NJC

Accepted Manuscript

This is an *Accepted Manuscript*, which has been through the Royal Society of Chemistry peer review process and has been accepted for publication.

Accepted Manuscripts are published online shortly after acceptance, before technical editing, formatting and proof reading. Using this free service, authors can make their results available to the community, in citable form, before we publish the edited article. We will replace this *Accepted Manuscript* with the edited and formatted *Advance Article* as soon as it is available.

You can find more information about *Accepted Manuscripts* in the Information for Authors.

Please note that technical editing may introduce minor changes to the text and/or graphics, which may alter content. The journal's standard Terms & Conditions and the Ethical guidelines still apply. In no event shall the Royal Society of Chemistry be held responsible for any errors or omissions in this *Accepted Manuscript* or any consequences arising from the use of any information it contains.

ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/njc

New Journal of Chemistry RSCPublishing

ARTICLE

Extraction and electrochemical detection of capsaicin and ascorbic acid from fresh chilli using ionic liquids

Cite this: DOI: 10.1039/xoxxoooooox

Benjamin B.Y. Lau, Janjira Panchompoo and Leigh Aldous*

School of Chemistry, UNSW Australia, Sydney, NSW 2052, Australia

Received ooth January 2012,
Accepted ooth January 2012

DOI: 10.1039/xoxxoooooox

www.rsc.org/

Ionic liquids can be used as non-volatile, tunable solvents, extractants and electrolytes. This work investigates their ability to extract an organic analyte, capsaicin, from fresh *Capsicum annuum* Bird's eye chilli peppers (also known as Thai chillies), followed by the electroanalytical quantification of the extracted analyte. Ascorbic acid (Vitamin C) was also extracted and quantified. Two ionic liquids were identified to extract capsaicin from fresh chilli more effectively than the conventional ethanol-based process; the inherently basic 1-ethyl-3-methylimidazolium acetate, [Emim][OAc], and the inherently acidic 1-ethyl-3-methylimidazolium hydrogen sulfate, [Emim][HSO_4]. The ionic liquid extracts could be electrochemically quantified using a bare glassy carbon electrode either in the pure ionic liquid (one pot extraction and quantification) or after dilution with water/ethanol to assist resolution of the capsaicin (flavour indicator) and ascorbic acid (freshness indicator) features.

1. Introduction

Capsaicin (and other compounds in the capsaicinoids family) result in the spicy taste and strong flavour of chillies, hot peppers and other spicy foods.[1] Capsaicin can also be employed in medicines[2] and in anti-personnel sprays.[3] The Scoville Organoleptic Test is the traditional method used to measure the level of capsaicin in hot peppers, and is achieved by dilution of the solution of pepper extract into sugar water until a trained volunteer cannot taste the pungency.[4] The dilution factor required is referred to as the Scoville Unit. The accuracy of this method is arguable because it depends on the volunteer, who may have different sensitivity to the level of hotness.[5] Other methods such as High performance liquid chromatography (HPLC) ,[1, 5-7], gas chromatography (GC)[8] and gas-liquid chromatography (GLC)[9] have also been used for measuring capsaicin, although these techniques can only quantify what has been successfully extracted from the sample matrix.

In 2008 Kachoosangi *et al.* reported the electroanalytical quantification of capsaicin extracted from chilli sauces, using graphite electrodes modified with multiwalled carbon nanotubes.[5] Since then the voltammetric determination of pure capsaicin has been investigated at graphite pencil electrodes,[10] gold nanoparticle/multiwalled carbon nanotube/glassy carbon electrode composites,[11] amino-functionalized mesoporous silica-containing carbon paste,[12] and boron-doped diamond electrodes in the presence of sodium dodecylsulfate.[13] Although these sensitive analytical protocols have been extended to cover chilli sauce[5, 13] and chilli powders,[10, 12] they have not been extended to cover fresh chilli samples. In addition, the use of pre-accumulation of capsaicin followed by its irreversible oxidation was observed to foul the modified electrode surfaces used for quantification.[5, 10, 11]

Ascorbic acid, also known as vitamin C, is a well-known biological compound that is abundant in fruits and vegetables, and a regular intake is essential to maintain our well-being. Ascorbic acid plays a role in producing collagen which is a protein that maintains the structure of bones, teeth, gums, *etc*.[14] A deficiency of ascorbic acid increases the risk of developing cardiovascular disease.[15] The ascorbic acid content of various fruits and vegetables is known to vary as a function of genome, ripeness and age.[16, 17] Ascorbic acid had been previously quantified using a wide range of electrochemical techniques[18-20] and specifically in chilli peppers by other methods such as HPLC[21] and reagent-assisted UV-Vis spectroscopy.[22]

Ionic liquids (ILs) are liquids that contain almost exclusively ions, with the somewhat arbitrary definition of having melting points of 100°C or lower.[23] Typically ILs are organic salts or mixtures of an organic ion and an inorganic ion. Often the cations consist of quaternary nitrogen or phosphorus heterocycles, and the anions are much more diverse in structure and nature. ILs are suitable for electrochemical applications because of their inherent ionic

conductivity and frequently high electrochemical stability.[24] They can be used as pure electrolytes for electrochemistry, or dissolved in other solvents to act as a dilute electrolyte.[23]

In the processing of biomass for analytical purposes, extraction can be used in an attempt to remove much of the analyte, although this is highly dependent upon the size, shape and nature of the biomass sample. Alternatively aggressive digestion using corrosive acids, with long time, high temperature and high pressure can be used to destroy the sample matrix.[25] Recently, ILs have been noted to dissolve a wide range of materials under significantly milder, safer and more rapid conditions.[26, 27,28] Temperatures between room temperature and 195°C have been employed, with a dissolution time of *ca.* 5 min even for challenging matrices such as wood.[29] The ILs themselves are non-volatile and not particularly hazardous.[27] Addition of an appropriate anti-solvent (often water) subsequently precipitates the majority of the dissolved biomass, and the IL can be isolated and recycled.[30]

Electrochemistry is one of the most scalable and portable analytical tools, which can theoretically replace expensive HPLC methods for the quantification of capsaicin[10] and ascorbic acid.[14] Both techniques required the initial extraction of capsaicin from solid sample matrices. As inherently conductive liquids, ILs can make extremely effective electrolytes for electrochemical investigations, directly coupled to the extraction process.

