Use of Natural Deep Eutectic Solvents (NADES) for the Selective Fractionation of Bioactive Low Molecular Weight Carbohydrates

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Article

Use of Natural Deep Eutectic Solvents (NADES) for the Selective Fractionation of Bioactive Low Molecular Weight Carbohydrates

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Abstract: Natural deep eutectic solvents (NADES) have been shown as selective and environmentally friendly solvents for the extraction of bioactive compounds. However, studies on the solubility of low molecular weight carbohydrates (LMWC) in NADES are scarce. In this work, new solubility data of LMWC in NADES are provided and a new approach based on the use of these solvents for the efficient fractionation of bioactive carbohydrates was explored for the first time. Several mono- and disaccharides and three NADES based on choline chloride (ChCl) and different donors [2-ethylene glycol (EtG), glycerol (Gly) and ethanedioic acid dihydrate (Eth)] were considered. While degradation of carbohydrates, mainly ketoses, was detected with ChCl:Eth due to its acidic nature, ChCl:EtG and ChCl:Gly were found to be useful alternatives for selectively separating bioactive ketoses and their corresponding aldoses (e.g. lactulose/lactose and tagatose/galactose) present in equimolar binary mixtures. In addition, the usefulness of ChCl:EtG for the selective enrichment of lactulose to be used as food ingredient or nutraceutical was proven (from a 25% in the reaction mixture to a 56% in the purified sample). NADES could be used for the selective fractionation of value-added carbohydrates from interfering sugars for several applications including food science, engineering or pharmaceuticals.

Keywords: Natural Deep Eutectic Solvents (NADES); ketoses; aldoses; fractionation; low molecular weight carbohydrates

1. Introduction

Low molecular weight carbohydrates (LMWC) are important components of foods as they belong to the group of basic nutrients involved in nutrition and metabolism. They may be naturally present or may be added to improve the sensory, functional and technological properties of food. In addition, certain carbohydrates present functional properties beneficial to human health (i.e. prebiotic effect, glycosidase inhibitors, treatment of conditions related to insulin resistance, etc.). However, in natural sources these LMWC coexist with other sugars that may interfere with their bioactive or technological properties [1]. Selective fractionation of these bioactive carbohydrates is essential for their analytical characterization, use in the food industry or evaluation of their bioactivity [2]. However, this is a challenging process due to the similarity and complexity of their structures as well as their varying concentrations in natural samples.

Several techniques based on the use of membranes (such as ultrafiltration, nanofiltration, etc.) have been proposed for selective separation of carbohydrates [2]; however, their efficiency for LMWC is limited [3]. Chromatographic techniques (such as ion exchange chromatography, size exclusion chromatography, simulated moving bead chromatography, etc.) are highly selective for the fractionation of carbohydrate mixtures with different degrees of polymerization (DPs) [3], but the

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fractionation of LMWC with the same DP and different monosaccharide composition or glycosidic linkages remains a challenge [2].

Differences in the solubility of carbohydrates in conventional solvents (ethanol, methanol, 1-propanol, etc.), which cause some of them to precipitate, have been used for their selective separation [4]. Despite the potential utility of these methods, their high solvent consumption and low selectivity have motivated the search for greener and cost-effective alternatives, such as ionic liquids (ILs) or deep eutectic solvents (DES) [5]. The tunability of these solvents and their unique physicochemical properties (negligible vapour pressure over a wide temperature range, high thermal stability, etc.) open up a range of possibilities for the selective extraction of bioactive compounds such as carbohydrates.

The solubility of LMWC in ILs has been extensively studied in recent years [6–8]. These solvents have also been proposed for the selective fractionation of bioactive carbohydrates such as bioactive ketoses/aldoses [9] and value-added polyols/sugars [1]. However, some studies suggest that, depending on their chemical structure, ILs may be as toxic as or even more dangerous than organic solvents and more expensive [10].

