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Ionic liquid-mediated recovery of carotenoids from the *Bactris gasipaes* fruit waste and their application in food-packaging chitosan films

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5 2 **and their application in food-packaging chitosan films**
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51 20 **Supporting Information:** 7 tables and 6 figures with some additional details.
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2
3 23 **Abstract:** In this work, the extraction and purification of carotenoids from the fruit
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5 24 *Bactris gasipaes* was developed. Ethanolic and aqueous solutions of ionic liquids (ILs),
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7
8 25 and surfactants were evaluated on the extraction of these pigments. Thus, we developed
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10 26 an optimized sustainable downstream process mediated by the best solvent with further
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12 27 isolation of the carotenoids and the recyclability of the IL used. The process was
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14 28 characterized, not only in terms of efficiency but also regarding its environmental impact.
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16 29 The recyclability of the solvents, as well as the high efficiency (maximum yield of
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18 30 extraction of carotenoids = $88.7 \pm 0.9 \mu\text{g}_{\text{carotenoids}} \cdot \text{g}_{\text{dried biomass}}^{-1}$) and the low environmental
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20 31 impact of the integrated process developed in this work, were demonstrated. In the end,
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22 32 in order to incorporate functional activity for an alternative food-packaging material,
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24 33 carotenoids were successfully applied on the preparation of chitosan-based films with
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26 34 excellent results regarding their mechanical parameters and antioxidant activity.
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33 36 **Keywords:** all-*trans*- β -carotene; sustainable downstream process; ionic liquid; bio-based
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35 37 material; sustainable material;
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45 **Introduction**

46 The exploration of natural resources to produce consumer goods and commodities is
47 being deeply investigated. New or improved production technologies have been
48 developed to mitigate the residues disposal, while simultaneously enhancing the
49 valorization of by-products.¹ There is nowadays a real demand for the creation of more
50 sustainable and efficient downstream processes able to recover bio compounds usually
51 discarded in biological waste, in spite of their potential for a large range of commercial
52 applications.^{2,3} Food waste is, in this scenario, one of the major problems worldwide. The
53 European commission is calling the attention for their impacts in a daily-base. It is
54 estimated that in EU, around 88 million tons of food wastes are generated annually with
55 associated costs around the 143 billion euros.⁴ As it has been successively explained by
56 authorities worldwide, wasting food has serious ethic and economic impacts. While the
57 demand for food increases proportionally with the human population, being estimated to
58 reach 9.1 billion by 2050,⁵ the environmental impact of the residues generated is growing,
59 severely damaging the environment and negatively impacting the climate (food alone is
60 responsible for about 8% of Global Gas Emissions).⁶ While reducing the food losses and
61 wastes will help achieving the Sustainable Development Goals, the design of more
62 sustainable downstream processes will allow the wastes industrial valorization, meeting
63 the demands of both Circular Economy and Biorefinery strategies.⁷⁻⁹

64 In addition to Europe, for many developing countries, such as Brazil, India and China,
65 with high social heterogeneity and cyclical economic crises, the reduction of food wastes
66 is a huge challenge.¹⁰ In Brazil, 41 million tons of food are wasted every year.¹⁰ In
67 contrast, Brazil has a massive food biodiversity, with 18% of the plants of the world,¹¹
68 and therefore, many unused raw materials to be explored, contributing thus for the
69 creation of new bio-based products.¹² In this context, the business of palm heart (*Bactris*

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3 70 *gasipaes*) is a very good example of an important agro-food activity from the Brazilian
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5 71 Amazonian region, originally cultivated by indigenous populations.¹³ Palm hearts, known
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7 72 in Brazil as “*palmito*”, are the edible stipe-apical meristem of the palm tree, the main
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9 73 agro-product of this species. Known as a predatory crop, the Brazilian Ministry of
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11 74 Agriculture (EMBRAPA), recommends *palmito* to be harvested after the fruit ripening.¹⁴
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13 75 However, despite this recommendation, their fruits are usually discarded.¹⁵ *Bactris*
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15 76 *gasipaes* fruits, popularly known as peach palm, are an excellent source of carotenoids
16
17 77 and other secondary metabolites.¹⁶ Carotenoids are natural pigments with high
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19 78 antioxidant and anti-inflammatory activity, and therefore, with commercial interest.¹⁷
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21 79 Depending of the application, the choice of the carotenoid method-extraction from food
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23 80 matrices is crucial to ensure safety for the end product.¹⁸ Nevertheless, the most common
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25 81 and efficient industrial downstream processes to produce carotenoids are usually
26
27 82 mediated by organic synthesis; or in some cases (lycopene and astaxanthin) by extraction
28
29 83 mediated by conventional organic solvents. Despite the different natural sources available
30
31 84 rich in carotenoids, their recovery from the biomass is not easy.¹⁹ Different strategies
32
33 85 employed on the recovery and purification of natural products have been reviewed in
34
35 86 literature, these including the use of membranes, imprinted polymers, chromatography
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37 87 and alternative solvents (e.g. ionic liquids and deep eutectic solvents).^{20,21} Synthetic dyes,
38
39 88 which are considered as cheaper and more stable than their natural counterparts are
40
41 89 usually reported as carcinogenic and allergenic for humans.²² Thus, considering the rapid
42
43 90 growth of the market of antioxidant products and formulations, there is a high demand
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45 91 for new strategies capable to recover natural, thermally stable and low toxic
46
47 92 carotenoids.^{23–25}

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49 93 Bio-based materials with antioxidant properties are nowadays one of the hottest topics
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51 94 in food sector.²⁶ Non-ecofriendly packages have been considered as one of the most
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3 95 important environmental problems to be solved, mainly considering the excessive use of
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5 96 plastic.²⁷ Thinking on a Circular Economy perspective, chitosan-based films are good
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8 97 candidates to replace the widespread use of non-degradable materials. According to
9
10 98 Guillard et al.,²⁸ the replacement of 50% of plastic food packaging by alternative materials
11
12 99 can generate savings of more than 56 million tons of plastic/year. Chitosan is the second
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14 100 most abundant polysaccharide in nature. It is a renewable matrix, easy to obtain, eco-
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16 101 friendly and nontoxic.²⁷ Despite the chitosan-based films poor mechanical properties,²⁹
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18 102 they are an excellent basis to develop packaging materials with antioxidant and
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20 103 antimicrobial activities that would improve the food shelf-life and the preservation of its
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22 104 organoleptic properties.^{30,31}

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26 105 This work addresses the development of the integrated process for the recovery of
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28 106 natural carotenoids from *Bactris gasipaes* fruits, namely the all-*trans*- β -carotene, all-
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30 107 *trans*-lycopene and the all-*trans*- γ -carotene.^{16,32} Various ethanolic and aqueous solutions
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32 108 of ionic liquids (ILs), with and without tensioactive nature, and two common surfactants
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34 109 were evaluated. After the selection of the best solvent to extract the carotenoids, the
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36 110 process conditions of the solid-liquid extraction, including the solid-liquid ratio $R_{(S/L)}$,
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38 111 the time of extraction, and the concentration of IL ($Conc_{IL}$), were studied and optimized.
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40 112 The carotenoids isolation was investigated by applying water as anti-solvent. The
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42 113 environmental impact of the proposed process was evaluated through the analysis of
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44 114 carbon footprint and complete E-factor. In the end, the carotenoids purified and isolated
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46 115 were utilized on the preparation of chitosan-based films. The mechanical properties of
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48 116 the membranes prepared were tested, namely the tensile strength, Young's modulus,
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50 117 elongation at break, thickness and elasticity. Moreover, the wettability and solubility of
51
52 118 the membranes in water were also studied.

