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Effective Liquid–Liquid Extraction for the Recovery of Grape Pomace Polyphenols from Natural Deep Eutectic Solvents (NaDES)

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Abstract: Natural deep eutectic solvents (NaDESs) are emerging solvents for their yield when used for extraction of different molecules, including polyphenols. NaDESs are a cutting-edge technology that offers numerous advantages, including cheap cost, safety, effectiveness and environmental friendliness. However, due to NaDES' high boiling point, the recovery and separation of compounds after the extraction is the bottleneck of the process. In this work, two affordable methods were tested for the recovery of phenolic compounds from three binary NaDESs (composed of choline chloride mixed separately with lactic acid, tartaric acid or glycerol as hydrogen bond donors): the antisolvent and the liquid–liquid extraction methods. The former was assessed by diluting the extracts with different aliquots of water, employed as antisolvent, which was ineffective. For the liquid–liquid extraction method, ethyl acetate (EtOAc), acetonitrile (ACN), 2-chlorobutane (2-CB) and 2-methyltetrahydrofuran (2-MeTHF) were compared. Except for ACN, all solvents were perfectly immiscible with the three NaDESs, forming biphasic systems that were analyzed by colorimetric assays and HPLC/MS. 2-MeTHF applied on a 10-fold water dilution of the NaDES extract reached recovery percentages higher than 90% for most of the non-anthocyanin phenols and good recovery (up to 80%) for some anthocyanins. 2-MeTHF appears to be the first known solvent capable of extracting anthocyanins from NaDESs. Finally, a two-step liquid–liquid extraction performed firstly with EtOAc and subsequently with 2-MeTHF is proposed for the separation of different phenolic fractions.

Keywords: circular economy; green chemistry; grape by-products; antioxidant extraction

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1. Introduction

Natural deep eutectic solvents (NaDESs) were introduced by Choi et al. [1] and consist of deep eutectic solvents (DES) [2] exclusively based on natural products. NaDESs can be used for several applications in different fields of chemistry [3] and attracted incredible interest in few years, due to their ease of preparation, safety, low-cost and ecologically friendly nature [4].

One of the most promising applications is the use as extraction solvents, broadly exploited for the extraction of antioxidant compounds from natural sources [5–8]. For this purpose, NaDESs are typically composed of a quaternary cation (usually choline chloride) as hydrogen bond acceptor and acids, sugars, alcohols or amides as hydrogen bond

donors [9]. The physicochemical features of NaDESs are a direct consequence of the characteristics of the individual starting compounds and depend on the molecular interactions between the components [10]. Since most of the substances used for NaDESs are natural-based and solid at room temperature, with high melting points, the resulting mixtures consequently present low vapor pressure, making them safe and stable [11]. However, these characteristics make it challenging to isolate and extract chemicals from NaDESs.

Often, NaDESs are indicated as safety mixtures [12], but their healthiness towards living beings is not completely assessed and contrasting evidence was collected [13]. This fact could limit the possibility of integrating in food products or medicines the whole mixture composed of NaDESs, and valuable compounds could be excluded due to contrasting results regarding the NaDES' safety towards living beings [13]. Several authors reported that different NaDESs showed toxicity to human cell lines [14–18], microorganisms [19–21] and plants [15,22]. Additionally, even for analytical purposes, the research of easy, cheap and time-saving methods for the separation and purification of the extracted compounds from NaDESs constitutes a current challenge [23,24]. In conventional extraction, organic solvents are easily evaporated thanks to their low boiling point, and the solid compounds are recovered, which is not feasible when using NaDESs. Several methods for the recovery of extracted compounds from NaDESs were proposed, but the most effective, such as solid–liquid extraction, are completely unaffordable for an industrial application [25]; for this reason, the main solutions concerning a large-scale implementation involve the following two methods: the antisolvent method and the liquid–liquid extraction method [26]. The use of an antisolvent for the recovery of phenolic compounds involves using solvents (usually water) to break the hydrogen bonds that the NaDES forms, thereby precipitating compounds that are poorly soluble in water. The method has the advantage of being very cost-effective and sustainable and could enable the recovery of a specific type of compound, as demonstrated by Nam et al. [27], Huang et al. [28], Patil et al. [29] and Hang et al. [25].

Liquid–liquid extraction, on the other hand, is a commonly used method for the recovery of analytes from NaDESs [26] and would appear to be effective for certain classes of compounds relying on the use of a solvent immiscible with the NaDES, through the creation of a biphasic system where the compounds migrate from the NaDES to the organic solvent as a function of the partition coefficient. The upper phase is then evaporated, allowing for recovery of the extract. This method has the disadvantage of including organic solvents in the process, but unlike direct extraction on biomass, it is possible to work in a closed system that allows the recovery and reuse of solvents, avoiding contact directly with biomass and allowing for the recovery of additional fractions. The substances identified so far for this purpose have proven to be specific for certain classes of compounds but no references describing the use of this approach for the recovery and separation of anthocyanins were found. For other target compounds, ethyl acetate (EtOAc) was used for the recovery of quercetin [30], catechin [31] apigenin and luteolin [25], i.e., less polar phenols, such as aglycones or simple phenols. De Souza Bezerra tested different solvents for the recovery of phenolic compounds from NaDESs, finding ethyl acetate and acetonitrile as the most effective [32]. With n-butanol, hesperidin, a flavanon, was recovered [33], while Pal and Jadeja used chloroform for the recovery of mangiferin, belonging to xanthonoid class [34]. Taking this into account, this approach is still partially unexplored, providing an opportunity to test new solvents for this purpose.

For the recovery of grape pomace polyphenols, including anthocyanins, the optimum solvent should be highly polar, approaching the dielectric constant of ethanol while remaining immiscible with the NaDES. For example, 2-methyltetrahydrofuran (2-MeTHF) is a state-of-the-art organic solvent that is considered the green alternative to tetrahydrofuran [35]. It is obtainable from natural sources, as it is a derivative of levulinic acid, which is

found in large quantities in waste biomass [36]. In addition, 2-MeTHF is readily biodegradable and immiscible with water, and has been tested for the recovery of polar compounds from aqueous matrices [37], but it was not yet employed for the recovery of polyphenols from NaDESs. Alternatives are 2-Chlorobutane (2CB), a completely unexplored solvent for this function that is a water-immiscible compound with a dielectric constant of 8.56, and acetonitrile (ACN), a solvent with an intermediate polarity, whose miscibility with NaDESs we decided to verify, based on evidence from other authors [32].

In previous research on grape by-products [38], three choline chloride-based NaDES (combined with lactic acid, tartaric acid and glycerol as hydrogen bond donors, respectively) were tested for the extraction of phenolic compounds from grape pomace. Those extracts were used as a foundation for the subsequent study reported here, which involved the recovery of chemicals from NaDESs. Thus, the aim of this work is to test different methods for the recovery of metabolites from the NaDES extracts obtained by Frontini et al. [38], in light of the need of separating the extracted compounds from NaDES for a safe integration in commercial products. Two methods were employed: the antisolvent method and the liquid–liquid extraction method. Water was used as antisolvent, and different dilution factors were compared to assess the most effective dilution. EtOAc, ACN, 2-CB and 2-MeTHF were utilized in the liquid–liquid extraction to determine their ability to form a biphasic system with the NaDES and their affinity for phenolic chemicals. The evidence obtained could serve as basis for future studies and industrial/analytical applications.

2. Materials and Methods

2.1. Chemicals

Chemical products and reagents used in the work were provided as described in the following: choline chloride (≥98%) was obtained from PanReac Química SLU (Castellar del Vallès, Barcelona, Spain); 2-CB, 2-MeTHF, ACN, (+)catechin, DL-lactic acid (≥90%), Folin-Ciocâlteu reagent, gallic acid, glycerol (≥99%), kaempferol-3-*O*-glucoside, L(+)-tartaric acid (≥99.5%), malvidin-3-*O*-glucoside, and quercetin-3-β-D glucoside were purchased from Sigma-Aldrich (St. Louis, MO, USA); bi-distilled water, ethanol, EtOAc and formic acid were obtained from VWR SAS (Fontenay-sous-Bois, France); sodium carbonate was purchased from Carlo Erba reagent SpA (Rodano, Milano, Italy); astilbin and resveratrol were obtained from Cayman Chemicals (Ann Arbor, MI, USA).