We have therefore investigated the ability of various ILs to extract capsaicin from fresh chilli samples. Electrochemical quantification of the extracted capsaicin was facile, and it was observed that inherently acidic or basic ILs were more effective at extracting capsaicin than the conventional ethanol method. In addition, ascorbic acid could also be effectively extracted from the fresh chilli and quantified. Thus ILs could be employed for the one-pot extraction and quantification of capsaicin (flavour indicator) and ascorbic acid (freshness indicator). The high extraction values meant that a bare glassy carbon electrode could be used, without having to resort to elaborately modified, easily fouled electrode surfaces.

2. Experimental

2.1 Reagents and equipment

All chemicals were of analytical grade and were used as received without any further purification. All aqueous solution were made using Milli Q water with resistivity not less than 18.2 MΩ. All of the electroanalysis experiments were conducted using an Autolab PGSTAT (Ecochemie, the Netherlands) and a three electrode system, which consisted of a glassy carbon working electrode (GC, 3 mm in diameter), a coiled Pt counter electrode and a Ag⏐AgCl (3 M NaCl) reference electrode. For voltammograms recorded in the pure ionic liquids, the reference electrode was a non-aqueous reference electrode kit (BASI Analytical, USA) containing an Ag wire immersed in 0.01M $Ag[NO_3]$ in $[Emim][NTf_2]$.[31] The potentials reported are with respect to the reference electrode. The voltammogram were produced with the software NOVA 1.10 and Origin 8. All cyclic voltammograms displayed as Figures in this work were recorded at a scan rate of 50 mV s^{-1}.

The ionic liquids (ILs) investigated include 1-ethyl-3-methylimidazolium acetate ([Emim][OAc]), 1-ethyl-3-methylimidazolium hydrogen sulfate ($[Emim][HSO_4]$). 1-ethyl-3-methylimidazolium trifluoromethylsulfonate ($[Emim][CF_3SO_3]$), 1-ethyl-3-methylimidazolium bis(trifluoromethylsulfonyl)imide ($[Emim][N(CF_3SO_2)_2]$), and 1-butyl-3-methylimidazolium chloride ([Bmim]Cl). All were purchased from IoLiTec (Germany) and used as received.

The chilli peppers employed were fresh green *Capsicum annuum* Bird's eye chillies (Thailand), also known as Thai chillies. Ultra Death Chilli sauce (Blair's Sauces and Snacks, USA) was used as purchased.

2.2 Method

A new extraction procedure was developed for contacting the chilli with the IL. First of all, the required number of chillies were cut into pieces and thoroughly mixed, in order to avoid discrepancies in strength. This means that individual series of experiments reported below (*e.g.* those shown on the same Figure) are internally consistent, but the total capsaicin concentration in the biomass samples might have varied between different stages of testing (*e.g.* different Figures).

The chopped chillies were added to a glass vessel along with the required amount of ILs; in most experiments a weight ratio of chilli and IL *ca.* 1:0.85 was employed. After the extraction, the ILs was filtered using millipore 0.45μm nylon filter paper. For voltammetry, the filtered solution was made up to 50 mL using 40/60 v/v ethanol/water containing 0.2 M HCl. Each mixture was measured in triplicate. The average value of the peak currents in three measurements was taken and the standard deviation was used as the error bars. Solutions were prepared to contain 0.1 M IL, unless otherwise specified. For example, in order to result in 0.1 M [Emim][OAc], 0.85g of IL was mixed with 1 g chilli, treated as described in the text, and then made up to 50 mL such that it resulted in a solution with 0.1M [Emim][OAc], 0.2 M HCl and 0.02 g chilli mL^{-1} (unless otherwise noted). Slight changes in weight were necessary for the different ILs employed, but as a result the number of moles of IL per gram of chilli was kept constant.

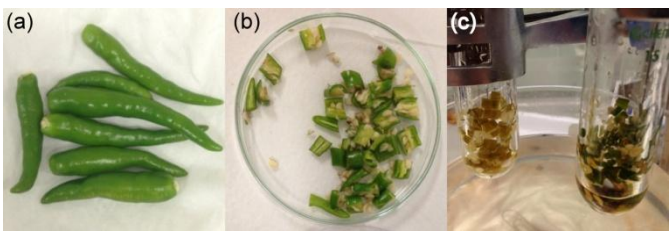

Fig. 1 Extraction scheme showing (a) fresh green *Capsicum annuum* Bird's eye chillies with stems removed, (b) sliced chillies combined to normalise values across experiments, and (c) extraction using different ionic liquids in glass vessels. Ethanol extraction used round bottom flasks with Liebig condensing columns (not shown)

For ethanol, a similar extraction could not be employed. Ethanol was far too volatile; use in a pressure vessel would run the risk of explosion, while to use as small a quantity as was employed for the

IL resulted in rapid drying out. Instead 20 mL of ethanol was used per g of chilli. This was refluxed for 40 min in a round bottom flask with condenser, following the protocol of Barbero *et al.*,[6] made up to its original volume after extraction was complete (to account for evaporation), then diluted with 30 mL of a HCl stock solution.

Enhanced stability and reproducibility in terms of peak shape and peak potential could be achieved before measurements by placing the GC in a 1mM capsaicin solution and scanning between 0 V to 1 V for 20 cycles in order to precondition the electrode surface to yield reproducible results. This resulted in the optimal reproducibility, by the prior interaction of the capsaicin with any reactive oxygen functionality present on the GC electrode surface.

