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Synergistic use of NADES and assisted extraction technologies for recovery and stabilization of bioactive compounds in *Beta vulgaris* L. peels

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ABSTRACT

This work concerns the use of Natural Deep Eutectic Systems (NADES) as sustainable extraction solvents to obtain a bioactive extract, from beetroot peels (BRP), that can be used in food or cosmetic applications. BRP are considered a agrifood waste and often underutilized, although they are rich in phytochemicals with bioactive properties (antioxidant, anti-inflammatory, neuroprotective, and anti-carcinogenic). In this work, the objective was to develop a sustainable, efficient, and extraction method of those valuable compounds, using NADES as solvents and efficient extraction methods. Furthermore, the role of NADES in the stabilization and preservation of these bioactive compounds was also studied. Among the various NADES tested, the one composed of betaine and glycerol (BG), showed the highest extraction efficiency and resulted in extracts with superior antioxidant activity. Optimized extraction conditions were found to be 40°C using a 1:20 solid-to-liquid ratio, with BG. The use of NADES not only improved extraction yield, but also improved the stability of BRP bioactive compounds over a longer period of time. This allows for the extract to be used together with NADES, as a liquid, without the need to remove the extraction solvent. The results confirm that NADES are a promising alternative to conventional solvents and have potential uses in nutraceutical or functional food applications. The study underscores the potential of BRP as a sustainable source of natural antioxidants and highlights a circular approach to waste utilization.

1. Introduction

Beetroot (*Beta vulgaris L.*), also known as common beet or garden beet is a root vegetable that is highly sought after because of its nutritional and health benefits (Clifford et al., 2015; Paulauskienė et al., 2023). Red beet high demand is also due to its red water-soluble dyes (Babarykin et al., 2019; Clifford et al., 2015; Sagar et al., 2018). Even though beetroots are highly valued, a substantial amount of waste is produced during its industrial processing, which is mainly composed by the leaves, peels and pomace that are still rich in bioactive phytochemicals (Babarykin et al., 2019; Sagar et al., 2018). These phytochemicals are bioactive compounds, mainly phenolic acids, flavonoids, and betalains. They have high antioxidant activity as well as other health benefits such as anti-inflammatory or anti-carcinogenic properties (Paulauskienė et al., 2023), and have been used as food additives to improve the health benefits of some products as well as nutraceuticals. The betalains present in beetroots are water-soluble pigments, that have betalamic acid as a base and can be divided depending on their structural composition in red-violet pigments- betacyanins, or yellow-orange pigments- betaxanthins. Betacyanins' ultraviolet-visible (UV-Vis) spectra show an absorption maximum at 536 nm and are the result of the complexation of belatamic acid with cyclo-dihydroxyphenylalanine (cyclo-DOPA). In contrast, betaxanthins are a derivative of betalamic acid and amino acids, showing an absorption maximum at 480 nm in its UV-Vis spectra (Azeredo, 2009; Gandía-Herrero and García-Carmona, 2013). In *Beta vulgaris L.*, the betanin and vulgaxanthin-I account for about 90 % of the total betacyanins and betaxanthins content, respectively, and these have been used as natural dyes in the food industry.

The purpose of this work is to use sustainable extraction methods and solvents, to obtain and stabilize these compounds from upcycled beetroot peels (BRP). These extracts rich in bioactive compounds have the potential to be used in cosmetic and nutraceutical formulations, either as pigments or as an antioxidant ingredient.

The extraction of phytochemicals from plants or agri-food waste is not new, even from beetroots, and is well described in literature.

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(Al-Harbi et al., 2021; Borjan et al., 2022; Eyshi et al., 2024; Singh et al., 2025). Different methods have also been used to extract these phytochemicals with bioactivity from beetroots, particularly betalains. The most conventional method is to use heat and stirring together with an extraction solvent (usually an organic solvent such as methanol, ethanol or acetone (Singh et al., 2025). Although this method is simple, it generally uses a high amount of volatile solvents, that cannot be recovered or re-used, while also requiring a high energy input. Because these solvents are highly volatile, they have a negative environmental impact. In this sense, these extraction processes require additional separation and purification steps, to separate the extract from the solvent, which has a negative environmental and economic impact.

Alternative to the heating and stirring (HS) method using volatile organic solvents, are more sophisticated methods such as Pulsed Electric Field (PEF) extraction, Microwave Assisted extraction (MAE), enzymatic extraction or Ultrasound assisted extraction (UAE). These alternative methods present advantages, but in the cases of PEF, MAE and enzymatic extraction, the cost of the equipment and enzymes can hinder its application as an industrial process (Singh et al., 2025). On the other hand, the use of UAE method, can offer the advantage of being a simple method that can reduce substantially extraction times (Hoseini Ghiasvand et al., 2025; Nabi et al., 2025). The solvent that is used for the extraction of bioactives from beetroot (betalain for example), can be selected from volatile organic solvents to ionic liquids (Coutinho and Freire, 2023) or supercritical fluids (Goyeneche et al., 2020). There are examples in literature that show that these solvents are efficient the extraction of bioactive compounds from beetroot, but still, their high cost can also prevent their use.

An alternative class of solvents that can be used, and that address some of these issues, are natural deep eutectic systems (NADES) (Hernández-Aguirre et al., 2021; Kaba et al., 2024). NADES are homogeneous mixtures of naturally occurring compounds such as organic acids, amino acids and alcohols, that when mixed in certain ratios results in a depression of the melting point of the mixture, becoming liquid at room temperature (Álvarez and Zhang, 2019). This depression is a result of intermolecular interactions established between the hydrogen bond acceptor (HBA) and a hydrogen bond donor (HBD) component of NADES. The HBA/HBD intermolecular interactions, mostly hydrogen bonding, are responsible for the physicochemical properties of the systems and as such, changes in the ratio of HBA/HBD or a change in one of the starting components influences the properties of the NADES, such as polarity, chemical and thermal stability, miscibility, and solubilizing ability (Mbous et al., 2017; Pena-Pereira and Namieśnik, 2014; Smith et al., 2014; Zainal-Abidin et al., 2017; Zhang et al., 2012). This shows that NADES can be tailor-made for a wide range of applications. NADES have been widely used as extraction solvents of bioactive compounds from plants and foods (García-Roldán et al., 2023; Huang et al., 2025; Nabi et al., 2025) such as antioxidants, that can be used in cosmetic, nutraceutical, food or pharmaceutical products. Most of the works that refer the use of NADES in bioactive compounds' extraction, report the use of choline chloride as an HBA (Rente et al., 2021). However, according to the European Commission database for the regulation of cosmetic products, the use of choline chloride has been banned in cosmetic formulations, and as such systems based on choline chloride were not used under the scope of this work (Couteau and Coiffard, 2010). As an alternative, betaine was used as an HBA, due to the similarity in chemical structure and its ability to form NADES (Jeong et al., 2017; Krisanti et al., 2019; Panić et al., 2019; Zhuang et al., 2017).