DES, a new generation of green solvents, are obtained by the complexation of a hydrogen acceptor (HBA), often a quaternary ammonium salt such as choline chloride, and a hydrogen bond donor (HBD), which can be carboxylic acids, alcohols, amines, polyols, acid amides or carbohydrates [11]. If the compounds that make up the DES are primary metabolites, such as organic acids, amino acids, choline derivatives, or sugars, they are known as natural deep eutectic solvents (NADES) [12,13]. NADES have been shown to be environmentally friendly and selective for the extraction of certain compounds, mainly phenolic compounds [14–16] and polysaccharides [17,18]. However, to the best of our knowledge, there is only one study related to the solubility of three LMWC in NADES (glucose, sucrose, and erythritol) [19], while these solvents have not been used for the selective fractionation of specific LMWC yet. Only, Gomez et al. [20] proposed the combined use of NADES and microwave-assisted extraction to remove soluble sugars from banana puree to obtain an enriched non-starch polysaccharide fraction.

In the present work, the solubility of various LMWCs (including mono- and disaccharides, aldoses and ketoses) in choline chloride-based NADES and their usefulness for the separation of bioactive ketoses (i.e. tagatose or lactulose) from their corresponding aldoses (i.e. galactose or lactose) was evaluated for the first time. Finally, the potential of NADES as a new alternative for the selective separation of carbohydrates in real and more complex samples was considered by applying the proposed method to the enrichment of lactulose in a lactose isomerization reaction mixture.

2. Materials and Methods

2.1. Standards and reagents

Carbohydrate standards: fructose (Fru), galactose (Gal), glucose (Glc), lactose (La), lactulose (Lu), maltose (Mal), sucrose (Suc), tagatose (Tg) and trehalose (Trh), DES constituents: choline chloride (ChCl), 2-ethylene glycol (EtG), glycerol (Gly) and ethanedioic acid dehydrate (Eth), and 5-hydroxymethyl-2-furaldehyde (HMF) were purchased from Sigma Chemical Co. (St. Louis, USA).

2.2. Synthesis and characterization of NADES

NADES were synthesized as indicated by Hakkinen and Abbott [19] with slight modifications. In brief, HBD (EtG, Gly and EthAc) were mixed with ChCl at 2:1 molar ratio for ChCl:EtG (NADES1, ethaline) and ChCl:Gly (NADES2, glyceline) and 1:1 molar ratio for Eth:ChCl (NADES3, oxaline) in a water bath at 50°C under stirring up to a homogeneous liquid was formed. Characteristics of these NADES are shown in Table 1.

Dynamic viscosity (η) of synthesized NADES was determined following the Poiseuille's law (Equation (1)) as indicated by Geurink et al. [21]. An Agilent 7100 CE instrument with a diode array detector (DAD) and an Agilent bare fused silica (BFS) capillary of 50 μm id, total length 57.5 cm, and with acetone as tracer was used. A hydrodynamic pressure (ΔP) of 4 bar was applied on one side of

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the capillary [length (L) 57.5 cm, inner diameter (d) 50 μm], homogeneously filled with the BGE solution. The velocity (υ_{gem}) of the tracer was calculated by measuring the migration time (t_m) over an effective length (L_{eff}) of 8.5 cm.

$$\eta=(\Delta P \cdot d\text{^}2)/(32 \cdot \upsilon_{gem} \cdot L)=(\Delta P \cdot d\text{^}2)/(32 \cdot L_{eff}/t_m \cdot L) \quad (1)$$

The water content of the NADES was measured using a C20 Compact Karl Fischer coulometer (Mettler Toledo; Ohio, US) with HYDRANAL®-Coulomat AG (Sigma Chemical Co.) as the reagent for the volumetric titration.

Table 1. Viscosity and water content of synthesised NADES.

	Hydrogen Bond Acceptor (HBA)	Hydrogen Bond Donnor (HBD)	Molar ratio HBA:HBD	Viscosity (cP)	Water content (%)
NADES1	Choline chloride (ChCl)	2-Ethylene glycol (EtG)	1:2	35.6 (0.9)*	0.29
NADES2		Glycerol (Gly)	1:2	309.2 (0.9)	0.23
NADES3		Ethanedioic acid dihydrate (Eth)	1:1	143.8 (0.7)	9.96

*Standard deviation in parenthesis (n=3).

