but also the colour stability and preservation of anthocyanins [9, 67]. However, it has been demonstrated that excessive acidity has the capacity to compromise the structural stability of flavonoids and anthocyanins [63]. Furthermore, elevated viscosity has been shown to restrict mass transfer and reduce extraction efficiency. For instance, Li et al. [49] reported that high viscosity in NADES significantly lowers diffusion rates of phenolic compounds, while Guzmán-Lorite et al. [33] found that adding water to NADES reduced viscosity and thus enhanced mass transfer and yield.

Glycerol-based NADES demonstrated notable efficacy in TMAC and TPAC assays, indicative of their substantial capacity to solubilise anthocyanins and proanthocyanidins. The tri-hydroxyl structure of glycerol has been shown to create extensive hydrogen-bonding networks, which have been demonstrated to improve pigment solubility [27]. A recent study by glycerol extraction of blueberry pomace revealed that glycerol's multi-hydroxyl group structure significantly contributes to the maintenance of anthocyanin stability and higher pigment recovery [28]. However, the high intrinsic viscosity of the substance remains a significant limitation in its application, often necessitating the use of US or MW assistance to reduce diffusion resistance and enhance yields [14, 57].

Glycine-based NADES consistently exhibited the lowest extraction yields across TPC, TFC, TMAC, and TPAC. This phenomenon can be attributed to the limited functional group diversity exhibited by glycine, which comprises a single amino and a single carboxyl group. This structural limitation results in a deficiency in hydrogen bonding strength

Table 3 Average properties of NADES extracts by HBD type

Analysis	Urea	Citric acid	Glycerol	Glycine
TPC (g GAE/kg dm)	46.25±0.60 ^{a†}	34.65±0.60 ^c	40.54±0.60 ^b	24.16±0.60 ^d
DPPH (g TEAA/kg dm)	172.31±1.16 ^a	98.48±1.16 ^c	132.66±1.16 ^b	94.11±1.16 ^d
FRAP (g TEAA/kg dm)	143.69±3.63 ^a	122.14±3.63 ^b	124.62±3.63 ^b	106.22±3.63 ^c
CUPRAC (g TEAA/kg dm)	137.12±1.13 ^a	106.23±1.13 ^d	118.85±1.13 ^b	114.97±1.13 ^c
TFC (g CE/kg dm)	3171.01±6.02 ^b	2933.70±6.02 ^c	3389.57±6.02 ^a	1971.32±6.02 ^d
TMAC (mg C3G/kg dm)	4236.84±20.49 ^d	5466.31±20.49 ^b	6859.17±20.49 ^a	5402.54±20.49 ^c
TPAC (mg C3G/kg dm)	28.09±0.50 ^a	23.56±0.50 ^b	29.55±0.50 ^a	20.44±0.50 ^c

*Mean±standard deviation (n=3)

†Different lowercase letters within the same row indicate statistically significant differences among HBD types ($P \leq 0.05$) according to Duncan's multiple range test. Statistical results obtained from Duncan methods

and an impaired effectiveness in solute–solvent interactions when compared to polyols or polycarboxylic acids [85]. Recent studies with fruit matrices have confirmed these limitations: lactic acid–glycine NADES displayed lower polyphenol recovery from pitaya extracts compared to other acid-based systems [60]. Furthermore, polarity mismatches and viscosity effects restrict their ability to efficiently extract flavonoids and anthocyanins [5, 54].

Impact of applied methods on extraction efficiency

The method of NADES preparation had a substantial impact on the properties of the solvents, which in turn affected the phenolic profiles and antioxidant activities of the extracts (Table 4). MW-prepared solvents yielded the highest TPC (37.60 g GAE/kg dm), FRAP (137.66 g TEAA/kg dm), and TFC (3068.33 mg CE/kg dm). These results are consistent with previous findings that MW promotes rapid homogeneous solvent formation and enhances extraction efficiency [48, 61]. US-prepared solvents produced intermediate TFC (2920.19 mg CE/kg dm) values, attributable to cavitation-driven homogenization that facilitated flavonoid solubilization [65]. However, US-prepared solvents showed lower TMAC and TPAC values, suggesting that cavitation may negatively affect anthocyanin and tannin stability. WB-prepared solvents provided the highest CUPRAC (127.69 g TEAA/kg dm) and TPAC (27.28 mg cyanidin/kg dm), in

line with reports that WB ensures a stable solvent environment under controlled heating [57, 64]. Earlier studies [16, 21] highlighted that MW and US shorten solvent formation time, reduce energy consumption, and improve solvent uniformity. NADES can be prepared rapidly by microwave (MW) or ultrasound (US): MW irradiation can yield homogeneous liquids within seconds, and ultrasonic-bath sonication (~ 40 kHz, ~ 50 °C) reliably affords clear NADES; both routes are well documented [13, 30, 61, 65, 82].

