Use of room temperature ionic liquids for the selective fractionation of bioactive ketoses from aldoses

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Abstract

This work deals with the effective fractionation of bioactive ketoses, i.e. lactulose and tagatose, from their corresponding aldoses, lactose and galactose, in equimolar binary mixtures driven by room temperature ionic liquids, i.e. 1-ethyl-3-methylimidazolium dicyanamide ([EMIM][DCA]) and 1-butyl-3-methylimidazolium methyl sulphate ([BMIM][$MeSO_4$]), respectively. Under assayed conditions, tagatose was found to be 6-fold more soluble on [BMIM][$MeSO_4$] than galactose; meanwhile lactulose was 3 times more soluble than lactose on [EMIM][DCA]. As an application example in a more complex sample, a lactose isomerization mixture containing in addition lactulose and monosaccharides was enriched in this ketose by using [EMIM][DCA]. Carbohydrates were then successfully recovered from the ionic liquid following an activated charcoal-based treatment. Overall, lactulose content was enriched from a 24% in the initial isomerization reaction mixture to a 62% in the purified sample. These experimental results demonstrated the potential of ionic liquids as green alternative solvents for the selective fractionation of bioactive ketoses from their corresponding aldoses in food and beverage production.

Keywords: room temperature ionic liquids, ketoses, aldoses, fractionation, activated charcoal

1. Introduction

Fractionation of food carbohydrates is considered a challenging task due to the complexity of the mixtures and the structural similarity among them. Most of the available procedures are suitable for the fractionation of carbohydrate mixtures with different degree of polymerization [1]. However, the fractionation of carbohydrates having the same molecular weight but different monomeric composition, glycosidic linkages and/or carbonyl group position (e.g., aldoses and ketoses) is particularly difficult.

Ketoses, such as tagatose or lactulose, are considered bioactive carbohydrates with potential pharmaceutical and/or food applications due to their functional properties, which include prebiotic activity among others [2, 3]. Both carbohydrates can be obtained by alkaline isomerization or by enzymatic treatment from their corresponding non bioactive aldoses, i.e. galactose or lactose, respectively. However, subsequent isolation of these carbohydrates from the synthesis mixtures remains as a difficult task. Montañés *et al*. [4] studied the individual solubility of three aldoses (glucose, galactose and lactose) and their respective ketoses (fructose, tagatose and lactulose) in different alcohols (methanol, ethanol, 1-propanol and 2-propanol) at several temperatures (295, 303 and 313 K). In general, ketoses were found to be more soluble than aldoses in these solvents. These authors also applied thermodynamic models to predict the solubility of sugars to further select the best solvent to fractionate these ketoses from mixtures with other carbohydrates. Despite the usefulness of these methods, they usually require large volumes of organic solvents, which are in sharp contrast to the increasing demand for more cost-effective and green analytical methodologies involving small solvent volumes.

During the last years, environmental friendly techniques based on supercritical fluid (SFE) and pressurized liquid (PLE) extraction have been evaluated for the selective fractionation of food carbohydrates. As an example, Montañes *et al*. [5, 6] efficiently separated tagatose or lactulose from binary mixtures with different aldoses using supercritical carbon dioxide with different co-solvents (ethanol/water mixtures, isopropanol, methanol, etc.) to increase the carbohydrate solubility. Under the experimental conditions proposed, purities above 90% of ketoses and recoveries higher than 75% were obtained. PLE has also been employed with successful results for the fractionation of lactulose from lactose with a purity of 97% and a yield of 64% [7].

Room temperature ionic liquids (RTILs or simply ILs) are solvents constituted by organic cations (imidazolium, piridinium, pirrolidinium, phosphonium, etc) and different organic and inorganic anions (acetate, trifluoroacetate, tetrafluoroborate, bromide, etc). These solvents show melting points below 373 K, are considered environmentally friendly, and have many extra advantageous features, including low volatility and viscosity, tuned selectivity, capacity to dissolve compounds of different nature and recycling feasibility [8]. In consequence, ILs could be considered a good and safe alternative to the use of traditional organic volatile solvents in carbohydrate chemistry [9]. However, the solubility of carbohydrates of low molecular weight in different ILs has only been evaluated in few studies [10-14]. Al-Nashef *et al*. [15] patented a method to separate fructose from glucose in binary mixtures based on their different solubility in 1,3-dimethylimidazolium dimethylphosphate and 1-ethyl-3-methylamidazolium ethylsulfate at room temperature. Recently, the individual solubilities of lactulose, lactose, tagatose and galactose, among others, in different ILs (i.e., 1-ethyl-3-methylimidazolium dicyanamide, 1-hexyl-3-methylimidazolium chloride and 1-butyl-3-methylimidazolium methyl sulfate) have been determined [16]. In

general, ketoses were found to be more soluble in ILs than aldoses, a finding that pointed out the potential of ILs as alternative solvents for the efficient fractionation of low molecular weight carbohydrates. The main objective of this work is to evaluate the feasibility of three ILs, 1-hexyl-3-methylimidazolium chloride, 1-butyl-3-methylimidazolium methyl sulphate and 1-ethyl-3-methylimidazolium dicyanamide, for the selective separation of ketoses with potential pharmaceutical and/or food applications such as lactulose, fructose and tagatose from their corresponding aldoses (i.e., lactose, glucose and galactose) in binary mixtures. The proposed methodology has been applied for the fractionation of lactulose from lactose isomerization reaction mixtures and the final recovery of this ketose from IL was also evaluated.