3. Results and discussion

3.1 Electrochemical behaviour of capsaicin in ionic liquid-containing aqueous systems

First, it was necessary to study the electrochemical response of capsaicin in the presence and absence of various ionic liquids (ILs). As capsaicin is water-insoluble, an electrolyte consisting of 0.1 M HCl in 60/40 v/v water/ethanol was chosen. Figure 2 displays the cyclic voltammetry of 1 mM capsaicin at a glassy carbon electrode (GC). Oxidation features can be clearly observed on the first cycle at *ca.* +0.75 V, with a reversible redox couple appearing on the second cycle centred at *ca.* +0.6 V. The observation of these two oxidation features is consistent with what has been previously reported for capsaicin oxidation at CNT-modified graphite electrodes.[5] Namely, capsaicin is firstly oxidised at *ca.* +0.75 V to result in a carbocation intermediate, adjacent to the 2-methoxy group (Figure 3). Next, capsaicin undergoes an irreversible hydrolysis step of the 2-methoxyl group, resulting in the formation of an *o*-benzoquinone unit. This is rapidly oxidised further to result in a catechol-like compound. It is the catechol/benzoquinone redox couple which can be observed on second and subsequent scans at *ca.* +0.6 V.[5]

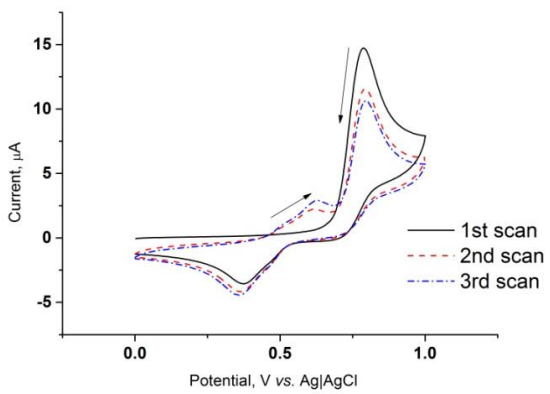

Fig. 2 Three consecutive cyclic voltammograms of 1.0 mM capsaicin at a glassy carbon (GC) electrode in a 60/40 %v/v water/ethanol mixture containing 0.1 M HCl. This and all subsequent cyclic voltammograms correspond to a scan rate of 50 mV s^{-1}.

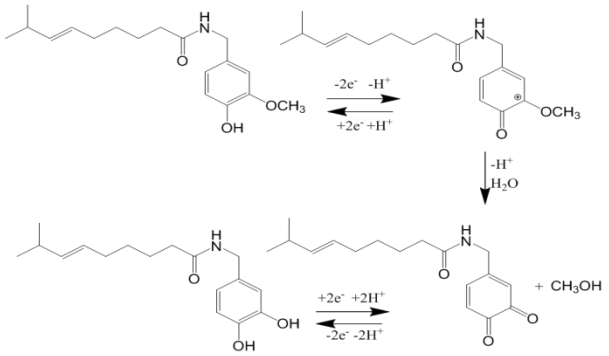

Fig. 3 Proposed reaction scheme of capsaicin (top left), with its electrochemical oxidation to a carbocation intermediate (top right) followed by rapid decomposition to yield an electrochemically reversible quinone/catechol couple, as reported by Kachoosangi *et al.*[5]

The voltammetry was unaffected by waiting time, indicating that physisorption of capsaicin was not significant. Additionally, a scan rate study gave a linear increase in peak current with respect to the square root of the scan rate, indicating that the process is under diffusional control rather than adsorption.[5, 11, 32] No fouling of the electrode was observed upon repeated scanning, *e.g.* up to 20 consecutive measurements could be performed in the same system with essentially identical peak currents each time. All of this differs from the adsorption-controlled, easily fouled conditions noted in prior work; the latter work used µM quantities and modified electrodes,[5, 10, 11] whereas this work used an unmodified electrode and mM quantities. A linear current response of 10.6 (±0.5) µA mM^{-1} capsaicin was observed at a scan rate of 50 mV s^{-1} between 0.1 mM and 10 mM.

Some IL (0.1 M) was added to 1 mM capsaicin solutions to observe if the IL had any quantitative effect upon the capsaicin voltammetry. Figure 4 displays the overlaid cyclic voltammograms of 1 mM capsaicin in the absence and presence of the ILs [Emim][OAc] and [Emim][HSO_4]. It can be seen that there was no significant alteration in the voltammogram before and after the addition [Emim][HSO_4], which was also observed for the ILs [Emim][CF_3SO_3], [Emim][$N(CF_3SO_2)_2$] and [Bmim][Cl] (not shown). However, the addition of [Emim][OAc], one of the most common ILs for biomass dissolution and pretreatment,[30] resulted in a reduction in the peak current, a broadening of the peak and an increase in the peak to peak separation. Addition of acetic acid had no effect upon the voltammetry, while addition of sodium acetate resulted in similar changes, indicating the changes in the pH were responsible. This is consistent with the fact that the electrochemical and chemical pathways involved in capsaicin oxidation are proton-dependant, and the previously observed pH dependence of 100 µM capsaicin at a CNT-graphite electrode.[5] For this reason, subsequent experiments utilised 0.2 M HCl (relative to 0.1 M IL) in order to remove this effect from systems containing [Emim][OAc].

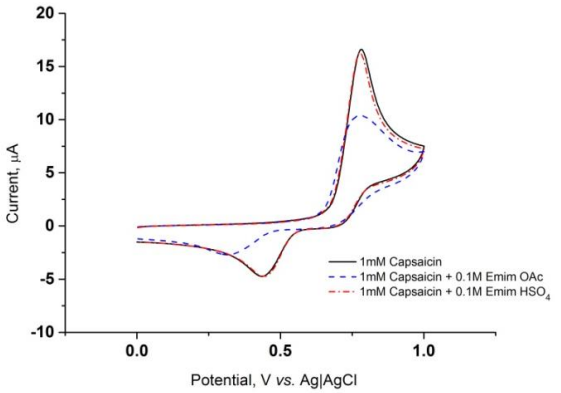

Fig. 4 Overlaid cyclic voltammograms of 1.0 mM capsaisin at a GC electrode in a 60/40 %v/v water/ethanol mixture containing 0.1 M HCl, in the absence (solid) and presence of 0.1 M [Emim][HSO_4] (dot dash) and 0.1 M [Emim][OAc] (dash).