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120 **Material and methods**

121 **Fruits and raw materials.** The *Bactris gasipaes* fruits studied were collected in the
122 Bahia State, northeast region of Brazil (Ilhéus city: 14°50'00.47''S, 39°01'51.98''W).
123 The fruits belong to the same batch previously studied,³³ and their respective samples
124 were pretreated as follows. Briefly, after manual seed removal, the peach palm edible
125 parts were immediately frozen at -100 °C, lyophilized for 48 h and then stored at -40°C.
126 The biomass composition was performed following the proximal characterization method
127 according to Association of Official Analytical Chemists (AOAC).³⁴

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129 **Chemicals.** 1-hexyl-3-methylimidazolium chloride ([C₆mim]Cl), 1-octyl-3-
130 methylimidazolium chloride ([C₈mim]Cl), 1-decyl-3-methylimidazolium chloride
131 ([C₁₀mim]Cl), 1-dodecyl-3-methylimidazolium chloride ([C₁₂mim]Cl), 1-tetradecyl-3-
132 methylimidazolium chloride ([C₁₄mim]Cl), 1-butyl-3-methylimidazolium
133 tetrafluoroborate ([C₄mim][BF₄]) were purchased from IOLITEC, with purities > 98%.
134 1-dodecyl-trimethylammonium bromide ([N_{1,1,1,12}]Br) and 1-tetradecyl-
135 trimethylammonium bromide ([N_{1,1,1,14}]Br) were purchased from Alfa Aesar, with
136 purities higher than 98%. 1-decyl-trimethylammonium bromide ([N_{1,1,1,10}]Br) and 1-
137 decyl-trimethylammonium chloride ([N_{1,1,1,10}]Cl) (> 98% of purity) were purchased from
138 TCI. SDS and Tween 20 were purchased from PanReac AppliChem, with purities > 98%.
139 To prepare the bio-based films, chitosan from shrimp of medium molecular weight with
140 a degree of deacetylation of 85% and glycerol were supplied by Sigma-Aldrich (St Louis,
141 MO, USA). The antioxidant activity was performed using anhydrous sodium carbonate
142 (99%) and 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic) (ABTS, 99%) were
143 purchased from Fluka (St Louis, MO). All other reagents used were of analytical grade.

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3 145 **Solid-liquid extraction using organic solvents.** A conventional approach with pure
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5 146 acetone and ethanol, organic solvents applied as controls, was performed to compare the
6
7 147 yield of carotenoids extraction with the alternative extraction mediated by ILs and two
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10 148 traditional surfactants. The peach palm fruits were incubated for 90 min with a solid-
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12 149 liquid ratio of 0.1, meaning 0.5 g of biomass in 5 mL of solvent, at room temperature with
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14 150 homogenization at 80 RPM. After, the solutions were centrifuged (5000 RPM, 4 °C, 30
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16 151 min) and the supernatant was stored at – 40°C for further analysis. The extracts were
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18 152 performed in triplicated being presented for discussion, the average and respective
19
20 153 standard deviations. The purification of the carotenoids obtained by these processes were
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22 154 mediated also by organic solvents, namely diethyl and petroleum ether.^{16,33}
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28 156 **Alternative solid-liquid extraction using ILs and common surfactants.** The potential
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30 157 of extraction of different ethanolic and aqueous solutions of surface-active ILs and
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32 158 common surfactants were evaluated regarding the recovery of carotenoids from the dried
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34 159 biomass. Included in the set of solvents tested are aqueous and ethanolic solutions of ILs
35
36 160 from the imidazolium, ammonium and phosphonium families, polysorbate 20 (Tween 20)
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38 161 and sodium lauryl sulfate (SDS). For the solvents' screening, the concentration of 250
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40 162 mM, $R_{(S/L)}$ of 0.1 and 90 min of time of exposure, were the conditions fixed. The samples
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42 163 were centrifuged at 80 RPM. The yield of extraction of carotenoids ($\mu\text{g}_{\text{carotenoids}} \cdot \text{g}_{\text{dried}}$
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44 164 biomass^{-1}) was determined by HPLC-DAD using the method described by de Souza
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46 165 Mesquita et al. (2019).³³ The assays were performed in triplicate for each system and each
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48 166 condition tested and the results expressed as the mean with the respective standard
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50 167 deviations.
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56 168 After the screening with the alternative solvents, the most promising system, meaning
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58 169 the one having the highest yield of extraction for carotenoids from the peach palm fruit,
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3 170 was selected. The optimization of the process was executed by applying a central
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5 171 composite rotatable design (CCRD; 2^3 plus axial) with six replicates at the central point,
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7 172 totalizing 20 extractions. The independent variables optimized were the solvent
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9 173 concentration (C_{IL}), time of extraction (t) and solid-liquid ratio ($R_{(S/L)}$). The results
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11 174 obtained were statistically verified for a confidence level of 95%. The surface responses
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13 175 were plotted changing two variables within the experimental range. The dependent
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15 176 variable here evaluated was the total yield of extraction of carotenoids, considering the
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17 177 amount of all-*trans*-lycopene, all-*trans*- β -carotene, and the all-*trans*- γ -carotene extracted,
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19 178 since these are the most abundant in the biomass. After analyzing the RSM results, the
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21 179 best conditions for the carotenoids' extraction and for the best solvent were determined.
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23 180 The model was validated in triplicate (predicted x experimentally data). The Statistica
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25 181 12.0 software was used to analyze the results allowing the determination of the response
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27 182 surfaces.
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35 184 **Carotenoids polishing and recycling of the best solvent.** After the validation and
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37 185 respective optimization of the alternative process, the carotenoid's recovery was
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39 186 performed by using water as an anti-solvent, allowing the consequent precipitation of
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41 187 carotenoids. Five conditions were tested considering the determination of the amount of
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43 188 water required, namely 2x, 3x, 5x, 10x and 50x more water added to the carotenoids-rich
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45 189 extract with an initial volume of 5 mL. After isolation of the main solvents, consecutive
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47 190 cycles of extraction of carotenoids from new biomass samples were tested aiming at to
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49 191 evaluate the performance of the best solvent after being recycled. In this work, 3 new
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51 192 cycles were carried and its performance evaluated by the analysis of the extraction
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53 193 efficiency ($\mu\text{g}_{\text{carotenoids}} \cdot \text{g}_{\text{biomass}}^{-1}$).
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3 195 **IL quantification.** The IL quantification was performed by ionic chromatography
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5 196 (bromide detection). The analysis of the samples was carried on a Dionex 2000i/SP Ion
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7 197 Chromatograph with conductivity detector. The bromide was analysed on an AS4A SC
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9 198 (25 cm X 4mm I.D) with an AG4A-SC ground column 4 mm I.D. It was detected by a
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11 199 suppressed conductivity detector using an Anion Micro-Membrane AMMS-I with
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13 200 regenerant of 25 mN of sulfuric acid. The injection volume was 10 μ L, and the flow rate
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15 201 was 2.0 mL.min⁻¹. The chromatograms were recorded on a Chromjet integrator from
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17 202 Dionex.
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24 204 **Thermal gravimetric assay (TGA).** TGA assays (SETSYS Evolution 1750 analyser -
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26 205 Setaram Instrumentation) were performed. Briefly, each sample was heated at a constant
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28 206 rate of 10 °C.min⁻¹ from 20 to 540 °C under a nitrogen flow of 200 cm³.min⁻¹.
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33 208 **Monosaccharides analysis.** Neutral monosaccharides were determined as alditol
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35 209 acetates by gas chromatography.³⁵ Briefly, the samples were hydrolyzed using a solution
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37 210 of sulfuric acid (2 M at 120 °C for 1 h). The monosaccharides were reduced with sodium
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39 211 borohydride and acetylated using acetic anhydride. 2-Deoxyglucose was used as an
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41 212 internal standard. The alditol acetates were analyzed by gas chromatography (GC) in a
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43 213 30 m capillary column DB-225 (J&W Scientific, Folsom, CA, USA), with an internal
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45 214 diameter of 0.25 mm and a film thickness of 0.15 μ m. The GC was equipped with a flame
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47 215 ionization detector (GC-FID Clarus 400, Perkin Elmer, MA, USA) and the
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49 216 monosaccharides were identified by retention time by comparison with the values
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51 217 obtained for the external standards. The analyses were performed in triplicate. The uronic
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53 218 acids were determined according to a modification of the 3-phenylphenol colorimetric
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55 219 method.³⁵ The samples (2 mg) were hydrolyzed using sulfuric acid (1 M at 100 °C for 1
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3 220 h). A calibration curve was made with D-galacturonic acid (0-200 mg mL⁻¹). The analyses
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5 221 were performed in triplicate.
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11 223 **Environmental evaluation by determination of carbon footprint and complete E-**
12 **factor.** The carbon footprint consists in the sum of greenhouse gas (GHG) emissions
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14 224 **factor.** The carbon footprint consists in the sum of greenhouse gas (GHG) emissions
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16 225 expressed as carbon dioxide equivalent (CO₂ eq) from a life cycle perspective, while the
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18 226 complete E-factor assesses the efficiency of a process by measuring the total amount of
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20 227 waste generated during the process, including water, relative to each isolated product. In
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22 228 both cases, the stages of fruit preparation, extraction and polishing were assessed. The
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24 229 carbon footprint considers the GHG emissions from the production of all chemicals, water
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26 230 and electricity consumed during the three stages. All data required for the environmental
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28 231 evaluation regarding chemicals, water and electricity consumed were obtained
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30 232 experimentally (Table S1 from ESI) and the GHG emission factors were taken from from
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32 233 Ecoinvent database version 3.5 (Ecoinvent, 2018) (Table S2 from ESI).³⁶ To calculate the
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34 234 complete E-factor, data on the amounts of waste generated from the use of [N_{1,1,1,10}]Br,
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36 235 acetone, ethanol, water, petroleum ether and diethyl ether in the integrated system were
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38 236 obtained during the experiments. The analysis was performed considering 4 distinct
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40 237 scenarios, which are different from each other considering the amount of raw materials,
41
42 238 as well as, their energy dependence. The scenarios 1 and 2 are the conditions mediated
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44 239 by IL-extraction, without and with recycling of the solvents, respectively. The scenarios
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46 240 3 and 4 are different from each other considering the conventional organic solvent used,
47
48 241 respectively acetone and ethanol, however, in both, the carotenoids isolation was
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50 242 performed using ether mixtures.^{16,33} Further details on the calculation of carbon footprint
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52 243 and complete E-factor can be found in the ESI. The carbon footprint values were
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54 244 compared
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3 245 applying analysis of variance (ANOVA) using the degree of significance
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5 246 of 95% ($p < 0.05$).
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10 248 **Chitosan biofilms preparation.** The chitosan solutions were prepared by dissolving
11
12 249 1.5% (w:v) of chitosan in 5% (v:v) of acetic acid aqueous solution under stirring for 16h
13
14 250 at room temperature. The films were divided into 3 samples. In each film sample, a
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16 251 different amount of carotenoids was added, namely 0.025% (w:w), 0.050% (w:w) and
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18 252 0.100% (w:w), meaning the amount of carotenoids *per* total amount of chitosan. In the
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20 253 biofilms preparation, the carotenoids used were those obtained after purification with the
21
22 254 best solvent and acetone. To prepare the chitosan-based films ($n = 6$), after the addition
23
24 255 and homogenization of glycerol (0.75 %, w:w), the pure carotenoids in ethanol were
25
26 256 added to the mixture and homogenized by ultra-dispersor at 19000 RPM for 3 min. After,
27
28 257 the biofilms were filtrated, degassed and transferred to a pexiglass plate (144 cm² with 3
29
30 258 mm deep). The plates were placed in the oven for 16h at 35°C to form the film by solvent
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32 259 casting. Biofilms without carotenoids were done by the same procedure to be evaluated
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34 260 as controls.
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41 42 262 **Chitosan biofilms characterization**

