



HAL
open science

Sequential one-pot NaDES assisted extraction and biotransformation of rice bran: A new strategy to boost antioxidant activity of natural extracts

Millena Cristina Barros Santos, Nathalie Barouh, Bruno Barea, Pierre Villeneuve, Claire Bourlieu-Lacanal, Mariana Simões Larraz Ferreira, Erwann Durand

► To cite this version:

Millena Cristina Barros Santos, Nathalie Barouh, Bruno Barea, Pierre Villeneuve, Claire Bourlieu-Lacanal, et al.. Sequential one-pot NaDES assisted extraction and biotransformation of rice bran: A new strategy to boost antioxidant activity of natural extracts. *Process Biochemistry*, 2022, 117, pp.110-116. 10.1016/j.procbio.2022.03.021 . hal-03625486

HAL Id: hal-03625486

<https://hal.science/hal-03625486v1>

Submitted on 22 Jul 2024

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial 4.0 International License

1 **Sequential one-pot NaDES assisted extraction and biotransformation of rice**
2 **bran: A new strategy to boost antioxidant activity of natural extracts**

3 Millena Cristina Barros Santos¹, Nathalie Barouh^{2,3}, Bruno Baréa^{2,3}, Pierre Villeneuve^{2,3},
4 Claire Bourlieu-Lacanal⁴, Mariana Simões Larraz Ferreira^{1*} and Erwann Durand^{2,3*}

5
6 ¹Laboratory of Bioactives (LabBio), Food and Nutrition Graduate Program (PPGAN), Federal
7 University of the State of Rio de Janeiro (UNIRIO), Rio de Janeiro, Brazil

8 ²CIRAD, UMR QualiSud, F-34398, France

9 ³Qualisud, Univ Montpellier, CIRAD, Institut Agro, IRD, Université de la Réunion, Montpellier,
10 France

11 ⁴IATE, Univ Montpellier, INRAE, Institut Agro, Montpellier, France

12 Corresponding authors: erwann.durand@cirad.fr; mariana.ferreira@unirio.br

13 **Abstract**

14 Natural Deep Eutectic solvents (NaDES) was used to produce, after sequential one-pot
15 extraction and biotransformation of naturally pigmented rice bran (RB), ready-to-use extracts
16 that improve the oxidative stability of lipids in an oil-in-water emulsion. Two NaDES-based
17 strategies were implemented. Extraction of phenolics content varied according to the NaDES.
18 The shiftable NaDES (obtained from the acidic-NaDES based on ChCl and xylitol) stands out
19 by showing content of 1525 mg eq gallic acid/100 g RB. The sequential one-pot
20 biotransformation with immobilized *Candida antarctica* lipase B after increasing pH, was
21 marked by a decrease in phenolics content and a lipids profile variation. These enriched
22 extracts could be used as natural additives or ready-to-use extracts, as confirmed by the
23 encouraging antioxidant results observed in emulsion. Omics tools should be applied to
24 elucidate the structural rearrangement between compounds and the pool of extracted
25 antioxidant molecules that may explain the protection against lipid oxidation.

26 **Keywords:** NaDES, iCALB lipase, rice coproducts, read-to-use extracts, antioxidant, lipid
27 oxidation.

28 1. Introduction

29 Natural deep eutectic solvents (NaDES) are liquid mixtures metabolites that are abundantly
30 present in nature, such as sugars, organic acids, amino acids, organic bases, or vitamins -
31 which besides its natural origin, may be suspected to have a presence and role in living
32 organisms [1, 2]. The strong solubilization capacity of NaDES was already used to provide
33 enriched extracts with high phytochemical concentration and molecular diversity [3, 4].
34 NaDES showed several advantages over conventional solvents, such as an enhanced
35 biological activity or an excellent capacity to stabilize and protect (macro)molecules from
36 degradation, mostly due to the supramolecular network of tightly interconnected molecules
37 [5]. These liquid properties make NaDES very attractive for extraction processes of bioactive
38 compounds. However, the difficulty to separate, and to recover dissolved molecules from
39 NaDES, mostly due to their low vapor pressure and strong intermolecular connections, have
40 limited up to now new applications and industrial transfers. NaDES offer all characteristics to
41 design atom economy, efficient, low cost and sustainable development for ready-to-use
42 formulation, and depending on the nature of starting components, they may be fully
43 compatible with food and feed applications [6]. NaDES were already used to provide ready-
44 to-use extracts with higher interest [5, 6]. Besides the great capacity to extract bioactive
45 compounds, these solvents can also be used as medium for enzymatic activity [7, 8].
46 Naturally occurring antioxidants obtained from agroindustry processing may be efficiently
47 used to prevent lipid oxidation in food (*e.g.* fruits [9] and cereals[10]) and cosmetic products.
48 Rice is one of the most important staple food and its processing lead to millions of tons of rice
49 bran (RB) annually. RB is usually underutilized, destined to animal feed. The sustainable
50 valorization of rice coproducts for improving human health has become a priority due to the
51 presence of bioactive phytochemicals, such as phenolic compounds (PCs) [11], vitamins,
52 fibers, proteins, minerals such as iron, but also γ -oryzanol and the presence of unsaturated

