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Ionic liquid-high performance extractive approach to recover carotenoids from *Bactris gasipaes* fruits†

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Nowadays, one of the biggest challenges for society is the development of appropriate technologies to process the waste residue produced worldwide. In the food sector, the generated waste is estimated to be nearly billions of tons annually. Brazil is one of the most representative examples of the economic and industrial potential of underexplored residues and raw materials. The palm heart, scientifically known as *Bactris gasipaes*, mainly its fruits, is one of the many examples found in Brazilian flora. The fruits have significant amounts of carotenoids, namely, the all-*trans*-β-carotene, all-*trans*-lycopene and the rare all-*trans*-γ-carotene, which are considered as excellent raw materials of commercial interest. However, the main challenge that remains is their efficient recovery. This work proposes the development of a performant process of extraction mediated by the use of ionic liquid (IL)-based ethanolic solutions. Four ILs were examined, as well as the solid–liquid ratio $R_{(S/L)}$, number of extractions, the time of extraction, the co-solvent-ratio $R_{(IL/E)}$ and the homogenization method employed. After selecting the best solvent ($[C_4mim][BF_4]$) and process conditions (extraction yield of 172 ± 18 $\mu g_{carotenoids}\ g_{dried\ biomass}^{-1}$), the IL-ethanolic solution recyclability was tested by freezing/precipitating the IL (maximum of 94% of IL recovered), proving its success for at least 10 cycles while decreasing the process carbon footprint by 50% compared with the conventional method using acetone.

Introduction

The residue derived from worldwide biodiversity is massive. In the food industry, waste generation is estimated at billions of tons annually.[1] Brazil is one of the most representative examples of the economic and industrial potential of underexplored residues, where the exploitation of the palm heart, scientifically known as *Bactris gasipaes*, stands out. This is a tropical species from the American continent belonging to the Arecaceae family, originally cultivated by indigenous populations.[2] The by-products generated from palm heart exploitation result in additional costs to the producer, since they must provide adequate disposal of the waste materials, which are environmentally and economically problematic.[3] Indeed, one of the most abundant residues is the fruit, popularly known as peach palm.[4] Despite their biochemical importance, namely in terms of carotenoids, they are not being significantly explored. Examples of their carotenoids are the all-*trans*-β-carotene, all-*trans*-lycopene and the rare all-*trans*-γ-carotene,[5] which are therefore an excellent source for the generation of new high-value products, applicable not only in the food industry, but also in the cosmetic and pharmaceutical fields. Currently, there is great demand to reduce the use of synthetic colorants, mainly in the food industry, due to the risk of allergies and respiratory and epidermal diseases.[6] Therefore, natural pigments are of great interest for industry; specifically, carotenoids have proved to be suitable replacements for synthetic reddish-yellow dyes.[7]

Carotenoids are natural pigments widely distributed in fruits and vegetables, with high protective action against photo-oxidative damage of cells.[8] Moreover, carotenoids are generally recognized by their anti-inflammatory activity and anti-cancer properties.[9] They are defined as lipophilic compounds that possess a long chain of conjugated double bonds, each able to form geometrical stereoisomers with different chemical and physical properties.[10] Due to their hydrophobic character, the extraction and purification steps to obtain pure carotenoids are usually performed using petroleum-derived volatile organic solvents, like methanol and chloroform, or solvents chemically prepared through complex synthetic pro-

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cesses.[11] Currently, the main challenge for industry is the development of efficient and low-cost downstream (extraction and purification) processes for carotenoids and, in general, natural-based bioactive compounds while maintaining their physicochemical and colorant properties.[12] Recently, there have been efforts to develop "more sustainable" isolation processes of natural products from various raw materials, namely, using membranes, imprinted polymers, ionic liquids, deep eutectic solvents, and chromatographic techniques.[13–16]

In the last 15 years, ionic liquids (ILs) have been recurrently investigated in different fields due to their unique properties. In fact, contrary to what was considered in the first decade of investigating ILs as solvents,[17] the paradigm about their "green" nature has changed. The idea that the ILs were green because of their non-volatility was challenged on several occasions, particularly by proving their toxicity and environmental persistence.[18] However, this claim is more complicated when ILs are used in the development of chemical/industrial processes. Considering the 12 principles of Green Chemistry, the development of a "green" process is a difficult task. However, the research community believes that the sustainability of the processes can be crucially improved by considering their performance in the overall process.[19] For sustainability, the IL research community identified three main topics, namely, their (i) increased capacity to improve the efficiency of the processes, (ii) higher biocompatibility with the compounds being extracted and, (iii) if/when possible, decreasing the economic impact of the processes, for example, by IL recyclability.[14,16] Hence, ILs seem to be advantageous solvents when correctly applied to the extraction and purification of a plethora of natural compounds in organic and aqueous media.[20,21] Some examples have been reported in the literature in which different compounds were processed from various raw materials, carotenoids being one such example.[20,21] These were recovered from different sources by applying ILs;[11,12] their advantageous application over conventional organic solvents while maintaining the pigments' chemical structure and main properties has been reported. Considering the high number of value-added compounds accumulated in nature, it seems promising to recover maximum possible bioactive compounds from residues and underexplored raw materials. Indeed, in circular economic thinking, the conversion of natural compounds to new bio-based products can improve economic growth from (primary) resources use.

This work proposes the development of an efficient process of extraction mediated using IL-based ethanolic solutions. For this purpose, different ILs were tested and several conditions were optimized, namely, the solid–liquid ratio $R_{(S/L)}$, the number of extractions, time of extraction, the co-solvent-ratio $R_{(IL/E)}$ and the homogenization method applied. This analysis was done by applying factorial planning, where the software-assisted optimization *via* the design of experiments (DoE) was used.[12,16,22] This approach does not require extensive theoretical knowledge, allowing us to reduce the number of experiments, and can be easily employed by academia and industry. After the proper optimization, the most efficient solvent was selected, considering not only its higher extraction yield but also its higher biocompatibility with carotenoids in terms of thermal stability and antioxidant activity. In the end, the industrial potential of this process was assessed not only by studying recycling of the IL (10 cycles) but also by assessing the environmental impact of the alternative process in terms of carbon footprint.

Results and discussion

Solid–liquid extraction of carotenoids

Selection of IL. As a first step, the screening of ILs was performed, aiming to identify the solvent with the highest extractive performance. Four ionic compounds were tested, namely $[C_4mim][BF_4]$, $[C_4mim][PF_6]$, $[C_4mim]Cl$ and $[C_6mim]Cl$, meaning two hydrophilic- and two hydrophobic-based ILs. In this work, the studies were carried out in ethanolic media instead of aqueous solution, thus avoiding any degradation of the fluorinated ILs, where the production of acids occurs by hydrolysis.[23] In addition to ethanol's efficient action in the extraction of carotenoids from marine raw materials, it has also been advantageously approved in most of the industrial fields applying carotenoids.