Besides studying the use of betaine-based NADES as effective solvents for the extraction of bioactive compounds from beetroot peels, we also carried out stability studies of the obtained extracts, to show that NADES can have an additional stabilizing ability of these compounds.

The work here presented addresses the environmental and processing challenges associated with the extraction methods of bioactive compounds and suggests an alternative to the extraction using volatile organic solvents. It reports the use of NADES as extraction solvents, to obtain bioactive compounds from upcycled beetroot peels, using extraction methods such as HS an UAE. Different extraction parameters were studied and optimized as well as the use of different NADES, to choose the best combination for a high extraction yield.

It is expected that the use of betaine-based NADES will efficiently extract phenolic compounds and betalains from beetroot peel waste. The use of NADES as extraction solvent is also hypothesized to improve the stability of the extract, for longer periods of time. This will also be studied in this work, if the extract in the NADES can have prolonged stability. By following this strategy to use NADES, BRP extracts can be obtained without the need for post-extraction purification, allowing the extract to be used directly in food or nutraceutical formulations. This reduces solvent and energy consumption, valorises a food industry by-product and contributes to waste reduction and circular economy goals. Because NADES can be designed to be compliant with food and cosmetic regulations, this approach ensures practical applicability in real-world product development.

Table 1
Composition of the different prepared NADES, as well as their molar ratio and abbreviation.

NADES composition				
component 1	component 2	component 3	molar ratio	Abbreviation
Betaine	Citric Acid	Water	1:1:3	BCW
Betaine	Glycerol	-	1:2	BG
Betaine	Lactic Acid	-	1:2	BLA
Betaine	D-Sorbitol	Water	1:1:3	BSW

2. Materials and methods

2.1. Reagents and chemicals

All the reagents used in this work were obtained with the maximum purity level available. Betaine anhydrous (99 %, CAS 107–43–7), D-sorbitol (98 %, CAS 50–70–4), DL-lactic acid (85 %, CAS 50–21–5), and Nile Red dye (CAS 7385–67–3) were obtained from Sigma-Aldrich. Glycerol (99.5 %, CAS 56–81–5) was acquired from Scharlab and citric acid monohydrate (99.5 %, CAS 5949–29–1) was acquired from Panreac.

2.2. Beta vulgaris L. plant material

The beetroots were obtained locally (Caparica, Portugal), washed and then peeled. The beetroot peels (BRP) were then rapidly frozen with liquid nitrogen and lyophilized in a Christ Alpha 1–4 freeze drier (Germany) at T = -55ºC, P = 0.02 bar). When the BRP was completely dried, they were ground to a fine powder, using an IKA tube mill C S000 (Netherlands). BRP powder was stored in an airtight amber flask and away from light and heat sources.

2.3. NADES preparation

The composition of the NADES used in this work is described in Table 1. The systems were prepared using the heating and stirring method first described by Abbot *et al.* (Abbott et al., 2004). The components of each NADES were weighed according to their molar ratio onto a flask. After that they were placed in a water bath, at the controlled temperature of 60°C, and stirred at 300 rpm, for a period of 2 h. After a clear and homogeneous liquid was formed, NADES were cooled down to room temperature and stored at room temperature in sealed 100 mL Schott flasks. The prepared NADES remained homogeneous liquid mixtures, without any precipitation being observed.

2.4. NADES characterization

2.4.1. Water content determination

The water content of the prepared NADES was measured by Karl Fisher titration using an 831 KF Metrohm Coulometer without diaphragm (Metrohom, Switzerland). The results are an average of at least three independent measurements for each NADES

The water content of the BPP used in extractions, was measured using a moisture analyser DAB (Kern, Germany) and was found to be 7 wt%.

2.4.2. Polarity measurement

The polarity of the prepared NADES was measured using a spectrophotometric method previously described by Craveiro *et al.* (Craveiro et al., 2016), using Nile Red as a solvatochromic probe. For this assay, a Nile Red stock solution of 1 g/L was prepared in ethanol. To a 1 cm^2 quartz cuvette, 1 mL of each NADES was added, and a blank spectrum was recorded. After that, 5 μL of Nile Red solution were added to the cuvette and properly mixed with the NADES, after which the UV-Visible spectrum was obtained, between 200 and 800 nm, in a UV-Vis spectrophotometer (Thermo Scientific Genesys 50, USA).

2.4.3. Viscosity and density measurements

The viscosity and density values for the prepared NADES, were obtained using an Anton Paar SVM 3001 viscosimeter (Anton Paar, Austria). Measurements were carried out in the range of temperatures between 20 °C and 60 °C.

2.5. Extraction of bioactive compounds from BRP- Heat and stirring method (HS)

An initial screening on NADES' ability to extract bioactive compounds from BRP was performed. The extractions were performed using water, ethanol and NADES as extraction solvents, at 60 °C, with stirring speed of 450 rpm (using a heat and stirring plate- Velp scientifical, Italy), for a period of 45 min. The studied solid to liquid ratio (S/L) was 1:20 (0.2 g of BRP were placed in 4 g of each solvent).

Briefly, the mass of BRP was placed in a flask with the correspondent mass of NADES and extraction is carried out. After this, the BRP powder+NADES mixture is filtered, to remove the solid phase. The obtained samples (liquid phase) were then centrifuged for 5 min at 6000 rpm, to precipitate any remaining BRP powder. Both solid and liquid phases were stored in the dark at 4 °C when not in use. All extractions were performed in triplicate. Later in this work, extractions with S/L ratio of 1:10 were also carried out, using this method.