2.3. Synthesis of lactulose

Lactulose was obtained by isomerization of lactose according to the method of Montilla et al. [22] with some modifications. Briefly, 2 mL of a solution of lactose (250 mg mL^{-1}) and powdered egg shell (final concentration, 30 mg mL^{-1}) were added to 8 mL of Milli-Q water and kept at 125 °C under reflux for 4 h. The reaction was stopped by immersion in an ice bath, the egg shell was removed by filtration through a 0.4 μm paper filter (Millipore) and the sample was lyophilized.

2.4. Solubility of LMWC in NADES

One gram of each NADES was added to an excess amount of the individual carbohydrates. These mixtures were magnetically stirred at 200 rpm at 25°C (and at 45°C for NADES2) for 24 h and left to stand for a further 24 h at the same temperature. Preliminary experiments were carried out to confirm that the stirring time was sufficient to achieve reproducibility and accuracy and to determine the amounts of each carbohydrate required (from 20 mg to 1000 mg). Aliquots were collected from the upper layer and diluted between 1:1,000 and 1:40,000 (v/v) with Milli-Q water. Samples were analyzed by high performance liquid chromatography coupled to mass spectrometry (HILIC-MS). All solubility assays were made in triplicate.

2.5. Solubility of binary mixtures of LMWC in NADES

One gram each of NADES1 and NADES2 were respectively added to 1:1 (w/w) binary mixtures containing lactose:lactulose, and galactose:tagatose. The samples were stirred at 200 rpm for 24 h and allow to stand for a further 24 h at 25°C. Aliquots of each mixture were diluted as required with Milli-Q water and analyzed by HILIC-MS.

2.6. NADES treatment of the lactose isomerization mixture

NADES1 (0.5 g) was mixed with 500 mg of the lyophilized isomerization mixture and subjected to the same treatment as described in section 2.5 for the dilution of binary mixtures. Aliquots of the supernatant were analyzed by HILIC-MS.

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2.7. Evaluation of carbohydrate degradation

Colour development of NADES1 and NADES3 samples was assessed using Spectra Max Plus 384 Microplaque Reader (Molecular Devices, California, United States) by the absorbance at 420 nm according to the method of Meydav et al. (1977) [23]. Samples were diluted [from 1:1 to 1:10 (v/v)] in sodium phosphate buffer (PBS, 1 mM; pH, 7.02; control value).

The formation of HMF was determined by high performance liquid chromatography using an HPLC-UV 1100 series with G1314B VWD detector (Agilent Technologies, Santa Clara, CA, US), an XDB-C18 column (Zorbax®, 5 µm particle size and 80 Å pore size, 150 x 4.6 mm i.d., Agilent Technologies) and a binary gradient consisting of methanol:water as indicated by Viñas et al. [24]. An external standard calibration curve (0.025 – 0.1 mg mL^{-1}) was used for quantitative analysis.

2.8. HILIC-MS analysis

Hydrophilic interaction liquid chromatography coupled to mass spectrometry (HILIC-MS) was used for the analysis of LMWC using a 1260 Infinity II Prime LC System equipped with a diode array detector coupled to a 6125 single quadrupole mass detector (Agilent Technologies) provided with an electrospray ionization (ESI) source as indicated by Hernández-Hernández et al. [25]. An ethylene bridge hybrid with trifunctionally-bonded amide phase column (150 mm × 4.6 mm; 3.5 µm particle size, 135 Å pore size, BEH X-Bridge, Waters, Hertfordshire, UK) and a gradient of acetonitrile : water with addition of 0.1% of ammonium hydroxide were used. The ESI source was operated under positive polarity and mono-sodiated adducts $[M+Na]^+$ were formed for the different carbohydrates and monitored in SIM mode. Data were processed using OpenLAB CDS Software (v.2.19.20, Agilent Technologies). External calibration curves of the different LMWC (0.0001-0.5 mg mL^{-1}) were performed in triplicate.

2.9. Statistical analysis

Statistical assays were carried out using Statistica 7.0 (StatSoft Inc., Tulsa, OK, USA). Analysis of variance (ANOVA, Tuckey test) was followed to evaluate significance ($P < 0.05$) of differences among solubility values.