All extracts were prepared in triplicate and by applying the different ILs under the same conditions of $R_{(S/L)}$, time of extraction and $R_{(IL/E)}$. From Fig. 1, it can be observed that for both $[C_4mim]Cl$ and $[C_4mim][BF_4]$ with ultrasonic probe homogenization, the obtained extraction yields of carotenoids were greater than that obtained with the conventional solvent, *i.e.* acetone (88 ± 7 $\mu g_{carotenoids}\ g_{dried\ biomass}^{-1}$), at 117.7 ± 0.4 $\mu g_{carotenoids}\ g_{dried\ biomass}^{-1}$ and 135 ± 3 $\mu g_{carotenoids}\ g_{dried\ biomass}^{-1}$, respectively. On the contrary, $[C_4mim][PF_6]$ (yield of extraction = 67.5 ± 0.7 $\mu g_{carotenoids}\ g_{dried\ biomass}^{-1}$) and $[C_6mim]Cl$ (yield of extraction = 63.5 ± 5.8 $\mu g_{carotenoids}\ g_{dried\ biomass}^{-1}$) were not able to surpass the yield of extraction obtained by applying acetone, a result independent of the method of homogenization (ANOVA: $p > 0.0001$). When the method of homogenization was analyzed, it seems from Fig. 1 that ultrasonic probe homogenization is more efficient than ultra-dispersion extraction. Therefore, $[C_4mim]Cl$ and $[C_4mim][BF_4]$ as well as the ultrasonic-assisted probe were chosen for further the optimization tests.

Optimization of the solid–liquid extraction step. The extractions under different experimental conditions were carried out by applying a factorial planning design 2^{5-1}. The yields of extraction of carotenoids for the different assays proposed by factorial planning are shown in Table S1 (ESI†). The experimental data indicate that the yield of extraction of carotenoids ranged from 38.13 $\mu g_{carotenoids}\ g_{dried\ biomass}^{-1}$ to a maximum of 132.71 $\mu g_{carotenoids}\ g_{dried\ biomass}^{-1}$. $[C_4mim][BF_4]$ increased the yield of carotenoid extraction by 25 ± 8 $\mu g_{carotenoids}\ g_{dried\ biomass}^{-1}$ (Fig. 2A). The experimental data also indicate that the number of extractions (repetitions) increases the yield of extraction of carotenoids (ANOVA: $p < 0.05$) (Fig. 2A). Despite being non-significant, the 2^{5-1} design indicated that when the time of extraction increased and $R_{(IL/E)}$ decreased, the yield of extraction seems to increase as well (Fig. 2A). For the all-*trans*-β-carotene content, the only significant variable was the type of IL, where

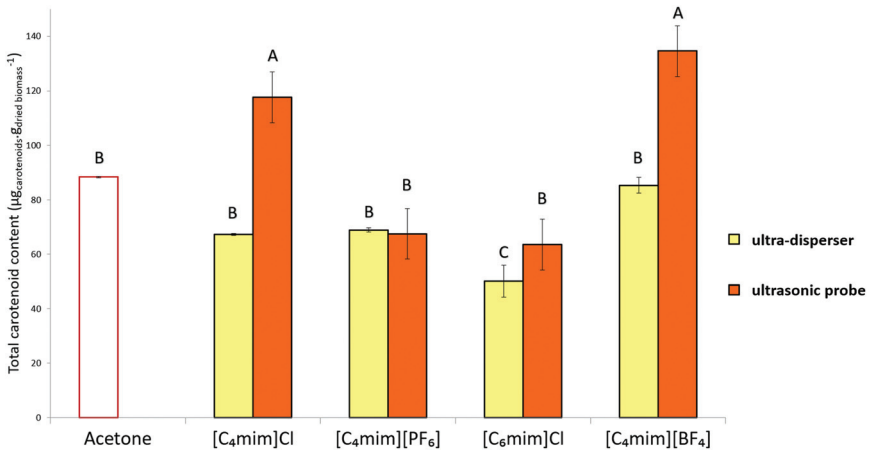

Fig. 1 Screening of different ethanolic IL solutions on the extraction of carotenoids from peach palm fruit using two homogenization methods, the ultrasonic probe and ultra-dispersion. Letters represent statistically equivalent results. Acetone is used as the control (white bar).

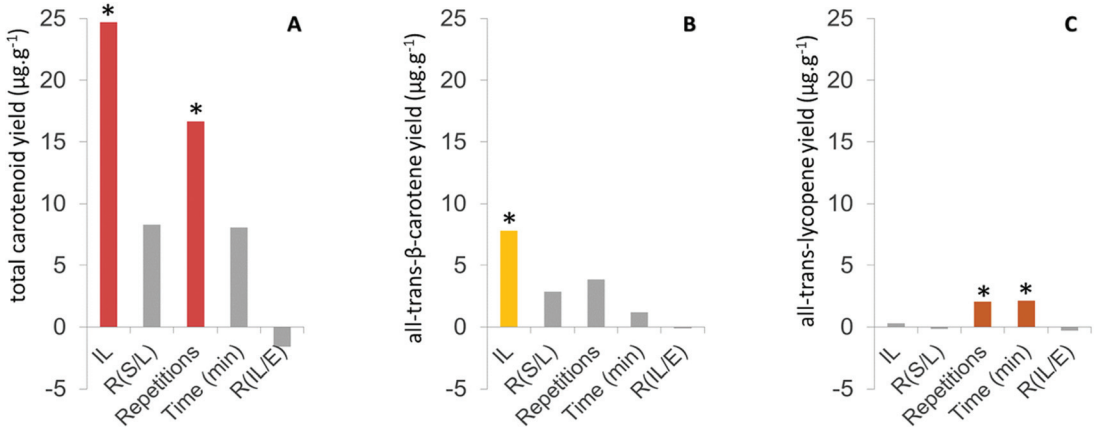

Fig. 2 Experimental results obtained for the factorial planning design (2^{5-1}). The main effects of each variable are described for (A) total carotenoids, (B) all-*trans*-β-carotene and (C) all-*trans*-lycopene contents.

