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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 sulfate ([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.

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

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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 sulfate 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 (Fig. 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 °C and analyzed as indicated in Section 2.5.1. This procedure was also applied to the isomerization mixture dissolved in [EMIM][DCA].