53 lipids [12]. Natural extracts from RB can generate different industrial applications, especially
54 for human consumption. Even more so, for the pigmented rice, which presents the highest
55 total phenolic content and antioxidant capacity [13]. Recently, NaDES were used to extract
56 PCs from pigmented RB and showed specificity, confirmed by omic tools, to extract distinct
57 compounds in comparison to the ethanol/water mixture [14].

58 In addition, the structural modification of antioxidant molecules has been recognized as an
59 efficient strategy to strengthen their capacity to retard oxidation of heterogeneous lipid-based
60 systems. Increasing the lipophilic nature (so-called lipophilization) of antioxidants dispersed
61 in water phase lead to a positive change in their capacity to stabilize lipid against oxidation in
62 oil-in-water (O/W) emulsions [15, 16]. This has been observed with chlorogenate [17, 18],
63 gallate [19], ferulate [20], or caffeate [21] alkyl esters, in various colloidal systems. Actually,
64 the physical location of antioxidants, and thus, their distribution and mobility when different
65 phases co-exist (*e.g.* emulsions) is fundamental and matrices-dependent. This structural
66 modification of PCs can be even more interesting, although more challenging, if the
67 modification takes place enzymatically (*e.g.* with lipase EC 3.1.1.3) because of milder reaction
68 conditions, higher selectivity, less by-products and purification steps, in line with this green
69 chemistry era [22-24]. *Candida antarctica* lipase B has been pointed out as a great and
70 versatile biocatalyst for lipophilization reaction of PCs in different media, including DES
71 [22].

72 Although the increasing use of NaDES in food research [2, 25-27], its scale-up is hardly
73 explored and the possibilities for its application for human consumption still need to be
74 developed [28]. In this context, we went to investigate how the antioxidant activity of natural
75 occurring molecules in RB may be improved by NaDES. The strategy was based on NaDES
76 as a potential media to develop a sustainable one-pot [29] extraction and biotransformation
77 environment to (i) promote antioxidant enrichment of a natural extract, and (ii) enzymatically

78 modify the structure of extracted compounds by lipase-catalyzed reactions. Therefore, the
79 samples were tested and compared for their ability to prevent lipid oxidation in an O/W
80 emulsion system.

81 **2. Material and methods**

82 **2.1. Materials and chemicals**

83 Choline chloride ($\geq 99\%$), lactic acid ($\sim 90\%$), 1,2-propanediol ($\geq 99\%$), xylitol ($\geq 99\%$),
84 methanol (HPLC grade), water (HPLC grade), ethanol, isooctane, isopropanol, sodium
85 dodecyl sulfate (SDS), potassium hydroxide (KOH), iron sulfate, ammonium thiocyanate,
86 1,1,3,3-tetramethoxypropane, cumene hydroperoxide, trichloroacetic acid, thiobarbituric acid,
87 mono and dibasic sodium phosphate, 1-octanol (anhydrous $\geq 99\%$), hydrochloric acid (HCl),
88 immobilized lipase B from *C. antarctica* (Novozym® 435, 11900 propyl laurate units/g)
89 (iCALB), the lipids standards: triacylglycerols (TAG), diacylglycerols (DAG),
90 monoacylglycerols (MAG), free fatty acids (FFA), monogalactosyldiacylglycerols (MGDG),
91 digalactosyldiacylglycerols (DGDG), phosphatidylcholine [11], and phosphatidylserine [30],
92 and gallic acid (GA) were obtained from Sigma-Aldrich (St. Louis, USA). Hexane (99%) was
93 obtained from Labover (Montpellier, France) and vinyl laurate ($\geq 99\%$) from Fluka (Saint
94 Quentin, France).