$[C_4mim][BF_4]$ increased the yield of extraction by 8 ± 2 $\mu g_{carotenoids}\ g_{dried\ biomass}^{-1}$ (ANOVA: $p = 0.0568$) (Fig. 2B). Considering all-*trans*-lycopene, the results showed that a high number of extractions over a long time increased the yield of extraction of carotenoids by approximately 2 $\mu g_{carotenoids}\ g_{dried\ biomass}^{-1}$ (ANOVA: $p < 0.05$) (Fig. 2C). For all-*trans*-γ-carotene, the significant variables were the IL type and the number of extractions performed, while the variable $R_{(S/L)}$ showed no significant effect. Again, this suggested the higher extractive performance of $[C_4mim][BF_4]$ over the hydrophilic $[C_4mim]Cl$ (ANOVA: $p < 0.05$), which was already discussed by other authors. Indeed, the hydrophobic nature of $[C_4mim][BF_4]$ induces more favorable interactions with hydrophobic molecules, like the carotenoids discussed in this work,[21,24,25] in addition to the high affinity of this IL to dissolve membrane biopolymers.[26]

At the end of the factorial planning, the IL ($[C_4mim][BF_4]$) and $R_{(IL/E)}$ (1 : 1) were fixed and further explored in the CCRD (2^3). The results obtained for the carotenoid extraction process using $[C_4mim][BF_4]$ with different experimental conditions of the 2^3 are described in Table S2 (ESI†). The yields of the carotenoid extracts as well as the most abundant carotenoids composing the peach palm were used as response variables to prepare the predictive model. In the 2^3 planning design developed, the yield of extraction of carotenoids varied from 98.66 $\mu g_{carotenoids}\ g_{dried\ biomass}^{-1}$ to 157.65 $\mu g_{carotenoids}\ g_{dried\ biomass}^{-1}$. The main effects and interactions were estimated for the total carotenoid content extracted, resulting in eqn (1).

$$\text{total carotenoid}\ (\mu g\, g^{-1}) = 136.41 - 6.74(x1) - 7.45(x2)^2 + 10.20(x3) + 9.356(x2 \times x3) \quad (1)$$

An ANOVA test was applied to evaluate the adequacy of the data and verify the possibility of obtaining a model considering the different responses. The model confidence was determined by the R^2 value (0.75). The F value was approximately three times the F value tabulated for the total carotenoid content, exceeding the 95% confidence level.[27] It was observed that carotenoid extraction was maximized when $R_{(S/L)}$ presented the

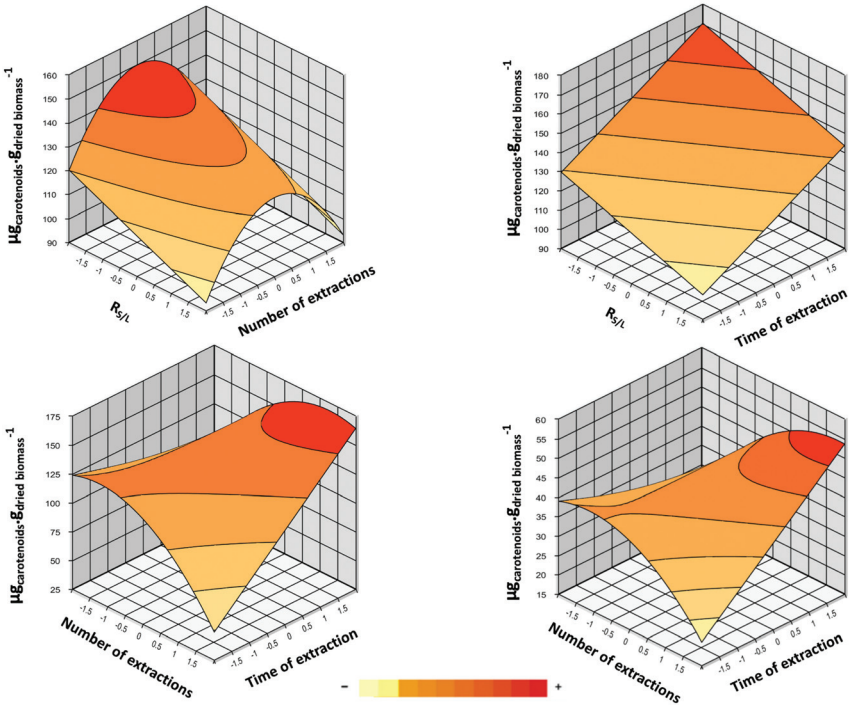

Fig. 3 Response surface plots obtained for the factorial planning design (2^3): the extraction yields of carotenoids (A–C) and all-*trans*-β-carotene (D). The conditions under study were combined considering the $R_{(S/L)}$, the number of extractions and the time of extraction (min).

lowest value and, thus, the optimal condition for this parameter was defined as being a 1 : 1 ratio (Fig. 3A and B). In this case, the yield of extraction was maximized by the minimization of the IL amount, which indicates a more economic extraction approach. After a careful analysis of the data obtained for planning 2^3, it was concluded that the optimum number of extractions of the carotenoids from the peach palm fruits is described by the central point (Fig. 3A and C). In addition, the response surface plots indicate that the time of extraction is the most significant condition. In both cases (Fig. 3B and C), the longer the extraction time, the higher the carotenoid content extracted (assay 14 with 157.65 $\mu g_{carotenoids}\ g_{dried\ biomass}{}^{-1}$). An ANOVA test was applied to statistically analyze the output response from all-*trans*-β-carotene according to the experimental design defined for $[C_4mim][BF_4]$. For the obtained model (p-value 0.05), the response was fitted to the input process variables through a regression analysis, considering the uncoded and coded terms according to eqn (2). The F value was approximately three times the F value tabulated for total all-*trans*-β-carotene content, exceeding the 90% confidence level at R^2 = 0.71. The accuracy and precision of the model were validated by comparing the experimental results with the theoretical data predicted by eqn (1) and (2). Moreover, Table S3 (ESI†) shows the yields of extraction of total carotenoids and the all-*trans*-β-carotene from the validation assays, performed in triplicate. The model was effectively validated with low relative deviation, evidencing its high confidence in the prediction of data (Fig. S1 from ESI†).

Finally, by analyzing Fig. 3, we concluded that the optimum conditions to maximize the extraction of carotenoids were achieved at $R_{(S/L)}$ of 1 : 1 (coded level −1.68), extraction time of 12 min (coded level +1.68) and when 4 extractions were performed by ultrasonication (central point).

$$\text{total all-}trans\text{-β-carotene}\ (\mu g\, g^{-1}) = 42.4 + 1.35(x2) - 1.95(x2)^2 + 2.32(x3) + 2.92 \quad (2)$$

Carotenoids characterization

Thermal stability. Despite the high efficiency of $[C_4mim][BF_4]$ in extracting the carotenoids from the peach palm fruits, it is necessary to guarantee the stability of their chemical structure;[28] for this, the thermal stability was analyzed for two distinct temperatures, 60 °C and 90 °C. Thermal stability is one of the most important properties that should be maintained by natural colorants used in the food industry. In fact, there is great demand for more thermally stable pigments with natural origins instead of synthetic pigments.[29] Here, aqueous and oily (or organic) media were both analyzed for thermal stability of carotenoids. These two environments were studied since they are representative of the media usually found in food formulations. Based on the obtained data, the carotenoids' thermal stability follows an exponential trend, best fitted by eqn (3), for both temperatures evaluated (60 and 90 °C) and both organic (or oily) and aqueous media.

$$y_t = A_1 e^{-\gamma_1 t} + A_1 e^{-\gamma_2 t} + y_\infty \quad (3)$$

Here, y_t and y_∞ are the carotenoid concentration values in real-time and infinite time, respectively. A_1 and A_2 are the pre-

exponential factors and γ_1 and γ_2 are the observed rate constants for fast and slow decays, respectively. The exponential thermal decay behavior verified for the carotenoids showed the same behaviors for fast and slow decays, suggesting the occurrence of a common degradation mechanism, possibly involving parallel irreversible and reversible coupled degradation reactions.

