# Use of ionic liquids in the study of fruit ripening by high-resolution $^{13}$C NMR spectroscopy: 'green' solvents meet green bananas

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**Banana pulps at any ripening stage can be completely dissolved in solvent systems based on the ionic liquid (IL) 1-*n*-butyl-3-methylimidazolium chloride ([C$_4$mim]Cl), and variations in the carbohydrate composition of the fruit analyzed directly on the resulting solutions using high-resolution $^{13}$C NMR spectroscopy.**

With more than 300 different species and 20 commercially cultivated varieties, bananas are extremely popular fruits and an important foodstaple in the normal diet of several tropical and sub-tropical cultures.[1] Bananas are normally eaten raw after ripening makes the pulp, composed mainly of water and sugars,[2] soft and sweet. The process that brings about these changes depends on numerous factors, and several investigations aimed at determining the variations in chemical composition and nutritional value of banana pulp with time and environmental conditions have been carried out.[2–5] All these studies reveal that during the initial stages of ripening virtually all the carbohydrate present in the pulp is in the form of starch (Fig. 1a). Enzymatic degradation of the polyglucans then leads to the gradual accumulation of sucrose, glucose, and fructose (Fig. 1b–d). The concentration of these sugars increases until the fruit has ripened fully, a stage at which most of the starch has been degraded.

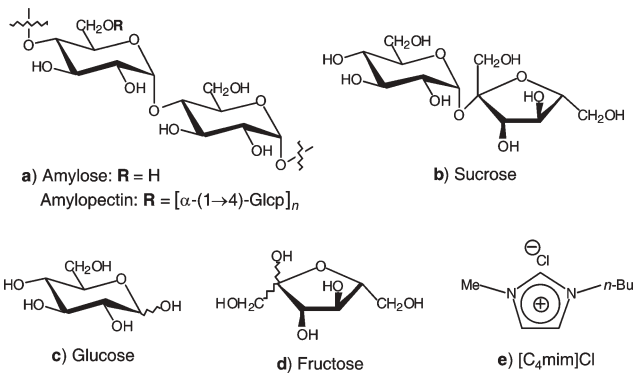


**Fig. 1** Structures of the major carbohydrates present in banana pulp (a–d) and 1-*n*-butyl-3-methylimidazolium chloride ([C$_4$mim]Cl, e).

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While well-established, the techniques employed in these analyses are laborious and time-consuming, and involve the extraction and derivatization of the carbohydrates present in the fruit prior to their quantification.[2–5] In this communication we show that following minimal sample preparation, banana pulps at any ripening stage can be fully dissolved in solvent systems based on the ionic liquid (IL) 1-*n*-butyl-3-methylimidazolium chloride ([C$_4$mim]Cl, Fig. 1e), and its carbohydrate composition determined directly through the use of conventional high-resolution $^{13}$C NMR experiments on the resulting solutions. The accuracy of the novel and notably simple method outlined here is comparable to that of reported protocols.

In addition to their now well-established role as potentially 'green' solvents with a plethora of applications in synthetic and analytical chemistry,[6] we and others have reported that ILs are capable of dissolving carbohydrates ranging from simple sugars to polysaccharides.[7,8] Some of the best results in this regard have been obtained with [C$_4$mim]Cl. As recently shown by us,[9] the non-hydrated chloride ions present in solutions of this IL solvate carbohydrates by forming hydrogen bonds with their hydroxyl groups. These interactions also disrupt the complex intermolecular hydrogen bonding network present in many polysaccharides and promote their dissolution. For example, cellulose solutions in concentrations of up to 25 wt% can be obtained with [C$_4$mim]Cl.[7] Furthermore, this IL displays no signals in the 55 to 120 ppm region of the $^{13}$C NMR spectrum typical of carbohydrates, and this has allowed us to perform detailed high-resolution $^{13}$C NMR studies of cellulose and related oligosaccharides dissolved in [C$_4$mim]Cl-based solvent systems.[10]