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44 263 **Thickness and mechanical assays.** The thickness of the films produced was evaluated
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46 264 after the complete drying of the material, one day after their preparation. These
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48 265 measurements were performed using a hand-held digital micrometer (Mitutoyo
49
50 266 Corporation). Six measurements were taken in random areas of the films. The results were
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52 267 expressed as the mean \pm respective standard deviations and used for the calculation of the
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54 268 contact area (mm²). The mechanical properties of the chitosan-based films, with and
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56 269 without carotenoids, were evaluated by uniaxial tensile tests at room temperature with
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3 270 monitored air humidity (model Ta.Hdi, Stable Micro Systems) equipped with fixed grips
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5 271 lined with thin rubber in the ends. The films were cut in strips with 90 mm length and 10
6
7 272 mm wide for the determination of their tensile properties. The terminal positions of the
8
9 273 films were fixed in the grips with an initial separation settled at 50 mm. The crosshead
10
11 274 speed was set at constant rate of 0.5 mm.s⁻¹. The contact area (mm²), Young's modulus
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13 275 (*E*), tensile strength or stress at break (σ_b) and elongation or strain at break (ε_b) were
14
15 276 determined from stress-strain curves obtained from uniaxial tensile testes to film failure.
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17 277 These parameters were calculated based on ASTM D 882-83 standard method. All the
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19 278 analysis were performed using at least 6 replicates and adopting the methodologies
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21 279 described elsewhere.³⁷
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28 281 **Water contact angle.** To estimate the wettability of the films, the contact angle of water
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30 282 molecules on the films' surface was performed using a contact angle measuring system
31
32 283 (OCA 20, Data-physics) at room temperature and with air humidity control. 3 μ L of
33
34 284 ultrapure water was dispensed as a drop in the surface of each film (1 x 10 cm) using a
35
36 285 microsyringe. The contact angles of the drops were calculated using an image obtained
37
38 286 by the software dataphysics SCA20_M4, using the Laplace-Young method. All the
39
40 287 analysis were performed at least 40 times in both sides of the chitosan-based films.
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47 289 **Solubility.** The film solubility was determined placing one square (4 cm²) of each film
48
49 290 prepared in 30 mL of 50:50 (V/V) water:ethanol mixture, at room temperature, with
50
51 291 orbital agitation (80 rpm) for a maximum of 7 days. Then, the films were placed in an
52
53 292 oven at 105 °C for 16 h. After cooling down to room temperature, the films were weighed.
54
55 293 The solubility was determined by the percentage of weight loss calculated as follows:

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$$\text{Weight loss (\%)} = 100 \times \frac{m_b - m_a}{m_b} \quad (\text{Eq.1})$$

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3 295 where m_b and m_a are the weight of dry film before and after being immersed in the
4
5 296 water:ethanol mixture, respectively. This determination was performed in triplicate. The
6
7 297 films moisture was determined in triplicate by measuring their loss of weight, upon drying
8
9
10 298 in an oven at 105 °C until reaching a constant weight (dry film weight).

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14 300 **Antioxidant activity.** The antioxidant analysis of the films was evaluated by the method
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16 301 of 2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid), ABTS, described in
17
18 302 literature.³⁸ The same approach was already used in chitosan-based films in previous
19
20 303 works of the group.³⁷⁻³⁹ Briefly, one square (1 cm²) of each film was placed in 3 mL of
21
22 304 ABTS⁺ solution and after 15 min (T0) the absorbance of the solution at 734 nm (Jenway
23
24 305 6405 UV/Vis) was measured. The antioxidant activity of the films was monitored after
25
26 306 01, 05 and 20 days post preparation. All measurements were performed at least 4 times.
27
28 307 The antioxidant activity was expressed by the percentage of inhibition of the ABTS⁺,
29
30 308 calculated by equation 1:

33 309
$$\text{inhibition ratio (\%)} = 100 \times \frac{A_b - A_f}{A_b} \quad (\text{Eq. 2})$$

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35
36 310 where A_b and A_f are the absorbance of the blank (without any film) and films'
37
38 311 solutions, respectively.