2.2. NaDES Starting Samples (NaDES Extracts)

NaDES starting extracts from grape pomace were obtained from rosé vinification of Primitivo grapes in 2023 in Apollonio Casa Vinicola (Monteroni di Lecce, Lecce, Italy) winery and results were published and made available as open access source [38]. Briefly, three NaDESs were employed, with choline chloride used as hydrogen bond acceptor (HBA), and lactic acid, tartaric acid and glycerol employed as hydrogen bond donors (HBD), respectively (Table 1). An extraction with ethanol was used as benchmark for comparison of NaDES with a traditional solvent. The results [38] are reported as Supplementary Material for quicker consultation (Table S1 for total phenolic content and Tables S2 and S3 for the quantification of anthocyanins and of the main phenolic compounds, respectively). Such data were utilized as reference values and correspond to the maximum achievable recovery percentage, as explained in the following sections.

Table 1. List of NaDESs selected and their composition [38].

NaDES Name	HBA	HBD	Molar Ratio	Water Content (*v*/*v*)	pH
NaDES-Lac	Choline chloride	Lactic acid	1:1	50%	1.47
NaDES-Tar	Choline chloride	Tartaric acid	1:1	50%	0.56
NaDES-Gly	Choline chloride	Glycerol	1:1	50%	2.76

2.3. Recovery of Compounds from NaDES Extracts

2.3.1. Antisolvent Extraction Method

The three NaDESs extracts obtained from Primitivo pomace [38] were separately diluted with different aliquots of water (employed as antisolvent) to estimate the effect of water content on the recovery ability of the compounds by precipitation. Specifically, the NaDES extracts (which already contained 50% water) were diluted with water at dilution factors of 5×, 10×, 20×, 30×, 40× and 50×; then, the mixtures were left overnight at 5 °C and centrifuged for 30 min at 4 °C at 15,000× *g*, the liquid phase was discarded and the pellet was resuspended with methanol acidified with 0.1% formic acid. The test was conducted in triplicate. Then, the total phenolic content of the precipitate was quantified by the Folin–Ciocâlteu assay [39]. Briefly, the suspended precipitates were diluted with bi-distilled water, so that each had the same dilution factor; subsequently, they were mixed with the Folin–Ciocâlteu reagent, and the pH of the solution was adjusted with 1 M sodium carbonate (Na_2CO_3). Finally, the absorbance at 765 nm of the solution was registered against blank after 1 h, with a JASCO V-550 UV/VIS spectrophotometer (JASCO Corporation, Tokyo, Japan). The results are expressed as gallic acid equivalents (GAE), calculated with standard solutions of gallic acid at different dilutions. The percentage recovery of phenolic compounds was calculated as follows:

$$\text{Recovery (\%)} = \text{TPC}_{\text{POST}} \times 100/\text{TPC}_{\text{NaDES}} \quad (1)$$

where TPC_{POST} corresponds to the total phenolic content in the sample after recovery and $\text{TPC}_{\text{NaDES}}$ corresponds to the total phenolic content of the original sample reported in Table S1.

2.3.2. Liquid–Liquid Extraction Method

Based on literature search [23,26,33,40–42] and the physicochemical characteristics of different substances, four solvents were selected: acetonitrile (ACN), ethyl acetate (EtOAc), 2-chlorobutane (2-CB) and 2-methyltetrahydrofuran (2-MeTHF) (Table 2).

To carry out the extraction, 1 mL of each of the four solvents was added to 1 mL of NaDES extracts [38]. The tubes were orbitally shaken (DAS 12,000, Continental Equipment, Singapore, SG) at 200 rpm for 20 min, then centrifuged at 15,000× *g* for 10 min. In the initial analysis, miscibility was evaluated and, if found to be immiscible, the staining of the two phases was observed. As for the solvents found to be immiscible, the upper phase (organic phase) was taken, evaporated with an integrated vacuum concentrator (model SpeedVac SPD2030, ThermoFisher Scientific Inc., Waltham, MA, USA) set at 45 °C and 5.1 Torr and resuspended with methanol acidified with 0.1% formic acid. The test was performed in triplicate. Total phenolic content was quantified by the Folin–Ciocâlteu method (as in Section 2.3.1), and the recovery percentage was calculated as described above (Section 2.3.1). The following tests were realized by EtOAc and 2-MeTHF because the two solvents appeared better performing. Thus, the undiluted NaDES (originally containing 50% water) was compared with the NaDES diluted with water at 1.5×, 4×, 5×, 10× and 50×, corresponding to final water content of 66%, 87.5%, 90%, 95% and 99%. For each of

them, 1 mL of the diluted NaDES was mixed with 1 mL of EtOAc or 2-MeTHF, separately, and the process was performed as described above. Each dilution was tested in triplicate. Total phenols were quantified by the Folin–Ciocâlteu method and the recovery calculated as previously indicated.

The dilution of 10×, which proved to be effective without adding an excessive volume of water to NaDES (95% final water content), was used for additional liquid–liquid extractions. Both EtOAc and 2-MeTHF were employed for two-step liquid–liquid extraction, as illustrated in Figure 1. Liquid–liquid extraction A (LLE-A): 1 mL of EtOAc was added to a 1 mL aliquot of 10-fold diluted NaDES extract (95% of water content), in triplicate. The tubes were orbitally shaken (DAS 12,000, Continental Equipment, Singapore, SG) at 200 rpm for 20 min, then centrifuged at 15,000× *g* for 10 min. The upper phase (organic phase) was taken, evaporated with an integrated vacuum concentrator (model SpeedVac SPD2030, ThermoFisher Scientific Inc., Waltham, MA, USA) set at 45 °C and 5.1 Torr and resuspended with methanol acidified with 0.1% formic acid. Then, on the same aliquot of NaDES extract, residual after the removal of EtOAc, 1 mL of 2-MeTHF was added and the operation repeated as before. For liquid–liquid extraction B (LLE-B), in parallel, the same procedure was carried out on a separate 1 mL aliquot of 10-fold diluted NaDES extract but reversing the order of EtOAc and 2-MeTHF, thus using first 2-MeTHF and then EtOAc (Figure 1). The four aliquots (in triplicate), represented by EtOAc and 2-MeTHF, the first and second step of LLE-A, respectively, and 2-MeTHF and EtOAc, the first and second step of LLE-B, respectively, were analyzed by HPLC/MS.

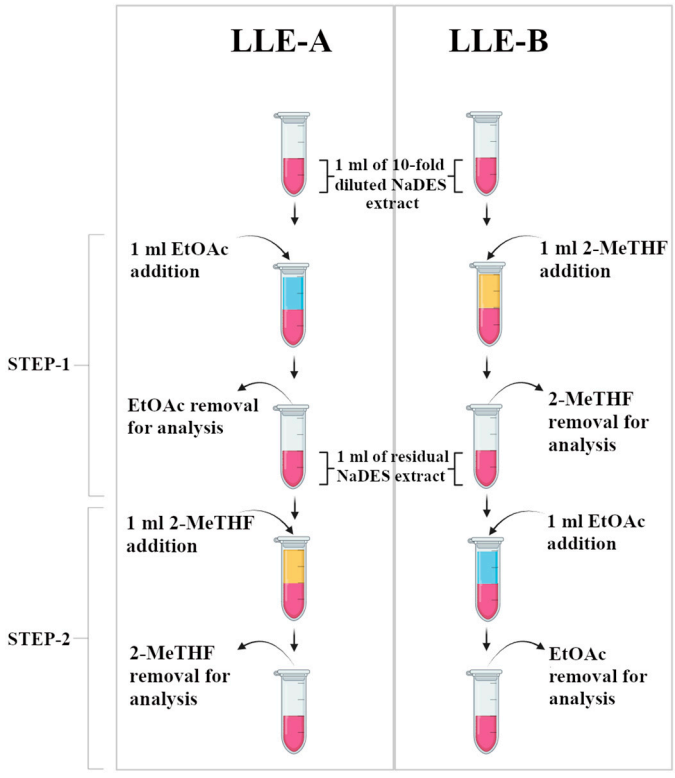

Figure 1. Graphical description of the two-step liquid–liquid extractions consisting of two-step liquid–liquid extraction with first EtOAc and then 2-MeTHF or vice versa. LLE-A: liquid–liquid extraction A; LLE-B: liquid–liquid extraction B. In each tube, the lower phase (pink) represents the NaDES extract, the light-blue upper phase EtOAc and the upper yellow phase 2-MeTHF.