2. Materials and methods

2.1. Chemicals and reagents

Analytical standards of fructose, glucose, galactose, tagatose, lactose, lactulose, phenyl-β-D-glucoside and activated charcoal (Darco G60, 100 mesh) were obtained from Sigma-Aldrich (St. Louis, USA), and tetracosane from Polyscience Corp (Illinois, USA). The three assayed ionic liquids, [HMIM][Cl], [BMIM][$MeSO_4$], [EMIM][DCA], dichloromethane and trimethylsilylimidazole (TMSI) were also purchased from Sigma-Aldrich. *n*-Heptane was from Merck (Darmstadt, Germany), acetone from Carlo Erba Reagents (Val de Reuil, France), and ethyl acetate, absolute ethanol, methanol and isopropanol extra pure from Scharlab (Sentmenat, Spain).

2.2. Dissolution of ketose:aldose mixtures in the test ILs

For solubility studies, binary mixtures of fructose:glucose, tagatose:galactose and lactulose:lactose (50%, w/w, of each carbohydrate) were dissolved in 100 mg of the test IL with slight excess (a 10% above the corresponding limit of solubility). Samples were stirred at 12,100 *g* using a Thermomixer (Eppendorf, Germany) during 24 h at 299 K and left to stand for another 24 h at this temperature. Then, an aliquot of the solution mixture was extracted from the upper liquid layer and analyzed by gas chromatography with flame ionization detector (GC-FID) and/or high performance liquid chromatography with refractive index detector (LC-RID) as indicated in section 2.5.

2.3. Synthesis of lactulose and subsequent fractionation with ILs

Isomerization of lactose was carried out following the method of Montilla *et al*. [17]. In brief, 2 mL of a 250 mg/mL solution of lactose were added to 8 mL of potassium phosphate buffer 0.05 M, pH 6.6. Pulverized egg shell was added to this solution (final concentration, 30 mg/mL) to act as catalyst for lactose isomerization. The mixture was heated at 398 K in a bath of glycerol under continuous stirring and reflux for 150 min. Reaction was stopped by immersion in an ice bath. Finally, egg shell was removed by filtration through a 0.4 μm paper filter (Millipore) and the sample was freeze-dried.

[EMIM][DCA] at 299 K was used for the fractionation of lactulose from the isomerization mixture. For this, 600 mg of [EMIM][DCA] was mixed with 320 mg of the freeze-dried isomerization mixture following the method described in section 2.2 for the dissolution of binary mixtures of ketoses and aldoses. Aliquots of supernatant were analyzed by GC-FID according to section 2.5.

2.4. Extraction of lactulose from IL

Different methods were evaluated and optimized for the extraction of lactulose from IL.

2.4.1. Effect of cooling

Binary mixtures of lactose:lactulose dissolved in [EMIM][DCA] were kept at temperatures of 277, 253 and 193 K, respectively, up to one week. Aliquots of the corresponding supernatants were taken at different times and subjected to analysis for the evaluation of the precipitation of carbohydrates.

2.4.2. Solvent treatment

Miscibility of [EMIM][DCA] on ethyl acetate, ethanol, isopropanol, and hexane was firstly evaluated.

Binary mixtures of lactose:lactulose dissolved in [EMIM][DCA] were vigorously stirred at 298 K for 15 min with the immiscible solvents, i.e. either ethyl acetate or hexane in a solvent:IL ratio of 10:1 (w/w), and then left to stand during 3 min. Thereafter, aliquots of 100 μL of the organic layer were taken for further analyses.

The antisolvent method was also evaluated following the method described by Hassan *et al*. [11]. Briefly, solubility of binary mixtures of lactose:lactulose was evaluated in ethanol and isopropanol, which were miscible solvents with [EMIM][DCA], by using a solvent:IL ratio of 10:1 (w/w). Mixtures were homogenized

at 313 K by stirring for 1 h and centrifuged at 12,100 *g* for 5 min. Finally, the supernatant was recovered and dried before analysis as indicated in section 2.5.1.