2.6. Extraction of bioactive compounds from BRP- ultrasound assisted extraction method (UAE)

In UAE experiments, extractions were carried out for 45 min, 40 kHz-60 W, at 40 °C and 60 °C and a S/L ratio of 1:20 (0.2 of BRP were placed in 4 g of each solvent), using a Grant XUB5 sonicator (Grant instruments, England). The extraction and post-extraction processing is the same as the one described for HS extraction. When optimizing BRP extraction, a S/L ratio of 1:10 was also tested.

2.7. Evaluation of BRP extract's antioxidant activity

The antioxidant activity of the obtained extracts was measured using the DPPH (2,2-Diphenyl-1-picrylhydrazyl) method, as described previously by other authors and adapted to samples that contain NADES (Rebocho et al., 2022). Briefly, a methanolic DPPH solution, called "mother solution", is prepared, using 24 mg of DPPH Powder (Sigma Aldrich, CAS 1898-66-4) in a 100 mL volumetric flask filled with methanol and covered with aluminium foil due to the light-sensitive nature of DPPH. After being stored in the freezer for at least 2 h, this solution is then diluted with methanol, with the ratio 2:9 (v/v), to obtain the "daily solution" with an UV absorbance must be between 1.0 and 1.1 at the start of the assay, at 517 nm. A series of dilutions of the extract sample are then prepared and added to DPPH solution in amber vials to minimize light exposure. After 40 min of reaction time, the absorbance of each sample is measured using UV-Vis spectrophotometry at 517 nm.

All the measurements were performed in triplicate (three individual samples for each measured point).

2.8. BRP Extract's stability studies

To study the stability of the bioactive compounds extracted from BRP, their degradation was studied by the measuring their antioxidant activity (DPPH assay), after different time periods. Also, the effect of temperature, extraction time, and extraction method in the stability were evaluated. For this, during each different extraction that was performed, a liquid sample was collected at defined timed intervals. Tests were conducted using both HS and UAE methods at 40 °C and 60 °C, in a 1:20 S/L ratio.

The stability and subsequent degradation kinetics of the activity of the obtained extracts, was also studied. The obtained solid extracts were dissolved in water and placed in a thermostatic bath at 37 °C and 60 °C. Samples were collected in 2 h intervals up to 10 h. Afterwards samples were collected at 24 h, 30 h, 48 h and 72 h. This test was followed by measuring the concentration of betalains, using UV-Vis spectroscopy and using the Nilsson equations (Elbe, 2001). Kinetic constants were calculated using the following formulas:

$$[N] = \frac{C_t}{C_0} \tag{1}$$

$$\ln([N]) = -kt \tag{2}$$

$$t_{1/2} = \frac{\ln(2)}{k}(\text{hours}) \tag{3}$$

where, [N] is the normalized concentration of betanin or vulgaxanthin-I, C_t is the concentration at a specific time (in mg/L), C_0 is the initial concentration (in mg/L), k is the degradation constant, and $t_{1/2}$ is the half-life time of the compound (in hours).

2.9. Quantification of bioactive compounds in the BRP extract by UV-Vis spectroscopy

The amount of total phenolics in each extract, was quantified using the Folin-Ciocalteu method, as previously described (Rebocho et al., 2022), and measured as gallic acid equivalents (GAE mg/L). Galic acid was used to build a calibration curve (10–1000 mg/L).

To determine the total amount of flavonoids, present in each extract, the aluminium chloride complexation method described by Pękal *et al.* (Pękal and Pyrzynska, 2014) was adapted for use in 96 wells microplates. To each well, 100 μL of extract was added followed by 50 μL of 10 % $AlCl_3$ and 50 μL of distilled water. The microplate was mixed in the microplate reader and left to incubate for 10 min. Absorbance was then measured at 425 nm. The results are expressed as quercetin equivalents (QE), and a quercetin calibration curve was built (5–150 mg/L).

The betalain content of the extracts was quantified using a previously reported method. The absorbance values at 476, 538 and 600 nm were recorded. The amounts of betanin and vulgaxanthin-I were determined according to the methodology previously described (Elbe, 2001) where

$$x = 1.095(\alpha - c) \tag{4}$$

$$y = b - z - x\,3.1 \tag{5}$$

$$z = \alpha - x \tag{6}$$

where a is the absorbance value at 538 nm, b is the absorbance at

Table 2
Amount of water in the different NADES prepared and used in this work.

NADES	Water content (wt%)
BCW	34.74 ± 1.4
BG	1.31 ± 0.18
BLA	15.64 ± 1.56
BSW	27.11 ± 2.67

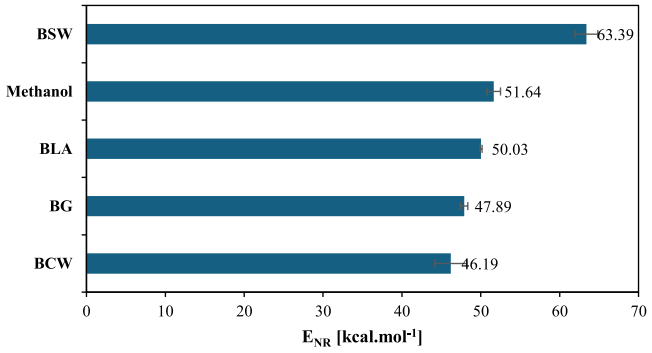

Fig. 1. Polarity of the different NADES using Nile Red as a solvatochromic probe.

476 nm and *c* is the absorbance at 600 nm. Also, *x* refers to the absorbance of betacyanin, *y* to the absorbance of betaxanthins and *z* is the absorbance of impurities in the extracts.

All the analysis were made in triplicate.

2.10. Quantification of bioactive compounds by high performance liquid chromatography (HPLC)

HPLC was also used to identify and quantify the phenolic acids and flavonoids present in the extracts, such as chlorogenic acid, p-coumaric acid, ferulic acid, rutin, quercetin, and luteolin. Samples were analysed using an Agilent 1100 HPLC equipped with a Novapack C18 150 × 4.64 μm with a pre column (Agilent, USA). Mobile phase was 10 % methanol with 2 % acetic acid (eluent A) and 90 % methanol with 2 % acetic acid (eluent B) with a flow rate of 0.5 mL/min. Data was acquired at 280 nm, with injections of 20 μL at 30°C.