Table 2. Main physicochemical characteristics of the organic solvents employed for the liquid–liquid extraction for the recovery of phenolic compounds from NaDESs.

Ethyl Acetate	Acetonitrile	2-Chlorobutane	2-Methyltetrahydrofuran	Ethyl Acetate
Boiling point (°C)	77.1	82	70	78
Dielectric constant (20 °C)	6.0	37.5	8.56	6.97
Density (g/mL)	0.902	0.786	0.873	0.854

Subsequently, a single step liquid–liquid extraction was performed adding only 1 mL of 2-MeTHF to 1 mL of 10-fold diluted NaDES extract. The 2-MeTHF phase was taken and processed as described before. This was analyzed by HPLC/MS along with the residual NaDES remaining after the removal of 2-MeTHF and the original NaDES extract 10-fold diluted.

2.3.3. HPLC/MS

The samples were characterized by an Agilent 1200 Liquid Chromatography system (Agilent Technologies, Palo Alto, CA, USA) equipped with a standard autosampler. The HPLC column was an Agilent Zorbax Extended C18 (1.8 µm, 2.1 × 50 mm). Separation was carried out at 40 °C with a gradient elution program at a flow rate of 0.3 mL/min. The mobile phases consisted of water plus 0.1% formic acid (A) and ACN (B). The following multistep linear gradient was applied: 0 min, 5% B; 13 min, 25% B; 19 min, 40% B. The injection volume in the HPLC system was 5 µL. The HPLC system was coupled to a DAD (Agilent Technologies, Palo Alto, CA, USA) set at 280 nm and an Agilent 6320 TOF mass spectrometer equipped with a dual electrospray ionization (ESI) interface (Agilent Technologies, Palo Alto, CA, USA) operating in negative ion mode. Detection was carried out within a mass range of 50–1700 m/z. Accurate measurements of the mass corresponding to each total ionic current (TIC) peak were obtained with a pump (Agilent G1310B) introducing a low flow (20 µL/min) of a calibration solution containing internal reference masses at m/z 112.9856, 301.9981, 601.9790 and 1033.9881 and using a dual nebulizer ESI source in negative ion mode [39]. The anthocyanins were identified with the same chromatography system. Phase A was water plus 2% formic acid, and phase B was can–water–formic acid (53:45:2). The HPLC column was an Agilent Zorbax Extended C18 (1.8 µm, 2.1 × 50 mm). Separation was carried out at 40 °C with a gradient elution program at a 0.3 mL/min flow rate. The following multistep linear gradient was applied: 0 min, 5% B; 12 min, 15% B; 20 min, 30% B; 35 min. 45% B. The injection volume in the HPLC system was 5 µL. TOF operated with positive ionization, using the internal reference masses at m/z 121.0508, 149.0233, 322.0481 and 922.0097. Finally, the wavelength for DAD detection was 520 nm. For both phenolic and anthocyanins, characterization mass spectrometer conditions were as follows: capillary voltage, 3.0 kV in negative mode and 3.5 kV in positive mode; nitrogen used as the nebulizer and desolvation gas; drying gas temperature, 300 °C; drying gas flow, 12 L/min; nebulizing gas pressure, 40 psig; source temperature, 120 °C. Mass Hunter software (B.07.00 version; Agilent Technologies, Palo Alto, CA, USA) was used to process the mass data of the molecular ions. The compounds were quantified using calibration curves of authentic standards (catechin, quercetin 3-β-D-glucoside, astilbin, kaempferol 3-O-glucoside, resveratrol and malvidin 3-*O*-glucoside).

The quantification result of each of these compounds was related to the value obtained in Frontini et al. [38], as reported in Tables S2 and S3, and the recovery percentage was calculated as follows:

$$\text{Recovery (\%)} = Q_{POST} \times 100/Q_{NaDES} \quad (2)$$

where Q_{POST} corresponds to the quantity of each specific compound recovered with EtOAc and 2-MeTHF, separately, and Q_{NaDES} corresponds to the quantity of the same compound in the original sample reported in Tables S2 and S3 (anthocyanin and non-anthocyanin phenols, respectively), separately for each NaDES.

2.4. Statistical Analysis

Statistical tests were conducted on both total phenolic content and on HPLC/MS quantitative analysis results; a one-way ANOVA was performed to assess differences, followed by a post hoc Tukey's test for honestly significant differences (HSD). In the case of quantitative analysis results, different tests were conducted separately for each compound. All data were reported as mean ± standard deviation. Regarding the results reported as chart, standard deviation is indicated as error bars. The analyses were performed using the R software, 4.4.2 version [43].

3. Results

3.1. Antisolvent Extraction

The use of water as antisolvent on NaDES-Lac, NaDES-Tar and NaDES-Gly previously employed for metabolite extraction from pomace [38] was unsatisfactory (Figure 2), as the total phenol recovery from the NaDES extracts did not exceed 10% in all cases. The higher the dilution factor, the greater the percentage of recovery from NaDESs in the relative precipitate; however, even at a dilution 50×, the recovery rate was not adequate.

3.2. Liquid–Liquid Extraction

Focusing next on the liquid–liquid extraction method, the first aspect considered was the miscibility of the solvents tested (Figure 3). Three of the four organic solvents employed were actually immiscible with the three NaDESs. In the case of ACN, in fact, phase separation was only partial, as can be seen in Figure 3; it is possible to see that approximately more than 30% of the organic phase of ACN merged with the eutectic mixture, preventing the formation of two distinct phases. In contrast, EtOAc, 2-CB and 2-MeTHF remained immiscible with each NaDES. In addition, as ACN, 2-MeTHF surprised for turning pinkish red in contact with two NaDESs (except in the case of NaDES-Gly).

Thus, a quantification of the phenols recovered only by the immiscible solvents (EtOAc, 2-CB and 2-MeTHF) was carried out to estimate the distribution of phenolic compounds between the two phases. ACN was not included due to the inability to form two distinct phases with NaDESs, as summarized in Table 3. For 2-CB, it was immediately apparent that the solvent shows no affinity for this class of molecules (recovery rate between 0 and 0.2%). Therefore, it was deemed appropriate to proceed further exclusively with EtOAc and 2-MeTHF.

Table 3. Main physicochemical characteristics of the organic solvents employed for the liquid–liquid extraction for the recovery (R) percentage of phenolic compounds from NaDESs. Different letters within columns indicate a statistical difference at $p < 0.05$.

Solvent	NaDES-Lac		NaDES-Tar		NaDES-Gly	
	Immiscibility	R (%)	Immiscibility	R (%)	Immiscibility	R (%)
EtOAc	Yes	1.3 ± 0.1 [b]	Yes	1.5 ± 0.0 [b]	Yes	2.2 ± 0.3 [b]
ACN	Partial	/	Partial	/	Partial	/
2-CB	Yes	0.1 ± 0.0 [c]	Yes	0.0 ± 0.0 [c]	Yes	0.2 ± 0.0 [c]
2-MeTHF	Yes	6.7 ± 0.4 [a]	Yes	4.8 0.3 [a]	Yes	5.1 ± 0.4 [a]

