

Enhanced extraction of caffeine from guaraná seeds using aqueous solutions of ionic liquids†

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The extraction of caffeine from bioresources using more benign and cost-effective processes is of fundamental relevance towards the finding of alternative (bio)pesticides. Classically, the best attempts to extract caffeine from biomass have resulted in low efficiency and in a large consumption of hazardous organic solvents and/or energy requirements. Here, we report an enhanced and selective extraction of caffeine from guaraná (*Paullinia cupana*, Sapindaceae) seeds using aqueous solutions of ionic liquids. Several ionic liquids composed of imidazolium or pyrrolidinium cations combined with the chloride, acetate and tosylate anions were investigated. Furthermore, the effect of the cation alkyl side chain length and the presence of functionalized groups were also addressed. Additional conditions such as the ionic liquid concentration, the contact time, the solid–liquid ratio and temperature were further optimized by a response surface methodology. Outstanding extraction yields (up to 9 wt% of caffeine per guaraná dry weight) were obtained at a moderate temperature and in a short-time. The recyclability and reusability of the ionic liquids were also confirmed. For the first time it is shown that aqueous solutions of ionic liquids are superior alternatives for the solid–liquid extraction of caffeine from biomass samples and, as a result, the development of an ionic-liquid-based process is straightforwardly envisaged.

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Introduction

Guaraná (*Paullinia cupana*, Sapindaceae) is a Brazilian native plant and its seeds are the only part suitable for human consumption.¹ Guaraná seeds are the richest vegetable source of caffeine with a concentration two to three times higher than those of coffee beans, tea leaves and cola nuts. The concentration of caffeine on a dry basis generally ranges from 3 to 7%.² Nevertheless, the place and method of cultivation and the drying procedure can lead to different amounts of caffeine. Guaraná also possesses other methylxanthines, tannins, saponins, starch and fats.³ As a result of its rich composition, guaraná is typically used as a medicinal plant, either in natural products or by pharmaceutical companies, as well as in the food industry.⁴

Caffeine (1,3,7-trimethylxanthine) belongs to the alkaloids family, more specifically to xanthines (2,6-dihydroxypurine). Theobromine (3,7-dimethylxanthine) and theophylline (1,3-

dimethylxanthine), albeit in lower amounts, are the two other xanthines that can be found in guaraná.³

Caffeine (Fig. 1) is the bioactive ingredient mostly ingested by humans. It is also used in pharmaceutical products for therapeutic purposes because of its stimulant effects on the nervous, muscular and cardiovascular systems.⁵ Nonetheless, besides these particular properties, caffeine also displays anti-bacterial and antifungal features.⁶ It can act as a natural pesticide protecting plants – principal function in guaraná, coffee, tea and cocoa. Taking into account the harmful and hazardous nature of pesticides commonly used in farming, caffeine can be foreseen as an alternative (bio)pesticide. Several works reported on the use of caffeine as a natural pesticide effective in killing/repelling slugs, snails, birds and insects.⁶ Therefore, caffeine can be used as a repellent or a more benign pesticide

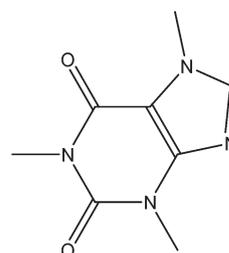


Fig. 1 Chemical structure of caffeine.

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† Electronic supplementary information (ESI) available: Chemical structure of the studied ionic liquids, data for the first factorial planning obtained with pure water, experimental points used in the factorial planning, model equations, yields of caffeine obtained experimentally and respective calculated values and statistical analysis connected to the response surface methodology. See DOI: 10.1039/c3gc40437d

while being an efficient economic alternative if extracted from residues, such as spent coffee or low added-value bioresources such as guaraná seeds. In this context, the extraction and purification of xanthines (mainly caffeine) from natural plants, *e.g.* guaraná, through economic and benign processes, is of considerable interest for their further applications as greener substitutes to common pesticides.

Currently, there is a great demand for natural ingredients and the discrimination among natural and synthetic caffeine has attracted enormous attention. In the past few years, the Food and Drug Administration (FDA) regulated that any added caffeine must be labelled on products for human consumption owing to possible adulteration and health concerns.⁷ Actually, caffeine extraction (essentially for decaffeination purposes and human intake) is carried out using supercritical carbon dioxide or related mixtures making use of co-solvents, such as alcohols and water, to improve the extraction yields.⁸ Other conventional methods include the extraction of caffeine by water at high temperatures,⁹ the use of organic solvents,¹⁰ namely chloroform, methylene chloride and ethyl acetate, and the use of water-organic solvent mixtures (water-ethanol, water-methanol and water-acetone).¹¹ These methods have some disadvantages since they require several hours of extraction and result in low yields of caffeine.⁸⁻¹¹ Furthermore, the organic compounds commonly employed are volatile, toxic and flammable leading to several human risks and safety issues and to a poor environmental performance.