95 **2.2. Pigmented rice bran**

96 The red RB from Madagascar were cultivated in Ampitatafika (-19.565717, 47.362911),
97 obtained by abrasive dehulling (polishing) in a single pass rice mill and stabilized (100/110
98 °C - 5 min). The RB was sieved to eliminate particles larger than 1 mm size and then milled
99 for 5 min with a ball mill (Fritsch Pulverizer Mill, Germany) to obtain a homogeneous
100 fraction.

101 **2.3. Preparation of Natural Deep Eutectic Solvents**

102 Choline-chloride (ChCl) based NaDES in combination with lactic acid, 1,2-propanediol and
103 xylitol were selected based on GRAS (Generally Recognized as Safe) classification [27].
104 NaDES were prepared by heating at 50 °C with orbital shaking at 400 rpm (IKA KS 4000 I
105 control, Staufen, Germany) after mixing the components (according to specific molar ratios)
106 in a closed bottle for 45 min until a clear liquid is formed. pH was monitored with a pH-meter
107 (edge® pH, Hanna instruments, Lingo Tanneries). NaDES composition and mole ratio were
108 as follows: NaDES1 (ChCl:1,2-propanediol:water, 1:1:1), NaDES2 (ChCl:lactic acid, 1:10),
109 NaDES3 (ChCl:xylitol:water, 1:1:1). Shiftable NaDES (shiftNaDES) refers to a sequential
110 strategy based on the capacity of a NaDES medium, such as NaDES3, to shift from acid to
111 alkaline pH, or *vice versa*. For shiftNaDES3, the addition of HCl 2M in water was performed
112 for the NaDES3 formulation to lead to acidic-NaDES3, then the addition of KOH was
113 performed in acidic-NaDES3 (9.59 mg per mL of NaDES) to lead to NaDES3-KCl.

114 **2.4. Assessment of the lipase activity and stability in NaDES**

115 The lipase-catalyzed activity and stability evaluated after 24 h of iCALB pre-incubation were
116 carried out in all NaDES (1 mL), during 30 min at 50 °C according to Durand, et al. [7].

117 **2.5. One-pot extraction and biotransformation of phenolic compounds and lipids**

118 RB were extracted (25 mg/mL) with NaDES in a closed amber glass flask (60 min, 40 °C,
119 200 rpm orbital agitation) (Thermo Scientific Poly 15, Ville, China). Then, samples were
120 cooled to room temperature (RT, ~20 °C) and centrifuged (5 min, 4000 rpm) (CR412
121 centrifuge; Jouan, Winchester, VA). The iCALB (10 mg/mL) was directly added for the
122 biotransformation reaction (biotransformed extracts) and left for 24h of reaction. Control
123 samples without addition of the enzyme were also performed in this step. After centrifugation,
124 aliquots (250 µL) of the supernatants of each extract were immediately extract by the Folch
125 method [31], where aqueous and organic phases were separated, collected, and evaporated
126 with a nitrogen flow. The aqueous phase was destined for PCs characterization by HPLC

127 and the organic phase for lipids identification to by thin layer chromatography (TLC). Beside
128 this, aliquots of 500 μ L of the supernatants, after centrifugation for biotransformed extracts,
129 were destined to the antioxidant activity in O/W emulsion.

130 **2.6. Phenolic compounds analysis by HPLC-DAD**

131 The aqueous phase of the Folch extraction was evaporated with nitrogen flow, resuspended
132 with 1 mL of MeOH/water (2:1, v/v), and filtered (0.45 μ m, cellulose filter). Samples (40 μ L)
133 were injected in the HPLC (LC-20AD equipped with oven CTO-10ASvp and detector DAD
134 SPO-M20, Shimadzu, Noisiel, France) [32] with the gradient method (mobile phase A:
135 MeOH and B: water, both with 0.1% acetic acid): 0-5 min: isocratic at 10% of B; 5-20 min:
136 linear gradient up to 100% B; 20-30 min isocratic at 100 % B; 30-35 min linear gradient up to
137 10% B; 35-42 min: equilibration at 10% B. The detection was performed at 280 nm and 330
138 nm. Gallic acid (GA) was used for the calibration curve quantification, and data were
139 expressed in mg eq of GA.