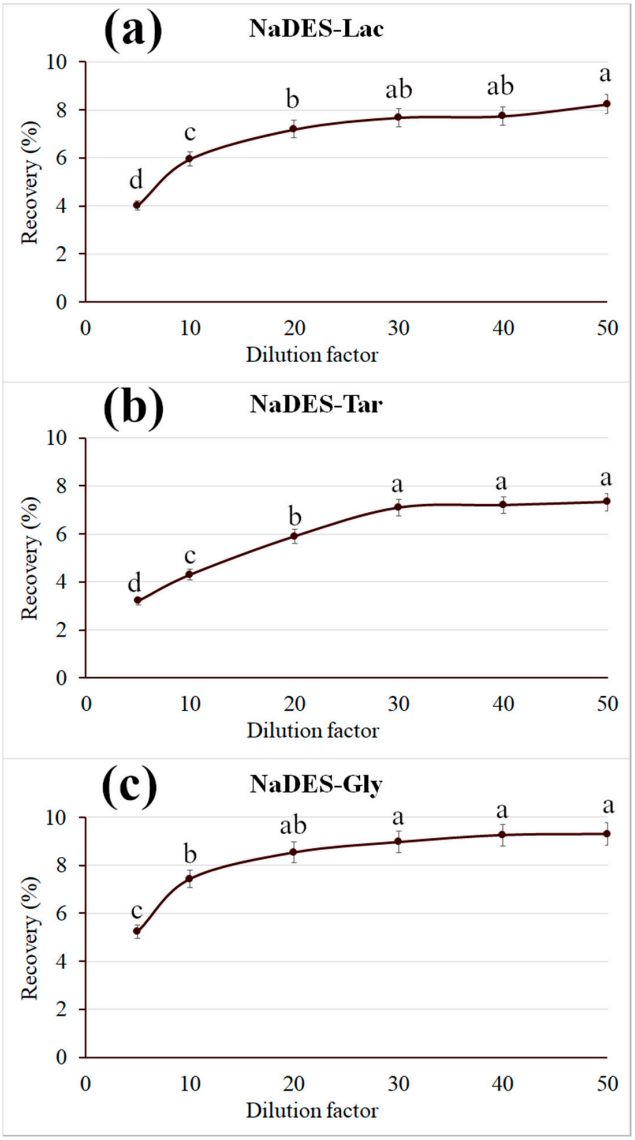

Figure 2. Recovery of total phenols by antisolvent method after dilution of NaDES starting samples. (**a**) Recovery of compounds from NaDES-Lac; (**b**) recovery of compounds from NaDES-Tar; (**c**) recovery of compounds from NaDES-Gly. For each NaDES, the recovery is calculated as percentage of the corresponding value reported in Table S1. Bars indicate standard deviation (n = 3), while different letters indicate a statistical difference ($p < 0.05$).

Then, the influence of water content in eutectic mixtures on the partitioning of compounds between the two phases was evaluated. In fact, as can be seen from Figure 4, the increase in water content substantially improves the recovery rate of the compounds (especially in the case of 2-MeTHF). For EtOAc, on the other hand, the recovery still did not go beyond 20 percent.

In any case, for the following tests, the dilution factor 10 (corresponding to a final water content of 95%) was chosen with respect to the original extract. Although dilution factor 50 (99% final water content) provided slightly higher recovery percentages, it was discarded because dilution factor 10 allowed us to obtain similar results by using five times less water. In fact, no significant differences were observed among them (Figure 4).

Therefore, two-step liquid–liquid extractions (LLE-A and LLE-B) were carried out for each NaDES extract: For each step, the main compounds were quantified, and the concentration was compared with that of the initial extract (Tables S2 and S3), estimating the recovery. Comparing the first step of both LLE-A and LLE-B, the quantitative analysis

of anthocyanins (Table 4) highlighted that 2-MeTHF was more effective than EtOAc for this purpose (2-MeTHF recovered up to 87.6% of malvidin 3-*O*-glucoside 4 vinylphenol and 81.7% of malvidin 3-*O*-glucoside vinylguaiacol from NaDES-Lac). EtOAc was almost unable to solubilize them, achieving a recovery percentage ranging from 0.2% to a maximum of 28.7%. The second step of LLE-A and LLE-B confirms, somehow, what was observed in the first step. In the case of LLE-A, 2-MeTHF extracted a quantity of anthocyanins from the residual NaDES mixture that was very similar to what was achieved in the first step of LLE-B. On the other hand, EtOAc, employed in the second step of LLE-B, was able to extract a lower amount of anthocyanins than in the step 1 of LLE-A due to the high efficiency of the previous extraction with 2-MeTHF.

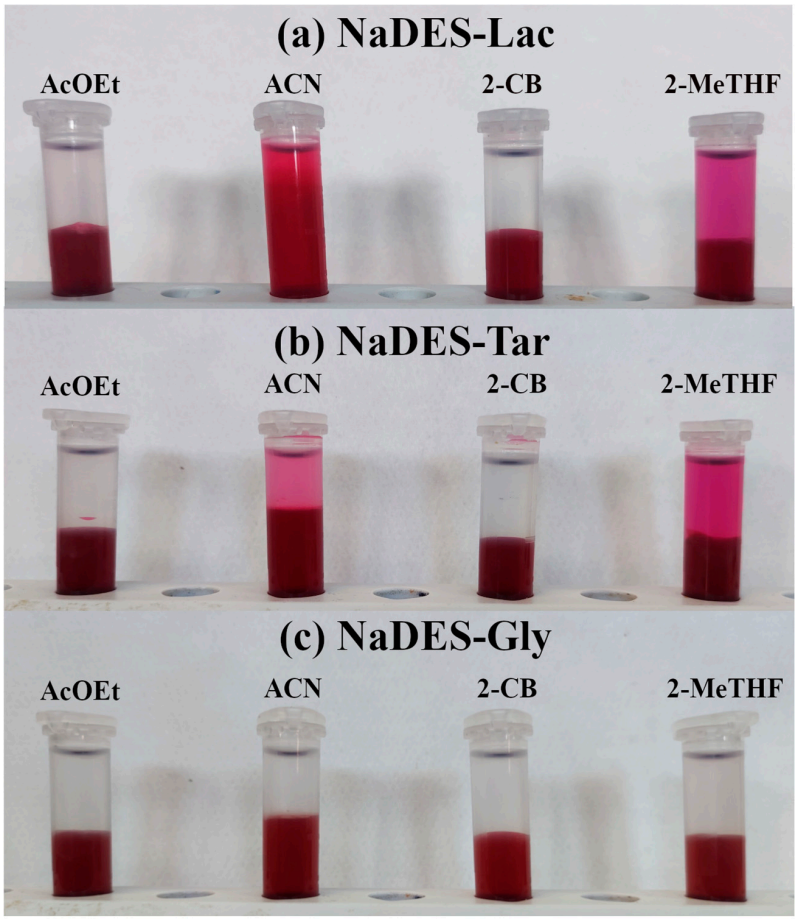

Figure 3. NaDES extracts (1 mL) after the addition of 1 mL of organic solvents. (**a**) NaDES-Lac, (**b**) NaDES-Tar and (**c**) NaDES-Gly. EtOAc, ethyl acetate; ACN, acetonitrile; 2CB, 2-chlorobutane; 2MeTHF, 2-metiltetrahydrofuran.

Focusing next on the quantitative analysis of non-anthocyanin compounds (reported in Table 5), in LLE-A, EtOAc solubilized a fair amount of each compound, with recovery percentages ranging from 38.8% (epicatechin from NaDES-Tar) to 81.9% (resveratrol from NaDES-Lac). Then, 2-MeTHF effectively recovered compounds for the remaining percentage value.

The novelty is the high recovery by 2-MeTHF in the first step of LLE-B. Except for resveratrol, the only stilbene quantified, in all other cases, the recovery rate with 2-MeTHF was more than 85% on average (and frequently more than 90%), which should be considered optimal. Obviously, the second step of LLE-B with EtOAc resulted in very low recovery percentages, because 2-MeTHF, in the first step, recovered almost the entire amount of the

compounds listed in Table 5. In addition, no remarkable differences were observed among different NaDESs.