In recent years ionic liquids (ILs) have emerged as alternative solvents for separation processes.¹² In particular, the applications of ionic liquids in the extraction of essential oils from orange peels, in the reactive dissolution of star anise seeds towards the isolation of Shikimic acid for the production of Tamiflu, in the sequestration of suberin from cork, in the extraction of lactones, tannins and phenolic compounds from medicinal plants, in the extraction of the bioactive alkaloid glaucine from plant material, in the isolation of pharmaceutically active betulin from birch bark and in the pretreatment of lignocellulosic biomass have been reported.¹³ Besides the minimization of the environmental footprint due to their negligible vapour pressures, tailored ionic liquids possess further advantageous properties. These include a high solvation ability for target biomolecules, non-flammability and high thermal and chemical stabilities. All these characteristics are dependent on the appropriate selection of the cation and/or the anion which comprise a specific fluid and that allows their properties to be tuned. Although ionic liquids present favourable environmental benefits compared to conventional organic solvents, particularly with regards to atmospheric pollution, their toxicity and biodegradability cannot be neglected and should be addressed. Important review manuscripts have been published considering both subjects.¹⁴ Moreover, the exploration of ionic liquids by academia and industry as “green solvents” has been comprehensively evaluated.¹⁵

Taking into account the large content of caffeine in guaraná seeds and its non-effective, non-selective or poor extraction by conventional methods and/or solvents, and based

on our previous experience in the extraction of alkaloids,¹⁶ we propose here, for the first time, the use of ionic liquids' aqueous solutions as alternative solvents for the selective extraction of caffeine from bioresources. An initial screening of the ionic liquids' structural characteristics was conducted followed by a response surface methodology aiming at optimizing the operational conditions, namely the ionic liquid concentration ([IL]), the solid-liquid ratio (S/L ratio), the extraction time (*t*) and temperature (*T*). Improved extraction yields of caffeine were attained and the recyclability and reusability of the ionic liquids were also demonstrated.

Results and discussion

Initial screening: concentration and structural features of ionic liquids and guaraná' particles diameter

Apart from the unique solvent properties of ionic liquids for biomass processing, namely cellulose, chitin and wood,¹⁷ the ability of ionic liquids to swell and dissolve raw biomass can lead to an improved access to the valuable ingredients embedded in biopolymer matrices. In particular, the use of aqueous solutions of ionic liquids can avoid the cellulosic matrix dissolution¹⁷ while allowing the extraction of target compounds, *e.g.* caffeine. On the other hand, there is already experimental evidence that water can act as a co-solvent (with supercritical CO₂) to improve the selective extraction of caffeine from crude biomass.¹⁸

With the goal of selectively extracting high yields of caffeine from guaraná seeds, several aqueous solutions of ionic liquids were therefore investigated. A list of the studied fluids, including a definition of their acronyms, is provided as an endnote.† The chemical structure of the ionic liquids considered is shown in ESI.†

As a first screening test only aqueous solutions of [C₄mim]-Cl were used. For comparison purposes, we also accomplished the extraction of caffeine with water and aqueous solutions of NaCl. The same operational conditions were kept in all experiments, namely a guaraná-solvent ratio of 1 : 10, an extraction time of 30 min at 70 °C and with guaraná particles diameter within 0.4–1.0 mm. The effects of the ionic liquid and NaCl concentration on the extraction yield of caffeine are depicted in Fig. 2. To confirm that caffeine is the predominant methylxanthine being extracted and to prove the selectivity of the aqueous solutions involving ionic liquids we used UV-Vis and nuclear magnetic resonance (NMR) spectroscopies and capillary electrophoresis to analyse the aqueous extract of several samples. It is safe to admit that other methylxanthines are not being extracted from the guaraná seeds, at least at concentrations higher than the detection limits of the equipment.

†Acronyms of ionic liquids: 1-butyl-3-methylimidazolium chloride, [C₄mim]Cl; 1-butyl-3-methylimidazolium tosylate, [C₄mim][Tos]; 1-butyl-1-methylpyrrolidinium chloride, [C₄mpyr]Cl; 1-ethyl-3-methylimidazolium chloride, [C₂mim]Cl; 1-hydroxyethyl-3-methylimidazolium chloride, [OHC₂mim]Cl; and 1-ethyl-3-methylimidazolium acetate, [C₂mim][CH₃CO₂].

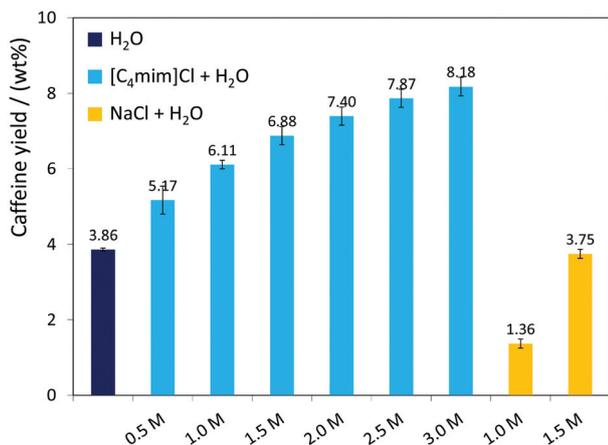


Fig. 2 Yield of caffeine extracted from guaraná seeds (guaraná' particles diameter within 0.4–1.0 mm, $T = 70\text{ }^{\circ}\text{C}$, S/L ratio = 0.10 and $t = 30\text{ min}$).

The yield of caffeine strongly depends on the salt employed. As shown in Fig. 2, the presence of ionic liquids has a huge influence on the extraction of caffeine from the bioresource with extraction yields up to 8 wt%. This value is significantly higher than those achieved with the extractions with water or the aqueous solutions of NaCl (used as model electrolyte solutions). In both salt solutions the extraction yield increases with the concentration of NaCl or [C₄mim]Cl. However, unlike what is observed with NaCl, the presence of ionic liquids always leads to increased extraction efficiencies when compared with pure water. The NaCl seems to be acting as a moderately salting-out agent reducing therefore the saturation solubility of caffeine in aqueous media.¹⁹ In general, the combined results gathered with both NaCl and [C₄mim]Cl show that the ionic strength is not a relevant factor regarding the caffeine extraction. Aqueous solutions of [C₄mim]Cl, or other ionic liquids as shown below, prove to be suitable and enhanced extractive solvents for caffeine, and the best results are achieved with the higher concentrations of ionic liquids (2.5–3.0 M). The low viscosity of aqueous solutions of ionic liquids, at least at the concentrations used, allows an easier penetration of the solvent into the sample matrix and a rapid mass transfer of the desired extracts into the liquid phase.