3.2 Electrochemical detection of capsaicin extracted from chilli sauce and fresh chilli

Capsaicin extraction was performed on commercially available chilli sauce and fresh chilli using ethanol, which has been used in previous studies to extract capsaicin from fresh chillies for HPLC analysis[6] and from sauces for electrochemical analysis.[5] Figures 5(a) and 5(b) highlight the results for chilli sauce and fresh chilli, respectively.

Ultra Death Chilli sauce demonstrated a higher concentration of capsaicin (*e.g.* higher peak current), consistent with its reported value of 800,000 Scoville units,[33] and gave a response consistent with pure capsaicin. The fresh chilli, corresponding to an estimated 50,000-100,000 Scoville units,[34] gave a proportionately smaller value. In addition, an oxidation feature was consistently observed on the first scan which has not been previously noted for prior work utilising chilli sauces and powders. Screening of various compounds subsequently identified it as ascorbic acid; the inset in Figure 5(b) displays the voltammetry of 0.18 mM ascorbic acid under the same conditions, which was oxidised at *ca.* +0.7 V. Ascorbic acid has been reported to be electrochemically oxidised to dehydroascorbic acid with further irreversible oxidation to 2,3-diketo-L-gulonic acid.[22] Fresh fruits and vegetables such as fresh chillies are typically rich in ascorbic acid, and ascorbic acid content decreases in foods as a function of time and thermal processing,[35, 36] explaining its absence in the much more highly processed and aged chill sauce. This represents the first simultaneous extraction and electroanalytical quantification of both capsaicin (flavour measure) and ascorbic acid (freshness indicator) from chilli.

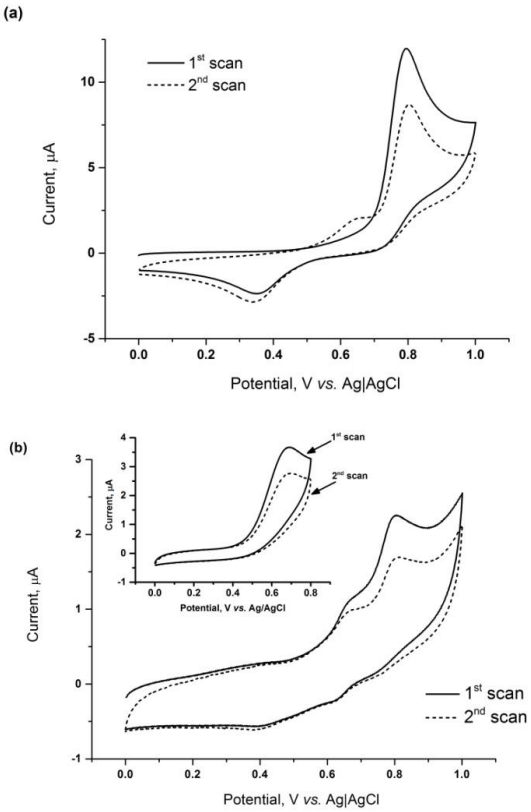

Fig. 5 Two consecutive cyclic voltammograms of the ethanol extracts from (a) 1 g of Ultra Death Chilli Sauce and (b) 1 g of fresh chilli, recorded at a GC electrode in a 60/40 %v/v water/ethanol mixture containing 0.2 M HCl. **Inset to (b)** Cyclic voltammograms of 0.18 mM ascorbic acid at a GC electrode in a 60/40 %v/v water/ethanol mixture containing 0.2 M HCl.

The ethanol-based extraction (1 g chilli, 20 mL EtOH, 40 min, reflux) represented 0.02 g chilli per ml in the final electrolyte after dilution with aqueous HCl. In order for this to be quantitatively compared with IL extractions, many factors had to be normalised in order to account for the various dilution factors, different viscosities, *etc*. To this end, first 1 g chilli was stirred with IL (*ca.* 0.85 g to 1 g IL, based upon molecular weight) for 40 min at 100 ℃, whereafter the IL was diluted to exist as 0.1 M IL in 0.2 M HCl in 60/40 v/v water/ethanol. Thus the quantity of chilli (0.02 g chilli per ml in the final electrolyte) was kept identical, and pH and viscosity differences were kept to a minimum. The only major difference was that the initial ethanol extraction used a *ca.* 20-fold larger volume of extractant. Thus the extraction processes could be quantitatively compared, and Figure 6 displays that [Emim][OAc] resulted in the highest peak current (*e.g.* most complete extraction), followed by [Emim][HSO_4], then the *ca.* 20-fold larger volume of ethanol using the reported optimised process.[6] [Emim][OAc] is known for its ability to dissolve a range of lignocellulosic biomass.[37, 38] The bottom half of Figure 6 numerically displays the above results, as well as those for [Bmim]Cl (equivalent to ethanol) and the ILs [Emim][$N(CF_3SO_2)_2$] and [Emim][CF_3SO_3] (both inferior to ethanol). Interestingly, all ILs were effective at extracting the carotene from the chillies (demonstrated by a significant green colour forming) regardless of their capsaicin extraction ability, but

experiments with beta-carotene demonstrated its lack of electrochemical activity under the utilised conditions.