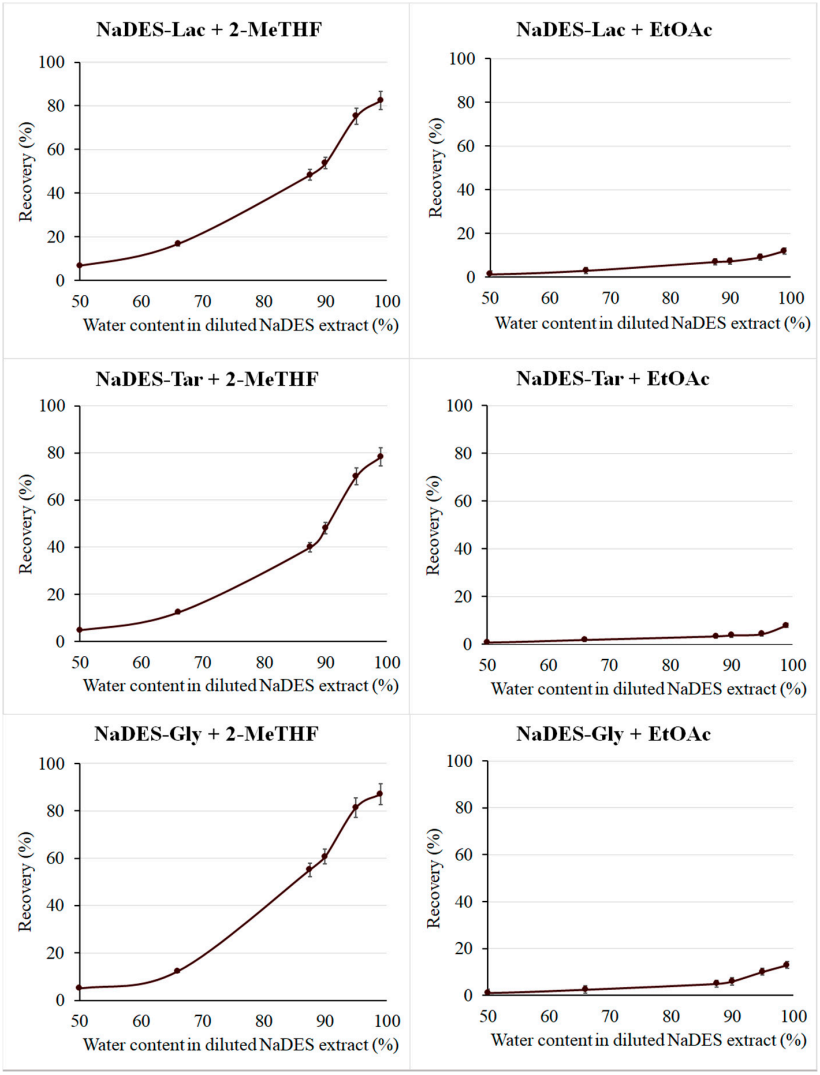

Figure 4. Recovery of total phenols from NaDES extracts with different organic solvents (2-MeTHF on the left and EtOAc on the right) related to water content (%) of the NaDES mixtures after dilution. The starting point of the x-axis is 50%, because NaDES extracts already contain 50% water. The water contents compared are 50%, 66%, 87.5%, 90%, 95% and 99%. 2-MeTHF, 2-methyltetrahydrofuran; EtOAc, ethyl acetate. For each NaDES, the recovery is calculated as percentage of the corresponding value reported in Table S1. Bars indicate standard deviation (n = 3).

To support previous observations, we measured, in the residual diluted NaDES extract (after one-step liquid–liquid extraction with 2-MeTHF), the presence of representative compounds of interest. Considering, for instance, catechin and epicatechin (chosen because they are among the most abundant non-anthocyanin phenolic compounds extracted), their level was close to zero (Figure 5), confirming the outcome.

Finally, Figure 6 shows the amount of each of four compounds of interest (two abundant anthocyanins, malvidin 3-*O*-glucoside and malvidin 3-*O*-glucoside 4-vinylguaiacol; two non-anthocyanin, catechin and astilbin, chosen as the most suitable to highlight the method's potential), expressed for DW, in the original NaDES extract [38] (red histogram), in 2-MeTHF applied on 10× diluted NaDES extract (blue histogram), and in ethanol extract (gray histogram). Except for malvidin 3-*O*-glucoside, the most abundant anthocyanin in the

samples, in all other cases, it is possible to obtain, using NaDESs and subsequent recovery with 2-MeTHF, an amount close to that obtained with ethanol.

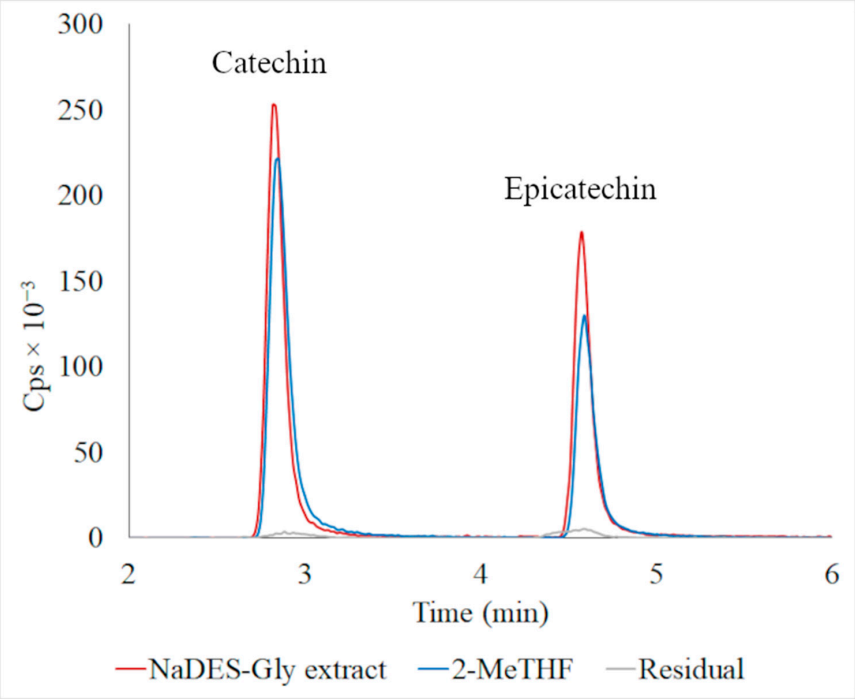

Figure 5. Partial representative chromatograms extrapolated from complete chromatograms showing the presence of catechin and epicatechin in the original NaDES-Gly extract (red), in 2-methyltetrahydrofuran (2-MeTHF) after single-step liquid–liquid extraction of the NaDES-Gly extract (blue), and in the residual NaDES-Gly liquid mixture after the liquid–liquid extraction (grey). Each of them was analyzed at the same dilution factor (10-fold dilution from the original NaDES extract).

Table 4. Anthocyanins recovery (quantified by HPLC/MS) from NaDES extracts after dilution 10× (95% water) with two-step liquid–liquid extraction. LLE-A: liquid–liquid extraction A, performed first with EtOAc and then with 2-MeTHF; LLE-B: liquid–liquid extraction B, performed with first 2-MeTHF and then with EtOAc. For each compound and each NaDES type, the recovery is calculated as percentage of the corresponding value in the original NaDES extract, reported in Table S2. EtOAc: ethyl acetate; 2-MeTHF:2-methyltetrahydrofuran. Values are the averages of three replicates ± standard deviation (n = 3). For each compound and for each individual NaDES, the means in a row with different superscript letters differ at $p < 0.05$.

		Recovery (%)			
		LLE-A		**LLE-B**	
		Step 1	**Step 2**	**Step 1**	**Step 2**
		EtOAc	**2-MeTHF**	**2-MeTHF**	**EtOAc**
Delphinidin 3-*O*-glucoside [1]	NaDES-Lac	1.0 ± 0.1 ^{b}	9.5 ± 1.4 ^{a}	6.6 ± 0.7 ^{a}	1.0 ± 0.2 ^{b}
	NaDES-Tar	0.2 ± 0.0 ^{b}	4.3 ± 0.5 ^{a}	4.0 ± 1.7 ^{a}	0.2 ± 0.0 ^{b}
	NaDES-Gly	1.5 ± 0.1 ^{c}	36.3 ± 4.1 ^{a}	20.5 ± 2.4 ^{b}	0.2 ± 0.0 ^{d}
Cyanidin 3-*O*-glucoside [1]	NaDES-Lac	5.8 ± 0.7 ^{c}	37.6 ± 3.6 ^{a}	11.9 ± 1.4 ^{b}	8.6 ± 1.5 ^{c}
	NaDES-Tar	0.5 ± 0.0 ^{b}	8.7 ± 1.7 ^{a}	7.6 ± 1.9 ^{a}	0.5 ± 0.0 ^{b}
	NaDES-Gly	2.7 ± 0.2 ^{b}	26.8 ± 1.9 ^{a}	22.9 ± 2.2 ^{a}	0.8 ± 0.1 ^{c}
Petunidin 3-*O*-glucoside [1]	NaDES-Lac	0.2 ± 0.0 ^{b}	9.4 ± 0.6 a	8.2 ± 0.6 ^{a}	0.2 ± 0.0 ^{b}
	NaDES-Tar	0.3 ± 0.0 ^{b}	5.4 ± 0.8 ^{a}	4.3 ± 0.6 ^{a}	0.1 ± 0.0 ^{b}
	NaDES-Gly	1.4 ± 0.4 ^{c}	32.3 ± 3.5 ^{a}	20.9 ± 2.2 ^{b}	0.1 ± 0.0 ^{d}