2.11. Extraction kinetics studies

For the kinetic studies of the extraction essays, during each different extraction, an aliquot of the extraction medium was collected at regular time intervals. Studies were conducted using both HS and UAE methods at 40°C and 60°C, using a 1:20 S/L ratio.

3. Results and discussion

This work is focused on using NADES to efficiently extract and recover bioactive compounds present in beetroot waste, in particular beetroot peels (BRP), which are often discarded but can be upcycled, and its extracts can have used in cosmetic and food applications. NADES based on choline chloride as an HBA, are some of the most referred and widely used in extraction processes using such solvents. But the use of choline chloride may pose a problem when considering the above-mentioned applications. Being an ammonium-based salt that like choline chloride is able to form NADES, betaine was used as an alternative (Aroso et al., 2017).

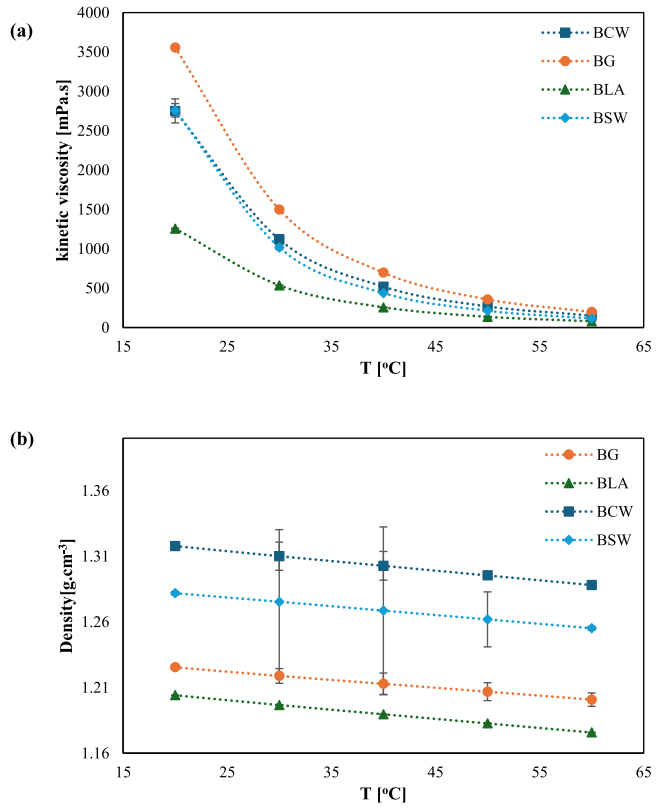

Fig. 2. Viscosity **(a)** and density **(b)** of the prepared NADES, measured in the 20–60°C temperature range.

3.1. NADES characterization

The NADES that were studied as potential solvents for the extraction and stabilization of BRP bioactive compounds are all composed by betaine, that acts as HBA. The selected HBD's for the betaine-based NADES under study (glycerol, citric acid, sorbitol, and lactic acid) were selected based on reported literature (Rente et al., 2021).

Upon NADES successful preparation, some of its physico-chemical properties were determined. The water content on each of the prepared NADES presented in Table 2. It is important to measure this parameter, since the amount of water in a NADES can influence its physical properties such as polarity and viscosity (Gabriele et al., 2019).

When considering a solvent for an extraction process, its polarity is also an important property. In a solid-liquid extraction process, if the solvent polarity is close to the one of the target compounds to be extracted, this improves the extraction yield. The polarity of the prepared NADES was measured and is presented in Fig. 1, as the value of E_{NR}. According to this method, the lower the value of E_{NR} the higher the polarity of the solvent (Craveiro et al., 2016).

Taking into consideration Fig. 1, NADES BCW is the one with the highest polarity. In this sense, this NADES would perform better as extraction solvent for the phytochemicals present in BRP, since the main bioactive compounds present in BRP (flavonoids, phenolic acids and betalains) are polar compounds. On the other hand, the use of NADES BSW would favour the extraction of phytochemicals with lower polarities.

Viscosity is another solvent property that plays an important role in extraction processes, mainly related with mass transfer phenomena. If a solvent's viscosity is too high, this may hinder mass transfer from solvent to solute. In this sense, knowing how the viscosity of NADES varies with temperature will allow to choose the most appropriate temperature for extraction. Fig. 2 shows the results for viscosity measurements carried out in a temperature range from 20 to 60ºC. It can be observed that

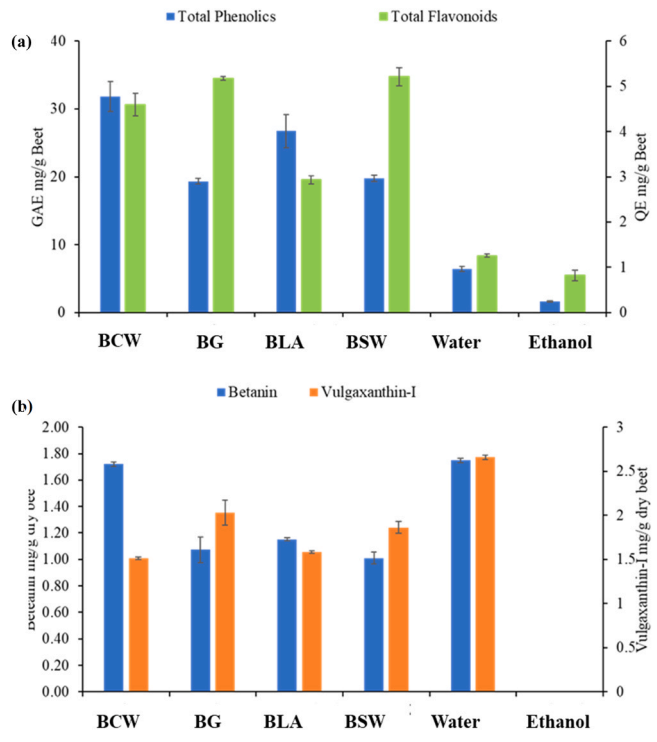

Fig. 3. Results on the total amount phenolics and total amount flavonoids in the obtained extracts (a); betanin and vulgaxanthin total amount content in the obtained extracts (b) (in the extraction carried out with ethanol, betanin and vulgoxanthin were not detected).

after 55ºC the temperature influence on systems' viscosity is not so noticeable, and as such, the extraction temperature was set at 60ºC. NADES density ranged from 1.20 g/cm^3 to 1.32 g/cm^3, and follows the order

BLA < BG < BSW < BCW,

at 20°C and a slight decrease can be seen with increased temperature.