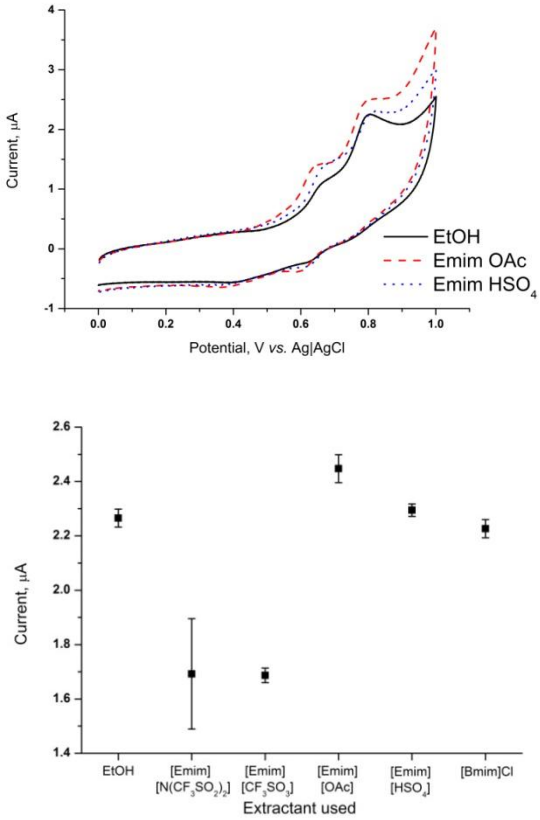

Fig. 6 The top figure displays cyclic voltammograms of 1 g of fresh chilli extracted in ethanol (EtOH, solid line), [Emim][OAc] (long dash) and [Emim][HSO_4] (short dash) at 100 °C for 40 min, recorded at a GC electrode in a water/ethanol 60/40 %v/v mixture containing 0.2 M HCl (+ 0.1 M of the relevant IL). The bottom figure displays the peak currents obtained for EtOH extraction and the five ILs investigated.

By comparing the peak currents obtained for capsaicin and ascorbic acid extracted using [Emim][OAc] with the previously noted linear calibration for pure capsaicin (10.6 µA mM^{-1} capsaicin) and another calibration obtained for ascorbic acid (20.0 µA mM^{-1} ascorbic acid), it was possible to quantify the amount extracted. The values for [Emim][OAc] correspond to 3.53 mg capsaicin per g chilli. Converting the quoted heat values for *Capsicum annuum* Bird's eye chilli peppers (50,000-100,000 Scoville units[34]) using a reported conversion factor of 16,000 Scoville units per mg of capsaicin,[9] this equates to a recovery of between 56% to 113% capsaicin relative to the anticipated content. This also compares to previously reported values of 3.1 mg to 5.1 mg capsaicin per g chilli for *Capsicum annuum* chillies of similar heat values, as quantified by HPLC[39] and gas chromography (GC).[8] Also extracted was 0.62 mg ascorbic acid per g chilli, which represents between 24% and 326% recovery compared to that previously quantified (spectrophotometrically) for green *Capsicum annuum* chilli peppers.[16, 17]

3.3 Effect of chilli extraction temperature on the extraction and electrochemical determination of capsaicin

[Emim][OAc] was chosen as the target IL for capsaicin and ascorbic acid extraction as it has been consistently effective in biomass pretreatment, and gave the highest peak current in Figure 6. The optimum temperature was then investigated (extraction time 40 min) and Figure 7 summarises the results. Improved extraction was oberved at 50 ℃ when compared to that at 25 ℃. The peak size at 50 ℃ and 100 ℃ were similar, except the peaks were significantly more poorly resolved at the higher temperature, resulting in a lower peak current at 100 ℃ after baseline correction. At 150 ℃ the absolute peak current increased overall, but peak resolution was extremely poor and baseline extrapolation was not possible. After extraction at 150 ℃ the chilli pieces were significantly darkened, and side reactions likely occurred. The temperature of 50 ℃ was therefore chosen as the optimum temperature.

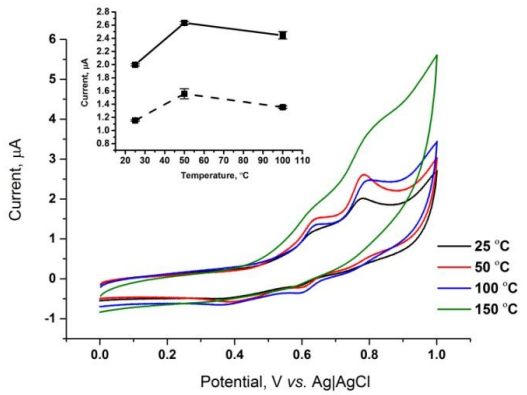

Fig. 7 Overlaid cyclic voltammograms of 1 g of fresh chilli extracted in 0.1 M [Emim][OAc] for 40 min at 25, 50, 100 and 150 °C, recorded at a GC electrode in a 0.1 M [Emim][OAc]-water/ethanol 60/40 %v/v mixture containing 0.2 M HCl. **Inset** Plot of peak current vs. chilli extraction temperature for the reduction peaks of capsaicin (solid) and ascorbic acid (dashed).

To find out the optimum extraction time once the chilli was exposed to the IL, experiments were performed as a function of extraction time. However, the results were initially irreproducible, and it was observed that the most significant factor was actually the time between chopping the chillies and extraction; the extraction efficiency decreased (regardless of extraction time, for both capsaicin and ascorbic acid) as a function of time passing since chopping the chilli. This has not been previously discussed in HPLC and GC investigations of fresh chillies[39,8] (likely due to a lack of awareness of this factor) and is potentially due to some enzymatic reaction initiated by exposure of the interior of the chilli to oxygen. Such reactions are widely known for other foods, such as the rapid enzymatic generation of garlic's characteristic flavour and fragrance only after chopping,[40] and the much slower development of vanillin from vanilla pods by enzymatic degradation of its glucoside precursor.[41]

Thereafter, chillies were chopped and immediately extracted (1 g chilli with 0.85 g [Emim][OAc], diluted to 0.1 M [Emim][OAc] before measurement) at 50 ℃ between 20 mins and 24 hours (Figure

8). The extraction trend was similar to the trend observed as a function of chopping time, with a clear maximum in extraction for both capsaicin and ascorbic acid after 1 hour, followed by a gradual decrease (to *ca.* 65% of its peak value) at longer times, indicating the enzymatic process was ongoing during extraction. This trend was consistently observed in repeat extractions, and the capsaicin peak current was found to be indefinitely stable but only after filtration to remove the chilli slices.