The enhanced extraction yields obtained with [C₄mim]Cl further motivated the study of other ionic liquid solutions as extractive solvents for caffeine. Based on the tailored ability of ionic liquids, their chemical structures were varied so that the cation core, the alkyl side chain length, the presence of functionalized groups and the anion nature effects could be evaluated. In addition, the influence of the guaraná' particles diameter was also investigated. The results obtained for the aqueous solutions of [C₄mim]Cl, [C₄mim][Tos], [C₄mpyr]Cl, [C₂mim]Cl, [OHC₂mim]Cl and [C₂mim][CH₃CO₂] at 0.5 M are depicted in Fig. 4. For the systems composed of [C₄mim]Cl and [C₄mim][Tos] the effect of the particles diameter is also shown.

For the larger guaraná' particles the obtained results reveal that the percentage of extracted caffeine is similar for the

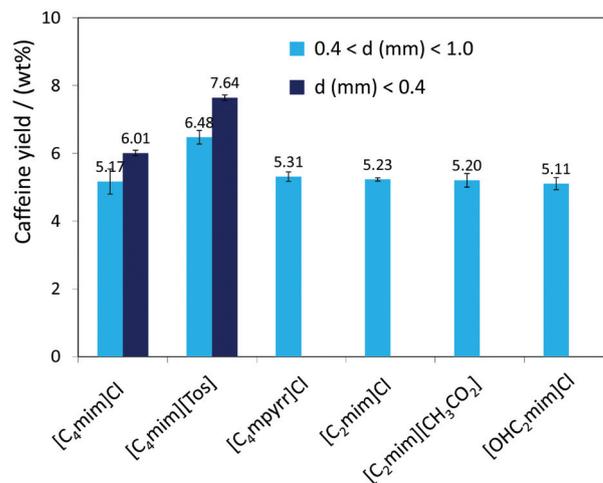


Fig. 3 Yield of caffeine extracted from guaraná seeds with different ionic liquids ([IL] = 0.5 M, $T = 70\text{ }^{\circ}\text{C}$, S/L ratio = 0.10 and $t = 30\text{ min}$).

various ionic liquids. One exception was observed with [C₄mim][Tos] where an increase in the caffeine yield was observed due to its anion aromatic nature. Indeed, non-covalent $\pi\cdots\pi$ interactions between caffeine and various aromatic compounds in physiological media have been reported.²⁰ Nevertheless, the influence of an aromatic ring at the cation seems to be more limited since [C₄mim]Cl and [C₄mpyr]Cl do not show significant differences in the extraction yields of caffeine within the associated uncertainty.

It was already demonstrated that the choice of the ionic liquid anion determines the dissolution process of crude biomass.²¹ However, in this work, and besides the data obtained with [C₄mim][Tos], there is no significant effect of the anion nature, when comparing the chloride- with the acetate-based ionic liquid, on the selective extraction of caffeine even though it was already shown that [CH₃CO₂]-based ionic liquids are better solvents for the dissolution of cellulose.¹⁷ Furthermore, an increase in the cation alkyl side chain length (from [C₂mim]Cl to [C₄mim]Cl) or the inclusion of a hydroxyl group at the longest aliphatic chain ([OHC₂mim]Cl) does not lead to significant variations in the caffeine extraction yields meaning that dispersive-type interactions and hydrogen-bonding are not playing a crucial role. Therefore, the extraction mechanism must be mainly related with the enhanced solubility and the ionic liquid effect on the biomass structure as discussed below. On the other hand, as illustrated in Fig. 3, the size of the guaraná particles is of more significance. Smaller particles of guaraná have a larger surface area, and thus, higher amounts of caffeine are extracted.

Optimization of the operational conditions: response surface methodology

The univariate method for the optimization of the operational conditions does not consider the interaction among the factors and may not correspond to the overall optimized

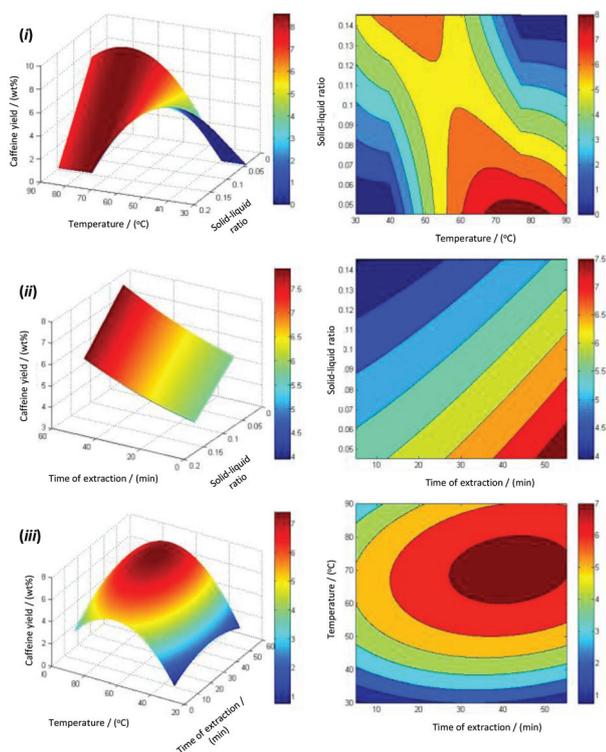


Fig. 4 Response surface plots (left) and contour plots (right) on the yield of caffeine with the combined effects of (i) T and S/L ratio, (ii) t and S/L ratio and (iii) T and t using aqueous solutions of $[C_4mim]Cl$ at 1.0 M and guaraná particles with a diameter within 0.4–1.0 mm.