3.2. Beetroot peel (BRP) extraction of bioactive compounds

An initial screening on the ability of the selected NADES to extract the compounds of interest was performed. The extractions were carried out at 60ºC for 45 min, using powdered BRP in a 1:20 w/w solid to liquid ratio. Water and ethanol were used as solvents for control experiments, using the heating and stirring (HS) extraction method. Fig. 3 shows that the NADES under study have higher extraction abilities than both water and ethanol.

As expected, the NADES BSW extracted the higher amounts of flavonoids. These results also suggest that the functional group of the HBD plays a role on the type of compound extracted. Both BCW and BLA, which are NADES with organic acid based HBD's, extract a higher amount of total phenolics in general, and both BG and BSW (NADES with alcohol based HBD's), extract higher amounts of total flavonoids. This shows that the depending on the NADES used, the extraction of bioactive compounds can be selective. Regarding the extraction of betalains (Fig. 3(b)) there are no significant differences between the amount of betacyanins and betaxanthins extracted with water or NADES. However, NADES based on organic acids perform better in the extraction of betacyanins, and alcohol-based systems showed better results in the extraction of betaxanthins.

Table 3 presents the results obtained from HPLC quantification, regarding the amounts of extracted phenolic compounds, mainly chlorogenic acid, *p*-coumaric acid, ferulic acid, rutin, quercetin, and luteolin. While all the NADES tested performed better than water, the system that showed higher extraction yield of these components was BG.

After the quantification of the BRP extract's phenolic, flavonoid, and betalain contents, the antioxidant activity of the systems NADES/extract was studied using the DPPH assay. Table 4 shows the EC_{50} values obtained for each NADES/extract system. The EC_{50} value refers to the concentration of antioxidants needed to scavage 50 % of free radicals in solution, so the lower this value is, the higher the NADES/extract antioxidant activity.

Here it is observed that BG/extract system has the lowest EC_{50} and therefore, the highest antioxidant activity of the extracts tested. While it could be expected that the BCW extract had a higher antioxidant activity (since it has the highest amount of total phenolics), BG extract has higher amounts of total flavonoids and betaxanthins. A combined effect of phenolics and flavonoids in BG extract may justify its higher antioxidant activity.

3.3. Stability of the obtained BRP extracts

Besides the optimization of the extraction conditions, one of the goals of this work was to show that NADES can be used as a stabilizing media for the extracted compounds, creating a NADES/extract system that can have a longer stability and activity over time.

By acting as a stabilizer, the NADES can be a part of the bioactive extract, in such a way that it can be incorporated into the final product, either as a cosmetic or a food ingredient. This strategy, of course, reduces the need for downstream purification steps, commonly required when using conventional volatile organic solvents, thus making the extraction process more sustainable (Rente et al., 2022).

Previous work reported by Dai *et al.* (Dai et al., 2014), has employed

Table 4
Antioxidant activity of the beetroot peel extract in different NADES and in water, expressed as EC_{50} in mg gallic acid equivalents (GAE) per mg of DPPH.

Solvent used in extraction	EC_{50} (mg_{GAE}/mg_{DPPH})
BCW	0.214 ± 0.03
BG	0.130 ± 0.02
BLA	0.189 ± 0.04
BSW	0.205 ± 0.01
Water	0.184 ± 0.06
Ascorbic acid (control)	*0.087 ± 0.03*

Table 3
HPLC results of the different systems tested to extract bioactive compound from beetroot peels, expressed in mg/L.

NADES	chlorogenic acid (mg/L)	*p*-coumaric (mg/L)	ferulic acid (mg/L)	rutin (mg/L)	quercetin (mg/L)	luteolin (mg/L)
BCW	1.91 ± 0.04	0.34 ± 0.02	0.66 ± 0.04	11.58 ± 0.20	3.72 ± 0.78	2.64 ± 0.1
BG	0.72 ± 0.07	*nd*	0.34 ± 0.03	16.83 ± 0.33	4.80 ± 0.30	1.33 ± 0.09
BLA	1.34 ± 0.04	0.35 ± 0.02	*Nd*	10.21 ± 0.18	3.53 ± 0.16	1.93 ± 0.09
BSW	*nd*	*nd*	0.26 ± 0.03	9.23 ± 0.18	4.92 ± 0.16	1.19 ± 0.09
Water	1.19 ± 0.06	*nd*	0.47 ± 0.02	11.79 ± 0.25	0.94 ± 0.02	0.72 ± 0.01
Ethanol	1.38 ± 0.06	*nd*	0.54 ± 0.03	5.00 ± 0.28	2.84 ± 0.05	2.17 ± 0.04

nd- not detected.