As part of our efforts to extend the methods developed during earlier investigations to the study of carbohydrate mixtures embedded in highly structured matrixes, we decided to apply them to the analysis of banana pulp composition. Our initial trials involved the isolated sugars present in the pulp, including fructose, glucose, sucrose, and amylopectin, the main polyglucan found in banana starch.[2] As expected, [C$_4$mim]Cl dissolved the pure carbohydrates efficiently. 5 wt% solutions could be easily prepared by gently warming stirred suspensions of the sugars in the IL for less than an hour.† The high viscosity of the resulting samples, particularly in the case of amylopectin, precludes their direct use in high-resolution NMR experiments. However, addition of a 15 wt% of DMSO-$d_6$, combined with probe temperatures moderately above ambient, allows for well-resolved $^{13}$C spectra to be recorded and has no effect on the solubility of the carbohydrates.[10] Fructose showed minor but detectable decomposition when heated in [C$_4$mim]Cl to temperatures higher than 80 °C, and spectra for this monosaccharide were therefore recorded at 70 °C. The remaining

 

sugars were stable in the IL-based solvent system at 90 °C, and they were studied at this temperature. Some of the features of the spectra obtained under these conditions deserve attention. Signals for all five isomers of fructose can be observed (Fig. 2a), and analysis of the resonances corresponding to the C-2 carbon indicates that the α-furanose (98.5 ppm), β-furanose (105.5 ppm), β-pyranose (103.0 ppm), α-pyranose (99.7 ppm), and ketohexose (214.6 ppm) forms of this sugar exist in a 2 : 24 : 44 : 22 : 8 ratio. Similar analysis of the signals corresponding to the C-1 carbon of glucose indicates the presence of three of its mutarotamers (Fig. 2b), including the β-furanose (104.2 ppm), β-pyranose (97.8 ppm), and α-pyranose (93.0 ppm) forms, in a 2 : 57 : 41 ratio. The resonances for all carbons in sucrose are well-resolved (Fig. 2c), and can be easily assigned by comparison to spectral data for the disaccharide recorded in aqueous solution. Inspection of the amylopectin spectrum reveals large signals corresponding to carbons in the α-(1→4)-linked gluocose units of the polymer, flanked by smaller ones for $^{13}$C nuclei of residues at the α-(1→6) branching points (Fig. 2d). This is evident on signals corresponding to the C-1, C-4, and C-6 carbons at 100.3, 79.1, and 61.1 ppm, respectively.

We then focused our attention on the dissolution and $^{13}$C NMR analysis of banana pulps. While as many as eight different ripening stages have been identified for this fruit based on the color of its peel,[2,5] only four were considered in the present study. The corresponding samples were prepared using Cavendish bananas from a single bunch, which were processed at regular intervals over a three-week period and classified according to peel color as green, green/yellow, yellow, and yellow/brown. Sample preparation was limited to coarse mincing of the peeled fruit followed by complete removal of water by lyophilization. In spite of the rather complex physical structure and chemical composition of banana pulp, the resulting flour-like samples dissolved with ease in either neat [$C_4$mim]Cl or [$C_4$mim]Cl/DMSO-$d_6$ mixture. Clear 5 wt% solutions that had no residual particulate matter and were therefore suitable for high-resolution $^{13}$C NMR analysis could be obtained readily in all cases.† On the other hand, use of neat DMSO-$d_6$ under the same conditions led only to partial dissolution of the samples. We believe that the combined effects of freeze-drying, moderate heating, and the ionic strength of the IL disrupt the pulp amyloplasts and lead to complete dissolution of the samples. Experiments for pulps corresponding to the green and green/yellow ripening stages in [$C_4$mim]Cl/DMSO-$d_6$ solution were carried out at 90 °C. Higher concentrations of fructose were expected at later stages of ripening, a sugar which as mentioned earlier degrades slowly in this medium above 80 °C. Hence, spectra for samples corresponding to the yellow and yellow/brown ripening stages were recorded at 80 and 70 °C, respectively.

As shown in Fig. 3, the changes in carbohydrate composition throughout the ripening process can be easily followed by inspection of the $^{13}$C NMR spectra of the different samples. Signals for starch dominate at the beginning of the process (Fig. 3a), corroborating that these polyglucans are the main constituents of banana pulp in the early stages of ripening.[2–5] In agreement with reported findings,[2] comparison of the spectra presented in Figs. 2d and 3a also reveals that banana starch is composed mainly of amylopectin. The spectra in Figs. 3b, 3c, and 3d indicate that the polysaccharide levels decrease as ripening progresses until they are undetectable, and at the same time signals for sucrose, glucose, and fructose grow and become predominant.