conditions. With the goal of optimizing the operational conditions to obtain high extraction yields, *e.g.* identification of the most significant processing parameters, we used herein a response surface methodology (RSM). This type of strategy allows the exploitation of the relationship between the response (yield of caffeine) and the independent variables that can mould the extraction.

In this work, three 2^3 (3 factors and 2 levels) factorial planings were executed. The first factorial planning was carried out with pure water where the temperature, the solid-liquid ratio and time of extraction were optimized. The results obtained for water are presented in ESI†. In the second and third factorial planings aqueous solutions of $[C_4mim]Cl$ were employed. Although higher extraction yields were achieved with $[C_4mim][Tos]$, $[C_4mim]Cl$ was used in the optimization procedure because it is a cleaner (synthesis in one step), more stable and cheaper compound.

In the second factorial planning we varied the solid-liquid ratio, the extraction time and the extraction temperature. At this stage, the guaraná particles diameter was between 0.4 mm and 1.0 mm and the concentration of $[C_4mim]Cl$ was maintained at 1.0 M. The influence of the three variables towards the extraction yields of caffeine is illustrated in Fig. 4. The experimental points used in the second factorial planning, the model equation, the yield of caffeine obtained experimentally and the respective calculated values using the correlation

coefficients obtained in the statistical treatment, as well as all the statistical analyses, are shown in ESI†. The average relative deviation between the experimental and the predicted values is -0.16% supporting the good description of the experimental results by the statistical model.

According to the statistical analysis shown in ESI† and the data depicted in Fig. 4 it is evident that temperature is the most significant parameter leading to a region of maximum yield of extraction. Higher temperatures are profitable for the extraction of caffeine. Moreover, an increase in temperature contributes to a decrease in viscosity of the ionic liquid solutions and to enhance their swelling ability. The solid-liquid ratio also leads to different yields of caffeine. In general, the amount of extracted caffeine increases with the solvent volume. The time was the variable with the weakest influence on the extraction of the alkaloid. The optimized conditions occur at temperatures ranging from 65 °C to 85 °C and for low solid-liquid ratios and moderate extraction times (between 30 and 50 min). In fact, extraction yields up to 8 wt% are observed with low concentrations of ionic liquids by the simple adjustment of the operational conditions.

Since in the statistical analysis of the previous 2^3 factorial planning it was found that time is not a significant variable for the caffeine extraction, the third and last factorial planning was further carried out with the time fixed at 30 min. The variables investigated in this methodology were the temperature, the solid-liquid ratio and the ionic liquid ($[C_4mim]Cl$) concentration. In this optimization process particles of smaller diameter (<0.4 mm) were also used aiming at attaining the highest yields of extraction. All the statistical analysis is provided in ESI†. The average relative deviation between the model results and the experimental data is -0.50% . The response surface and contour plots are shown in Fig. 5.

Based on the statistical analysis provided in ESI† and the data depicted in Fig. 5, it can be concluded that the most significant parameters are temperature, and the combined effects of temperature – solid-liquid ratio and ionic liquid concentration – solid-liquid ratio (P -value <0.05).

Outstandingly, from the results obtained in this last factorial planning, extraction yields of caffeine up to 9 wt% were attained. This high value of caffeine was achieved within 30 min at 70 °C using an aqueous solution of $[C_4mim]Cl$ at 2.34 M and a solid-liquid ratio of 0.1. At this optimum point the predicted extraction yield of caffeine by the statistical approach is 9.49 wt% whereas the experimental value is 9.43 wt%.

To support the high yields obtained, the extraction of caffeine from the same biomass sample using methylene chloride and Soxhlet extraction during 270 min was also carried out for comparison purposes. The maximum yield of caffeine achieved was 4.30 wt%. Despite the use of a hazardous organic solvent and for an extended time, the extraction of caffeine by this conventional approach is significantly less efficient when compared with the enhanced results achieved with the ionic liquid aqueous solutions.