Table 4. *Cont.*

		Recovery (%)			
		LLE-A		LLE-B	
		Step 1	Step 2	Step 1	Step 2
		EtOAc	2-MeTHF	2-MeTHF	EtOAc
Peonidin 3-*O*-glucoside [1]	NaDES-Lac	1.6 ± 0.4 [b]	19.2 ± 1.4 [a]	16.8 ±1.6 [a]	1.8 ± 0.1 [b]
	NaDES-Tar	0.6 ± 0.1 [b]	9.5 ± 1.2 [a]	7.1 ± 0.6 [a]	1.0 ± 0.1 [b]
	NaDES-Gly	3.8 ± 0.3 [b]	27.7 ± 2.0 [a]	26.1 ± 1.7 [a]	2.9 ± 0.3 [b]
Malvidin 3-*O*-glucoside	NaDES-Lac	0.9 ± 0.1 [b]	13.4 ± 0.8 [a]	10.8 ± 0.7 [a]	1.3 ± 0.1 [b]
	NaDES-Tar	0.3 ± 0.0 [b]	6.3 ± 0.7 [a]	4.7 ± 0.4 [a]	0.6 ± 0.0 [b]
	NaDES-Gly	1.8 ± 0.2 [c]	17.3 ± 0.4 [a]	14.4 ± 0.7 [b]	1.8 ± 0.2 [c]
Malvidin 3-*O*-(6′-acetyl)-glucoside [1]	NaDES-Lac	5.4 ± 0.6 [c]	40.3 ± 1.8 [a]	32.8 ± 1.9 [b]	4.6 ± 0.4 [c]
	NaDES-Tar	2.9 ± 0.5 [b]	26.0 ± 3.0 [a]	20.7 ± 1.1 [a]	3.1 ± 0.1 [b]
	NaDES-Gly	9.0 ± 1.0 [b]	42.9 ± 1.8 [a]	41.8 ± 2.3 [a]	6.3 ± 0.5 [c]
Malvidin 3-*O*-(6′-caffeoyl)-glucoside [1]	NaDES-Lac	7.2 ± 0.5 [b]	64.2 ± 6.5 [a]	69.9 ± 2.9 [a]	1.4 ± 0.1 [c]
	NaDES-Tar	2.5 ± 0.3 [c]	44.1 ± 3.7 [b]	55.9 ±2.7 [a]	2.6 ± 0.1 [c]
	NaDES-Gly	13.4 ± 1.3 [c]	54.0 ± 5.1 [b]	72.2 ± 7.6 [a]	1.4 ± 0.3 [d]
Malvidin 3-*O*-glucoside 4 vinylphenol [1]	NaDES-Lac	14.9 ± 1.3 [c]	64.9 ± 6.6 [b]	87.6 ± 3.8 [a]	4.8 ± 0.6 [d]
	NaDES-Tar	10.0 ± 0.9 [c]	61.9 ± 5.7 [b]	77.7 ± 1.1 [a]	4.8 ± 0.3 [d]
	NaDES-Gly	23.7 ± 2.7 [c]	38.7 ± 2.5 [b]	84.4 ± 5.1 [a]	4.2 ± 0.5 [d]
Malvidin 3-*O*-glucoside 4 vinylguaiacol [1]	NaDES-Lac	19.3 ± 2.1 [c]	69.1 ± 5.1 [b]	81.7 ± 3.0 [a]	0.3 ± 0.0 [d]
	NaDES-Tar	7.0 ± 0.5 [c]	58.0 ± 2.3 [b]	69.9 ± 2.6 [a]	0.4 ± 0.0 [d]
	NaDES-Gly	28.7 ± 3.4 [c]	45.1 ± 6.5 [b]	80.1 ± 6.1 [a]	3.4 ± 0.4 [d]

[1] Quantified as malvidin 3-*O*-glucoside equivalent.

Table 5. Non-anthocyanin compounds recovery (quantified by HPLC/MS) from NaDES extracts after dilution 10× (95% water) with two-step liquid–liquid extraction. LLE-A: liquid–liquid extraction A, performed first with EtOAc and then with 2-MeTHF; LLE-B: liquid–liquid extraction B, performed with first 2-MeTHF and then with EtOAc. For each compound and each NaDES, the recovery is calculated as percentage of the corresponding value in the original NaDES extract, reported in Table S3. EtOAc: ethyl acetate; 2-MeTHF: 2-methyltetrahydrofuran. Values are the averages of three replicates ± standard deviation (n = 3). For each compound and for each individual NaDES, the means in a row with different superscript letters differ at $p < 0.05$.

		Recovery (%)			
		LLE-A		LLE-B	
		Step 1	Step 2	Step 1	Step 2
		EtOAc	2-MeTHF	2-MeTHF	EtOAc
Catechin	NaDES-Lac	60.9 ± 5.0 [b]	35.1 ± 3.2 [c]	94.6 ± 3.3 [a]	5.4 ± 0.7 [d]
	NaDES-Tar	44.8 ± 5.3 [b]	51.2 ± 3.8 [b]	95.5 ± 2.2 [a]	3.4 ± 0.5 [c]
	NaDES-Gly	63.7 ± 2.9 [b]	32.7 ± 1.0 [c]	95.9 ± 1.9 [a]	4.0 ± 0.7 [d]
Epicatechin [1]	NaDES-Lac	44.4 ± 8.5 [b]	44.6 ± 5.4 [b]	77.6 ± 9.2 [a]	10.5 ± 0.9 [c]
	NaDES-Tar	38.8 ± 4.8 [c]	54.0 ± 5.2 [b]	90.0 ± 2.2 [a]	3.0 ± 0.7 [d]
	NaDES-Gly	57.5 ± 3.9 [b]	37.6 ± 2.2 [c]	89.9 ± 2.9 [a]	4.4 ± 0.8 [d]
Quercetin 3-*O*-hexuronide [2]	NaDES-Lac	55.4 ± 1.3 [b]	37.8 ± 2.1 [c]	96.8 ± 2.1 [a]	4.4 ± 0.5 [d]
	NaDES-Tar	54.3 ± 2.7 [b]	39.7 ± 4.0 [c]	92.7 ± 4.7 [a]	5.3 ± 0.7 [d]
	NaDES-Gly	42.7 ± 2.3 [b]	43.0 ± 3.1 [b]	85.7 ± 5.1 [a]	7.1 ± 0.8 [c]
Quercetin 3-β-D-glucoside	NaDES-Lac	61.0 ± 4.4 [b]	40.6 ± 3.0 [c]	85.9 ± 5.3 [a]	6.2 ± 0.8 [d]
	NaDES-Tar	56.4 ± 5.9 [b]	45.4 ± 5.4 [b]	94.6 ± 3.2 [a]	5.2 ± 0.8 [c]
	NaDES-Gly	61.0 ± 6.5 [b]	40.8 ± 3.3 [c]	96.1 ± 2.4 [a]	4.8 ± 0.3 [d]

Table 5. *Cont.*

		Recovery (%)			
		LLE-A		LLE-B	
		Step 1	Step 2	Step 1	Step 2
		EtOAc	2-MeTHF	2-MeTHF	EtOAc
Astilbin	NaDES-Lac	79.4 ± 2.9 [b]	15.7 ± 1.5 [c]	96.4 ± 2.1 [a]	3.1 ± 0.2 [d]
	NaDES-Tar	77.2 ± 1.8 [b]	19.7 ± 1.4 [c]	95.7 ± 2.3 [a]	4.1 ± 0.3 [d]
	NaDES-Gly	78.4 ± 2.1 [b]	17.0 ± 1.5 [c]	96.5 ± 1.6 [a]	3.3 ± 0.2 [d]
Kaempferol 3-*O*-glucoside	NaDES-Lac	77.3 ± 3.4 [b]	20.4 ± 4.4 [c]	95.3 ± 4.2 [a]	4.4 ± 0.2 [d]
	NaDES-Tar	73.1 ± 3.2 [b]	23.8 ± 1.9 [c]	95.0 ± 6.1 [a]	4.7 ± 0.4 [d]
	NaDES-Gly	80.2 ± 5.8 [b]	17.0 ± 5.4 [c]	93.3 ± 2.2 [a]	4.8 ± 0.4 [d]
Resveratrol	NaDES-Lac	81.9 ± 7.4 [a]	8.9 ± 0.9 [c]	73.4 ± 6.7 [a]	19.1 ± 1.7 [b]
	NaDES-Tar	62.2 ± 4.6 [a]	31.2 ± 3.1 [b]	73.5 ± 8.4 [a]	13.4 ± 0.9 [c]
	NaDES-Gly	63.1 ± 9.9 [a]	5.5 ± 4.7 [c]	79.6 ± 4.9 [a]	17.9 ± 1.4 [b]

[1] quantified as catechin equivalent; [2] quantified as quercetin-3-β-D-glucoside equivalent.