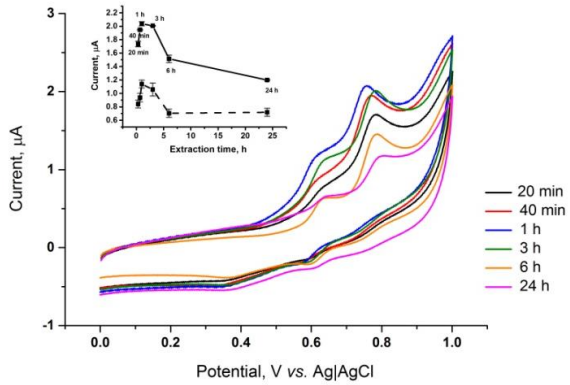

Fig. 8 Overlaid cyclic voltammograms of 1 g of fresh chilli extracted in 0.1 M [Emim][OAc] at 50 °C for a range of times (20 min to 24 h), recorded at a GC electrode in a 0.1 M [Emim][OAc]-water/ethanol 60/40 %v/v mixture containing 0.2 M HCl. **Inset** Plot of peak current vs. chilli extraction time for the reduction peaks of capsaicin (solid) and ascorbic acid (dashed).

3.4 Effect of chilli:ionic liquid ratios on the extraction and electrochemical determination of capsaicin in ionic liquid-based aqueous systems

To find out the optimum chilli:IL ratio, 1 g chilli was extracted with increasing quantities of [Emim][OAc]. Figure 9(a) highlights the six different IL:chilli ratios employed, as well as the equivalent concentration of IL once diluted to 50 mL (dilution factor maintained at 0.02 g chilli per ml electrolyte). For 0 M, the chilli was treated the same (*e.g.* placed in an empty vessel, heated, placed on the filter and washed with ethanol, before being diluted with aqueous HCl) and the very small capsaicin and ascorbic acid peaks corresponds to that removed by ethanol washing alone.

A clear maximum for both extractants can be observed at 0.25 M [Emim][OAc], or a ratio of 2.12g IL for 1 g chilli. Beyond this value the peak current decreases slightly, but this is attributed to the associated pH changes with increasing amounts of the basic[42] IL [Emim][OAc] resulting in a smaller peak current. Beneficially, the ascorbic acid peaks became much more well defined. Both ascorbic acid and capsaicin displayed Nernstian shifts of *ca.* 59 mV pH unit^{-1}, as both are two electron two proton oxidations[5, 43, 44] with the apparent rate of electron transfer increasing with increasing pH for ascorbic acid (up to a pH of *ca.* 4)[45], while the opposite applies for capsaicin.[5]

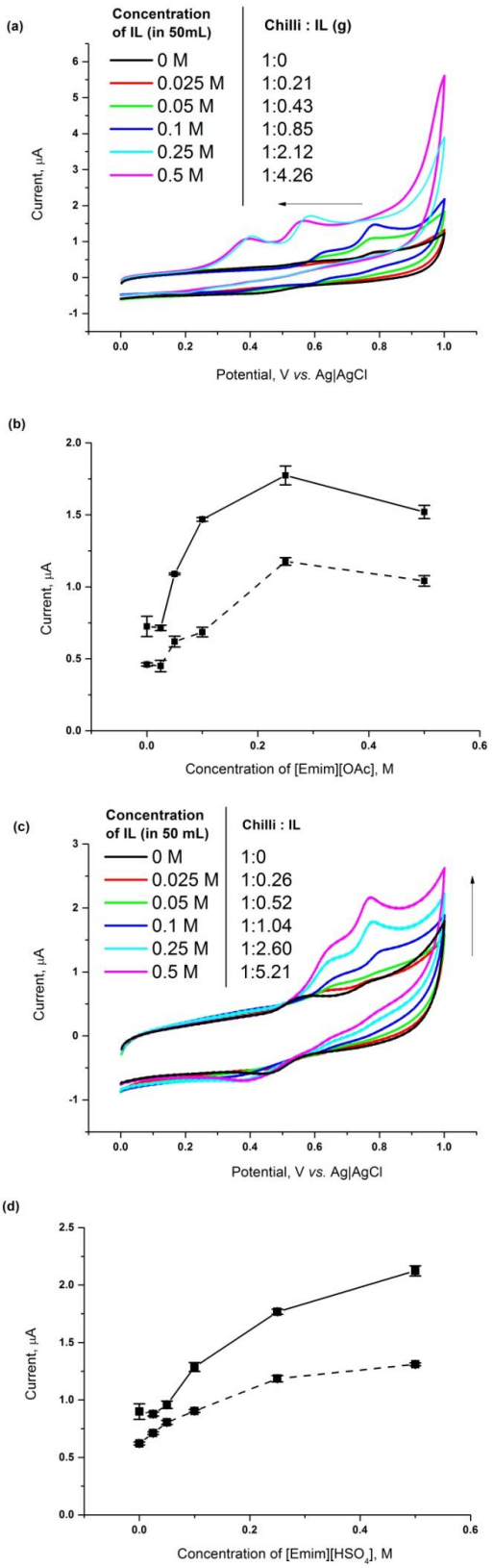

Fig. 9 Overlaid cyclic voltammograms of fresh chilli extracted in different amounts of either (a) [Emim][OAc] or (c) [Emim][HSO_4] and graph of current vs concentration for (b) [Emim][OAc] or (d) [Emim][HSO_4] at 50 °C for 1 h, recorded at a GC electrode in an IL-water/ethanol 60/40 %v/v mixture containing 0.2 M HCl. Also shown here are plots of peak current for capsaicin (solid) and ascorbic acid (dashed) vs. concentration of IL in the final electrolyte.