2.4.3. Active charcoal treatment

Binary mixtures of lactose:lactulose dissolved in [EMIM][DCA] were treated with activated charcoal as indicated by Hernandez *et al*. [18] but varying the solvent composition. In the optimized experiment, 165 mg of the carbohydrates mixtures dissolved in [EMIM][DCA] were treated with 655 mg of activated charcoal mixed with 3 mL of water (**Figure 1**). The slurry was stirred for 1 h to allow the adsorption of carbohydrates on the carbon surface. Then, the mixture was filtered through a Whatman No. 1 paper (Whatman International Ltd., Maidstone, UK) under negative pressure and the filtrate (IL + water) was removed. Activated charcoal was washed with 2 mL of water by stirring the slurry for 1 h to assure the complete IL removal and then filtered as indicated above. Desorption of carbohydrates from the activated charcoal was done by washing the sorbent with 12 mL of ethanol:water (50:50, v/v) under agitation for 1 h. Phase separation was done by filtration as previously indicated. One mL of the filtrate was finally evaporated under vacuum at 40 ºC and analysed as indicated in section 2.5.1. This procedure was also applied to the isomerization mixture dissolved in [EMIM][DCA].

2.5. Analytical methods

2.5.1. GC-FID analyses

The GC system was an HP 7890A equipped with a FID from Agilent Technologies (Palo Alto, CA, USA). The GC separation was performed on a silica capillary coated column DB-17 with 50% phenyl and 50% polysiloxane (30 m x 0.25 mm i.d. x 0.25 μm *df*; Agilent Technologies). Nitrogen was used as carrier gas at a constant flow of 0.677 mL/min. The GC oven temperature program started at 200 ºC and increased at 2 ºC/min up to 290°C. The inlet and detector temperatures were set at 300ºC. Samples were injected (1 μL) with a split ratio of 20:1.

In all cases, 10 mL of a solution containing phenyl-β-D-glycoside (internal standard) at a concentration level of 1 mg/mL in heptane were added to aliquots of 10 mg of the mixtures of carbohydrate and IL. Analytes derivatization to trimethylsilyl (TMS) ethers was done according to Ruiz-Aceituno *et al*. [19]. In brief, 100 μL of trimethylsilylimidazole (TMSI) were added to the samples and stirred at room temperature for 1 h. The reaction was stopped by adding 200 μL of water. Trimethylsilyl carbohydrates were extracted by liquid-liquid extraction (LLE) with *n*-heptane. The extraction was repeated twice to ensure total carbohydrate recovery.

Quantitation was carried out using the internal standard method. For this, solutions of carbohydrate standards dissolved in ILs in the 0.25 – 1 mg range were prepared. Calculated response factors for each carbohydrate relative to two internal standards (*n*-tetracosane and phenyl-β-D-glucoside) were used for quantitative analysis. All GC analyses were carried out, at least, in triplicate.

2.5.2. LC-RID analyses

LC analyses were performed using an Agilent Technologies 1220 Infinity LC System-1260 (Boeblingen, Germany) equipped with a RID. LC separation was carried

out on an amino column (100-NH_2, 250 mm x 4.6 mm, 5 µm particle size) from Kromasil (Bohus, Sweden) using isocratic elution with acetonitrile:water at 70:30 (v/v) as the mobile phase and at flow rate of 1.0 mL/min for 20 min.

Samples involved in the solubility studies were dissolved in a 1:1 (v/v) acetonitrile:water solution to yield a concentration of 10 mg/mL and 50 µL were injected in the LC system. Acquisition and processing were performed using the Agilent ChemStation software (Agilent Technologies). All LC analyses were carried out, at least, in triplicate.

Quantitation analyses were carried out using the external standard method. For this, solutions of 2-27 mg carbohydrate standards in 100 mg of ILs were diluted with acetonitrile:water (1:1, v/v) to 10 mL.

2.6. Statistical analysis

Data treatment was done using the software Statistica 7.0 (Stat Soft Inc., Tulsa, OK, USA). Differences were considered to be significant when $p < 0.05$; analyses of variance (ANOVA) using the Fisher test were used to evaluate significant differences.