Importantly, this study demonstrates for the first time that NADES preparation methodology is not merely a technical step but a critical design parameter that governs solvent structure, extraction efficiency, and compound stability. By systematically comparing water bath heating, ultrasound-assisted, and microwave-assisted preparation techniques, the present work provides novel mechanistic insights into how preparation-induced changes in hydrogen bonding dynamics, viscosity, and homogeneity translate into enhanced extraction performance. This integrated evaluation significantly advances the current understanding of NADES systems and offers a rational framework for designing sustainable extraction processes.

Correlation analysis of phenolics and antioxidant activities

Correlation analysis yielded a more profound understanding of the interrelationships between phenolic subclasses and antioxidant assays in the NADES extracts (Table 5). The most significant result was the strong positive correlation between TPC and DPPH ($r = 0.831$, $P < 0.01$) as well as between TPC and TFC ($r = 0.795$, $P < 0.01$). This finding clearly indicates that phenolics, particularly flavonoids, are the major contributors to the radical scavenging capacity of the extracts, as also reported in fruit-based systems [3, 79]. The positive correlation between TPC and FRAP ($r = 0.461$, $P < 0.01$) lends further support to the hypothesis that total phenolics enhance electron-transfer-based reducing activity, albeit to a lesser extent compared to radical scavenging. Another noteworthy trend was the significant association of TPAC with both TPC ($r = 0.687$, $p < 0.01$) and CUPRAC ($r = 0.673$, $p < 0.01$). This finding indicates that anthocyanins function as pivotal reducing agents, particularly in assays that are sensitive to conjugated structures and metal ion reduction. Conversely, CUPRAC exhibited a moderate correlation with FRAP ($r = 0.392$, $P < 0.05$), consistent with the methodological disparities inherent in the measurement of antioxidant power [2]. It was found that TMAC exhibited only moderate correlations with TPAC ($r = 0.506$, $P < 0.01$), indicating that while tannins share structural similarities with anthocyanins, their contribution to global antioxidant activity is more assay-dependent. This underscores the intricacy of plant extract matrices, wherein each subclass of phenolics

Table 4 Average properties of NADES extracts by applied methods

Analysis	WB	US	MW
TPC (g GAE/ kg dm)	36.55±0.52*ab†	35.06±0.52b	37.60±0.52a
DPPH (g TEAA/ kg dm)	127.84±1.00a	126.72±1.00a	118.62±1.00b
FRAP (g TEAA/ kg dm)	116.47±3.15b	118.37±3.15b	137.66±3.15a
CUPRAC (g TEAA/ kg dm)	127.69±0.98a	104.70±0.98b	125.49±0.98a
TFC (g CE/kg dm)	2610.68±5.21c	2920.19±5.21b	3068.33±5.21a
TMAC (mg C3Gkg dm)	5590.87±17.45a	4835.49±17.75c	5647.29±17.75b
TPAC (mg cyani- din/km dm)	27.28±0.44a	24.16±0.44b	24.71±0.44b

*Mean±standard deviation ($n=3$)

†Statistical differences in the same column are shown by different lower case letters ($P<0.05$). Statistical results obtained from Duncan methods

Table 5 Pearson correlation coefficient among TPC, DPPH, FRAP, CUPRAC, TFC, TMAC and TPAC assays of the NADES extractions

	TPC	DPPH	FRAP	CUPRAC	TFC	TMAC	TPAC
TPC	1						
DPPH	0.831**	1					
FRAP	0.461**	0.489**	1				
CUPRAC	0.542**	0.698**	0.392*	1			
TFC	0.795**	0.530**	0.378*	-	1		
TMAC	-	-	-	-	-	1	
TPAC	0.687**	0.729**	-	0.673**	0.539**	0.506**	1

**Correlation is significant at the 0.01 level

may prevail over distinct antioxidant mechanisms. The results of this study underscore the pivotal role of phenolics, particularly flavonoids and anthocyanins, in determining the antioxidant potential of NADES extracts. Comparable observations have been reported in previous research, which identified TPC and TFC as significant contributors to radical scavenging, while anthocyanins were found to be effective in reducing assays such as CUPRAC [12, 19].