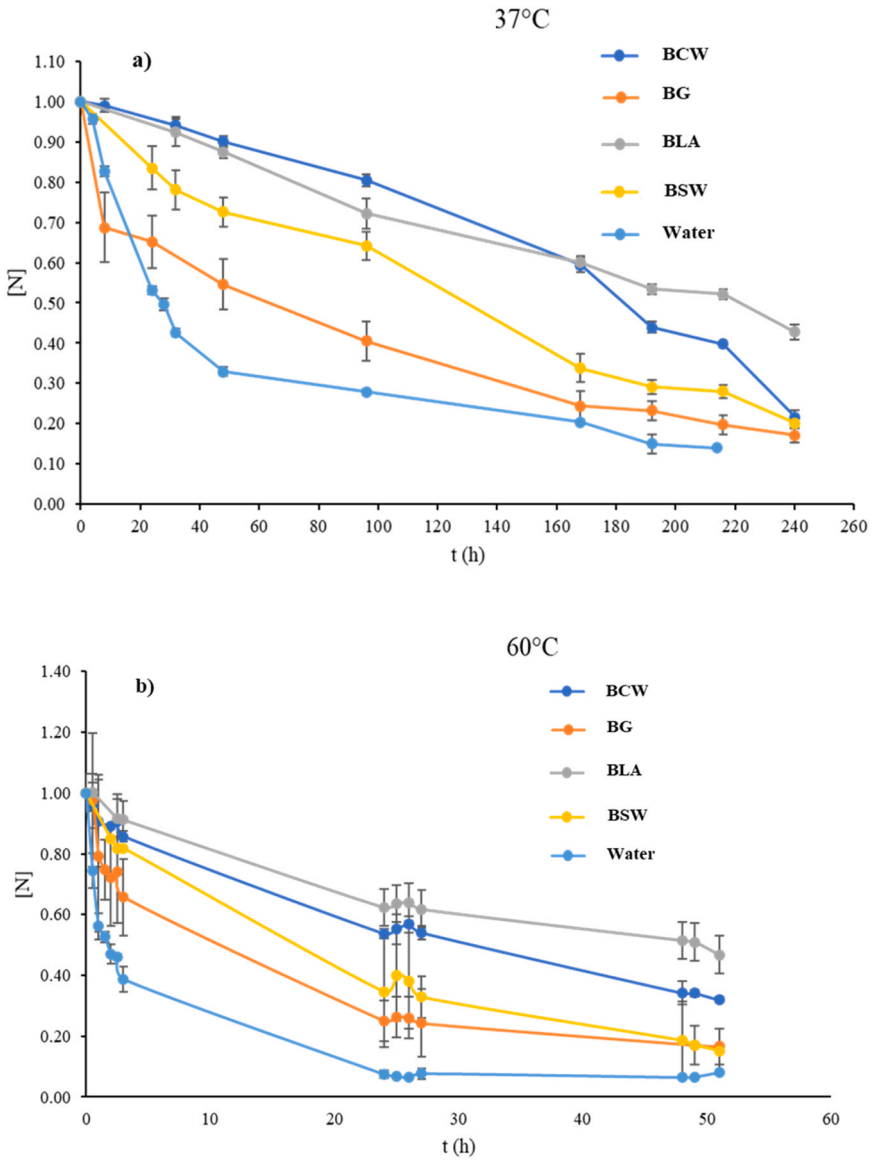

Fig. 4. Normalized data for the degradation curves of betanin at 37 °C and 60 °C.

Fourier transformed infrared spectroscopy (FTIR) to show that when dissolved in DES, the hydroxyl groups of quercetin create bonds with the hydroxyl groups of the DES, and change its conformation to a more stable one. This change in quercetin conformation also improved quercetin solubility in the NADES. This shows that NADES can have an important role in the stabilization of bioactive compounds. Other works reported in the literature (Hernández-Aguirre et al., 2021; Zin and Bánvölgyi, 2023), performed kinetic studies at high temperatures to prove that compounds that are dissolved in DES have higher half-life times than the same compounds dissolved in a common solvent such as water.

In this work, the degradation kinetics of both betacyanin and betaxanthin in the four NADES/extract systems under study, were followed at 37ºC and 60ºC, using the water/extract system was used as a comparison point.

Betalains' stability is dictated by four major agents: pH, light exposure, O_2 presence and temperature, with temperature having an increased effect on degradation the higher the temperature and exposure time (Azeredo, 2009). Betalains degradation usually follows a first order degradation kinetics (Chandran et al., 2014). Since the method for the quantification of betalains based on the Nilsson equations allows for the detection of both betanin and vulgaxanthin-I, the degradation of both compounds was followed. To accurately compare the results obtained during the degradation kinetic studies, the concentration of both betanin and vulgaxanthin-I was measured before the experiment started and used to normalize the compounds concentration at the defined time intervals.

Table 5
Kinetic data obtained from betanin stability studies, of the extracts obtained in different NADES and in water, in particular degradation constants (k) and half-life times ($t^{1/2}$) at 37°C and 60°C. R^2 is the determination coefficient associated with the linear regression of the linearized data.

Solvent	37°C			60°C		
	k	$t^{1/2}$ [h]	R^2	k	$t^{1/2}$ [h]	R^2
BCW	0.0042	165	0.9658	0.0207	34	0.9866
BG	0.0106	65	0.9501	0.0527	13	0.9478
BLA	0.0032	217	0.9935	0.0135	51	0.9641
BSW	0.0063	110	0.9829	0.0334	21	0.9859
Water	0.0246	28	0.9874	0.0871	8	0.966