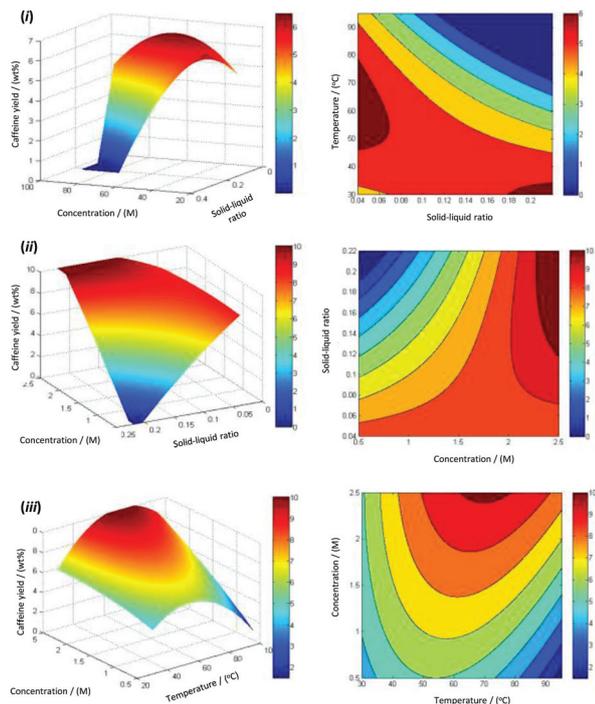


Fig. 5 Response surface plots (left) and contour plots (right) on the yield of caffeine with the combined effects of (i) T and S/L ratio, (ii) [IL] and S/L ratio and (iii) T and [IL] using aqueous solutions of $[C_4mim]Cl$ for 30 min of contact time and guaraná particles with a diameter inferior to 0.4 mm.

Recyclability and reusability of the solvent

Once the optimum conditions were identified, the isolation of caffeine from the aqueous media and the recyclability and reusability of the solvent were then investigated.

The first task consisted of the exploration of the reusability of the aqueous solutions without performing any pre-extraction of caffeine from the ionic-liquid-based medium. Thus, the aqueous solution of $[C_4mim]Cl$ at 2.34 M was used for three successive extractions under the optimized operational conditions (solid-liquid ratio of 0.1 for 30 min and at 70 °C). After each extraction the solid-liquid mixture was filtered and the aqueous solution was reused with a new sample of guaraná particles. Remarkably, in all samples, *circa* 9 wt% of caffeine was extracted with the overall solution reaching a caffeine concentration three times higher. The aqueous solution of ionic liquid can thus be reused at least three times without reaching saturation.

After settling the reusability of the ionic liquid aqueous solutions the next step was to confirm their recyclability. To this end, the first step consisted of the choice of a suitable solvent capable of re-extracting caffeine from the ionic liquid medium. At this stage, several organic solvents non-miscible with water were tested. The extraction was performed by placing the several organic solvents in contact with an aqueous solution containing caffeine at 22 g L⁻¹ (concentration similar to that found in the real experiments) and in a volume ratio of 1 : 3. This procedure was repeated three times.

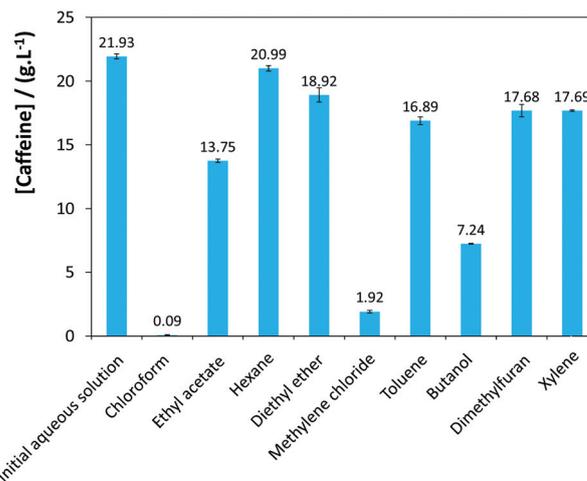


Fig. 6 Concentration of caffeine in the aqueous solution after the liquid-liquid extraction by different organic solvents non-miscible with water.

The amount of caffeine in the aqueous solution after the re-extraction procedure is depicted in Fig. 6.

The results shown in Fig. 6 represent an evaluation of the ability of several organic solvents to re-extract caffeine from aqueous solutions. The best extraction solvents are chloroform and methylene chloride. Indeed, chloroform is shown to be capable of completely extracting caffeine from aqueous solution. These solvents are in fact the fluids of choice to extract caffeine from bioresources.¹⁰ Nevertheless, we also found that butanol can be a good candidate for extracting caffeine and can replace the more toxic and hazardous ethyl acetate currently used.¹⁰

A scalable isolation process for the valuable ingredients from the ionic liquid aqueous media, as well as an efficient recovery strategy for the ionic liquids, are critical features for their scale-up implementation. Accordingly, after the selection of the best extractive solvents, chloroform and butanol were used to extract caffeine from the real samples containing caffeine extracted under the optimum operational conditions (aqueous solution of caffeine obtained from the guaraná seeds after 30 min at 70 °C and using an aqueous solution of $[C_4mim]Cl$ at 2.34 M and a solid-liquid ratio of 0.1). Even though chloroform is an organic, volatile and toxic solvent it should be emphasized that it was used here only with the intent of proving the recyclability of the ionic liquid aqueous solutions to extract caffeine from new guaraná seeds. More benign approaches, such as the use of supercritical CO₂ or butanol, are recommended at this stage.

Both the highly concentrated caffeine aqueous solution (after three extractions with new guaraná seeds) and a sample where a single extraction was performed were investigated. The aqueous solutions were placed in equilibrium with chloroform or butanol three times, as previously described, and reused for a new extraction under the optimized conditions. This process was repeated two more times and the aqueous solutions of $[C_4mim]Cl$ always shown to be capable of extracting the maximum 9 wt% of caffeine.