The results described above are better depicted in Fig. 4, which shows color-coded expansions of the anomeric carbon region of

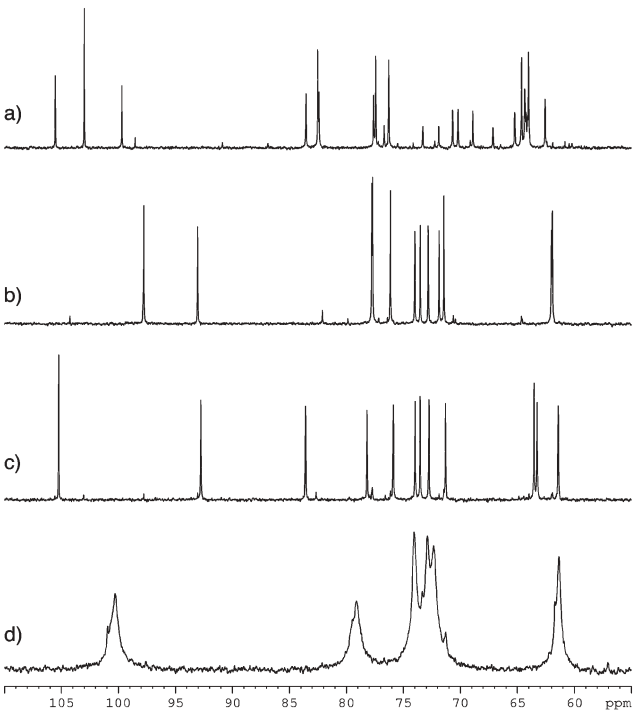


**Fig. 2** $^{13}$C NMR spectra of fructose (a), glucose (b), sucrose (c), and amylopectin (d) recorded in [$C_4$mim]Cl/DMSO-$d_6$ solution. In all cases the carbohydrate concentration was 5 wt%.

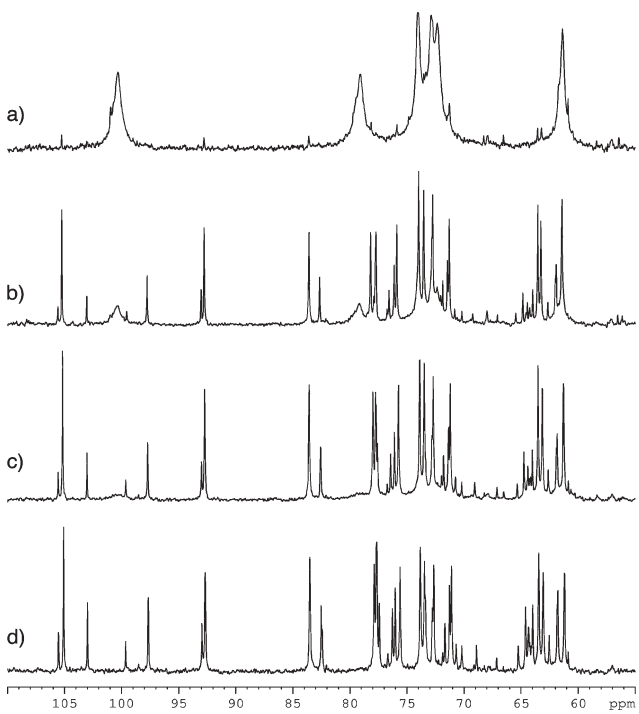


**Fig. 3** $^{13}$C NMR spectra of lyophilized pulp from green (a), green/yellow (b), yellow (c), and yellow/brown bananas (d) in [$C_4$mim]Cl/DMSO-$d_6$ solution. The sample concentration was 5 wt% in all cases.