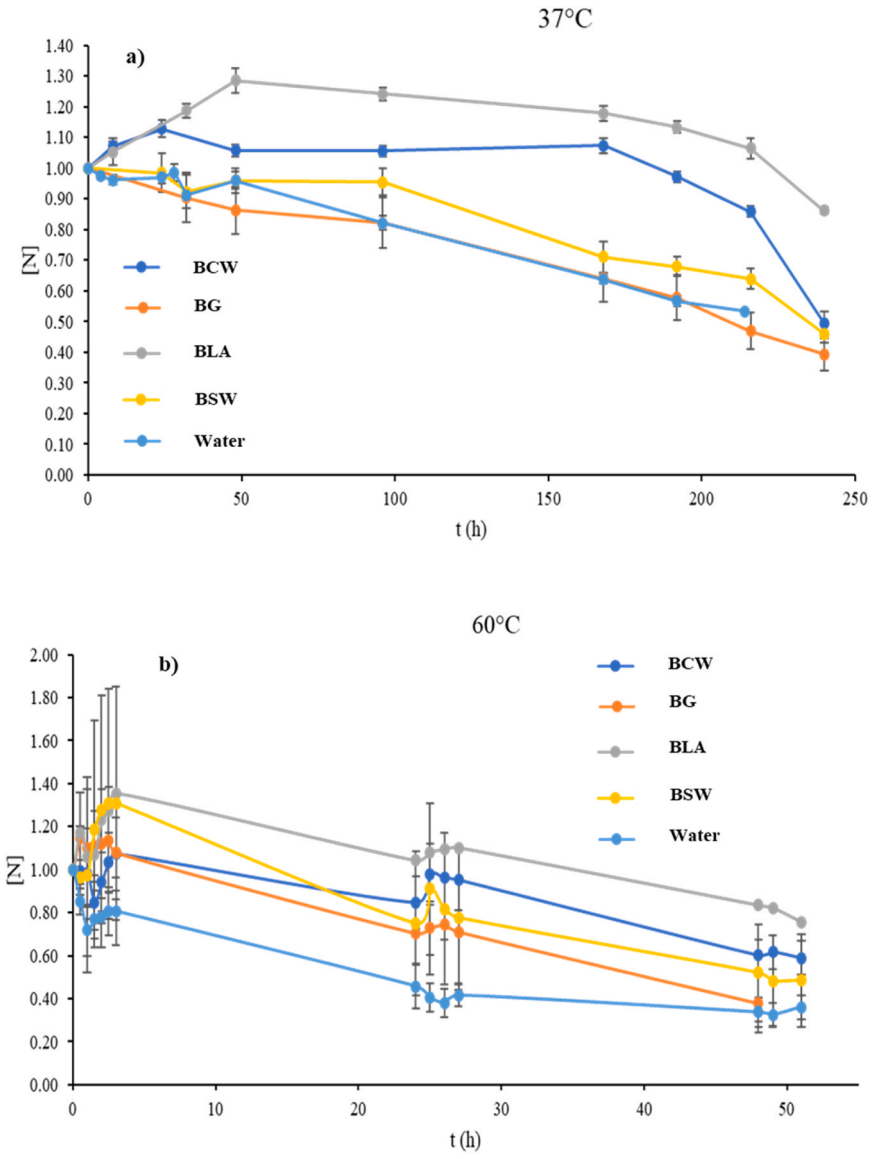

Fig. 5. Normalized degradation curves of vulgaxanthin-I at 37° and 60°C.

Table 6
Kinetic data obtained from vulgaxanthin-I stability studies, of the extracts obtained in different NADES and in water, in particular degradation constants (k) and half-life times ($t^{1/2}$) at 37°C and 60°C. R^2 is the determination coefficient associated with the linear regression of the linearized data.

Solvent	37°C			60°C		
	k	$t^{1/2}$ (h)	R^2	K	$t^{1/2}$ (h)	R^2
BG	0.0035	198	0.9369	-	-	-
BSW	0.0021	330	0.9175	-	-	-
Water	0.0029	239	0.9874	0.0284	24	0.9498

3.3.1. *Betanin degradation*

Betanin degradation can occur due to high temperatures, resulting either in oxidation or in the aldimine bond hydroxylation (forming betalamic acid and cyclo-DOPA) (Miguel, 2018; Sekiguchi et al., 2010). Results shown in Fig. 4, show that the degradation of betanin present in the obtained extracts, follows a first order degradation at both 37 °C and 60 °C. All four NADES extracts were less prone to degradation when compared to the water extracts, as it can be observed in Fig. 4.

Table 5 shows the data obtained from the degradation curves, for the studied extracts, in particular the degradation constant and half-life times (k and $t^{1/2}$). At both temperatures, we can observe that betanin extract stability is higher in all the tested NADES, when compared to water. This is more evident for NADES BLA, that reduces betanin degradation by 7.75 and 6.38 times, at 37°C and 60°C respectively.

3.3.2. *Vulgaxanthin-I degradation*

The degradation of vulgaxanthin-I at temperatures of 37 °C and 60 °C was also studied (Fig. 5), to evaluate the extract's stability. Table 6 shows that, while vulgaxanthin-I degradation in water and the NADES BG (at 37°C) followed the expected degradation behaviour. With the other NADES under study there was an increase in vulgaxanthin-I before its degradation. At 60°C there was an increase in of vulgaxanthin-I in every system except for water.

Betaxanthins are formed through the condensation of betalamic acid and amino acids/amines. This is a spontaneous reaction *in-vivo* and can be the explanation for these results (Gandía-Herrero and García--Carmona, 2013; Miguel, 2018).

Vulgaxanthin-I is formed by the condensation of betalamic acid (derived from betanin degradation) and glutamine (extracted from beetroot peel)(Gandía-Herrero and García-Carmona, 2013), which are

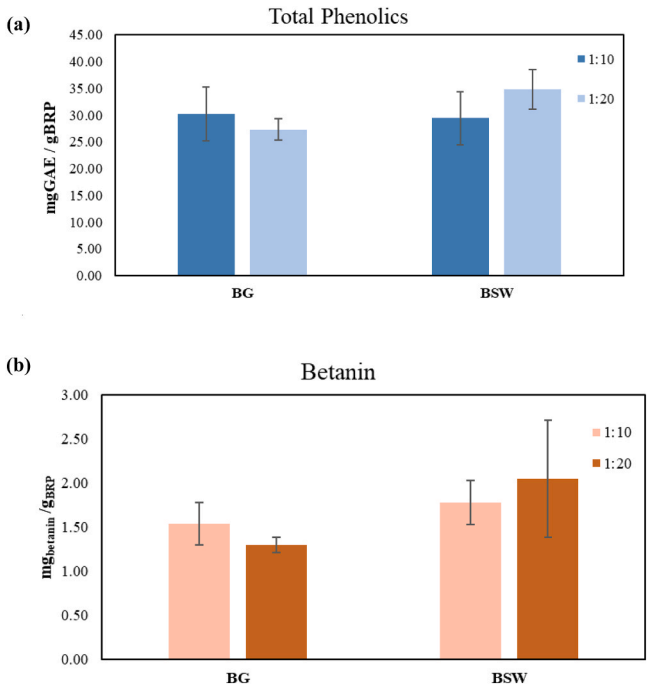

Fig. 6. Total amount of phenolics (a) and betanin (b), present in BG and BSW extracts, obtained using S/L 1:10 and 1:20.

both present in these extracts. Wherein it is hypothesized that vulgaxanthin-I formation in BCW and BLA, is due to the presence of the components and the lower acidity of the media. While this phenomenon also occurs in the other systems, it appears to be increased at higher temperatures. For the case of vulgaxanthin-I degradation, it was only possible to calculate the degradation constant and half-life time of vulgaxanthin-I for the systems with NADES BG and BSW at 37°C, the latter being more efficient in vulgaxanthin-I stabilization (Table 6).