The output fittings representing exponential decay reveal that the model has high precision with good correlation coefficients for all curves ($R^2 \geq 0.97$) (Fig. 5 and S2 from ESI†). Table S4 (ESI†) shows the kinetic parameters calculated by fitting the data from eqn (3), considering both media under evaluation. Despite the common first-order model generally identified when ILs are used as a solvent for carotenoids' thermal degradation,[11,30] in this work, a different trend was found, which could be justified by differences in the carotenoids' composition and structures, as already detailed in the literature.[11,31] The data of carotenoid degradation rate constants (k_d) as well as the $t_{1/2}$ values at 60 and 90 °C are presented in Table 1. These results suggest that the $t_{1/2}$ data are independent of the medium used and that the trends are maintained at both investigated temperatures. In summary, it is concluded that by using [C_4mim][BF_4] as solvent, not only is the extraction of the pigments more efficient but also their thermal stability is increased by almost 3-fold (results confirmed by the deactivation energy (E_a) data) when compared with those results obtained with acetone as solvent.[11] After carefully analyzing both our data and literature results,[32,33] we came out with the conclusion that the thermal degradation of carotenoids is highly dependent on the medium and their chemical structure. Moreover, as previously demonstrated,[34] the extraction processes using (more) hydrophobic ILs proved to be selective for pigments but not for other hydrophobic components present in the biomass. As previously detailed, the IL used in this work has hydrophobic characteristics and is even more hydrophobic than acetone, which was used in this work as a conventional solvent. Thus, the higher hydrophobicity of the IL implies the extraction of the most hydrophobic components of the biomass, namely the lipids, the class of compounds most abundant in this biomass, as already pointed out by its key role in heat stress management.[35]

Antioxidant activity. In this work, the thermal stability of the carotenoids extracted from the peach palm fruits with [C_4mim][BF_4]-based ethanolic solution was improved, allowing the potential application of these natural pigments in the food industry.[29] Nevertheless, the assurance that the antioxidant activity of carotenoids is not compromised by this IL-mediated extraction method is fundamental, since the key to the biological effect of these pigments is precisely their high capacity to protect cells against oxidative damage.[36] In this sense, the antioxidant activities of extracts of carotenoids obtained using both acetone (conventional system) and the ethanolic solution of [C_4mim][BF_4] were determined, with the main results depicted in Fig. S3 in ESI.† The results obtained were 5.09 μmol of α-tocopherol and 4.35 μmol of α-tocopherol, for the IL and conventional systems, respectively (Fig. S3 from ESI†). Although there is not a large difference between the results, it guaranteed the maintenance of antioxidant activity of the carotenoids extracted in presence of [C_4mim][BF_4], which is a very important condition when the food industry is considered. In the end, [C_4mim][BF_4] did not demonstrate a pro-oxidative effect. The small difference found for the antioxidant activity between both conventional (acetone) and alternative ([C_4mim][BF_4]-based ethanolic solution) extracts may be due to the simultaneous extraction of other antioxidant phytochemicals (*e.g.* isomers of tocopherol) present in *Bactris gasipaes* fruits[37] when the IL was applied.

Sequential extraction of carotenoids and IL′ recycling. The process of extraction proposed in this work seems to efficiently extract the carotenoids from peach palm fruits, simultaneously increasing their thermal stability and maintaining their antioxidant activity. However, when the industrialization potential of any process is envisioned,[38] the need for understanding how to improve the sustainability of the process is mandatory, mainly considering the recovery and reuse of the main solvents, a step less explored up to now.[39–42] In this work, the reuse of the IL and carotenoid polishing (or isolation) were the last two steps studied for the complete optimization of the process of extraction of these natural pigments. To optimize IL recycling and carotenoid isolation, 10 consecutive cycles of extraction were conducted.

The recycling of [C_4mim][BF_4] was thus tested in the same conditions previously selected. The IL-ethanolic solution rich in carotenoids was frozen at temperatures lower than −80 °C, allowing the IL to precipitate and separate from the ethanol supernatant enriched in carotenoids. Subsequently, the same IL was consecutively used in new (10) steps of extraction, as described in Fig. 4. The results suggest that the yield of extraction of carotenoids is guaranteed for at least 9 cycles of extraction, without significant IL′ losses (ANOVA: $F = 11.72$; $p <$

Table 1 Degradation constant (k_d) and half-life ($t_{1/2}$) values for carotenoids extracted from *Bactris gasipaes*

Media	Solvent	T (°C)	T (K)	k_d (min^{-1})	$t_{1/2}$ (min)	E_a (kJ mol^{-1})
Oily (organic)	Acetone	60	333.15	0.0001	6931.5	23.3
		90	363.15	0.0002	3465.7	
	[C_4mim][BF_4]	60	333.15	0.00003	23 104.9	28.4
		90	363.15	0.00007	9902.1	
Aqueous	Acetone	60	333.15	0.0121	57.3	12.6
		90	363.15	0.0176	39.4	
	[C_4mim][BF_4]	60	333.15	0.0046	150.7	15.0
		90	363.15	0.0072	96.3	