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313 **Results and discussion**

314 **Optimization of IL type and solid-liquid extraction conditions.** Considering the high
315 hydrophobicity of carotenoids and the purity levels required for their application on the
316 preparation of biodegradable chitosan-based membranes, the first step carried on this
317 work was the proper selection of the most efficient solvent to recover the carotenoids. In
318 our recent publication,³³ another method for the extraction and purification of the same
319 carotenoids was developed. If the aim of developing an efficient method to obtain pure

carotenoids is not new,³³ the search for different processes of extraction is still crucial since up to know, carotenoids are a class of compounds with more 700 natural-based structures scarcely explored and with too much commercial potential to be ignored.⁴⁰ Moreover, the 94% of purity obtained in our last work³³ are not enough for the demands of the food sector, and thus, new or improved methodologies are still demanded. In this context, in this work, we tested the extraction efficiency of eleven ILs (with and without tensioactive nature) and two common surfactants, both tested in aqueous and ethanolic solutions (Figure 1). The common tensioactive compounds, sodium lauryl sulfate (SDS, anionic) and polysorbate 20 (Tween 20, non-ionic), were selected since they were reported as efficient solvents on the recovery of carotenoids from other rich-carotenoids matrices.⁴¹⁻⁴⁴

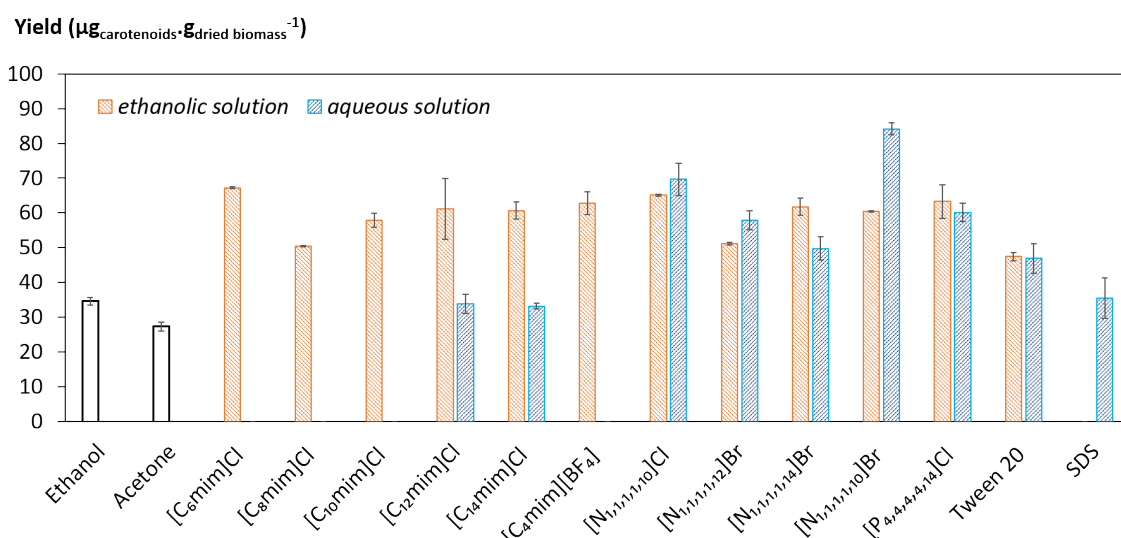


Figure 1. Screening of eleven ILs and two common surfactants on the extraction of carotenoids from peach palm fruits using aqueous (■) and ethanolic (■) solutions. Ethanol and acetone were used as controls (white bars).

The results depicted in Figure 1 suggest that, for the extraction of carotenoids, the use of aqueous solutions (blue bars) instead of ethanolic solutions (orange bars) is the best

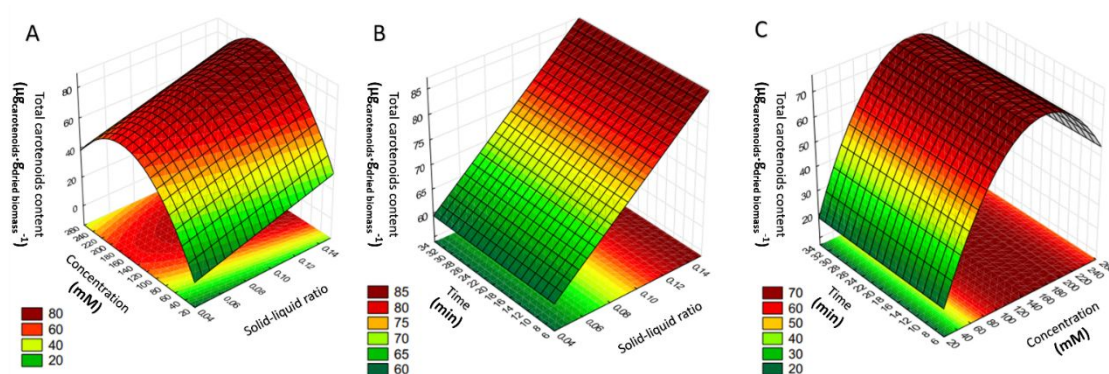
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3 338 approach. The conventional extractions by acetone and ethanol achieved the lowest yields
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5 339 of extraction obtained in the present work; $35 \pm 1 \mu\text{g}_{\text{carotenoids}} \cdot \text{g}_{\text{dried biomass}}^{-1}$ and 27 ± 1
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7 340 $\mu\text{g}_{\text{carotenoids}} \cdot \text{g}_{\text{dried biomass}}^{-1}$, respectively. Moreover, all aqueous solutions composed of ILs
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9 341 had yields of extraction equivalent or higher than those reported for conventional organic
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11 342 solvents. Actually, the aqueous solutions of non-tensioactive ILs, namely 1-hexyl-3-
12
13 343 methylimidazolium chloride $[\text{C}_6\text{mim}]\text{Cl}$, 1-octyl-3-methylimidazolium chloride
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15 344 $[\text{C}_8\text{mim}]\text{Cl}$, 1-decyl-3-methylimidazolium chloride $[\text{C}_{10}\text{mim}]\text{Cl}$, 1-butyl-3-
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17 345 methylimidazolium tetrafluoroborate $[\text{C}_4\text{mim}][\text{BF}_4]$, were not able to extract carotenoids
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19 346 using shaker homogenization.^{42,45}

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21
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23 347 The discussion about the poor capacity of non-tensioactive ILs to extract hydrophobic
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25 348 compounds is not new. It has been justified by the poor affinity on non-tensioactive
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27 349 solvents with the membranes of the cells and by their lower capacity to solubilize
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29 350 hydrophobic compounds in water, which has been overcome by the use of more
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31 351 aggressive homogenization techniques.^{33,46,47} Moreover, our results also suggest that
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33 352 aqueous solutions of $[\text{N}_{1,1,1,10}]\text{Br}$ represent the best system to extract carotenoids,
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35 353 achieving an extraction yield of $84 \pm 2 \mu\text{g}_{\text{carotenoids}} \cdot \text{g}_{\text{dried biomass}}^{-1}$.