The inherently acidic IL [Emim][HSO_4] was also evaluated. The results are summarised in Figure 9(b), and displays that the capsaicin peak current is roughly proportional with respect to the amount of [Emim][HSO_4] added, indicating better extraction as more IL was added. Due to the inherent acidity of the IL, no peak shifts were observed but this also resulted in poorly resolved ascorbic acid features.

3.5 Chilli extraction and measurement in pure ILs

Extraction and measurement was also performed in the pure ILs without subsequent dilution. In these experiments, 1 g of fresh chilli was placed into a sample vial with 1 g IL and extracted for 1 hour at 50 °C. The data is displayed in Figure 10, and highlights that despite the significantly higher viscosity of the ILs (relative to their diluted mixtures) the overall current response was much greater due to no dilution. Capsaicin oxidation was observed at *ca.* +1.2 V (*vs.* Ag/Ag^+ in IL) in the inherently acidic [Emim][HSO_4], and was correspondingly more cathodic and less well resolved in the inherently basic IL [Emim][OAc]. This is despite [Emim][OAc] having a superior extraction efficiency when all factors were normalised by dilution (as shown in Figure 4), and again derives from the inherent basicity of pure [Emim][OAc], which likely assists in the extraction of capsaicin but hinders its subsequent electrochemical quantification.

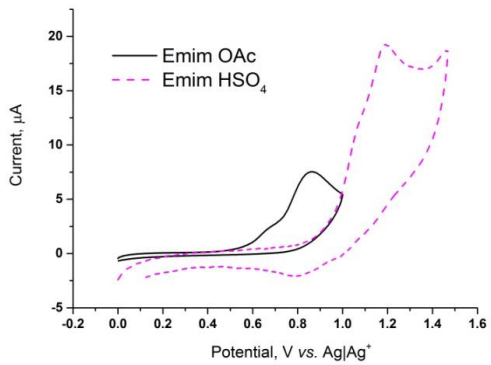

Fig. 10 Overlaid cyclic voltammogram of 1 g of fresh chilli extracted in 1 g of [Emim][OAc] (solid) and 1 g of [Emim][HSO_4] (dashed) by stirring at 50 °C for 1 h, recorded at a GC electrode in the pure IL system.

The superior extraction ability of the ILs, combined with their non-volatility means that analytical procedures aimed at the quantification of capsaicin can be designed to operate on a smaller scale, be completed over a shorter time, and occur under milder conditions than those which are currently available and use poorer extraction solvents than ILs. However, under these pure IL conditions simultaneous ascorbic acid quantification could not be performed due to the more poorly resolved oxidation features, likely due to higher viscosity and potentially slower electron transfer in the pure IL.[46] However, subsequent dilution and pH modification can allow the simultaneous quantification of both capsaicin (flavour indicator) and ascorbic acid (freshness indicator) present in fresh chilli (*c.f.* Figure 9(a)).

Having identified in the key roles of inherent acidity and basicity in the ILs, we are now working on developing ILs which can fully digest the chilli. Previous work has always quantified capsaicin and ascorbic acid based upon the material extracted and assumed 100% extraction. However, this extraction is clearly dependent upon the size and nature of the chilli pieces, and apparently (as demonstrated in this work) in a complicated manner with the period of time passed since being chopped. By moving to a complete digestion and extraction system, a one-pot, calibration-less method can be realised for the quantification of total capsaicin and ascorbic acid content in fresh chilli. This analytical route will also likely be successful in quantifying other key quality indicators for other food materials.

4. Conclusions

Two ionic liquids (ILs), [Emim][OAc] and [Emim][HSO_4], demonstrated superior extraction ability of capsaicin and ascorbic acid from fresh chilli, relative to a range of other ILs as well as the widely utilised ethanol extraction, likely due to the inherent acidity and basicity of these ILs, respectively. [Emim][HSO_4] was the optimum IL for extraction and electrochemical measurement of capsaicin without dilution. Extraction with either [Emim][OAc] or [Emim][HSO_4] followed by dilution with 0.2 M HCl in 60/40 v/v water/ethanol resulted in smaller peaks but improved resolution, allowing both capsaicin and ascorbic acid to be individually electrochemically quantified. By focussing upon extraction and by using a bare glassy carbon electrode, these species could be easily quantified without electrode fouling. A complicated relationship between chopping time and extraction ability was noted for the first time.

Under optimised conditions (1 hr, 50°C, *ca.* 5:1 IL:chilli ratio), acidic IL [Emim][HSO_4] resulted in the optimum extraction of capsaicin and ascorbic acid. Its extraction ability was superior to the optimised literature extraction method, which utilised a much larger volume of ethanol and higher temperatures.

5. Acknowledgements

The Australian Research Council (ARC DECRA DE130100770) is acknowledged for funding.

References

1. G. Barbero, A. Liazid, M. Palma and C. Barroso, *Food Chem*, 2008, **107**, 1276-1282.
2. G. C. Morris, S. J. Gibson and R. D. Helme, *Pain*, 1995, **63**, 93-101.
3. L. K. Pershing, C. A. Reilly, J. L. Corlett and D. J. Crouch, *J. Appl Toxicol*, 2006, **26**, 88-97.
4. W. Scoville, *J Am Pharm Assoc*, 1912, **1**, 453.
5. R. T. Kachoosangi, G. G. Wildgoose and R. G. Compton, *Analyst*, 2008, **133**, 888-895.
6. G. F. Barbero, M. Palma and C. G. Barroso, *Analytica chimica acta*, 2006, **578**, 227-233.