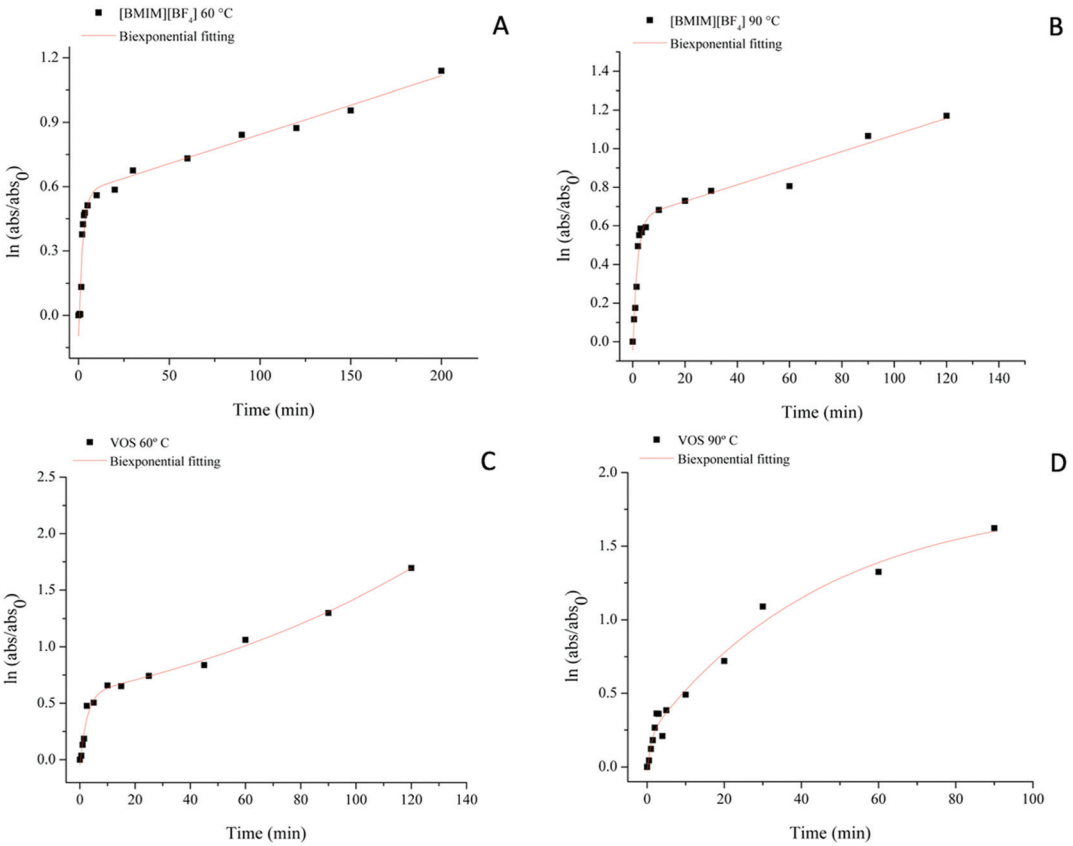

Fig. 4 Kinetics of degradation of the carotenoids extracted with $[C_4mim][BF_4]$ at 60 °C (A) and 90 °C (B) and with acetone at 60 °C (C) and 90 °C (D) in an organic medium. The coefficient of correlation for all curves is $R^2 \geq 0.97$. The kinetics of degradation of carotenoids in aqueous media is described in ESI Fig. S2.†

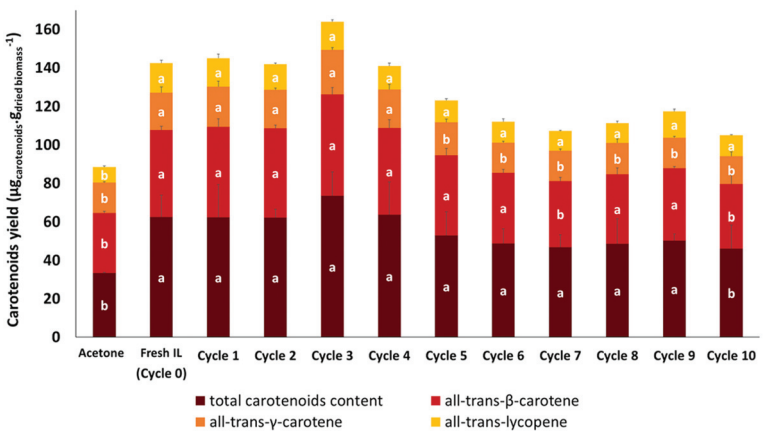

Fig. 5 Results of the yields of extraction of carotenoids from peach palm fruits using the recycled $[C_4mim][BF_4]$ solution for 10 cycles. The letters depicted in the bars represent statistically equivalent values in each test.

0.0001; Cohen's $f = 2.3181$). However, after cycle 9 (Cohen's for cycle 10 $f = -5.6640$), the yield of extraction of carotenoids was compromised, decreasing by 19.70% when compared with the maximum yield obtained with fresh IL, but still showed better results than acetone (conventional solvent) (Fig. 4; Table S5 from ESI†). Additionally, the results proved that it is worth reusing the IL for more than 10 cycles. Moreover, the isolation of carotenoids from ethanol was performed by evaporating and reusing the ethanol for new cycles of extraction. In the end, on average, around 94% of IL and 100% of ethanol were

recovered and recycled. Several approaches have been reported to recover the extracted compounds, including the use of supercritical carbon dioxide, recrystallization, nanofiltration, and anti-solvents.[6,14] Good results were obtained in this work compared with some polishing strategies reported in the literature, particularly the use of nanofiltration,[14] anti-solvent and solid-phase extraction-based strategies.[16] In the first example, nanofiltration was successfully used to separate the solvent (ethyl acetate) from the oleuropein, achieving a recovery of 97.5%.[14] In a second study, where deep eutectic solvents (DES) were used to recover flavonoids from *Flos sophorae*, the isolation of flavonoids from DES was performed by applying water as anti-solvent or solid-phase extraction with recovery values between 50 and 87%.[16] Taking into account the compared results, it is possible to define the process developed here as more efficient and less complex.

Regarding the most abundant carotenoids, the results show that the extraction yields of all-*trans*-β-carotene and all-*trans*-lycopene were not significantly reduced until 9 and 10 cycles, respectively. In contrast, for all-*trans*-γ-carotene, after cycle 5, the carotenoid extraction was compromised (ANOVA: $F = 6.395$; $p > 0.05$; Cohen's $f = 1.7121$) (Table S5 from ESI†).

Even with the low range of application of this method for IL recovery (IL precipitation by drastically decreasing the temperature), an average amount of 93.6 ± 2.6(%) of [C_4mim][BF_4] was recovered in each extraction cycle (Table S5 – ESI†), which was effective for at least 10 extraction cycles with low efficiency loss and low IL′ losses (Fig. 4), contrary to that occurring with the use of co-solvents, supercritical CO_2 (more expensive technique), the addition of inorganic salts (high environmental problems) and separation by membranes (more complex systems).[40,43,44] The IL-based extraction approach proposed in this work appears to be a sustainable, simple and effective method to extract carotenoids, particularly all-*trans*-β-carotene, all-*trans*-lycopene and all-*trans*-γ-carotene, from peach palm fruits, simultaneously maintaining the antioxidant activity while increasing their thermal stability. Given the high commercial interest for these natural pigments[45] and envisioning its industrial application, the complete process of extraction is depicted in Fig. 6. Briefly, the process proposed in Fig. 6 is represented by two main steps. (i) The solid–liquid extraction was repeated 3 times, as the carotenoids are recovered from the lyophilized biomass by applying ethanol-based [C_4mim][BF_4] solution as solvent. (ii) The carotenoids were isolated from the main solvents IL and ethanol. At the end of this process, it was possible to obtain a maximum of 143 $\mu g_{carotenoids}\ g_{dried\ biomass}^{-1}$.