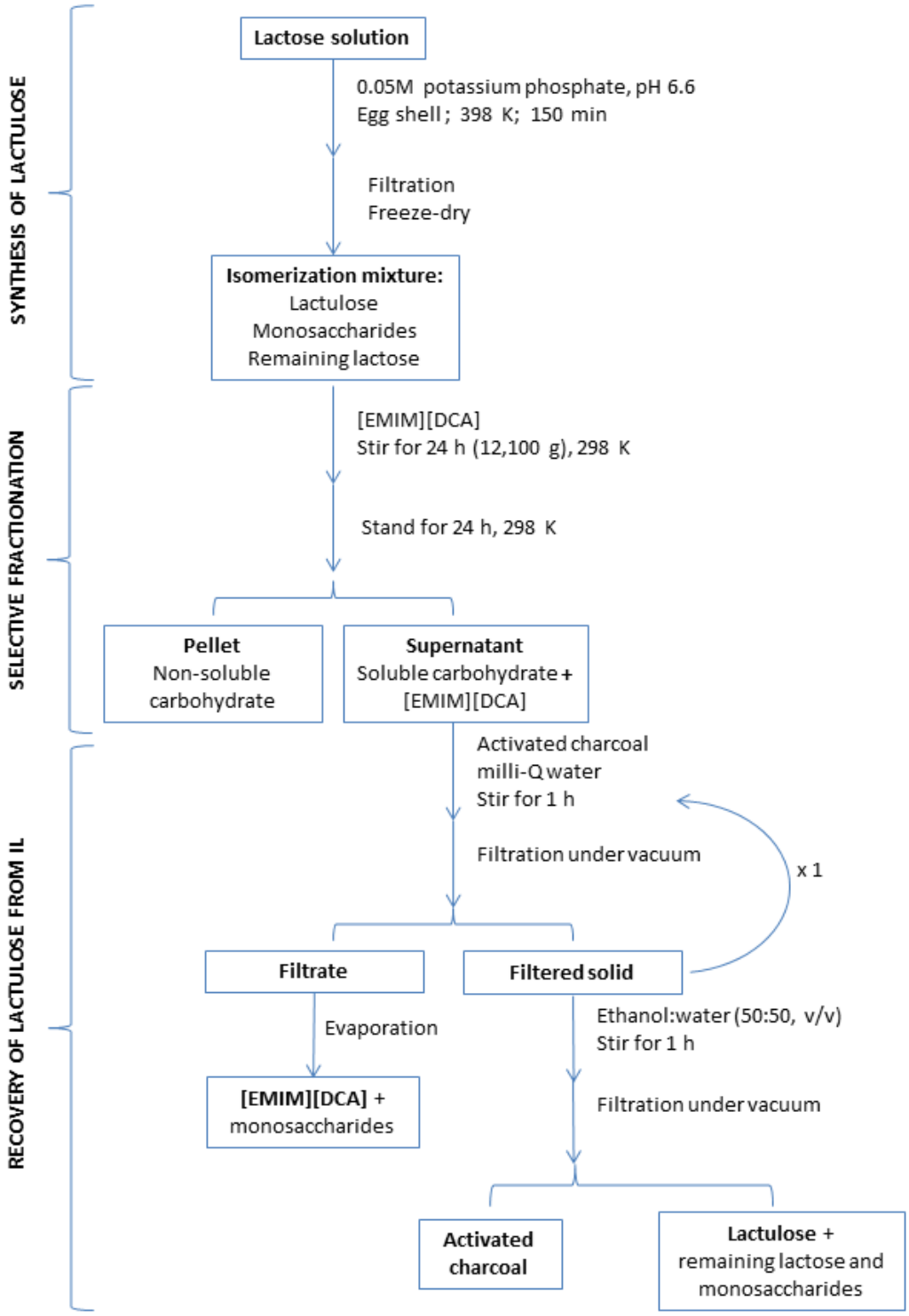

Figure 1: Scheme of the general process of synthesis, fractionation, and recovery of lactulose obtained from lactose. Pulverized egg shell was used as catalyst for lactose isomerization, [EMIM][DCA] as fractionation agent and active charcoal for the recovery of lactulose from IL.

3. Results and discussion

3.1. Fractionation of ketoses from aldoses in equimolar binary mixtures by ILs

ILs used in this work were chosen based on preliminary results published by Carrero-Carralero *et al*. [16], who determined the solubility of single aldoses and ketoses in selected ILs at different temperatures. Consequently, 1-hexyl-3-methylimidazolium chloride ([HMIM][Cl]), 1-butyl-3-methylimidazolium methyl sulphate ([BMIM][$MeSO_4$]) and 1-ethyl-3-methylimidazolium dicyanamide ([EMIM][DCA]) were selected for the fractionation of equimolar binary mixtures of fructose:glucose, tagatose:galactose and lactulose:lactose, respectively, at 299 K. These ILs showed the highest differences between individual solubility values calculated for a given ketose and its corresponding aldose which, in principle, could lead to effective fractionation in binary mixtures. According to reported solubility values, tagatose was 7 times more soluble than galactose in [BMIM][$MeSO_4$], lactulose was 4-fold more soluble than lactose in [EMIM][DCA], and fructose was 2-fold more soluble than glucose in [HMIM][Cl]. The temperature was set at 299 K to avoid ketose dehydration into 5-hydroxymethylfurfural [16].

Table 1 shows the solubility data (%, w/w) obtained for ketoses and aldoses in the equimolar binary mixtures in the corresponding studied IL. Solubility values of the investigated carbohydrates in [HMIM][Cl] and [EMIM][DCA] were determined by GC-FID after a derivatization step. Carbohydrates dissolved in [BMIM][$MeSO_4$] were only partially derivatized with the proposed methodology [19]. Therefore, these samples were analyzed by LC-RID.

Table 1. Solubility values, as % (w/w) of the carbohydrate in the mixture, of aldoses and ketoses in binary mixtures (1:1, w/w) on selected ILs at 299 K. Experimentally determined standard deviations (SD) are shown in parenthesis (n=3).

[EMIM][DCA][a]		**[HMIM][Cl][a]**		**[BMIM][$MeSO_4$][b]**	
Lactose	9.7 (1.7)	Glucose	9.0 (0.4)	Galactose	1.2 (0.2)
Lactulose	28.0 (1.3)	Fructose	9.4 (0.2)	Tagatose	7.1 (0.3)

[a] Solubility values determined by GC-FID analysis.

[b] Solubility values determined by LC-RID analysis.