3. Results and Discussion

NADES1 (ethaline), NADES2 (glyceline) and NADES3 (oxaline) were chosen in the present work based on results reported by Hakkinen and Abbott [19], who determined the solubility of glucose, sucrose and erythritol in these solvents. In addition to their good efficacy in solubilizing carbohydrates, choline-based NADES are also valued for their biological activity [26]. Characterization of synthetized NADES was carried out on the basis of their viscosity and water content, considering the relevance of these properties for their use as solvents. As shown in Table 1, the water content was below 0.3% for NADES1 and NADES2, while NADES3 contained a 10% of water. Regarding viscosity, NADES2 showed the highest values (309.2 cP) while NADES1 was the least viscous (35.6 cP). These values agreed with those described by Hakkinen and Abbot [19], except for NADES3, which showed a markedly lower viscosity (137.4 cP) than that reported (767 cP). However, this value was in good agreement with that described by Skulcová et al. (2017). Considering that the high viscosity of NADES is one of the main disadvantages of these solvents as it can reduce mass transfer during extraction, the NADES used in this work showed adequate viscosity values, which makes them promising solvents for carbohydrate solubility studies.

3.1. Stability of carbohydrates in NADES

Before measuring the solubility of the different carbohydrates in the selected NADES, their stability was evaluated. When some carbohydrates were dissolved in NADES3 at 25°C, a change in color from transparent to brown was observed. On the contrary, color changes were not observed for carbohydrates dissolved in NADES1 and NADES2 at this temperature.

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The change in color could be due to the non-enzymatic degradation of the carbohydrates dissolved in NADES3 [19]. To evaluate this reaction, absorbance at 420 nm was measured (Table 2). Before the measurement, 1:2 and 1:6 (v/v) dilutions in PBS were mandatory for fructose and tagatose solutions, respectively. Under the same conditions, a control assay was performed with each carbohydrate dissolved in PBS; the absorbance values were in all cases lower than 0.059 units of absorbance (UA). A noticeable increase in the absorbance was mainly found for tagatose, followed by fructose, sucrose and lactulose. Aldose solutions, such as glucose and lactose, also experimented a slight increase in the absorbance values, however, it was lower than that of ketoses. Changes in the color of maltose and galactose solutions were not observed.

Table 2. Measure of absorbance at 420 nm and 5-hydroxymethylfurfural content (%, w/w) of carbohydrate/NADES3 mixtures at 25°C. Absorbance data of carbohydrate solutions in phosphate buffered saline (PBS) is also included as reference.

LMWC	Absorbance at 420 nm (uA)			HMF (% w/w)
	Dilution	PBS	NADES3	
Tagatose	1/6	0.0467 (0.0003)*	0.5837 (0.0065)	6.23 (0.68)
Fructose	1/2	0.0491 (0.00017)	0.4631 (0.0018)	6.32 (0.16)
Glucose	1/1	0.0458 (0.0002)	0.1226 (0.0003)	0.02 (0.01)
Sucrose	1/1	0.0477 (0.0002)	0.4752 (0.0002)	4.00 (0.13)
Lactose	1/1	0.0584 (0.0002)	0.0970 (0.0005)	0.040 (0.001)
Lactulose	1/1	0.0587 (0.0001)	0.3595 (0.0004)	2.63 (0.08)

* Standard deviation in parenthesis (n=3).

Degradation of glucose and cellobiose in oxaline at 25°C has been previously reported, while erythritol remained stable under the same conditions [19], probably because it does not contain a reducing end. A similar behavior has been observed for some carbohydrates dissolved in acidic ILs; it has been described that fructose dissolved in these solvents is more prone to the formation of dehydration products (e.g. HMF) than glucose [8,27].

In this work, HMF was also determined in carbohydrate solutions that presented higher absorbance values (Table 2). The greatest HMF concentrations (ratios above 6%, w/w) were found for tagatose and fructose in NADES3, followed by sucrose (4.0 % w/w) and lactulose (2.6% w/w). On the contrary, the production of HMF by the degradation of the aldoses, glucose and lactose was very low (below 0.05%, w/w). Wu et al., (2018) indicated that, in comparison with neutral or basic DES, acidic DES can contribute to equilibrate a higher proportion of furanose forms, which have been described as the major forms responsible for converting ketoses such as fructose and tagatose into 5-HMF [28]. Based on these results, acidic NADES could be used as catalysts for these dehydration reactions, mainly for ketoses and, to a lesser extent, for some aldoses, likewise some imidazolium-based ILs [8,29].