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39 354 In an attempt to design a sustainable process, the aqueous solution of $[\text{N}_{1,1,1,10}]\text{Br}$,
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41 355 selected was further used on the optimization of the process conditions by applying a 2^3
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43 356 factorial planning. Here, the solid-liquid ratio $[\text{R}_{(\text{S/L})}]$, concentration of $[\text{N}_{1,1,1,10}]\text{Br}$
44
45 357 (Conc_{IL}) and time of extraction (time) were the conditions optimized (Table S3 from ESI).
46
47 358 The yield of extraction of carotenoids was used as the dependent variable in the definition
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49 359 of the predictive model represented by Equation 1. The yield of extraction of the
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51 360 carotenoids experimentally determined and predicted, as well as the statistical analyses
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53 361 performed, are shown in Table S3 in ESI. The model was adjusted with a confidence level
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55 362 of 95% and considered as a highly predictive model. Briefly, the results show that the
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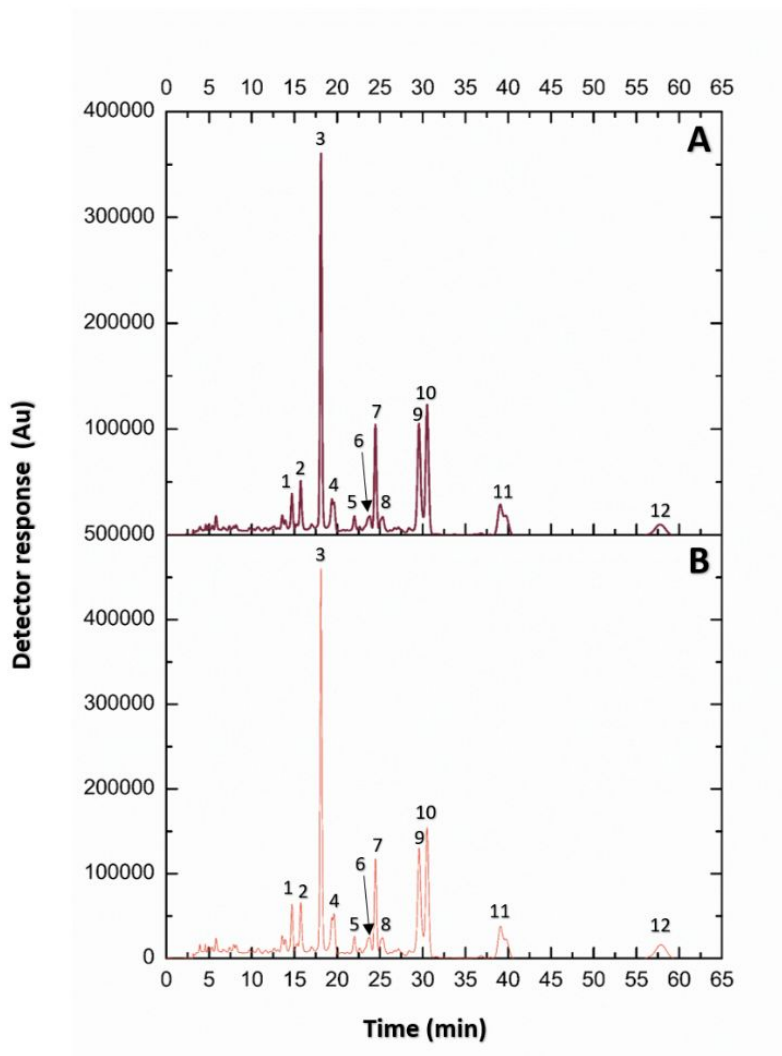
363 $R_{(S/L)}$ and the IL concentration were the independent variables with higher influence on
 364 the extraction yield. A maximum extraction yield of $92 \mu\text{g}_{\text{carotenoids}} \cdot \text{g}_{\text{dried biomass}}^{-1}$ was
 365 obtained for the conditions $R_{(S/L)} = 0.15$, $\text{Conc}_{\text{IL}} = 140 \text{ mM}$ at 20 min (Assay 10).
 366 Furthermore, the results showed the negligible impact of time on the yield of extraction,
 367 demonstrated by the extraction yields that do not change between 8.2 and 31.8 minutes
 368 (Figure 2 and Figure S1 from ESI), which is an advantage from the process point of view.
 369 The model was further validated using the optimal conditions ($R_{(S/L)} = 0.15$, $\text{Conc}_{\text{IL}} = 140$
 370 mM in aqueous solution for 8.2 minutes). Therefore, the average extraction yield obtained
 371 in the validation tests was $88.7 \pm 0.9 \mu\text{g}_{\text{carotenoids}} \cdot \text{g}_{\text{dried biomass}}^{-1}$, which corresponds to a
 372 relative deviation of 1.5%, evidencing the high confidence and accuracy of the model.
 373 Besides, comparing the optimized results using aqueous-solution of $[\text{N}_{1,1,1,10}]\text{Br}$ and
 374 acetone (as main conventional organic solvent), we achieved an equivalent HPLC-DAD
 375 profile for both extracts, showing that the alternative process developed in this work do
 376 not impair the extraction of any carotenoid commonly present in the peach palm (Figure
 377 3, Table S4 from ESI).

$$\text{total carotenoid content } (\mu\text{g} \cdot \text{g}^{-1}) = -23.61 + 252.18(x_1) + 0.90(x_2) - 0.003(x_2)^2 \quad (\text{Eq. 3})$$



381
 382 **Figure 2.** Response surface plots obtained for the factorial planning design (2^3)
 383 considering as independent variables the $R_{(S/L)}$, Conc_{IL} (mM) and time (min) towards the

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3 384 yield of extraction of carotenoids (total carotenoids content) as the dependent variable.
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5 385 The combination between independent variables were performed according to $R_{(S/L)}$ and
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7 386 $Conc_{IL}$ (A), Time and $R_{(S/L)}$ (B), $Conc_{IL}$ and Time (C).
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47 388 **Figure 3.** HPLC-DAD chromatograms (at 450 nm) of the carotenoids extracts obtained
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49 389 by the extraction using acetone (A) and aqueous solution of $[N_{1,1,1,10}]Br$ (B). The
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51 390 identification of the peaks is presented in Table S4 from ESI.
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57 392 **Carotenoids' polishing and IL' recycling.** To envision the industrial application of an
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59 393 extraction process, the solvents recyclability should be achieved. For this, two issues must
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3 394 be addressed, namely the complete isolation of the biomolecules from the solvents and
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5 395 the solvents recovery and reuse. In this work, considering the carotenoids hydrophobic
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7 396 nature and taking into account the structural features of $[N_{1,1,1,10}]Br$, the isolation of
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9 397 carotenoids from the aqueous solution was investigated by applying water as an anti-
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11 398 solvent.⁴⁸ In this case, the rationale was that the addition of water is able to decrease
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13 399 significantly the IL concentration in solution, thus reducing the solvation potential of
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15 400 $[N_{1,1,1,10}]Br$,^{49,50} and decreasing the solubility of carotenoids in the medium. The amount
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17 401 of water was optimized by evaluating the effect of diluting the extract rich in carotenoids
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19 402 by a factor of 2, 3, 5, 10, and 50. As depicted in Figure S2 from ESI, the dilution
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21 403 conditions by factors of 2 and 3, were not able to isolate the carotenoids from the solvents.
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23 404 However, for the dilutions by factors of 5, 10 and 50, a second thin layer was formed,
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25 405 clearly evidencing the separation of carotenoids concentrated in the top phase and the
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27 406 aqueous solution of $[N_{1,1,1,10}]Br$ as the bottom phase (Figure S2C). Instead of the
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29 407 precipitation of carotenoids (Figure S2B), a pigmented top phase was formed (Figure
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31 408 S2C), a result already reported in some works using anti-solvents.^{48,49,51} The “oily” layer
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33 409 formed, shows a high lipidic content of 15.6 ± 0.3 wt% (Table S5 from ESI). As shown
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35 410 in Figure S2, the carotenoids were suspended in the lipid’s solution, preventing them from
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37 411 being precipitated. According to literature,³² the carotenoids from peach palm fruits are
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39 412 in intracellular lipid bodies, which explains the lipids’ simultaneous extraction and
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41 413 presence in this second layer. From the $88.7 \pm 0.7 \mu g_{\text{carotenoids}} \cdot g_{\text{dried biomass}}^{-1}$ extracted by
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43 414 using the aqueous solution of $[N_{1,1,1,10}]Br$, 99.9 ± 0.1 % of the total carotenoids were
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45 415 recovered in the lipidic phase formed, while no carotenoids were detected in the IL-rich
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47 416 phase. Besides the successful isolation of carotenoids, the recovery of the $[N_{1,1,1,10}]Br$
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49 417 was also successfully achieved, with only 0.17 ± 0.02 % of IL in the carotenoids phase,
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51 418 while 93 ± 2 % could be recovered and reused in new cycles of extraction (the rest of the
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IL was lost in the biomass residues during extraction). Due to the economic interest in the extraction, purification, and processing of carotenoids,⁵² we have designed an integrated process, reproducible and with scale-up viability, depicted in Figure 3. After the isolation step, part of the water added was removed by evaporation and the aqueous solution of IL was reintroduced in a new cycle of (solid-liquid) extraction, to test the extractive viability of the solvent recycled. A total of 4 cycles (first extraction + 3 cycles of reutilization of the solvents) were carried as depicted in Figure S3 from ESI. The results show that in the first two cycles, the yields of extraction of all carotenoids (insets A, B, C and D of Figure S2 from ESI) were not compromised, however, after the third cycle, a decay on the extraction yield (ANOVA $p < 0.05$) was observed. Nevertheless, even with the decrease observed for the yield of extraction, after cycle 3, the results are still more than twice as higher as those obtained for ethanol and acetone (Figure S3 from ESI). To decrease even more the environmental impact of the final process, the water removed may be again used as anti-solvent as also depicted in Figure 4.