Remarkably, tagatose was 6-fold more soluble than galactose in [BMIM][$MeSO_4$], whereas lactulose was 3 times more soluble than lactose in [EMIM][DCA] (**Table 1**). These results agreed with the individual solubility data previously reported by Carrero-Carralero *et al*. [16] and point out the feasability of using these ILs for their efficient fractionation. However, solubility of fructose in [HMIM][Cl] in the presence of glucose (9.4 %) was meaningfully lower than that previously described for individual samples (20.2 %) [16]. This fact could be attributed to the high viscosity of [HMIM][Cl], determined as 7,500 cp [20], which could impair the solubilization of fructose [21].

3.2. Fractionation of lactulose from isomerization reaction mixtures

To evaluate the efficiency of ILs in the fractionation of a carbohydrate real mixture, the isomerization of lactose in basic media catalyzed by egg shell was carried out. **Figure 2A** displays the GC-FID chromatogram of this reaction mixture. This process had a yield of 24% in lactulose, and the rest of carbohydrate composition consisted of 48% lactose and 28% monosaccharides (galactose and glucose). The yield of lactulose was in accordance with the values reported by Montilla *et al*. [17].

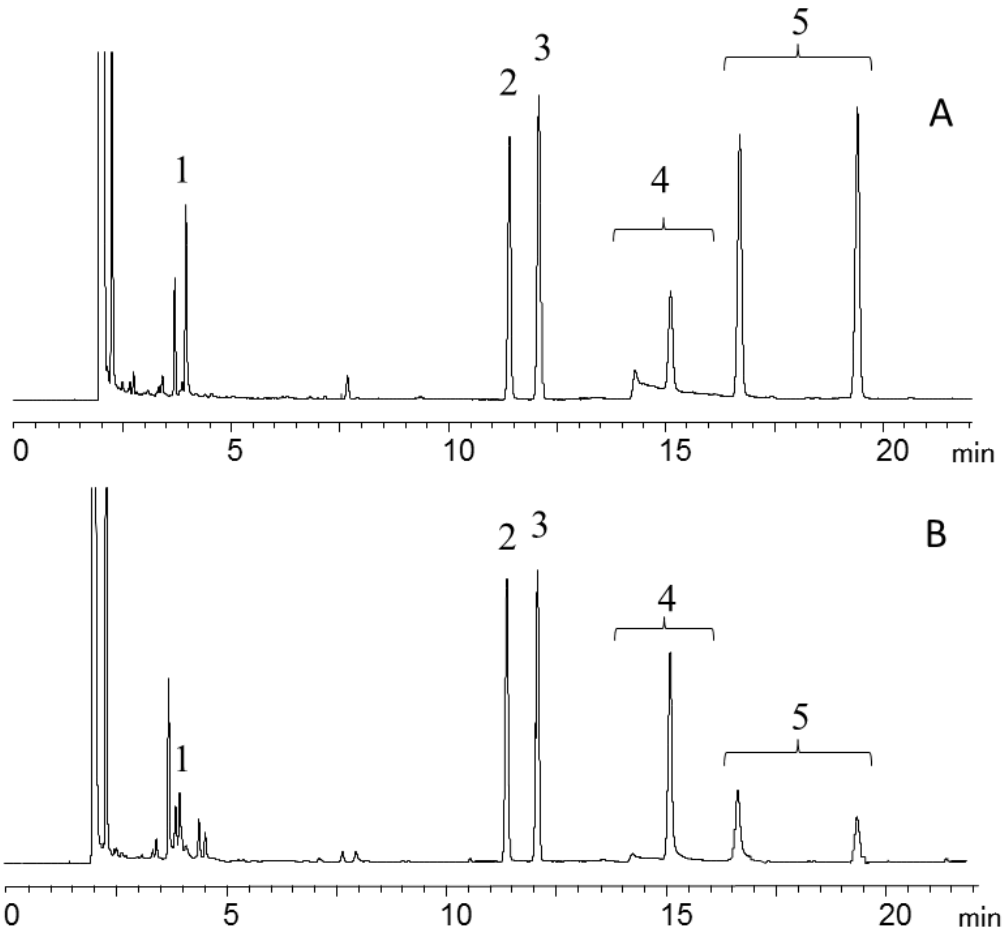

Figure 2: GC-FID profiles of the reaction mixture derived from the alkaline isomerization with egg shell of lactose to lactulose before (A) and after (B) fractionation with [EMIM][DCA]. Labelled peaks are as follows: (1) Monosaccharides, (2) tetracosane (non-derivatized internal standard), (3) phenyl-β-D-glucoside (derivatized

internal standard), (4) lactulose and (5) lactose. Both chromatograms use the same abundance scale.

Considering the data obtained from the study of solubility of the standard binary mixture of lactulose and lactose (**Table 1**), [EMIM][DCA] was used for the fractionation of lactulose from the isomerization reaction mixture at 299 K. As it can be observed in **Figure 2B**, the use of [EMIM][DCA] resulted in a noticeable enrichment of lactulose which became the predominant carbohydrate.