Considering these results and to provide reliable solubility data, NADES3 was discarded for the following studies.

3.2. Solubility of carbohydrates at 25°C

Table 3 shows the solubility data of the different carbohydrates in NADES1 and NADES2 at 25°C. In general, the solubility of monosaccharides was higher in NADES2, except for glucose, whereas that of disaccharides was higher in NADES1, except for trehalose.

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Table 3. Solubility (mg g^{-1}) of monosaccharides and disaccharides in NADES at 25°C and 45°C.

	LMWCs	NADES1	NADES2	
		25°C	25°C	45°C
Monosaccharides	Glucose	242 (16)*,f	135.7 (0.2)c	364 (1)c
	Galactose	31.4 (0.4)a	43 (4)a	71 (6)b
	Fructose	191 (1)d	245 (5)d	806 (16)f
	Tagatose	72 (2)b	150.0 (0.4)c	214 (7)b
Disaccharides	Lactose	147 (5)c	26.3 (2.9)a	92 (29)a,b
	Maltose	323 (10)g	280 (14)e	630 (90)e
	Trehalose	183 (3)d,e	252 (17)d	503 (13)d
	Sucrose	152 (3)c,e	82 (11)b	368 (15)c
	Lactulose	666 (25)h	135.1 (0.3)c	250 (11)b

* Standard deviation in parenthesis (n=3); a-h Different letters within the same column indicate significant ($p < 0.05$) differences for the CHs assayed.

Regarding monosaccharides, significant differences in the solubility of glucose and fructose were observed in both NADES. Solubility of fructose was higher than that of glucose in NADES2 (245 *vs*. 135.7 mg mL^{-1}). This ketohexose has also been found to be more soluble than glucose in different imidazolium-based ILs [7,8,30], considering its more stable structure and its lower melting temperature (378.1K *vs*. 423.1K for Fru and Glc, respectively) and enthalpy [7]. However, in this work, solubility of fructose in NADES1 (191 mg mL^{-1}) was significantly lower than that of its corresponding aldose (242 mg mL^{-1}). Moreover, great differences were observed between the solubility of glucose and its C-4 epimer (galactose) in both NADES (Glc/Gal: 7.7 in NADES1 and 3.2 in NADES2); the same behavior was observed for fructose and its C-4 epimer (tagatose), although differences were smaller (Fru/Tg: 2.6 in NADES1 and 1.6 in NADES2). These results point to these NADES as suitable solvents for the selective fractionation of these isomeric carbohydrates.

As for the disaccharides, large differences in solubility values were observed for both NADES. In this sense, lactulose (4-O-β-galactopyranosyl-D-fructofuranose) was the most soluble sugar in NADES1 (666 mg mL^{-1}), while the solubility of lactose (4-O-β-galactopyranosyl-D-glucopyranose) in this solvent was the lowest (147 mg mL^{-1}; Lu/La: 4.5). Similarly, lactulose (135.1 mg mL^{-1}) was more soluble than lactose (26.3 mg mL^{-1}; Lu/La: 5.1) in NADES2, although solubility values of both carbohydrates were lower than in NADES1. Hakkinen and Abbot [19] observed a similar behavior between sucrose (α-D-glucopyranosyl-β-D-fructofuranose) and cellobiose in ethaline (the former was fourteen times more soluble than the latter). They attributed these differences to the more rigid structure of cellobiose (disaccharide with two symmetrical glucose units linked with a 1-4 glycosidic linkage) that restricts bond rotation, while sucrose has a less symmetrical structure with more conformational freedom in solution, resulting in a higher solubility. However, maltose which is also constituted by two symmetrical glucose units linked with 1-4 linkage, showed a higher solubility values in both NADES than sucrose. Similarly, solubility of trehalose (1-O-α-D-glucopyranosyl-α-D-glucopyranose) was significantly higher than that of sucrose in NADES2, although differences were lower in NADES1. Probably different factors such as their folded structure in the solution or the greater hydrogen bond capacity [19,31] among others, could contribute to the solubility of these disaccharides in the NADES.

Considering that the HBA (choline chloride) of studied NADES is the same in both solvents, differences in solubility values observed for the different carbohydrates could be attributed to the HBD (2-ethylene glycol in NADES 1 and glycerol in NADES2).