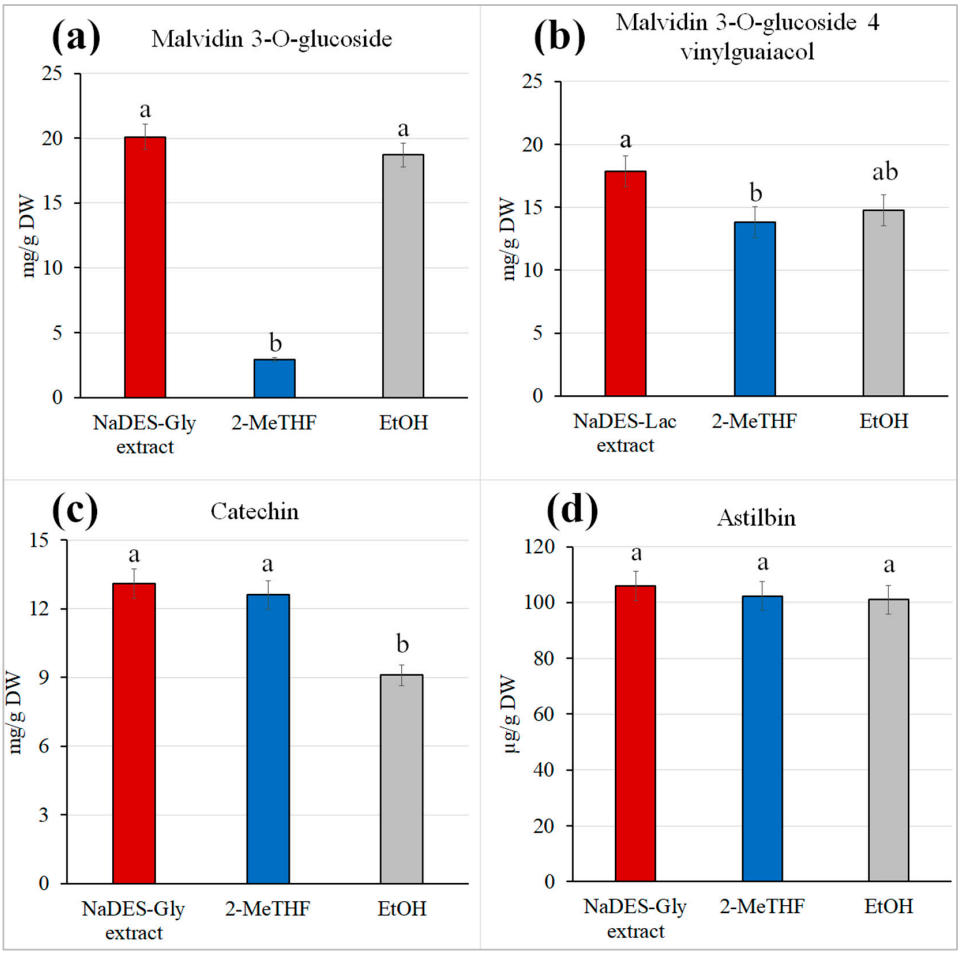

Figure 6. Yield of some compounds, quantified by HPLC/MS, after different extraction processes. NaDES extract (red histogram), 2-MeTHF: single-step liquid–liquid extraction with 2-MeTHF (blue histogram) and EtOH: ethanol extract (grey histogram). (**a**) Malvidin 3-*O*-glucoside; (**b**) malvidin 3-*O*-glucoside 4-vinylguaiacol; (**c**) catechin; (**d**) astilbin. Values are expressed per gram of dry weight of pomace. Different letters above the histograms indicate significantly different means ($p < 0.05$).

4. Discussion

From the results presented above, it is inferred that among the two methods tested, only one was effective. The use of water as antisolvent provided completely unsatisfactory results, as the recovery percentage remained below 10% (Figure 2). The antisolvent method is based on the concept that the eutectic mixture is effective only within a specific range of molar ratio between its components; consequently, adding a solvent (such as water) to a NaDES extract leads to the breakdown of the hydrogen bonds that allow the formation of a eutectic mixture [44]. The use of water for this purpose results in switching from a eutectic mixture to an aqueous solution, losing the solvation properties of NaDESs and precipitating the compounds soluble in NaDESs but insoluble in water. However, the glycosylated anthocyanins and flavonoids are fairly soluble in water and normally do not precipitate, even after breaking the NaDES-forming bonds. In fact, Nam et al. [27] observed recovery rates for quercetin, kaempferol and isorhamnetin glycoside that were below 50% by this method. For rutin, however, being nearly insoluble in water, the recovery rate was shown to be about 75%. Similarly, Huang et al. [28] were able to recover rutin from NaDESs by more than 90%, and Patil et al. [29] recovered curcuminoids from NaDESs through the addition of water, but, of course, these compounds are poorly water soluble. The same can be true for the more than 90% recovery of ellagic acid from NaDESs using choline chloride [45]. Not surprisingly, no studies were found regarding the recovery of anthocyanins or other glycosylated flavonoids by this method. In addition, Hang et al. [25] confirmed that this approach could only recover 24% and 27% of apigenin and luteolin, respectively. Contrary to all these observations, Zhou et al. [46] report recovery rates, through water addition alone, between 61% and 80% for chlorogenic acid, benzoic acid, catechinic acid, astragalin and rutin. Considering these findings, the antisolvent strategy was considered inefficient and abandoned.

Regarding the liquid–liquid extraction method, the first aspect considered was the miscibility of the solvents tested (Figure 3). Acetonitrile was only partially immiscible; EtOAc, 2-CB and 2-MeTHF resulted immiscible. 2-MeTHF, interestingly, assumed a pinkish-red staining, probably due to a partial solubilization of anthocyanins. No literature sources were found reporting this ability of 2-MeTHF towards anthocyanins and NaDESs. However, Koyu et al. [47] found that 2-MeTHF was more effective than ethanol in extracting total flavonoids, but less effective for total phenols. In another work [48], eucalyptus wood was exposed to a mixture of NaDES (choline chloride and lactic acid) and 2-MeTHF, allowing the two-phase separation for lignin recovery, but in this case, both NaDESs and 2-MeTHF were put in contact with the biomass. EtOAc, on the other hand, was found to be completely insoluble in water (Figure 3), and was used for the recovery of polyphenols by Pal and Jadeja [30], achieving complete phase separation. Xu et al. [31] tested different solvents for the recovery of phenolic compounds from citrus peels NaDES extract. The solvents used were hexane, acetone, n-propanol, EtOAc, chloroform and n-butanol; among these, EtOAc was the only one that proved to be completely immiscible with NaDESs regardless of the amount of water in the mixture. Li et al. [49] exploited a biphasic system with NaDESs and EtOAc to remove phenols recovered from coal tar by NaDESs, allowing them to reuse the eutectic mixture, indicating the value of this approach. Years earlier, Tan et al. [50] had also employed this method to recover phenolic compounds from ionic liquids.