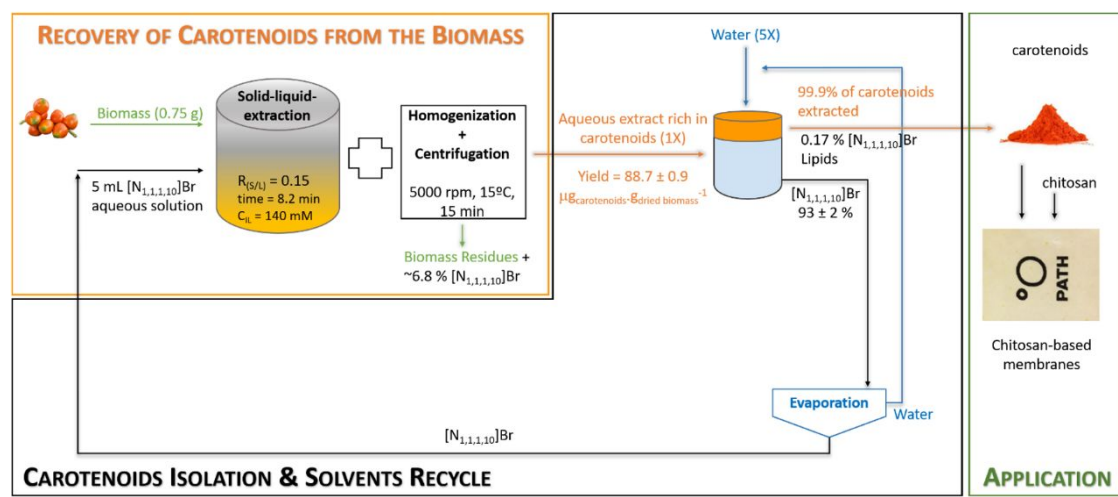


Figure 4. Schematic representation of the integrated process developed in this work contemplating the recovery of carotenoids from the biomass, followed by their isolation and solvents' recycle.

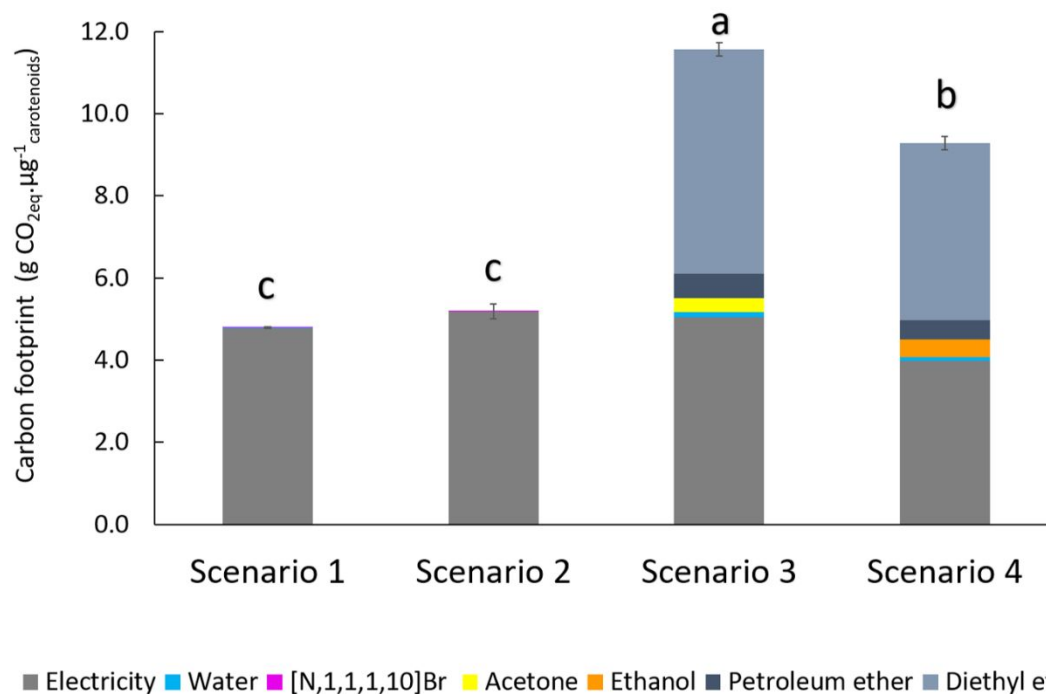
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3 438 In addition to the recyclability potential of the IL, its selectivity on the extraction
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5 439 process was also experimentally assessed. According to Cláudio et al. (2014),⁵³ the
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7 440 structural integrity of a biomass sample after the extraction process comprises the non-
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9 441 dissolution of significant amounts of carbohydrates, polysaccharides, fibers and other
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11 442 essential compounds present in the biomass. The thermo gravimetric profiles of our
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13 443 biomass before and after extraction mediated by the $[N_{1,1,1,10}]Br$ aqueous solution are
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15 444 identical (Figure S4 from ESI), thus confirming the integrity of the polymeric matrix, and
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17 445 consequently, the high selectivity of the IL applied in this process. Focusing the results
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19 446 obtained for the carbohydrates (the most abundant class of compounds in this biomass),
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21 447 no significant changes were detected on the amounts of xylose, glucose and uronic acids
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23 448 present after extraction (Table S6 from ESI).
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31 450 **Environmental evaluation of the carbon footprint and complete E-factor.** The
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33 451 environmental sustainability of the process developed in this work was evaluated through
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35 452 the carbon footprint and complete E-factor parameters. Data on the amounts of chemicals,
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37 453 water and electricity consumed were obtained during the experiments (Table S1 from
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39 454 ESI). Data on Greenhouse gas (GHG) emission factors from the production of chemicals,
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41 455 water and electricity were taken from Ecoinvent database version 3.5 (Ecoinvent, 2018)
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43 456 (Table S2 from ESI).³⁶ The results of both metrics are shown in Figure 5 and Table 1.
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46 457 Four scenarios were studied, with *Scenarios 1* and *2* representing the methods of
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48 458 extraction using aqueous solutions of $[N_{1,1,1,10}]Br$ without and with the cycles of IL and
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50 459 water reuse, respectively; and *Scenarios 3* and *4* for the conventional methods using
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52 460 acetone and ethanol, respectively. Since different extraction yields of carotenoids were
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54 461 obtained in each scenario, the metric results are expressed by 1 μg of extracted
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56 462 carotenoids, allowing thus the direct comparison between scenarios (Table S7 from ESI).
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3 463 In terms of carbon footprint, Figure 4 and Table S1 from ESI show that *Scenarios 1*
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5 464 and 2 have lower carbon footprint values than *Scenarios 3* and 4. *Scenario 2* considers
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7 465 the recovery and reuse of [N_{1,1,1,10}]Br and water, with equivalent results compared to
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9 466 *Scenario 1* (ANOVA $p > 0.05$), carbon footprint at 5.2 g CO_{2 eq}·μg⁻¹_{carotenoids} equivalent to
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11 467 *Scenario 1* (4.8 g CO_{2 eq}·μg⁻¹_{carotenoids}). This occurs because, despite the reduction of the IL
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13 468 used in *Scenario 2*, there is also a decrease in the carotenoids extraction yield through the
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15 469 three new cycles of extraction. The main contribution to the carbon footprint of *Scenarios*
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17 470 *1* and *2* comes from the electricity consumption, mainly from the evaporation and
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19 471 centrifugation units (53% and 47% of the total carbon footprint for both scenarios,
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21 472 respectively), while the contribution of the IL for both *Scenarios 1* and *2* is practically
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23 473 zero.

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25 474 The *Scenario 3* (conventional method using acetone) has the worst environmental
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27 475 performance, with a carbon footprint of 11.6 g CO_{2 eq}·μg⁻¹_{carotenoids}. In this scenario, the use
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29 476 of ethyl ether and the electricity consumption represent the major contributions to the
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31 477 total carbon footprint, with 47% and 43% (mostly from centrifugation), respectively. The
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33 478 acetone is only responsible for 3% of the total carbon footprint of this scenario. Finally,
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35 479 *Scenario 4* (conventional method using ethanol) has a total carbon footprint of
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37 480 9.3 g CO_{2 eq}·μg⁻¹_{carotenoids}. In this scenario, the ethyl ether and the electricity consumption
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39 481 are again the main contributors to the total carbon footprint, with 46% and 42% (mostly
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41 482 from centrifugation), respectively. The ethanol is only responsible for 5% of the total
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43 483 carbon footprint. These results suggest that, besides the IL-mediated downstream
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45 484 processes being excellent regarding the yield of extraction, the alternative carotenoids
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47 485 polishing performed by water as an anti-solvent is also extremely useful to mitigate the
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49 486 environmental impact of the whole process, comparing with the diethyl and petroleum
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51 487 ether polishing techniques.