Multivariate evaluation of phenolic composition and antioxidant activity by PCA

PCA was performed to simplify the complex dataset of antioxidant parameters (TPC, TFC, DPPH, FRAP, CUPRAC, TMAC, TPAC) and to investigate the relationships between phenolic composition and antioxidant activities within the NADES extracts, as widely applied in phenolic profiling and NADES-based extraction studies [3, 12, 13]. The dataset's suitability for PCA was confirmed by Bartlett's test of sphericity ($P < 0.001$), demonstrating significant inter-variable correlations, and the Kaiser–Meyer–Olkin measure indicated acceptable sampling adequacy. The first two principal components accounted for approximately 75% of the total variance (PC1: 53.03% and PC2: 20.42%), supporting effective dimensionality reduction while preserving the essential structure of the data, which is consistent with previous studies [19].

According to the rotated component matrix (Table 6), PC1 demonstrated a strong association with antioxidant-related

variables, exhibiting high positive loadings for DPPH (0.939), TPC (0.910), TFC (0.769), CUPRAC (0.687), and FRAP (0.625). This clustering indicates that PC1 represents a general antioxidant capacity axis, predominantly governed by total phenolics and flavonoids. The markedly elevated co-loading of TPC and DPPH indicates that radical scavenging activity is predominantly driven by phenolic hydroxyl groups with the capacity for hydrogen atom donation, a finding that is consistent with established antioxidant mechanisms [2, 41]. Furthermore, the integration of FRAP and CUPRAC within a unified component substantiates the notion that electron transfer-based antioxidant assays are predominantly influenced by a shared phenolic pool. However, it is important to note that these assays may exhibit divergent sensitivities to particular compounds [1, 12].

In contrast, PC2 was predominantly characterised by pigment-related phenolics, with TMAC exhibiting a notably high loading (0.936), while TPAC contributed to both PC1 (0.741) and PC2 (0.561). This finding suggests that anthocyanins constitute a discrete compositional dimension, exhibiting partial independence from the overall antioxidant pool. As demonstrated in the relevant literature, analogous PCA separations have been documented in the context of fruit and plant systems [5, 69]. In these systems, anthocyanins have been observed to form distinct clusters, a phenomenon attributable to their distinctive flavylum structure and stability characteristics.

The spatial distribution of variables in the rotated component plot (Fig. 2) lends further support to these interpretations. FRAP is positioned separately along Component 1, while DPPH and CUPRAC are closely clustered near the origin on the same component, collectively indicating that Component 1 predominantly captures antioxidant capacity. TPC, TFC, DPPH, and CUPRAC are tightly clustered along the positive axis of PC1, indicating strong positive correlations and a shared biochemical origin. On the other hand, TPC, TFC, TPAC, and TMAC are located higher along Component 2, reflecting a strong association with phenolic composition. The location of FRAP along PC2 suggests a partial mechanistic distinction, likely associated with ferric ion reduction specificity [2]. In contrast, TMAC is distinctly separated along PC2, confirming that anthocyanin-rich

Table 6 Rotated component loadings illustrating the relationships between phenolic composition and antioxidant activity in NADES extracts

Analysis	Rotated component matrix ^a	
	Component	
	x (PC1)	y (PC2)
TPC	0.910	-
DPPH	0.939	-
FRAP	0.625	-
CUPRAC	0.687	-
TFC	0.769	-
TMAC	-	0.936
TPAC	0.741	0.561

Principal component analysis with Varimax rotation. Only loadings ≥ 0.40 are shown

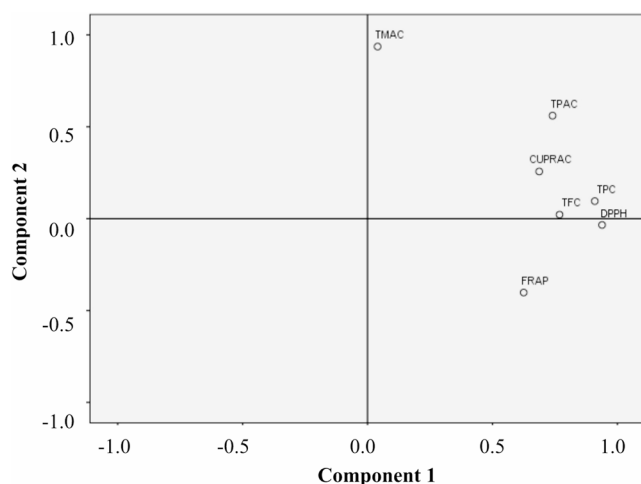


Fig. 2 Rotated component plot showing the distribution of variables along component 1 and component 2 obtained from PCA

fractions behave independently from total phenolics and flavonoids, while TPAC is positioned between these clusters, reinforcing its intermediate functional role. The clear spatial separation between these groups highlights the existence of two related yet biochemically distinct dimensions within the dataset.