Figure 3 shows the percentages of lactose, lactulose and monosaccharide before and after the fractionation using [EMIM][DCA] (grey and white bars, respectively). Lactulose percentage in the isomerization mixture increased more than 2-fold as compared to values obtained before treatment. On the contrary, lactose and monosaccharides percentages decreased notably after this treatment. Thus, the carbohydrate content of the isomerization reaction mixture after the fractionation with [EMIM][DCA] was 58% lactulose, 31% lactose and 11% monosaccharides. Regarding extraction yields, it can be mentioned that lactulose was totally dissolved in the IL whereas only 28% of lactose and 19% of monosaccharides remained in the treated mixture. These results demonstrated that treatment of the isomerization reaction mixture with [EMIM][DCA] resulted in a notable enrichment of lactulose.

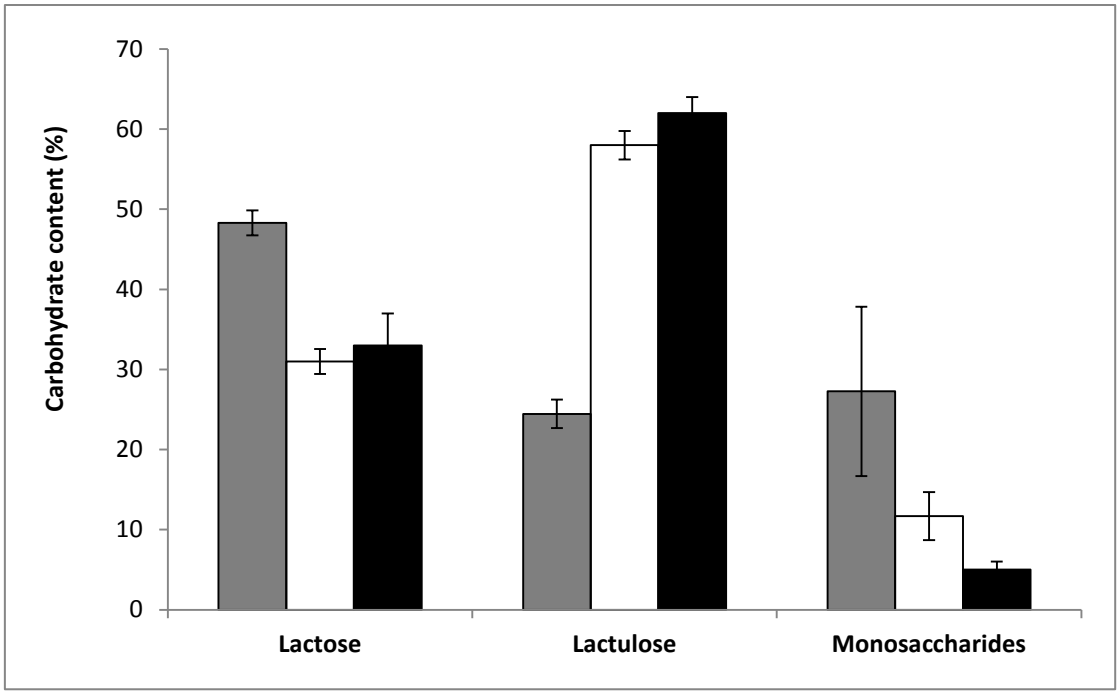

Figure 3: Content (%) of lactose, lactulose and monosaccharides in the alkaline isomerization mixture before (grey bars) and after treatment with [EMIM][DCA] (white bars), and after removing the IL by treatment with activated charcoal (black bars). SD are shown as error bars (n=3).

3.3. Recovery of lactulose from [EMIM][DCA]

Different procedures were evaluated to isolate lactulose from the corresponding IL, i.e. [EMIM][DCA]: (i) effect of cooling, (ii) solvent or antisolvent treatment, and (iii) adsorption on activated charcoal. Likewise, these treatments could simultaneously contribute to lactulose enrichment and the recovery of the IL for subsequent recycling.

As it was previously observed by Carrero-Carralero *et al*. [16], solubility of lactose and lactulose in [EMIM][DCA] decreased as the temperature does. Therefore, sample cooling could in principle lead to a higher precipitation of carbohydrates, so allowing their effective fractionation from the IL. Al-Nashef *et al*. [15], also proposed a

cooling procedure to separate glucose and fructose from 1-ethyl-3-methylimidazolium ethylsulfate or 1,3-dimethyl-imidazolium dimethylphosphate. In the present study, binary mixtures of lactose and lactulose dissolved in [EMIM][DCA] were kept at 279, 253 and 193 K up to one week. None of these treatments were effective for lactose and lactulose separation from IL by precipitation. Best separation was obtained at 193 K and resulted only in 4.3% of lactulose and 2.1% of lactose precipitation.