488 In terms of complete E-factor, Table 1 shows that the recovery and reuse of the IL-
 489 based aqueous solutions and water after the polishing steps resulted in a 99% reduction
 490 of the complete E-factor in comparison with *Scenario 1*. In this scenario, the complete E-
 491 factor is $0.3 \text{ g}_{\text{waste}} \cdot \mu\text{g}_{\text{carotenoids}}^{-1}$, because of the discarded IL and water. By applying the
 492 conventional methods using acetone (*Scenario 3*) and ethanol (*Scenario 4*), $185.1 \text{ g}_{\text{waste}} \cdot$
 493 $\mu\text{g}_{\text{carotenoids}}^{-1}$ and $146.1 \text{ g}_{\text{waste}} \cdot \mu\text{g}_{\text{carotenoids}}^{-1}$, were respectively generated. Finally, for
 494 *Scenarios 3* and *4*, the discarded water contributes to almost 100% of the complete E-
 495 factor.



496
 497 **Figure 5.** The carbon footprint of the four scenarios investigated in this work. *Scenarios*
 498 *1* and *2* for the methods of extraction using aqueous solutions of [N_{1,1,1,10}]Br without and
 499 with three cycles of IL and water reuse, respectively; and *Scenarios 3* and *4* for the
 500 conventional methods using acetone and ethanol, respectively. Equal letters represent
 501 statistically equivalent values of carbon footprint (ANOVA $p < 0.05$)

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Table 1. Complete E-factor of the integrated process for obtaining carotenoids in four different scenarios. *Scenario 1* – method with [N_{1,1,1,10}]Br without recovery and reuse of the IL-based aqueous solution and water from extraction and polishing stages; *Scenario 2* – method with [N_{1,1,1,10}]Br with recovery and reuse of the IL-based aqueous solution and water from extraction and polishing stages; *Scenario 3* – conventional method using acetone; *Scenario 4* – conventional method using ethanol.

| Complete E-factor (g _{waste} ·μg _{carotenoids} ⁻¹) | | | | |
|--|------------------------|------------------------------|-------------------|-------------------|
| Waste generated | <i>Scenario 1</i> | <i>Scenario 2</i> | <i>Scenario 3</i> | <i>Scenario 4</i> |
| [N _{1,1,1,10}]Br | 2.3 x 10 ⁻³ | 0 | – | – |
| Acetone | – | – | 0.1 | – |
| Ethanol | – | – | – | 0.1 |
| Water | 0.3 | 4.6 x 10 ⁻³ | 182.9 | 144.4 |
| Petroleum ether | – | – | 1.3 | 1.0 |
| Diethyl ether | – | – | 0.8 | 0.6 |
| Total | 0.3 | 4.6 x 10⁻³ | 185.1 | 146.1 |

Preparation and characterization of chitosan films. The development of an alternative food-packaging biomaterial with the incorporation of bioactive compounds into edible films and other biomaterials has become a feature nowadays.^{54,55} In addition, the Food and Drug Administration (FDA) recognizes as safe (GRAS status) the incorporation into biodegradable films of compounds derived from fruit pulps.⁵⁶ However, most studies focus on the use of phenolic compounds and their role in the biological properties of the films, such as antioxidant, antimicrobial and anti-inflammatory activities.^{57–59} Despite the great interest in this field, the incorporation in edible films of an extract rich in carotenoids has not yet been evaluated. In this work, chitosan films were used as a matrix for the incorporation of carotenoids from *B. gasipaes* fruits in three different doses [0.025 %, 0.050 %, and 0.100 % (w/w)], obtained from two types of extraction, the one proposed using the IL as solvent, and the extract obtained using acetone (Figure S5 from ESI).

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3 523 The mechanical properties of the films were determined to evaluate the influence of
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5 524 the carotenoids incorporation, since they could modify the structure of the polymer
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7 525 matrix, by weakening the inter-chain bonds⁶⁰ and, consequently, modifying the films
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9 526 mechanical properties.⁶¹ The chitosan-based films without carotenoids (control) had a
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11 527 tensile strength (σ_b) of 37 MPa, a Young's modulus 1.2 MPa and an elongation at break
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13 528 (E_b) of 32%. The effects of incorporating carotenoids in different concentrations on
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15 529 chitosan-based films are shown in Table 2. The film thickness was evaluated, since it is
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17 530 an important parameter that influences the mechanical properties of the films.⁶² The
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19 531 addition of carotenoids, regardless of dose, does not modify the thickness of the films
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21 532 compared to the control group (ANOVA $p > 0.05$), which reflects the equivalent contact
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23 533 area of the materials. All films composed of carotenoids have significant differences in
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25 534 the mechanical properties when compared to control (Table 3 and Figure S5 from ESI).
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27 535 The incorporation of the carotenoids obtained by the IL-based process, at the doses of
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29 536 0.025 % and 0.050 %, do not change the tensile strength limit of the films (ANOVA $p >$
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31 537 0.05). On the contrary, the incorporation of carotenoids at the dose of 0.100% obtained
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33 538 by using IL as solvent and carotenoids obtained by acetone extractions (all doses),
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35 539 decreased the tensile strength, meaning the decrease of the films' mechanical resistance.
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37 540 The differences between the mechanical behavior of the films could be justified by the
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39 541 effect of the carotenoids crystallization after the film storage process, which justifies that,
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41 542 for the highest dose of carotenoids extracted by IL (0.100%), the film has a lower tensile
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43 543 strength,⁶³ contrarily to what happened in the work of Liu and collaborators,⁶⁴ where the
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45 544 addition of curcumin to the chitosan-films increased the tensile strength.
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53 545 A higher elasticity (elongation at break) of the chitosan-based membranes was
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55 546 obtained by the incorporation of 0.100% of carotenoids obtained by the IL-based process
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57 547 (49 %) when compared with the control films (32 %), whereas the incorporation of
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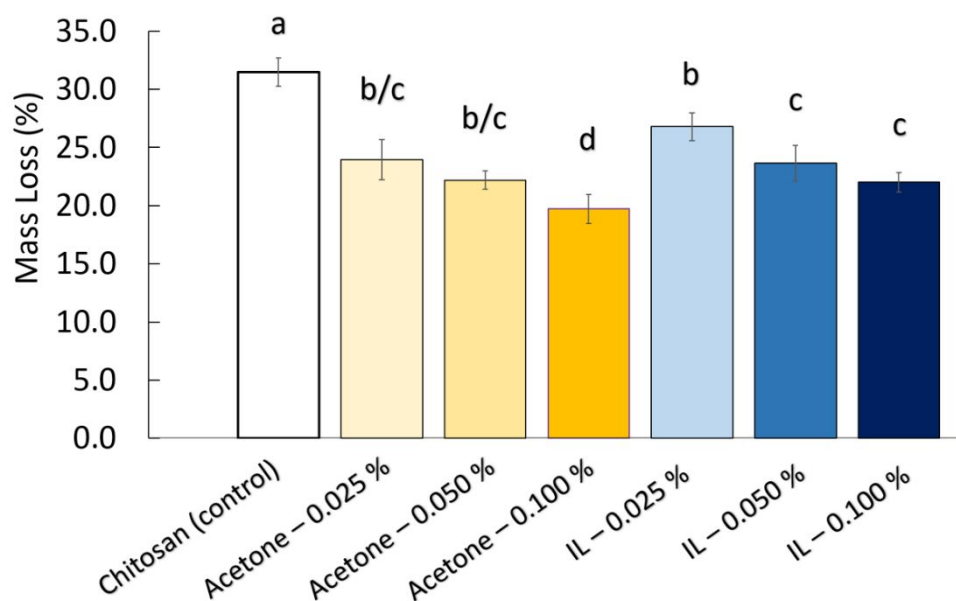
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3 548 0.100% of carotenoids extracted by acetone have the lowest elasticity (12 %). In addition,
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5 549 all films with carotenoids incorporated showed lower Young's modulus values in
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7 550 comparison with the control, which means their higher ductility. Although all films
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9 551 already have 0.1% of glycerol used as plasticizer, the addition of carotenoids influenced
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11 552 this parameter, which seems to be attributed to the plasticizer action that carotenoids
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13 553 could have on the carbohydrates network.⁶⁰
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17 554 Comparing the enriched films of carotenoids obtained with IL and acetone, it seems
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19 555 that the viscoelasticity properties are significantly different (Table 2). For example, at
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21 556 doses of 0.100 %, the films enriched with carotenoids extracted by IL resemble elastomers
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23 557 (E of 49 %), while the films enriched with carotenoids extracted by acetone have E of
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25 558 12%, besides low tensile strength. Despite the wide range of applications of natural
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27 559 biopolymers, the elastomer-like ones possess a robust interface,⁶⁵ since they can be
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29 560 molded by a wide range of processes, due to their softness, flexibility, and resilience.⁶⁶
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31 561 The surface wettability of the chitosan-based films incorporating the carotenoids was
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33 562 evaluated by contact angle analysis using ultrapure water. The contact angle of all films
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35 563 with different carotenoids doses are listed in Table 3. The films with carotenoids
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37 564 incorporated revealed a slightly lower contact angle value in both film phases. These
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39 565 results are somehow contrary to what was expected, a phenomenon that could be
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41 566 explained by the amorphous and crystalline surfaces obtained after the incorporation of
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43 567 carotenoids in the chitosan network bounds;⁶⁷ or by the spatial condition of the carbon
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45 568 skeleton between the chitosan bounds, as previously reported for the addition of curcumin
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47 569 also in chitosan-based membranes;⁶⁴ or even by the potential contamination of the
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49 570 extracts derived from acetone extraction (acetone is not a selective solvent).
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57 571 The films solubility was also determined after 7 days immersed in a mixture of 50:50
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59 572 (v/v) of water:ethanol under continuous stirring. Figure 6 shows the weight loss
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3 573 percentage for the films with carotenoids extracted with acetone and [N_{1,1,1,10}]Br as
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5 574 solvents, as well as one film prepared only with chitosan (without carotenoids
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7 575 incorporated) used as control. The control film presented the higher weight loss
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9 576 percentage (32%), comparing with the weight loss determined for films prepared with
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11 577 carotenoids obtained with acetone and IL as solvents (20-27%). The films prepared with
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13 578 the carotenoids derived from the use of acetone as solvent showed a slightly lower
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15 579 solubility, especially for the film with 0.100% of carotenoids extract (*T*-test: *p* < 0.05),
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17 580 when compared with the film incorporating the carotenoids extracted by the IL. Summing
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19 581 up, the carotenoids incorporation resulted in a decrease of the films' solubility, due to the
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21 582 hydrophobicity of carotenoids incorporated in the chitosan matrix. Fortunately, we
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23 583 proved that the chitosan-carotenoid biofilms were prepared safely, since the 0.17% of IL
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25 584 remaining still in the carotenoids extract is not released to the water, which was proved
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27 585 by the absence of bromide anions determined by ionic chromatography.