3.3. Solubility of carbohydrates at 45°C in NADES2

The effect of the temperature on carbohydrate solubility in NADES was investigated. While color changes in carbohydrate solutions at 45°C in NADES2 were not detected, some solutions in NADES1, mainly of ketoses, became darker. Then, NADES2 was selected for the following studies

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and results are shown in Table 3. As expected, most carbohydrate solubility values were significantly higher with increasing temperature from 25°C to 45°C, as previously observed with other solvents [8]. A moderate increase was found for the solubility of tagatose and galactose which increased by a factor of 1.4 and 1.6, respectively. However, higher differences were observed in the solubility of fructose, lactose and sucrose which increased by a factor of 3.3, 3.5 and 4.5, respectively. Intermediate increases in solubility values were found for other carbohydrates.

3.4. Fractionation of bioactive ketoses from aldoses in equimolar binary mixtures

Some ketoses such as tagatose and lactulose are used as additives or functional ingredients in food, cosmetic, and pharmaceutical formulations due to their texturizing and stabilizing behaviors and to their bioactive properties such as prebiotic effect [32,33]. These bioactive ketoses are usually synthesized from their corresponding aldoses (galactose and lactose) using different catalysts [22,34], which affect to their isomerization yields. The remaining aldoses must be removed from the product, requiring an efficient selective fractionation process. Considering the different solubility of aldoses and ketoses in the studied NADES, the feasibility of these solvents for the selectively fractionation of lactulose and tagatose from their corresponding aldoses (i.e., lactose and galactose) in binary mixtures was evaluated.

According to the solubility data shown in Table 3, NADES2 at 25°C was chosen for tagatose/galactose fractionation because it provided the highest differences between the individual solubility values of both sugars (Tg/Gal: 3.5), which, in principle, could lead to an effective fractionation. For lactulose/lactose, NADES1 at 25°C was selected, also taking into account the high solubility of lactulose in this solvent.

Figure 1 shows the solubilized and precipitated percentage of each carbohydrate of the equimolar binary mixtures after NADES treatment. Attending lactulose:lactose mixture (Figure 1A), all lactulose was dissolved in NADES1, while only 18.8% of lactose remained in this solvent. Then, lactose can be purely obtained in the precipitate, while lactulose is highly enriched in the solution (77%). Similarly, in the galactose:tagatose binary mixture (Figure 1B), 92.7% of the total tagatose was dissolved in NADES2, while only 13.5% of the galactose was found in the solution. High purity galactose could be separated in the precipitate, while an enriched solution of tagatose (16% galactose, 84% tagatose) was obtained. In both cases, ketoses were more soluble than aldoses, which agreed well with the individual solubility data reported in Table 3. These data point out the feasibility of using these green solvents for ketose/aldose fractionation.

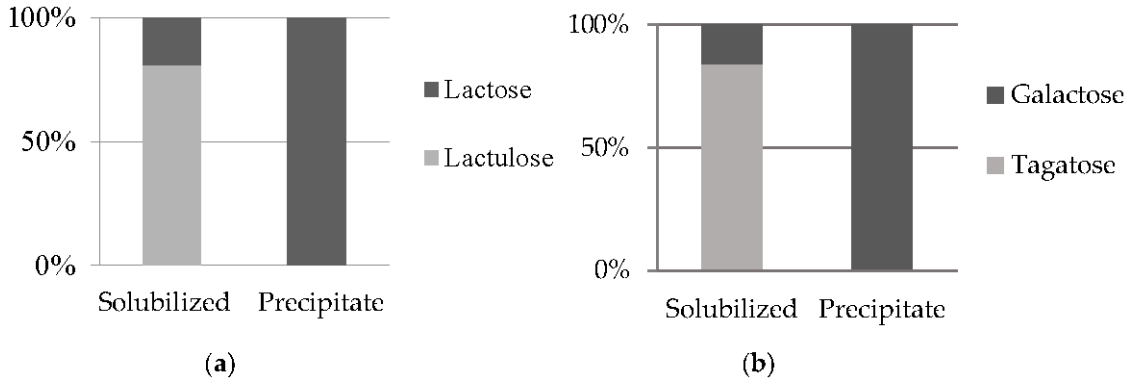

Figure 1. Content (as percentage, %) of lactulose and lactose (a) and tagatose and galactose (b) in 1:1 (w/w) binary mixtures in NADES1 and NADES2, respectively, determined as remaining in solution and precipitate after treatment at 25 °C.