2-CB was not able to recover phenolic compounds, confirming the lack of literature sources regarding the use of 2-CB in combination with NaDESs or their extraction from natural biomass; however, by virtue of its immiscibility with water and its dielectric constant of 8.56 (a value that suggested a polarity that could make it capable of solubilizing phenols), it was tested for phenol recovery. Instead, regarding EtOAc and 2-MeTHF, whereas both solvents provided unsatisfactory recovery percentages of total phenols (Table 3), the effect

of water content on the recovery capacity was investigated, relying on previous evidence suggesting a role of water in the distribution of polyphenols among the two phases [33,37]. Increasing the water content in NaDES extract allowed for a higher recovery percentage (Figure 4). It remained lower in the case of EtOAc, but it should be considered that anthocyanins are not soluble in this solvent. Consistently, Xu et al. [33] reported diluting the NaDES extract five times before recovering the compounds with EtOAc. It is quite interesting that in the present work, in all cases, maximum recovery occurs from the higher water dilution (99%), especially employing 2-MeTHF, which achieves extremely good percentages, as reported in Figure 4. Other authors have used 2-MeTHF for the recovery of phenolic acids from aqueous matrices containing phenols, achieving recovery percentages up to 100% [37], thus supporting the decisive influence of water content. Several authors had proposed a two-phase water/2-MeTHF system, but as pretreatment of lignocellulosic biomass to separate lignin and related compounds [48,51–57]. One explanation for this behavior could be sought in the affinity of these molecules toward different solvents. Since phenolic compounds are obviously more akin to NaDESs than to water, as shown by the significant higher phenolic extraction yield obtained with the former [58], increasing the water content leads to obtaining chemical and physical characteristics almost identical to those of pure water, effectively reducing the affinity toward polyphenols.

The EtOAc employed in the first step of LLE-A did not permit the recovery of anthocyanins (Table 4). The first step of LLE-B with 2-MeTHF, instead, resulted in recovery percentages ranging from 4.0% to 87.6%. Indeed, anthocyanins are highly water-soluble molecules [59], and 2-MeTHF had been identified as a solvent with characteristics as close to those of water as possible, but not so similar as to make it water-soluble. Generally, the weaker a compound's ability to form hydrogen bonds, the greater the transition to the organic phase [25]. With respect to compounds such as malvidin 3-*O*-(6′-caffeoyl)-glucoside, malvidin 3-*O*-glucoside 4 vinylphenol and malvidin 3-*O*-glucoside 4 vinylguaiacol, the percentage of recovery with 2-MeTHF can be considered more than acceptable because it varied approximately between 70% and 87%. It is well known that acylated anthocyanins are more soluble in organic solvents than the non-acylated form [60]. Furthermore, with reference to the decreasing polarity of the molecules analyzed, other authors have shown that 2-MeTHF represents a viable environmentally friendly alternative to apolar solvents (such as hexane) for the extraction of fat-soluble compounds such as carotenoids, oils and fats [61,62], but the observations here presented show a fair affinity toward polar and water-soluble compounds as well. It is certainly noteworthy that the recovery from NaDES-Tar is almost always lower than from the other two NaDESs, and that from NaDES-Gly we have obtained the highest recovery, probably depending on the different affinity of the three NaDESs to anthocyanins. The efficacy of 2-MeTHF and EtOAc was also observed in the second step of LLE-A and LLE-B extractions, respectively, confirming their different ability of solubilizing anthocyanins. On the other hand, the non-anthocyanin phenolic compounds were recovered from both EtOAc and 2-MeTHF (Table 5). Similar recovery values had already been observed for EtOAc [25,30–33,41,48,62,63], while the new utilization of 2-MeTHF appears to be quite successful in solubilizing non-anthocyanin phenolic compounds. Except for resveratrol, the only stilbene quantified, in all other cases, the recovery rate with 2-MeTHF was more than 85% on average (and frequently more than 90%), which should be considered optimal. In addition, it is widely recognized that in a biphasic system consisting of NaDESs and an aprotic solvent, most phenolic compounds tend to be retained in the NaDES phase [26]. Our findings indicate that 2-MeTHF could be used alone for a single-step liquid–liquid extraction of polyphenols from NaDESs, with great effectiveness. Indeed, the second step of LLE-B was completely useless, since the first step with 2-MeTHF outperformed for both anthocyanins and non-anthocyanin compounds.

On the other hand, LLE-A might make sense in view of separating the two fractions of phenolic compounds (anthocyanins and non-anthocyanin compounds). Specifically, in the fraction obtained with EtOAc, one would obtain a concentrate of non-anthocyanin phenols (glycosylated and non-glycosylated flavonoids, stilbenes and phenolic acids) without anthocyanins; on the other hand, in the fraction obtained with 2-MeTHF, one would obtain a fraction enriched in anthocyanins, with a fair amount of phenols (the residue of what did not migrate into EtOAc). Obviously, this method does not allow for accurate purification and separation of the individual molecules. In fact, each solvent extracts compounds from NaDESs with different efficiency and selectivity. Other authors have also opted for a multi-step system with different solvents to recover different classes of compounds with different solvents: Xu et al. [33] suggest utilizing EtOAc first to recover polymethoxylated flavonoids and n-butanol, then to recover hesperidin from a tertiary NaDES formed by choline chloride, levulinic acid and N-methylurea. Other authors, however, identified EtOAc as the best solvent for the recovery of phenolic compounds extracted from onion peels with a NaDES [30], and later showed that chloroform was more effective for the recovery of mangiferin extracted from mango peels [34]. Therefore, considering the separation of different phenolic fractions, 2-MeTHF was discovered to be the opportune solvent for the recovery of phenolic chemicals from NaDESs, exceeding expectations in several ways. The effectiveness of 2-MeTHF in recovering most phenols from NaDESs was proved by analyzing the residual NaDES after single-step liquid–liquid extraction (Figure 5), which resulted in a content of catechin, taken as example compound, close to zero. Similarly, Sun et al. [48] extracted lignin from agro-forestry biomass using 2-MeTHF and NaDESs at the same time, but the yield was low. Other authors, instead, have obtained equally promising results by mixing 2-MeTHF with winery wastewater in a biphasic system, achieving phenolic acid recovery rates of up to 96% in the case of gallic acid [64]. In addition to those observations, our study opens several prospects for the use of liquid–liquid method with 2-MeTHF for the recovery of bioactive chemicals from NaDESs after the extraction from plant biomasses. Cañadas et al. [42] extracted phenolics from white grape pomace with water and then recovered the compounds with 2-MeTHF, proposing it as an innovative and environmentally friendly method. However, the yield they obtained for each of the quantified compounds was less than half that obtained with an extraction with ethanol. On the contrary, adding a NaDES step to the process, as proposed in this work, yields a result comparable to that obtained with ethanol (Figure 6). It could be also a novelty, for example, for the removal of pollutants, such as phenols, from water derived from urban or industrial activities. In fact, Figure 6 indicates that the proposed method is a viable alternative to conventional extraction methods. A thorough investigation of the process's scalability is needed. In fact, the volume of water required and the use of an organic solvent in the process may limit its implementation. However, this process has the advantage of being faster and less expensive than other effective procedures, such as solid–liquid extraction utilizing adsorbent materials. Finally, the usage of water and solvents could be justified by the capacity to reuse any substance used in a closed system.

5. Conclusions

The comparison of the antisolvent method with the liquid–liquid extraction method for the recovery of compounds from NaDES extracts indicated that the liquid–liquid extraction is useful and satisfactory. The 2-MeTHF solvent was extremely effective and outperformed for the recovery of non-anthocyanin phenolics, allowing the recovery of a fair number of anthocyanins, even though the method requires a dilution with water (10×), which is recoverable at the end of the process. In addition, we proposed a sequential two-step process for the separation and recovery of different fractions of polyphenols, obtaining

high yields and partial purification. The collection of phenol fractions from grape pomace could provide an additional source of profits for winemakers while also improving both the sustainability of the oenological industry and of extraction operations. In parallel, this method could represent a cheap and time-saving alternative to other analytical methods requiring expensive equipment. Finally, 2-MeTHF appears to have been tested for the first time for the recovery of anthocyanins from NaDES extracts. Additional research could provide a better knowledge of the behavior of 2-MeTHF when combined with NaDESs or other aqueous matrices, while it would be fascinating to assess the lifespan of the recycled solvents.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/separations12060148/s1, Table S1: Total phenolic content of the extracts obtained with different NaDESs and ethanol from pomace obtained from rosé vinification of Primitivo grapes; Table S2: HPLC/MS analysis of the main anthocyanins extracted with different NaDESs and ethanol from pomace obtained from rosé vinification of Primitivo grapes; Table S3: HPLC/MS analysis of the main non-anthocyanin compounds extracted with different NaDESs and ethanol from pomace obtained from rosé vinification of Primitivo grapes.

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Conflicts of Interest: Authors Massimiliano Apollonio and Luigi De Bellis were employed by the company Apollonio Casa Vinicola. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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