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34 586 The antioxidant activity of the films was also tested for three different periods after
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36 587 their production, namely 1, 5 and 20 days (Figure S6 from ESI). Briefly, and as expected,
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38 588 the films with carotenoids showed higher antioxidant activity when compared with the
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40 589 control films. It is observed that the maximum dose of carotenoids incorporated in films
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42 590 (0.100%) have the highest antioxidant activity, for both strategies of extraction (IL and
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44 591 acetone). In all doses (0.025, 0.050 and 0.100 %) and for all times tested (15, 30, 60 and
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46 592 120 min) the films enriched with carotenoids obtained by using IL as solvent have the
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48 593 highest antioxidant activity data, which was maintained during the 20 days of evaluation.
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50 594 This can be justified by the highest selectivity of the IL carrying the extraction of
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52 595 carotenoids, when compared with the films incorporating the carotenoids extracted by
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54 596 acetone.^{37,38}



597

598 **Figure 6.** Weight loss percentage determined for the chitosan-based films solubility test,
 599 after 7 days in a water:ethanol mixture.

600

601 **Table 2.** Mechanical properties of films with and without carotenoids incorporated.
 602 Different letters in the same column indicate significant differences (Bonferroni pos-hoc
 603 test, $p < 0.05$)

| Films | Young's modulus (E) | Tensile strength (MPa) | Elongation at break (%) |
|------------------------|-------------------------|------------------------|-------------------------|
| Control | 1.2 ± 0.2^a | 37 ± 6^a | $32 \pm 4^{b/c}$ |
| IL 0.025 % | 0.6 ± 0.2^b | 34 ± 5^a | 35 ± 4^b |
| IL 0.050 % | 0.4 ± 0.1^b | 35 ± 8^a | 38 ± 5^b |
| IL 0.100 % | 0.05 ± 0.02^c | $18 \pm 1^{b/c}$ | 49 ± 6^a |
| Acetone 0.025 % | 0.4 ± 0.1^b | 22 ± 3^b | 29 ± 6^c |
| Acetone 0.050 % | 0.5 ± 0.1^b | 19 ± 7^b | 31 ± 8^c |
| Acetone 0.100 % | 0.53 ± 0.08^b | 13 ± 2^c | 12 ± 3^d |

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Table 3. Contact angle ($^{\circ}$) measures from chitosan-based films in both bottom and top surfaces. Different letters in the same column indicate significant differences (Bonferroni pos-hoc test, $p < 0.05$).

| Films | Bottom phase | Top phase |
|------------------------|------------------|------------------|
| Control | 108 ± 8^a | 100 ± 7^a |
| IL 0.025 % | 92 ± 4^b | 90 ± 5^b |
| IL 0.050 % | $85 \pm 6^{b/c}$ | 80 ± 4^b |
| IL 0.100 % | 87 ± 3^b | 76 ± 6^b |
| Acetone 0.025 % | 93 ± 7^b | 77 ± 9^b |
| Acetone 0.050 % | 91 ± 3^b | $72 \pm 6^{b/c}$ |
| Acetone 0.100 % | 72 ± 8^c | 69 ± 6^c |

Conclusions

An extraction process of all-*trans*- β -carotene, all-*trans*-lycopene and all-*trans*- γ -carotene from the fruit biomass of the Amazonian tree *Bactris gasipaes* was successfully developed in this work using an ionic liquid-based extraction. $[N_{1,1,1,10}]Br$ in water was selected as the most efficient solvent to maximize the selective extraction of carotenoids (yield of extraction of $88.7 \pm 0.9 \mu g_{\text{carotenoids}} \cdot g_{\text{dried biomass}}^{-1}$), for the optimum conditions of 8.2 minutes, 140 mM of IL and $R_{(S/L)}$ of 0.15. Besides the highest yield of extraction, the use of aqueous solutions of $[N_{1,1,1,10}]Br$ allowed the development of an integrated process, in which the isolation of the carotenoids and recyclability of the solvents, IL and water, were steps successfully and easily achieved by using water as an anti-solvent. In addition, the reuse of the IL for a total of four cycles of extraction was optimized, which helped to significantly decrease the environmental impact of the IL-based process, a result demonstrated by the carbon footprint and complete E-factor assessment. Proved the efficiency and low environmental impact of the process, and after isolation, the carotenoids were applied on the development and optimization of a task-specific

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3 630 chitosan-based film applicable in food packaging, with advantageous results obtained for
4
5 631 tensile strength, Young's modulus, elongation at break, elasticity, film thickness,
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7 632 wettability, solubility in water and, in particular for antioxidant activity, which was
8
9 633 maintained in the highest levels during 20 days of experiment.
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48 648 **Conflicts of interest**

49 649
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51 650 There are no conflicts to declare.
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53 651 54 55 652 **References**

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24 900 **Synopsis sentence**

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26 901 Sustainable process developed to obtain carotenoids from the *Bactris gasipaes* fruits and

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29 902 their application in a food-packaging biomaterial.
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