3.4. Optimization of BRP extraction process

Considering the different NADES under study, BLA and BCW yielded extracts with the highest total phenolic content, while also improving the stability of betanin.

Nevertheless, BRP extract obtained using the NADES BG is more specific towards the extraction of rutin and quercetin. Also, the extract obtained with this NADES shows higher antioxidant activity, and good betanin stability. Due to this reason NADES BG was chosen for optimization of the beetroot peel extraction process.

The parameters under study for this extraction optimization were the: solid to liquid ratio (S/L), extraction time, and the method of extraction. The S/L ratio is an important extraction parameter, because while higher S/L ratios may allow a higher surface area between the solids and the liquids and a better leaching of the phytochemicals from the solid into the liquid phase (higher extraction yields), it will also increase the costs of the whole process.

We tested different S/L ratios of 1:10, 1:20, and the results are presented in Fig. 6. From these results, it is observed that the extraction yields obtained using NADES BG with a 1:10 S/L ratio present better results than with 1:20 ratio. Despite this, for further testing the ratio of 1:20 was used due to easier handling.

3.5. Extraction kinetics

To study the effect of the extraction method as well as time and temperature, the extraction kinetics for betanin was followed for a 24-hour period, using BG as the extraction solvent. To compare the extraction methods, both the HS and the UAE methods were used. The effect of temperature was tested in both extraction methods, and the results are presented in Fig. 7.

The extractions performed using UAE method, were only monitored for a period of 5 h, which is why the kinetic curves do not show the plateau region. Despite this, it is noticeable that higher amounts of betanin were obtained when using the UAE method. Regarding the extraction temperature, for both extraction methods, the experiments carried out at 40°C performed better than at 60°C, since higher temperatures can degrade the betanins present in the obtained extracts (Saguy, 1979).

4. Conclusions

This work shows that betaine-based NADES can be used to extract bioactive compounds from beetroot peels, such as phenolics and betalains. When comparing the NADES extraction ability with conventional solvents such as water, an increase in performance can be observed, where the total amount of phenolic and flavonoid content can be 3 times

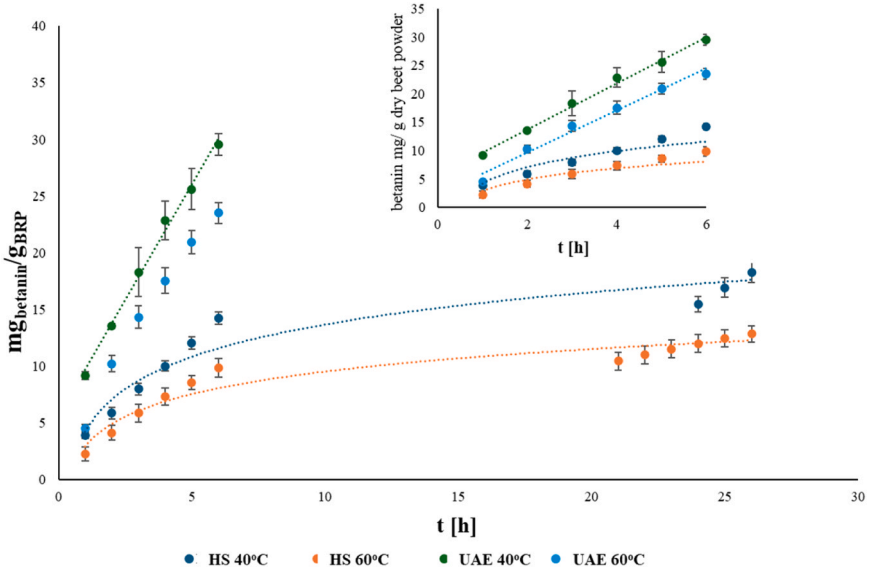

Fig. 7. Extraction kinetics of betanin using BGat 40° and 60°C, using both HS and UAE extraction method.

higher when using NADES as extraction solvent.

NADES composition, particularly the HBD component, appears to play an important role in the specific extracted compounds, with organic acid based HBD's favouring the extraction of phenolics, and betacyanins and alcohol based HBD favouring the extraction of flavonoids and betaxanthins. Contrary to what was expected, the extract with BG had the highest antioxidant activity, despite not being able to extract large amounts of total phenolics. This may be due to its ability to extract both flavonoids and betaxanthins more efficiently and their synergistic activity.

Regarding NADES ability to act as stabilizing agents, both BCW and BLA showed promising results for betanin stabilization. The activity of betanin is increase 6–7 times longer in NADES BLA (for example) than when compared with the aqueous extract. Still, the role of NADES in the stabilization vulgaxanthin-I is still not fully understood.

The optimal extraction conditions of BRP bioactive compounds were defined, and use NADES BG, a 1:20 S/L ratio, using UAE method and at a temperature of at 40°C, for the period of 5 h.

This is a promising extraction strategy, that could valorise a biomass that has low value and is usually regarded as waste, upcycling it and finding used for potential applications in cosmetic or food sectors. To achieve this, future work would include colour stability studies, as well as tests to guarantee the extract safe use as a cosmetic or a food additive, namely performing cytotoxicity and ecotoxicity testing, as well as guaranteeing regulatory compliance.

CRediT authorship contribution statement

Paiva Alexandre: Writing – review & editing, Validation, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Ana Rita C. Duarte:** Writing – review & editing, Validation, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Rente Duarte:** Writing – original draft, Methodology, Investigation, Formal analysis. **Craveiro Rita:** Writing – review & editing, Writing – original draft, Validation, Investigation, Formal analysis, Data curation.

Declaration of Competing Interest

The authors of the original paper submitted to Food and Bioproducts Processing, entitled "Synergistic use of NADES and assisted Extraction technologies for recovery and stabilization of bioactive compounds in *Beta vulgaris* L. peels." declare that they have no conflict of interest.

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