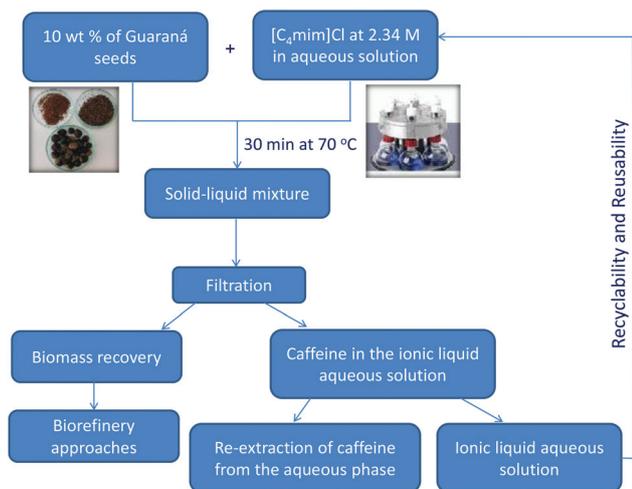


Fig. 7 Flowchart for the extraction of caffeine from guaraná seeds.

Due to the low-volatility of alkaloids, their separation from the feedstock has to be accomplished essentially by solid-liquid extraction methodologies. Although some progress in the use of ionic liquids as extractive media for added-value compounds from bioresources has been reported, these investigations are mostly restricted to the identification and quantification of the bioactive structures.²² A scalable isolation process for the target compounds from the ionic liquid solution as well as the recovery strategy of both the solute and the solvent are still critical for the scale-up implementation. Fig. 7 depicts the optimized strategy for the extraction of caffeine from guaraná seeds highlighting the recyclability and reusability of the ionic liquid aqueous solutions. Moreover, after the extraction of this biocompound, which has shown to be successful by ionic liquid aqueous solutions, and since the dry biomass consists mainly of carbohydrates, the processed guaraná can further proceed for biomass conversion approaches within an integrated biorefinery concept.

Feedstock analysis to infer the mechanism of extraction

The gathered results show that temperature is the main factor affecting the extraction of caffeine, followed by the combined effects of temperature – solid-liquid ratio and ionic liquid concentration – solid-liquid ratio. With the goal of inferring the reasons behind the enhanced extraction of caffeine by ionic liquid solutions we further applied scanning electron microscopy (SEM) to the guaraná samples after the extraction procedure. The SEM images of guaraná after the treatment with water and an aqueous solution of $[C_4mim]Cl$, under the optimal conditions described before, are shown in Fig. 8.

The sample structure that was in contact with pure water is less affected than the one treated with the ionic liquid aqueous solution. Albeit broken cells are seen in both samples, there is an increase in the ratio of broken cells to intact cells in the presence of the ionic liquid medium. This change in structure favours the extraction of caffeine into the aqueous phase. Hence, the extraction yield of the alkaloid is

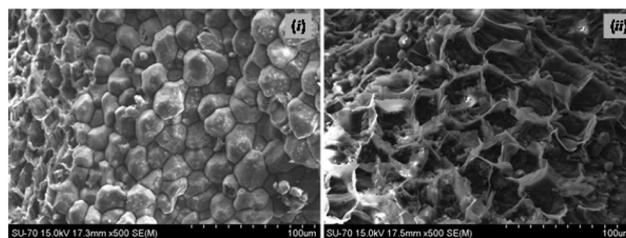


Fig. 8 SEM images of the guaraná samples after extraction with (i) water and (ii) an aqueous solution of $[C_4mim]Cl$.

greatly improved in the presence of ionic liquids since, besides enhancing the solubility of the alkaloid in aqueous medium, they further permit a better access to the valuable ingredients embedded in the biopolymer matrix.

As Fourier transform infrared spectroscopy (FTIR) provides useful information for identifying the presence of certain functional groups or chemical bonds it was additionally applied to investigate the changes in the chemical structure of guaraná seeds. Fig. 9 shows the results obtained for the original feedstock and after extraction with water and aqueous solutions of three different ionic liquids. Moreover, the FTIR spectrum of a caffeine standard is also included for comparison purposes.

The infra-red spectrum of caffeine displays two characteristic bands corresponding to the carbonyl ($C=O$) vibration frequency at *circa* 1655 and 1700 cm^{-1} .²³ Interestingly, the high relative strength band at 1655 cm^{-1} , indicative of caffeine, is seen in the spectrum of the guaraná sample before extraction; yet, it is not visible in the spectra of the guaraná seeds after the extraction procedures and that can be associated with the dissolution of caffeine by the aqueous media. In addition, the FTIR spectra show that the signal positions and intensity of the major absorption bands related to the polymeric matrix of guaraná do not significantly change after the extraction procedure. These results indicate that the chemical structures of the carbohydrates, and thus of the cellulose-polymeric matrix

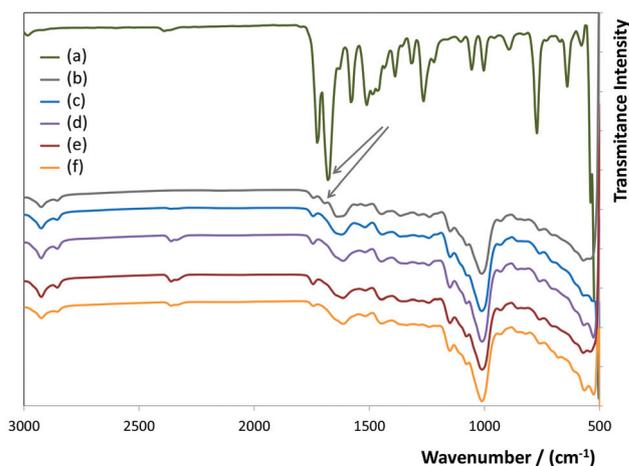


Fig. 9 FTIR spectra of caffeine (a), guaraná sample before extraction (b), and guaraná samples after extraction with water (c), and aqueous solutions of $[C_4mim]Cl$ (d), $[C_2mim]Cl$ (e) and $[C_4mim][Tos]$ (f).