7. G. F. Barbero, M. Palma and C. G. Barroso, *J Agr Food Chem.*, 2006, **54**, 3231-3236.
8. B. V. Thomas, A. A. Schreiber and C. P. Weisskopf, *J Agr Food Chem*, 1998, **46**, 2655-2663.
9. P. H. Todd, M. G. Bensinger and T. Biftu, *J Food Sci*, 1977, **42**, 660-665.
10. Y. Yardim and Z. Senturk, *Talanta*, 2013, **112**, 11-19.
11. T. Mpanza, M. I. Sabela, S. S. Mathenjwa, S. Kanchi and K. Bisetty, *Anal Lett*, 2014, DOI: 10.1080/00032719.2014.924010, 140623065410009.
12. Y. Ya, L. Mo, T. Wang, Y. Fan, J. Liao, Z. Chen, K. S. Manoj, F. Fang, C. Li and J. Liang, *Colloids Surf, B*, 2012, **95**, 90-95.
13. Y. Yardım, *Electroanal*, 2011, **23**, 2491-2497.
14. S. Yilmaz, M. Sadikoglu, G. Saglikoglu, S. Yagmur and G. Askin, *Int J Electrochem Sc*, 2008, **3**, 1534-1542.
15. P. Tveden-Nyborg and J. Lykkesfeldt, *Antioxid Redox Signaling*, 2013, **19**, 2084-2104.
16. B. M. Khadi, J. V. Goud and V. B. Patil, *Qual Plant*, 1987, **37**, 9-15.
17. O. A. Kumar and S. S. Tata, *Not Sci Biol*, 2009, **1**, 50-52.
18. J. A. Cooper, M. Wu and R. G. Compton, *Anal Chem*, 1998, **70**, 2922-2927.
19. D. Lowinsohn, P. T. Lee and R. G. Compton, *Int J Electrochem Sci*, 2014, **9**, 3458-3472.
20. J. A. Rodrigues, I. M. Valente, L. M. Goncalves, J. G. Pacheco and A. A. Barros, *Collect Czech Chem C*, 2010, **75**, 731-741.
21. R. Poon, I. Chu, P. Lecavalier, A. Bergman and D. C. Villeneuve, *J Biochem Toxicol*, 1994, **9**, 297-304.
22. W. Zeng, F. Martinuzzi and A. MacGregor, *J. Pharm Biomed Anal.*, 2005, **36**, 1107-1111.
23. M. Freemantle, *An Introduction to Ionic Liquids*, The Royal Society of Chemistry, Cambridge, 2010.
24. M. Galiński, A. Lewandowski and I. Stępniak, *Electrochimica Acta*, 2006, **51**, 5567-5580.
25. K. Shill, S. Padmanabhan, Q. Xin, J. M. Prausnitz, D. S. Clark and H. W. Blanch, *Biotech bioeng*, 2011, **108**, 511-520.
26. A. M. da Costa Lopes, K. G. João, A. R. C. Morais, E. Bogel-Łukasik and R. Bogel-Łukasik, *Sust Chem Processes*, 2013, **1**, 3.
27. A. Brandt, J. Gräsvik, J. P. Hallett and T. Welton, *Green Chem*, 2013, **15**, 550.
28. T. Vancov, A.-S. Alston, T. Brown and S. McIntosh, *Renew Energ*, 2012, **45**, 1-6.
29. W. Li, N. Sun, B. Stoner, X. Jiang, X. Lu and R. D. Rogers, *Green Chem*, 2011, **13**, 2038.
30. M. M. Hossain and L. Aldous, *Aus J Chem*, 2012, **65**, 1465.
31. M. M. Hossain, E. H. B. Anari and L. Aldous, *Electrochem Commun*, 2013, **34**, 331-334.
32. R. G. Compton and C. E. Banks, *Understanding voltammetry*, Imperial College Press, Singapore ; London, 2nd ed. edn., 2011.
33. How Hot Is My Hot Sauce, http://www.ushotstuff.com/hotSauceHeatScale.htm, (accessed August 2014).
34. Just How Hot Are My Chiles, http://www.ushotstuff.com/Heat.Scale.htm, (accessed August 2014).
35. R. Paul and U. Ghosh, *Indian J Biotechnol*, 2012, **11**, 309-313.
36. S. Martinez, M. Lopez, M. Gonzalez-Raurich and A. Bernardo Alvarez, *Int J Food Sci Nutr*, 2005, **56**, 45-51.
37. S. P. Magalhães da Silva, A. M. da Costa Lopes, L. B. Roseiro and R. Bogel-Łukasik, *RSC Adv*, 2013, **3**, 16040.
38. A. Sant'Ana da Silva, S. H. Lee, T. Endo and E. P. Bon, *Bioresource technol*, 2011, **102**, 10505-10509.
39. Z. A. Al Othman, Y. B. H. Ahmed, M. A. Habila and A. A. Ghafar, *Molecules*, 2011, **16**, 8919-8929.
40. B. C. M. Martindale, L. Aldous, N. V. Rees and R. G. Compton, *Analyst*, 2011, **136**, 128-133.
41. N. J. Gallage, E. H. Hansen, R. Kannangara, C. E. Olsen, M. S. Motawia, K. Jorgensen, I. Holme, K. Hebelstrup, M. Grisoni and B. L. Moller, *Nat Commun*, 2014, **5**.
42. Q. Huang, Q. Wang, Z. Gong, G. Jin, H. Shen, S. Xiao, H. Xie, S. Ye, J. Wang and Z. K. Zhao, *Bioresource technol*, 2013, **130**, 339-344.
43. F. H. Lin, J. Y. Lin, R. D. Gupta, J. A. Tournas, J. A. Burch, M. A. Selim, N. A. Monteiro-Riviere, J. M. Grichnik, J. Zielinski and S. R. Pinnell, *J Invest Dermatol*, 2005, **125**, 826-832.
44. R. J. Wilson, A. E. Beezer and J. C. Mitchell, *Thermochim Acta*, 1995, **264**, 27-40.
45. F. Wantz, C. E. Banks and R. G. Compton, *Electroanal*, 2005, **17**, 1529-1533.
46. S. R. Belding, N. V. Rees, L. Aldous, C. Hardacre and R. G. Compton, *J Phys Chem C*, 2008, **112**, 1650-1657.