Environmental assessment – carbon footprint

The environmental performance of the methods applied to extract carotenoids from *Bactris gasipaes* was evaluated through the calculation of the carbon footprint. In this work, three different scenarios were investigated: Scenario 1 – method using [C_4mim][BF_4] without recovery and reuse of IL-based ethanolic solution; Scenario 2 – method using [C_4mim][BF_4] but incorporating its recovery and reuse (10 cycles); and Scenario 3 – conventional method using acetone. Given that the systems studied in each scenario afforded different yields of carotenoids extracted, the results of the carbon footprint (Fig. 6) are expressed by μg of carotenoids extracted, allowing direct comparison among all scenarios.

The main results depicted in Fig. 7 and in Table S8 (ESI†) show Scenario 2 as the one with the best environmental performance, with a carbon footprint of 1.7 g $CO_{2\ eq.}$ $\mu g_{carotenoids}^{-1}$. In this case, the recovery and reuse of the main

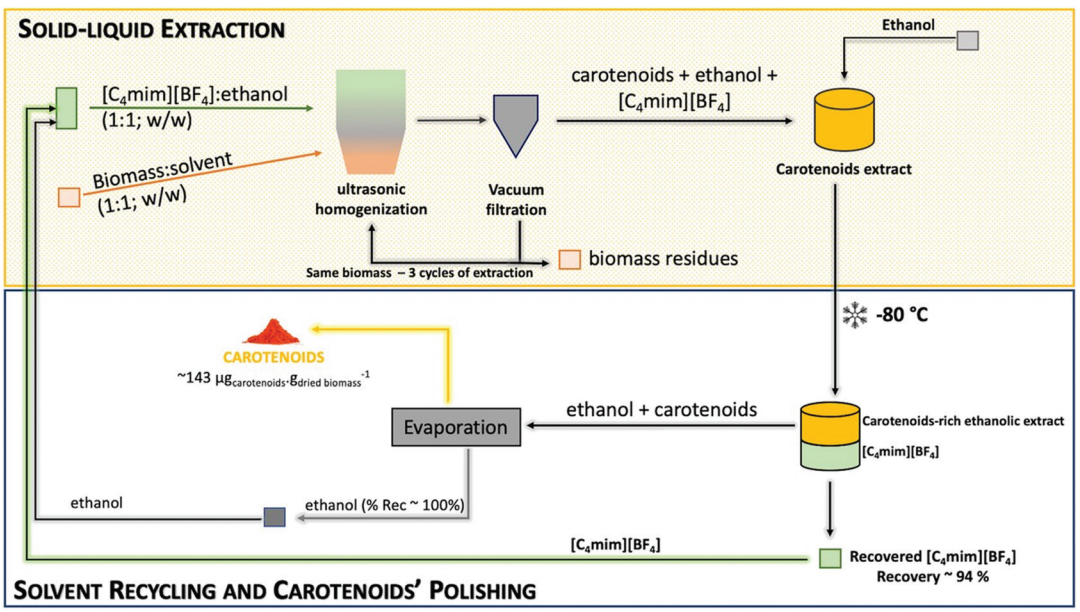

Fig. 6 Schematic of the integrated process optimized in this work, using [C_4mim][BF_4]-based ethanolic solution to extract the carotenoids from peach palm fruit biomass.

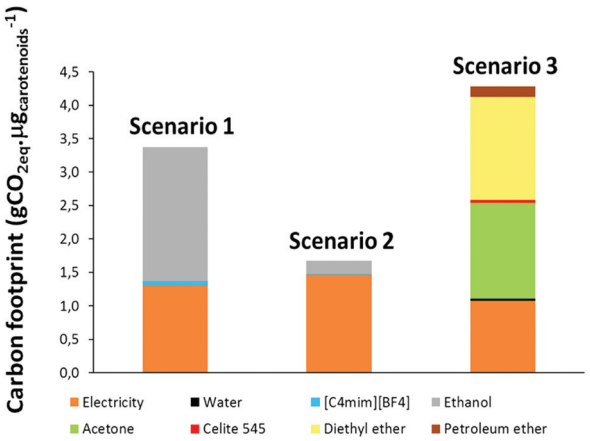

Fig. 7 The carbon footprint of the three scenarios assessed in this work. Scenario 1 – method with $[C_4mim][BF_4]$ without recovery and reuse of the IL-based ethanolic solution; Scenario 2 – method using $[C_4mim][BF_4]$ with recovery and reuse of the IL-based ethanolic solution (10 cycles); Scenario 3 – conventional method using acetone.

solvents used in the extraction of carotenoids from the biomass cause a decrease of 50% when compared with Scenario 1 (3.4 g $CO_{2\ eq}$ $\mu g_{carotenoids}^{-1}$). Indeed, despite the small increase in electricity consumption, this reduction of 50% is mainly contributed by the reduction of ethanol consumption. The majority contribution towards the carbon footprint of Scenario 2 is electricity consumption, mostly from use of the rotary evaporator and ultrasonic probe homogenization (55% and 31% of the total carbon footprint, respectively). In Scenario 1, almost 60% of the carbon footprint is associated with ethanol consumption, but electricity consumption (mainly used by the rotary evaporator and ultrasonic probe homogenization) is also an important parameter. Finally, the conventional method using acetone was also analyzed in this work, represented by Scenario 3. This last scenario has the worst environmental performance, with a carbon footprint of 4.3 g $CO_{2\ eq}$ $\mu g_{carotenoids}^{-1}$, which is 27% higher than Scenario 1 (using IL-ethanolic solution without recycling). In Scenario 3, the problem areas are the diethyl ether, acetone, and electricity consumption, representing 36%, 34% and 25% of the total carbon footprint, respectively.

Conclusions

This work describes the development and optimization of a simple and sustainable process mediated by using a hydrophobic IL in ethanol to recover carotenoids from peach palm fruits. The developed process doubled the yield of extraction of carotenoids when compared with the conventional extraction using pure acetone. The best performing IL, $[C_4mim][BF_4]$, was selected not only for its higher obtained extraction yield, but also its higher biocompatibility with carotenoids, assessed by investigation of the thermal stability and antioxidant activity of the pigments.

Envisioning industrial potential for this work, the reuse of the IL for 10 cycles and the environmental impact of this process were investigated. When three distinct scenarios were tested in terms of carbon footprint, it was proved that the most benign is the one where the carotenoids are extracted by applying the $[C_4mim][BF_4]$-based ethanolic solution and the IL solution is reused. In this case, the carbon footprint decreases by a total of 50%.

In the end, the process developed here represents a more sustainable extractive approach because it has an (i) increased capacity to improve the efficiency of the processes, (ii) a higher biocompatibility with the compounds being extracted and, possibly, (iii) the promise of decreasing the economic impact of the processes, for example, by IL recycling.

Experimental

Materials

Fruit raw material. *Bactris gasipaes* fruits were collected in the Bahia state of the northeast region of Brazil (Ilhéus city: 14°50′00.47″S, 39°01′51.98″W). All fruits were sanitized with tap water to remove sediments and possible contaminants. After manual seed removal, peach palm mesocarp and exocarp were immediately frozen at −100 °C, lyophilized for 48 h and then stored at −40 °C to preserve the carotenoid content for further use.