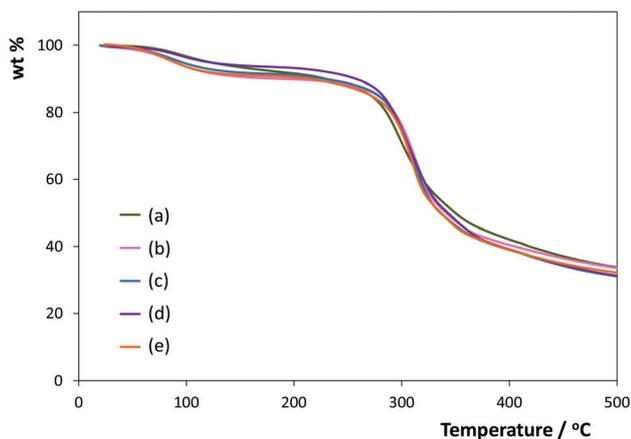


Fig. 10 TGA of a guaraná sample before extraction (a), and of guaraná samples after extraction with water (b), and aqueous solutions of $[C_4mim]Cl$ (c), $[C_2mim]Cl$ (d) and $[C_4mim][Tos]$ (e).

(intense characteristic bands in the fingerprint region from $900\text{--}1400\text{ cm}^{-1}$ of the mid-infrared spectral range), do not significantly change after the treatment with aqueous solutions. The bands in the region $900\text{--}1153\text{ cm}^{-1}$ are assigned to C–O and C–C stretching modes, while those in the $1400\text{--}1199\text{ cm}^{-1}$ region are due to O–C–H, C–C–H and C–O–H bending vibrational modes of the carbohydrates. These results disclose that the carbohydrate fraction is not significantly dissolved by the water–ionic–liquid mixtures.

Thermogravimetric assays (TGA) were also used to assess the structural integrity of the biomass sample and to support the non-dissolution of significant amounts of carbohydrates or polysaccharides from the guaraná seeds by the ionic liquids aqueous solutions. The TGA results are depicted in Fig. 10.

The thermal stabilities and the onset degradation temperatures of the guaraná samples after the extraction procedure are identical to the original sample. Likewise, no major differences are observed with the diverse samples exposed to different aqueous solutions. These results further confirm that the predominant polymeric matrix is not affected or significantly dissolved by the aqueous solutions of ionic liquids.

In summary, although ionic liquids were previously shown to be good candidates for the dissolution of biomass,¹⁷ the presence of water prevents the dissolution of the biopolymers while allowing the selective extraction of caffeine.

Conclusions

This work shows, for the first time, that aqueous solutions of ionic liquids are enhanced solvents for the extraction of caffeine from bioresources, *e.g.* guaraná seeds. Compared with traditional extraction methods this new strategy proves to be selective towards caffeine and capable of providing extraction yields up to 9 wt%.

The recovery and reusability of the ionic liquids were successfully demonstrated supporting the economic viability and

weak environmental footprint of the proposed methodology. The time and temperature of extraction were greatly reduced compared to conventional methods. These advantages coupled to the high extraction yields make the proposed technique promising for large-scale applications. In this context, the use of aqueous solutions of ionic liquids for the extraction of value-added ingredients from other biomass sources is straightforwardly envisaged.

Experimental

Materials

Guaraná seeds were purchased at a local market in Aracaju, Sergipe, Brazil. They were kept in sealed plastic bags at $4\text{ }^\circ\text{C}$ until use. The seeds preparation for extraction purposes involved their initial grinding with a commercial coffee grinder. The samples of ground guaraná were further divided and classified according to the particle size by means of stainless steel sieves. Two groups of different particles diameter (d) were isolated: $d < 0.4\text{ mm}$ and $0.4\text{ mm} < d < 1.0\text{ mm}$. The biomass samples were further dried at $105\text{ }^\circ\text{C}$ for $\sim 4\text{ h}$.

The solvents used for the extraction of caffeine from guaraná seeds include water, aqueous solutions of sodium chloride and aqueous solutions of ionic liquids. Double distilled water passed through a reverse osmosis system, and further treated with a Milli-Q plus 185 water purification equipment, was used in all experiments. NaCl, 99.9 wt% pure, was from Normapur.

The ionic liquids $[C_4mim]Cl$, $[C_4mim][Tos]$, $[C_4mpyrr]Cl$, $[C_2mim]Cl$, $[OHC_2mim]Cl$ and $[C_2mim][CH_3CO_2]$ were purchased from Iolitec. All ionic liquid samples were dried, at least for 24 h, under vacuum and at a moderate temperature ($\sim 323\text{ K}$) before use. Their purities were further confirmed by ^1H and ^{13}C NMR spectra and showed to be $\geq 98\text{--}99\text{ wt}\%$. The water content of all ionic liquids, after the drying procedure, was $< 1000\text{ ppm}$ as determined by Karl–Fischer titration.

The water immiscible solvents tested for the re-extraction of caffeine from an aqueous phase were chloroform, $>99\%$ pure from Carlos Erba, ethyl acetate, 99.5% pure from Fluka, hexane, 99.63% pure from Acros Organics, diethyl ether and toluene, 99.5% and 99.9% pure, respectively, from Prolabo, 1-butanol, 99.5% pure from Panreac, and xylene, 95% pure from Pronalys.