3.5. Fractionation of lactulose from the isomerization reaction mixture

The efficiency of NADES as solvent candidates for the fractionation of carbohydrates in real mixtures was evaluated. Lactulose was synthetized by the isomerization of lactose using egg shell as catalyzer; Figure 2A shows de HILIC-MS profile of this reaction mixture. The synthesis yielded 25%

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of lactulose, which is in good agreement with previously published results [9,22], 14% monosaccharides (glucose and galactose) and 3% epilactose; 60% lactose remained as unreacted sugar in the mixture.

Then, NADES1 at 25°C, previously chosen as selective solvent for the lactose:lactulose binary mixture, was used for the fractionation of lactulose from the other isomerization reaction products. As it is shown in Figure 2B, HILIC-MS profile of carbohydrates in the isomerization mixture after treating with this NADES changed. Lactulose became an abundant carbohydrate in the mixture, while a noticeable reduction of lactose was observed.

The extraction yields in NADES1 of the different carbohydrates present in the mixture were determined while 100% of lactulose was solubilized, only 25% of the available lactose was extracted. A high percentage of monosaccharides (55%) was also dissolved, mainly of glucose in agreement with the individual solubility of this carbohydrate in NADES1 (Table 3), while a 10% of epilactose was also solubilized. Percentages of these carbohydrates before and after treatment are shown in Figure 3. Lactulose percentage showed a 2-fold increase, while lactose and epilactose decreased notably and monosaccharides remained constant after this treatment. Then, the final mixture was constituted by 50% lactulose, 32% lactose, 17% monosaccharides and 1% epilactose. These results were quite similar to those achieved by Carrero-Carralero et al. (2015) for lactulose enrichment using ILs (58% lactulose, 31% lactose and 11% monosaccharides), but using a more natural solvent.

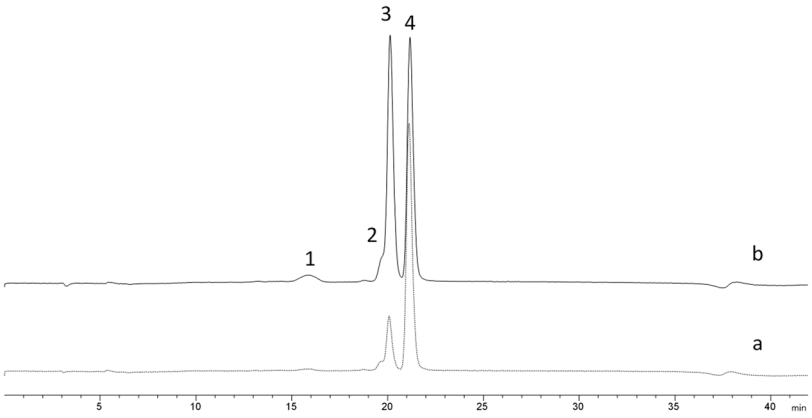

Figure 2. HPLC-MS profile of the isomerization with egg shell of lactose to lactulose before (a) and after (b) treatment with NADES1 at 25°C. Labelled peaks are as follows: (1) Monosaccharides, (2) Epilactose, (3) Lactulose and (4) Lactose.

4. Conclusions

In this work, new solubility data of various mono- and disaccharides in selected NADES (ChCl:EtG and ChCl:Gly) are presented for the first time. Differences were found between the solubility values of carbohydrates as a function of their structure. In general, the solubilities of the ketoses were higher than those of their corresponding aldoses in both solvents. These NADES proved to be promising alternatives for the selective fractionation of the binary mixtures galactose:tagatose and lactose:lactulose. Furthermore, the results obtained in this work provide the first evidence of the usefulness of ChCl:EtG (NADES 1) for the enrichment of lactulose in its synthesis mixture. They may contribute to the development of new applications based on the separation of value-added carbohydrates using this new generation of sustainable bio-solvents.

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Conflicts of Interest: The authors declare no conflict of interest.

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