Chemicals. 1-Hexyl-3-methylimidazolium chloride ($[C_6mim]Cl$) and 1-butyl-3-methylimidazolium tetrafluoroborate ($[C_4mim][BF_4]$) were purchased from Sigma-Aldrich, with >99% purity. For the synthesis of $[C_4mim]Cl$ and $[C_4mim][PF_6]$, the 1-chloro-butane (99.5%), 1-butyl-3-methylimidazole (>98.5%), hexafluorophosphate (>99%), and chloroform (>99.8%) were purchased from Sigma-Aldrich. The petroleum ether (100%), diethyl ether (100%) and ethanol (P.A.) were purchased from Labsynth.

Methods

Ionic liquids' synthesis. IL synthesis was done in accordance with standard protocols described in the literature[11] and in the ESI (section S1†).

Conventional solid–liquid extraction using acetone. Acetone was applied for carotenoid extraction as a control to compare and evaluate the extractive performance of the proposed alternative method in an ethanol-based IL solution. The conventional method is defined using pure acetone by pestle homogenization. 1 g of lyophilized fruit was put in contact with 70 mL of pure acetone (100%) and the carotenoids were extracted and transferred to a petroleum ether : diethyl ether (2 : 1 w : w) solution. Then, the organic mixture was concentrated to complete dryness using a rotary evaporator at temperatures <37 °C, under vacuum and for 15 minutes, in triplicate. The carotenoid extract was stored at −40 °C for further use.[5] The reported results were performed in triplicate and the respective associated standard deviations were calculated.

Alternative solid–liquid extraction using ethanol-based ILs solutions. The potential of four ILs in extracting carotenoids

was evaluated. The lyophilized pulp of *Bactris gasipaes* fruits was placed in contact with the ethanol-based solution of each IL, namely, [C_4mim]Cl, [C_4mim][PF_6], [C_6mim]Cl and [C_4mim][BF_4], and extraction was performed. During the experiments, the mass (g) of pulp fruit per mass of solution (g) equivalent to the solid–liquid ratio ($R_{(S/L)}$), was fixed at 1 : 3; the number of extractions fixed at 3 times; the time of homogenization fixed at 5 minutes; the co-solvent-ratio $R_{(IL/E)}$, meaning the mass (g) of IL per mass of ethanol (ethanol, 99%), fixed at 1 : 3; and the homogenization method used was ultra-dispersion or an ultrasonic probe. Then, the extracts of carotenoids were transferred to an organic solution of petroleum ether : diethyl ether (2 : 1 w : w) and concentrated to dryness using a rotary evaporator at temperatures <37 °C, under vacuum, for 15 minutes and in triplicate for quantification.

Experimental design to maximize the total carotenoid yield: response surface methodology (RSM)

The design of factorial planning with three central points (2^{5-1} was performed to evaluate the effects of different conditions on the yield of extraction of carotenoids using the two ILs with the highest extractive performance. The parameters evaluated are the same as those investigated in the assays of section solid–liquid extraction: alternative methodology: the proportion of IL1/IL2, $R_{(S/L)}$, number of extractions, time of extraction, and $R_{(IL/E)}$. The experiments of the 2^{5-1} factorial planning design were performed using three values for each of the independent variables (Table S6 from ESI†). An estimation of the main effects was obtained by evaluating the difference in the yield of extraction of carotenoids caused by a change from a low (−1) to a high (+1) level of the corresponding variable.

The focus of the fractional planning design was screening the significant variables over the total yield of extraction of carotenoids. After analysis of the data, the most influential variables were tested and a central composite rotatable design (CCRD; 2^3 plus axial) with three replicates at the central point was performed to optimize the extraction process. Accordingly, a total of 17 experiments were performed. Table S2 (ESI†) shows the values of the real and coded levels used in the CCRD.

The obtained results were statistically analyzed considering a minimum confidence level of 90%. The response was expressed in $\mu g_{carotenoids}\ g_{dried\ biomass}^{-1}$. The main response evaluated for optimization of the process was based on the total yield of carotenoids extracted. In addition, to preserve the average yield of each carotenoid and because independent factors may act in the individual extractions of compounds, the results were evaluated considering the yields of all-*trans*-β-carotene, all-*trans*-γ-carotene, and all-*trans*-lycopene, to obtain models for those responses as well. After analyzing the RSM results, the best conditions for the carotenoids' extractions with the best IL were determined and the model was validated in triplicate. Statistica 12.0 software was used to analyse the results and plot the response surfaces.

Identification and quantification of carotenoids

The identification and quantification of carotenoids were performed in triplicate using a Shimadzu HPLC (Kyoto, Japan) equipped with quaternary pumps (model LC-20AD), an on-line degasser, and a Rheodyne injection valve (Rheodyne LCC, Rohnert Park, Ca) in a loop with a capacity of 20 μL. The chromatographic resolution was obtained under the following conditions: C30 YMC column (3 μm, 250 × 4.6 mm i.d., Waters, Wilmington, MA) using a mobile phase of a linear gradient of MeOH/MTBE from 75 : 25 to 50 : 50 in 25 min, then 50 : 50 until the end (65 min). The flow rate was 0.9 mL min^{-1} with 22 °C as the column temperature. A PDA detector was connected to the LC system (Shimadzu, model SPD-M20A). The UV-visible emissions were monitored between 250 and 600 nm, with data processed at 450 nm (maximum peak of absorbance for the pigments under study). The carotenoids were identified according to the following information: elution order on the C30 column, co-chromatographic comparison with authentic standards, the UV-visible spectrum (λ_{max}), the spectral fine structure (%III/II), the peak *cis* intensity (%AB/AII), and comparison between the data collected and data available in literature[5] (Table S7 and Fig. S4 and S5 from ESI†). The carotenoids were quantified by HPLC-PDA using an external analytical curve (six points, in duplicate) for all-*trans*-β-carotene (2–50 μg mL^{-1}) and all-*trans*-lycopene (2–15 μg mL^{-1}), in accordance with Rosso & Mercadante (2007).[5] The average and standard deviation results obtained for the carotenoid content were analyzed by analysis of variance (ANOVA), calculated by STATISTICA 12.0.

Statistical analysis

The total carotenoid contents extracted by IL-ethanolic solutions (fresh-IL and recovered-IL) and acetone (as the control) were compared by applying the analysis of variance (ANOVA) using the degree of significance of 95% ($p < 0.05$), with Fisher's *post hoc* LSD test. Additionally, Cohen's *d* test was also employed.