The high purity caffeine, used as a standard in the several quantitative and qualitative techniques, was obtained from Marsing & Co. Ltd. with a nominal purity $\geq 98.5\text{ wt}\%$. The other two methylxanthines, theophylline and theobromine, used in capillary electrophoresis and spectroscopic analysis were from Sigma and $\geq 99.0\text{ wt}\%$ pure.

Methods

Extraction of caffeine. All the aqueous solutions were prepared gravimetrically within 10^{-4} g (using an analytical balance Mettler Toledo Excellence-XS205 Dual Range). Mixtures of specific amounts of guaraná ground seeds and

aqueous solutions were also prepared by weight and in specific sealed glass vials. Several concentrations of the solvent of extraction and different solid–liquid ratios were used according to the surface response methodology described below.

The extractions were carried out in a commercial Carousel Radleys Tech equipment able to both stir and maintain the temperature within ± 0.5 °C. In all experiments the stirring was kept constant at 250 rpm.

After the extractions, the overall solution and extract were filtered under vacuum using a $0.45\ \mu\text{m}$ cellulose membrane. After filtration, the extracted liquid solution was quantified through UV-spectroscopy, using a SHIMADZU UV-1700, Pharma-Spec Spectrometer, at a wavelength of 274 nm using a calibration curve previously established for caffeine. The effect of each ionic liquid on the quantification technique was evaluated and it was found to have no major influence taking into account the dilutions carried out. To confirm that caffeine is the predominant methylxanthine being extracted we further employed NMR and capillary electrophoresis analysis in the aqueous extract of several samples to evaluate if any other alkaloids, at least at concentrations higher than the detection limit of the equipment, were concomitantly extracted from the guaraná seeds. The NMR spectra and capillary electrophoresis data were acquired for pure caffeine, guaraná extract after the extraction with water, and guaraná extract after the extraction with $[\text{C}_4\text{mim}]\text{Cl}$ at a concentration of 0.5 M. The ^1H NMR spectra were recorded using a Bruker Avance 300 at 300.13 MHz using deuterated dimethylsulfoxide as a solvent. The capillary electrophoresis analysis was carried out using a P/ACE™ MDQ Beckman Coulter system in a 0.5 m (0.4 m to the detector) \times 50 μm I.D. fused silica capillary. A 0.5 M acetic acid + 100 mM sodium dodecyl sulphate (SDS) solution was used. The separation was achieved at 20 kV (reverse polarity) by direct UV detection at 270 nm. Both techniques revealed that no other alkaloids are being extracted in measurable amounts from the guaraná seeds and support the adequacy of the UV spectroscopy as the major quantification technique.

The amount of caffeine present in the dry seeds was calculated according to the weight of pure caffeine present in the extract divided by the total weight of dry biomass used. At least three individual samples were prepared, and three samples of each aqueous phase were quantified, allowing us to determine the average extraction yield and corresponding standard deviation.

Surface response methodology. The factorial planning allows the simultaneous analysis of various factors and more than one answer.²⁴ In the 2^k factorial planning there are k factors that can contribute to a different response and the data are treated according to a second order polynomial equation:

$$y = \beta_0 + \sum \beta_i \chi_i + \sum \beta_{ii} \chi_i^2 + \sum_{i < j} \beta_{ij} \chi_i \chi_j \quad (1)$$

where y is the response variable and β_0 , β_i , β_{ii} and β_{ij} are the adjusted coefficients for the intercept, linear, quadratic and

interaction terms, respectively, and χ_i and χ_j are independent variables. The 2^3 factorial planning is provided in ESI.† This mathematical model allows the drawing of surface response curves and through their analysis the optimal conditions can be determined.

In this work a 2^3 factorial planning was used with the goal of optimizing the amount of extracted caffeine from guaraná seeds as well as to identify the most significant parameters and their interactions. This planning has been defined by the central point (zero level), the factorial points (1 and -1 , level one) and the axial points (level α). The axial points are encoded at a distance α from the central point:²⁴

$$\alpha = (2^k)^{1/4} \quad (2)$$

The obtained results were statistically analysed with a confidence level of 95%. Student's t -test was used to check the statistical significance of the adjusted data.²⁵ The adequacy of the model was determined by evaluating the lack of fit, the regression coefficient (R^2) and the F -value obtained from the analysis of variance (ANOVA) that was generated.²⁵ Three-dimensional surface response plots were generated by varying two variables within the experimental range and holding the other factors constant at the central point.

In each factorial planning the central point was experimentally addressed at least in triplicate. Additional 12–20 experiments per factorial planning were conducted, for which several operational conditions were repeated to guarantee the accuracy of the data. The number of isolated experiments was limited by the operational conditions capability.

The Statsoft Statistica 8.0® software was used for all the statistical analysis, and Matlab R2010a, The MathWorks, was used for representing the response surfaces and contour plots.

SEM, FTIR and TGA. The SEM pictures, used to evaluate the morphology of the guaraná particles after extraction, were acquired using a Hitachi SU-70 microscope (after carbon evaporation) with a 15 kV acceleration voltage. The FTIR spectra, with a resolution of $4\ \text{cm}^{-1}$, were obtained using a Perkin Elmer BX spectrometer operating in the attenuated total reflection (ATR) mode (equipped with a single horizontal Golden Gate ATR cell). TGA assays were performed with an SETSYS Evolution 1750 analyser, from Setaram Instrumentation. Samples were heated at a constant rate of $10\ \text{°C min}^{-1}$ from 20 to 540 °C under a nitrogen flow of $200\ \text{cm}^3\ \text{min}^{-1}$.

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