Regarding the use of solvents, two different approaches were followed: LLE using solvents immiscible with [EMIM][DCA], and the antisolvent method, which involves the use of a solvent miscible with the selected IL, but in which lactose and lactulose were not at all or only partially soluble. Ethyl acetate and hexane were assayed as solvents for the former approach. Solubility of lactose and lactulose in *n*-hexane was almost negligible, whereas 47% of lactulose and 50% of lactose were dissolved in ethyl acetate and so recovered from [EMIM][DCA]. These results allowed a low recovery of lactulose, making difficult the potential recyclability of the IL for further usages.

Ethanol and isopropanol, solvents miscible with [EMIM][DCA], were evaluated as antisolvents to separate the binary mixtures of lactose and lactulose from this IL. Isopropanol allowed the highest removal of lactulose (66%) and lactose (96%) from [EMIM][DCA]; meanwhile, ethanol was not able to recover lactulose while 88% of lactose was extracted. According to these results, we conclude that the use of ethanol and isopropanol as antisolvents were not useful for the recovery of lactulose from IL mixtures since lactose was notably enriched in relation to lactulose. However, these results would indicate that these solvents could be of great interest in carbohydrate chemistry, mainly for lactose extraction. Previously, ethanol has been suggested as a good antisolvent to recover glucose from mixtures with 1-ethyl-3-methylimidazolium

thiocyanate [11] and with ILs based on 1-methyl-3-alkylimidazolium as cation and chloride, bromide, acetate, and hydrogen sulfate as anions [22].

Finally, the effect of activated charcoal on the separation of binary mixtures of lactose and lactulose in [EMIM][DCA] was evaluated and latter being applied to the treatment of the isomerization reaction mixture. Different ethanol:water ratios, i.e. 5:95, 1:99 and 0:100, (v/v) were assayed according to Hernandez *et al*. [18] to allow the maximum adsorption of carbohydrates in the charcoal and the IL removal. Treatments were carried out twice to assess the total IL removal. Ethanol:water 5:95 (v/v) resulted in a complete desorption of carbohydrates from the sorbent, whereas 33% lactose and 44% lactulose were removed with ethanol:water 1:99 (v/v). Best results were obtained using water as eluent, resulting only in a 10% removal of lactose and lactulose.

Recovery of disaccharides from charcoal using ethanol:water 50:50 (v/v) was remarkably high (89% and 90% for lactose and lactulose, respectively), whereas only 24% of monosaccharides remained in the eluate. Absence of detectable IL in these extracts was confirmed by pre-concentration of the extract to dryness and subsequent analysis. IL was recovered by evaporation of the filtrates derived from the two first washes in the lactulose recovery process with activated charcoal (**Figure 1**). In consequence, IL could be recycled for further uses in lactulose fractionation. This is an important aspect to be considered for the balance of the cost of the ILs and, consequently, for the viability of the whole process. Considering these results, carbohydrate mixtures were enriched in lactose and, mainly, lactulose, which accounted for 33 and 62% of total carbohydrates in the mixture (**Figure 3,** black bars).

To sum up, the overall process (including IL treatment and the activated charcoal step) allowed the recovery of 90% lactulose, 25% lactose and 4.6%

monosaccharides from the original isomerization mixture, whereas purity of this mixture was 62% lactulose, 33% lactose and 5% monosaccharides.

4. Conclusions

ILs are a promising alternative to conventional organic volatile solvents for the selective fractionation of aldoses and ketoses. The results reported in the present study are the first evidence of the usefulness of [EMIM][DCA] for the enrichment of lactulose in its product of synthesis by isomerization of lactose in basic media. Recovery of carbohydrates from ILs was also successfully achieved using an activated charcoal treatment. This last step could also facilitate the potential recycling of ILs favoring, thus, the development of a cost-effective process. The reported results demonstrate that this procedure was more effective than the antisolvent or the cooling method for the fractionation of ketoses from aldoses.

As a whole, the proposed methodology represents a novel, environmental friendly and valuable alternative to conventional organic solvent-based procedures in use for carbohydrates fractionation. Their positive features, such as simplicity, straightforward nature and efficiency, make to consider it an interesting methodology with potential for scaling up processes.

Acknowledgements

Authors thank Ministerio de Ciencia e Innovación (projects AGL2009-11909 and AGL2011-27884), Ministerio de Economía y Competitividad (project CTQ2012-32957) and the European founding from FEDER program for financial support. L. Ruiz-Aceituno is supported by CSIC by a JAE-Pre grant, co-financed by the European Social

Fund (ESF). C. Carrero-Carralero thanks Ministerio de Ciencia e Innovación (Spain) for a predoctoral contract.

References

[1] F.J. Moreno, C. Carrero-Carralero, O. Hernández-Hernández, M.L. Sanz, Fractionation of food bioactive oligosaccharides, in: F.J. Moreno, M.L. Sanz (Eds.), Food Oligosaccharides: Production, Analysis and Bioactivity, Wiley-Blackwell, Oxford, 2014, pp. 257-277.

[2] H. Bertelsen, B.B. Jensen, B. Buemann, D-tagatose--a novel low-calorie bulk sweetener with prebiotic properties, World Rev. Nutr. Diet 85 (1999) 98-109.