In addition to variable relationships, PCA also provides insight into sample behaviour and extraction selectivity. Although Fig. 2 presents variable loadings as opposed to score values, the observed structure enables the indirect interpretation of sample positioning. NADES extracts characterised by elevated TPC, TFC, and antioxidant activity – particularly urea-based systems such as N2 and N3 – are hypothesised to be associated with the positive side of PC1, reflecting their dominance in antioxidant capacity. Conversely, samples with elevated anthocyanin and proanthocyanidin contents, such as glycerol-based N8 and glycine-based N10, are predicted to exhibit a stronger correlation with PC2, corresponding to the pigment-related compositional axis. Conversely, low-performing extracts, such as glycine-based N11 or ethanol control N13, are likely to cluster near the origin, indicating negligible contributions to both components. Comparable PCA-based interpretations of extraction selectivity and bioactive clustering have been reported in NADES and plant phenolic studies [3, 73].

Overall, PCA revealed that the dataset is structured along two major biochemical dimensions: (i) a dominant antioxidant capacity axis (PC1), primarily governed by total phenolics and flavonoids, and (ii) a secondary compositional axis (PC2), driven by anthocyanins and condensed tannins. This distinction highlights that antioxidant activity in NADES extracts arises from a synergistic and mechanism-dependent contribution of multiple phenolic subclasses rather than a single compound group. Furthermore, the clear separation between these axes confirms that NADES composition and

preparation methods selectively influence both extraction efficiency and the functional profile of bioactive compounds.

This finding underscores the notion that a specific compound class does not solely govern the antioxidant activity observed in NADES extracts, but rather results from a multifaceted, mechanism-dependent contribution from multiple phenolic subclasses. Furthermore, the clear distinction between these axes confirms that NADES composition and preparation methods selectively modulate not only extraction efficiency but also the functional profile of the recovered compounds. The present findings are in accordance with those of recent studies, which have emphasised the importance of tailored NADES systems for the targeted recovery of bioactive substances [8, 86].

Conclusion

The present study demonstrated that sodium acetate-based NADES, prepared with different HBDs and formation methods, provide efficient and environmentally friendly alternatives to conventional solvents for the extraction of phenolics from red plum peels. Urea-based systems have been shown to outperform ethanol-based systems in terms of phenolic and flavonoid recovery, as well as antioxidant capacity. This finding highlights the potential of urea-based systems for bioactive enrichment. Glycerol-based NADES, when subjected to US, demonstrated remarkable efficiency in the extraction and stabilization of anthocyanins and proanthocyanidins. In contrast, citric acid-based systems yielded moderate outcomes, while glycine-based NADES exhibited the least effective performance. The preparation technique had a significant effect on the outcomes of the extraction process. MW-assisted NADES improved the overall recovery of phenolics, US promoted the solubilisation of flavonoids, and WB conditions preserved condensed tannins. The strong correlations between TPC, TFC, and antioxidant assays confirmed that flavonoids and anthocyanins are the primary contributors to radical scavenging and reducing activities. Collectively, these results provide new insights into the role of sodium acetate-based NADES as sustainable green solvents and establish their applicability for the valorisation of plum peel by-products into functional food and nutraceutical formulations. It is recommended that future studies expand the present approach to include different fruit by-products, whilst also exploring a wider range of sodium acetate-based NADES compositions. This would enable further optimisation of extraction efficiency and the potential broadening of applications. The study demonstrates that maximum extraction yield does not necessarily correspond to maximum greenness, and that balanced operational conditions provide a more sustainable extraction strategy.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s11694-026-04624-z>.

Author contributions Vildan Eyiz: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Writing – Review & Editing, Visualization.

Funding Open access funding provided by the Scientific and Technological Research Council of Türkiye (TÜBİTAK). This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Data availability Data will be made available on request.

Declarations

This work does not involve any animal experiments and does not violate any items required by research ethics.

Conflict of interest The author declared no conflict of interest.

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