Carotenoid polishing and solvent recycling

After the optimization of the solid–liquid extraction performed using the IL-ethanolic solution, the recycling and reuse of the solvents as well as the polishing (or isolation) of the carotenoids from the IL-media were investigated. The process of isolation of carotenoids was performed in two main steps. In the first step, the IL was frozen (at −80 °C for around 70 minutes) and then separated from the carotenoids-rich ethanolic extract. Secondly, the ethanol was evaporated under low pressure, allowing carotenoid isolation. After the carotenoids were collected and stored under refrigeration (−40 °C), the added ethanol was collected for a new cycle of extraction, as performed in this work. Even so, some carotenoids were still present in the IL-media. To recover those carotenoids, more ethanol was added to the flask, and the mixture was shaken to homogenize the frozen IL with the ethanol added, forming a new ethanolic solution containing IL + carotenoids.

The freezing/precipitation process (−80 °C) was repeated 2 or more times until the frozen IL became completely free of carotenoids.

Characterization of carotenoids obtained

Carotenoids' thermal stability. The determination of the carotenoids' stability was done for both the acetone-based and the extract in the highly performing IL-based ethanolic solution. So, for both extracts rich in carotenoids, the stability at 60 °C and 90 °C was evaluated following standard protocols described elsewhere.[31]

Furthermore, in the present study, the stability of the carotenoid extracts was evaluated in both an oily (sunflower oil) and an aqueous solution. For the assays in an oily medium, both extracts (obtained with acetone and IL-based ethanolic solution) were diluted in 60 mL of sunflower oil, yielding an initial solution with an absorbance of approximately 0.8 at 453 nm. On the other hand, for the evaluation in an aqueous medium, approximately 200 μg of carotenoids from both extracts was first dissolved in 5 mL of ethanol and then mixed with 25 mL of Milli-Q water. The stability data of both systems (aqueous and oily) for all carotenoid extracts were assessed by heating at 60 °C and 90 °C in a water bath, for different heating times, for a maximum of 48 h. After an aliquot of 2 mL was removed from the water bath, it was immediately cooled in an ice bath and analyzed. The carotenoid degradation was monitored by measuring the absorbance at 453 nm using a Cary 50 Bio Varian spectrophotometer (São Paulo, Brazil).

The thermal kinetics and an estimation of the deactivation energy of the carotenoid extracts were determined. From a semi-natural logarithmic plot of the residual carotenoid content *vs.* time, the degradation rate constants (k_d) were calculated and the half-life times estimated using eqn (4). The half-life time ($t_{1/2}$) is defined as the period taken for the residual carotenoid content to reach 50%, considering the initial concentration.

$$t_{1/2} = \frac{-\mathrm{Ln}0.5}{k_d} \quad (4)$$

The temperature dependence of k_d was analyzed using an Arrhenius plot; the activation energy was calculated from the Arrhenius equation as follows (eqn (5)):

$$\mathrm{Ln}\, k_d = \mathrm{Ln}A - \frac{E_a}{R}\frac{1}{T} \quad (5)$$

where E_a is the activation energy for the thermal degradation, A is a constant and R is the universal gas constant (8.314 × 10^{-3} kJ mol^{-1} K^{-1}). The value of the activation energy (E_a) was estimated from the slope of the plot of Ln k_d against $1/T$.

Antioxidant activity. The antioxidant activity of the carotenoid-rich extracts obtained by both conventional and alternative processes was determined by kinetic monitoring of fluorescein decay for 3 hours. Briefly, the extracts were dissolved in petroleum ether to prepare a stock solution of *circa* 177 μM. Ability to deactivate peroxyl radicals ($ROO^{\bullet}$) was evaluated for 5

Table 2 Data on inputs of chemicals, water and electricity and output of carotenoids in the three scenarios under study. Scenario 1 – method with [C_4mim][BF_4] without any step of recovery and reuse of the IL-based ethanolic solution; Scenario 2 – method with [C_4mim][BF_4] with recovery and reuse of the IL-based ethanolic solution (10 cycles); Scenario 3 – conventional method using acetone

	Scenario 1	Scenario 2	Scenario 3
Inputs			
Fruit preparation			
Fruit (g)	1	11	1
Water (mL)	0.5	5.5	0.5
Electricity (W h)	6.51×10^{-2}	0.716	6.51×10^{-2}
Extraction			
[C_4mim][BF_4] (g)	2	2	—
Ethanol (g)	2	2	—
Acetone (g)	—	—	54.88
Celite 545 (g)	—	—	35
Electricity (Wh)	253	2779	22.17
Polishing			
Ethanol (g)	120	120	—
Petroleum ether (g)	—	—	35.75
Diethyl ether (g)	—	—	21.39
Water (mL)	—	—	5000
Electricity (W h)	434	4771	325
Outputs			
Carotenoids (μg)	142.97	1411.13	88.37

Table 3 GHG emission factors and process names from Ecoinvent version 3.4 used in the calculation of the carbon footprint

Input	Reference unit	GHG emissions (kg $CO_{2\ eq}$/ reference unit)[a]	Name of the process in Ecoinvent
[C_4mim][BF_4]	kg	3.78	Sodium tetrafluoroborate production, global; imidazole production, global[b]
Ethanol	kg	2.35	Ethanol, without water, in 95% solution state, from fermentation, Brazil
Acetone	kg	2.31	Acetone production, liquid, rest of the world
Celite 545	kg	9.88×10^{-2}	Silica sand,[c] cryolite production, from fluosilicic acid, global
Petroleum ether	kg	0.385	Petroleum refinery operation, naphtha, rest of the world
Diethyl ether	kg	6.36	Diethyl ether production, without water, in 99.95% solution state, global
Water	kg	6.00×10^{-4}	The market for tap water, the rest of the world
Electricity	kW h	0.274	The market for electricity, low voltage, Brazil

[a] GHG into a mass of $CO_{2\ eq}$ are those recommended by the Intergovernmental Panel on Climate Change (IPCC)[48] for a time horizon of 100 years. [b] In the absence of data for the production of [C_4mim][BF_4], the average of these two processes was considered. [c] Ecoinvent considers silica sand as an approximation of silicon dioxide.

different concentrations of each extract (30, 60, 90, 120 and 170 μM). The assay was performed in a 96-well black polystyrene microplate according to the protocol established by Rodrigues *et al.* (2012).[46] The antioxidant activity of the extracts was expressed in μmol of α-tocopherol.

Environmental assessment

The carbon footprint is a measure of the greenhouse gas (GHG) emissions of a system, expressed as carbon dioxide equivalent ($CO_{2\ eq}$), from a life cycle perspective. In the three scenarios evaluated, the carbon footprint considers the production of all chemicals, water, and electricity consumed in the stages of fruit preparation, extraction and polishing. Data on the amounts of chemicals, water and electricity consumed were obtained during the experiments (Table 2), while GHG emissions from their production were taken from Ecoinvent database version 3.4[47] (Table 3). More details on the carbon footprint calculation are provided in section S2 of the ESI.†

Conflicts of interest

There are no conflicts to declare.

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