[3] V. De Preter, T. Coopmans, P. Rutgeerts, K. Verbeke, Influence of long-term administration of lactulose and Saccharomyces boulardii on the colonic generation of phenolic compounds in healthy human subjects, J. Am. Coll. Nutr. 25 (2006) 541-549.

[4] F. Montañés, A. Olano, E. Ibáñez, T. Fornari, Modeling solubilities of sugars in alcohols based on original experimental data, AIChE J. 53 (2007) 2411-2418.

[5] F. Montañés, T. Fornari, P. J. Martín-Álvarez, N. Corzo, A. Olano, E. Ibáñez, Selective recovery of tagatose from mixtures with galactose by direct extraction with supercritical CO2 and different cosolvents, J. Agric. Food Chem. 54 (2006) 8340-8345.

[6] F. Montañés, N. Corzo, A. Olano, G. Reglero, E. Ibáñez, T. Fornari, Selective fractionation of carbohydrate complex mixtures by supercritical extraction with CO2 and different co-solvents. J. Supercrit. Fluid 45 (2008) 189-194.

[7] A. I. Ruiz-Matute, Sanz, M.L.; N. Corzo, P.J. Martín-Álvarez, E. Ibáñez, I. Martínez-Castro, A. Olano, Purification of lactulose from mixtures with lactose using pressurized liquid extraction with ethanol-water at different temperatures, J. Agric. Food Chem. 55 (2007) 3346-3350.

[8] O. A. El Seoud, A. Koschella, L.C. Fidale, S. Dorn, T. Heinze, Applications of ionic liquids in carbohydrate chemistry: A window of opportunities. Biomacromolecules 8 (2007) 2629-2647.

[9] L. Ruiz-Aceituno, M. L. Sanz, L. Ramos, Use of ionic liquids in analytical sample preparation of organic compounds from food and environmental samples, TrAC - Trend Anal. Chem. 43 (2013) 121-145.

[10] A.A. Rosatella, L.C. Branco, C.A.M. Afonso, Studies on dissolution of carbohydrates in ionic liquids and extraction from aqueous phase, Green Chem. 11 (2009) 1406-1413.

[11] E.S.R.E. Hassan, F. Mutelet, S. Pontvianne, J.C. Moïse, Studies on the dissolution of glucose in ionic liquids and extraction using the antisolvent method, Environ. Sci. Technol. 47 (2013) 2809-2816.

[12] A.P. Carneiro, C. Held, O. Rodríguez, G. Sadowski, E.A. Macedo, Solubility of sugars and sugar alcohols in ionic liquids: Measurement and PC-SAFT modeling, J. Phys. Chem. 117 (2013) 9980-9995.

[13] L.J.A. Conceiao, E. Bogel-Łukasik, R. Bogel-Łukasik, A new outlook on solubility of carbohydrates and sugar alcohols in ionic liquids, RSC Advances 2 [2012] 1846-1855.

[14] M.E. Zakrzewska, E. Bogel-Yukasik, R. Bogel-Yukasik, Solubility of carbohydrates in ionic liquids, Energ. Fuel. 24 [2010] 737-745.

[15] I.M. Al-Nashef, M.H. Gaily, S.M. Al-Zahrani, A.E Abasaeed, Method for separating fructose and glucose. US Pat., US 2009/0283093 A1, May 17, 2011.

[16] C. Carrero-Carralero, L. Ruiz-Aceituno, L. Ramos, F.J. Moreno, M.L. Sanz, Influence of chemical structure on the solubility of low molecular weight

carbohydrates in room temperature ionic liquids, Ind. Eng. Chem. Res. 53 (2014) 13843-13850.

[17] A. Montilla, M.D. Del-Castillo, M.L. Sanz, A. Olano, Egg shell as catalyst of lactose isomerisation to lactulose. Food Chem. 90 (2005) 883-890.

[18] O. Hernández, A.I. Ruiz-Matute, A. Olano, F.J. Moreno, M.L. Sanz, Comparison of fractionation techniques to obtain prebiotic galactooligosaccharides, Int. Dairy J. 19 (2009) 531-536.

[19] L. Ruiz-Aceituno, C. Carrero-Carralero, L. Ramos, I. Martinez-Castro, M.L. Sanz, Development of a carbohydrate silylation method in ionic liquids for their gas chromatographic analysis, Anal. Chim. Acta, 787 (2013) 87-92.

[20] A. Berthod, M.J. Ruiz-Ángel, S. Carda-Broch, Ionic liquids in separation techniques, J. Chromatogr. A. 1184 (2008) 6-18.

[21] A.P. Carneiro, O. Rodríguez, E. A. MacEdo, Fructose and glucose dissolution in ionic liquids: Solubility and thermodynamic modeling, Ind. Eng. Chem. Res. 52 (2013) 3424-3435.

[22] W. Liu, Y. Hou, W. Wu, S. Ren, Y. Jing, B. Zhang, Solubility of glucose in in ionic liquid + antisolvent mixtures, Ind. Eng. Chem. Res. 50 (2011) 6952-6956.