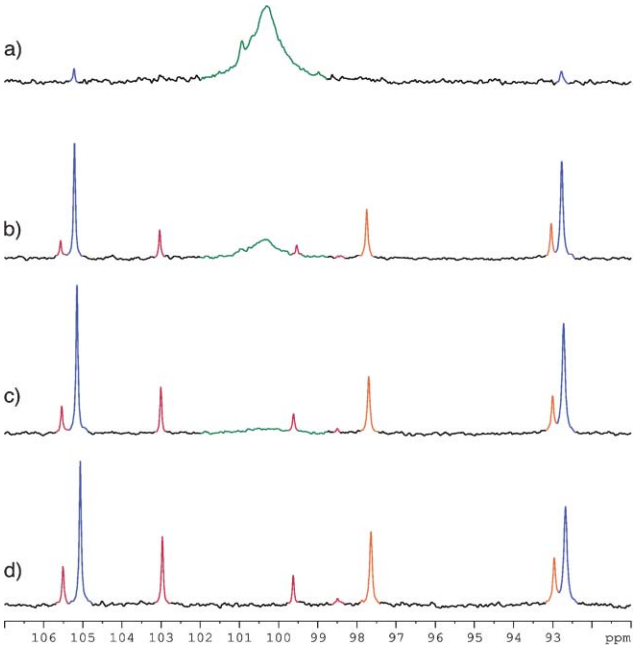


**Fig. 4** Anomeric carbon region of the spectra shown in Fig. 3. The signals corresponding to starch, sucrose, glucose, and fructose in each sample are highlighted in green, blue, orange, and red, respectively.

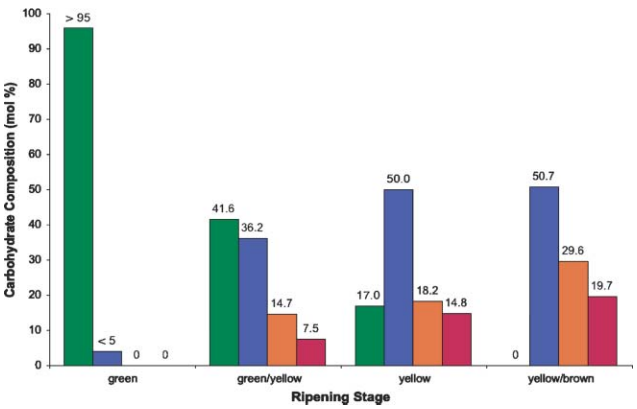


**Fig. 5** Changes in the carbohydrate molar composition of banana pulp at different ripening stages. The color code corresponds with that of Fig. 4.

the banana pulp spectra. Since signals corresponding to the different sugars in this spectral window are well-resolved, they can be used to quantitate the variation in the carbohydrate molar ratios as the fruit ripens. Taking into account all the mutarotamers observed for reducing sugars and their ratios (*vide supra*), judicious integration of the anomeric carbon resonances yields the molar composition estimates presented in Fig. 5. Our quantitation methods have some limitations that are worth noting. First, the wt% composition in samples containing starch cannot be calculated from the molar ratios because the molecular weight of the polyglucan mixture is not accurately known. In addition, the signal intensities for anomeric carbons on different sugars can be affected differently by relaxation and NOE effects, which could lead to errors in the molar ratios derived by direct integration of these resonances. These drawbacks can be overcome through the application of common quantitative NMR techniques, such as the use of calibration curves against internal weight standards for the different carbohydrates present in banana pulp. While this approach was not followed here, our results compare remarkably well with published data. For example, a sucrose to reducing sugars mass ratio of 66 : 34 can be computed from the molar composition at the yellow/brown stage (Fig. 5), which is in excellent agreement with the ratio of 62 : 38 reported for fully ripe bananas by Lii and co-workers.[5]

In summary, the results presented above show that IL-based solvent systems are capable of fully dissolving banana pulps with virtually no preconditioning, yielding solutions that can be conveniently studied through the use of standard $^{13}C$ NMR techniques. The approach can be easily extended to the analysis of carbohydrate mixture composition in diverse forms of biomass, including other fruits and grains, as well as more complex lignocellulosic materials. More importantly, the methods described here constitute the first steps towards the development of environmentally-friendly alternatives for the extraction and processing of polysaccharides from natural sources.

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## Notes and references

† 5 wt% solutions of glucose, fructose, sucrose, amylopectin, and powdered lyophilized banana pulps in [$C_4$mim]Cl/DMSO-$d_6$ were prepared by mixing 50 mg of the samples, 800 mg of the IL, and 150 mg of DMSO-$d_6$, and gently heating the resulting viscous suspensions with constant stirring.[7,10] Upon complete dissolution, the samples were transferred to 5 mm NMR tubes. Proton-decoupled $^{13}C$ spectra were collected at temperatures between 70 and 90 °C on a Bruker AVANCE 400 NMR spectrometer operating at a $^{13}C$ frequency of 100.61 MHz. A total of 5,000 and 20,000 scans were collected for the analytical samples of the simple sugars and amylopectin, respectively. 40,000 accumulations were required for all banana pulp samples except for the one corresponding to the yellow/brown ripening stage, for which 20,000 scans were collected. A line-broadening factor of 5 Hz was employed to process all spectra.

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