140 **2.7. Lipids analysis with thin layer chromatography (TLC)**

141 Organic phases from Folch extraction method were resuspended with 500 μ L of
142 CHCl_3 :MeOH (2:1, v/v) and spotted on silica gel HPTLC plates (Merck, Saint-Quentin
143 Fallavier, France) using an automatic sampler (ATS4, Camag, Muttenz, Switzerland). Lipid
144 standards (TAG, DAG, MAG, FFA, MGDG, DGDG, PC, and PS) were also spotted. The
145 elution was performed in double migration using an automatic developing chamber ADC2
146 (Camag, Muttenz, Switzerland) as follows: 40 mm with CHCl_3 /MeOH/water (19:4:0.5, v/v/v)
147 and 80 mm with hexane/diethyl ether/formic acid (14:6:0.2, v/v/v). After elution, plates were
148 dried (RT), dipped into $\text{CuSO}_4/\text{H}_3\text{PO}_4$ reagent using a Chromatogram Immersion Device 3
149 (Camag, Muttenz, Switzerland), dried again (RT), and placed in an oven (180 $^\circ\text{C}$, 10 min).
150 Absorbances of revealed spots were measured at 500 nm (TLC Scanner 3, Camag, Muttenz,

151 Switzerland), analyzed by VISIONCAT software, and qualitatively evaluated in comparison
152 with lipid standards.

153 **2.8. Antioxidant activity in oil-in-water (O/W) nanoemulsion**

154 An O/W nanoemulsion of rapeseed oil in PBS (10 mM, pH 7.0) with SDS 20 mM and 0.02 %
155 sodium azide [33] was prepared. Briefly, O/W nanoemulsions were prepared by
156 homogenization of 1 g of rapeseed oil with 99 g of aqueous phase. The aqueous phase
157 consisted of phosphate buffer solution (10 mM, pH 7.0) and SDS at 20 mmol/l in the final
158 emulsion. For nanoemulsion preparation, oil and aqueous phases were initially mixed using
159 an Ultra-Turrax IKA (Janke & Kunkel IKA-Labortechnik, Staufen, Germany) for 2 min at
160 5400 rpm speed. The resulting coarse emulsions were then homogenized on a Microfluidizer
161 (9 K, Microfluidics, Newton, MA, USA) at an operating pressure of 40 MPa for 3 cycles. The
162 antioxidant solutions were incorporated in the aqueous phase after the homogenization step.
163 Aliquots (500 μ L) of NaDES extracts and biotransformed NaDES extracts (after
164 centrifugation) were added to 25 mL of nanoemulsions. RB powder (12.5 mg), considering
165 that all rice samples had the final concentration of 500 ppm in the emulsion, and NaDES
166 alone (500 μ L), were analyzed as control. The oxidation assays were carried out in triplicate
167 into 25-mL screw-capped amber vials and incubated at 40 °C for 20 days under gentle orbital
168 stirring (110 rpm) (IKA KS 4000i control, Germany). Samples were collected at 1, 3, 6, 9, 13,
169 16, and 20 days to follow lipid oxidation.

170 **2.9. Determination of lipid oxidation**

171 The evaluation of lipid oxidation in the nanoemulsions was carried out by monitoring the
172 formation of primary (peroxide value, PV) and secondary (2-thiobarbituric acid reactive
173 substances, TBARS) oxidation products [33].
174 Briefly, PV were determined on 100 mg of extract with isooctane/isopropanol solution (3:1
175 v/v). An aliquot of this extract was then added to MeOH/BuOH (3:7 v/v) to give a final

176 volume of 260 μL in the microplate well (Greiner Bio-One International, Austria). Then, 2.5
177 μL of 30% ammonium thiocyanate solution and 2.5 μL of ferrous solution (0.144 M) were
178 added, and the mixture was incubated (RT, 10 min). After incubation, the absorbance was
179 measured at 510 nm (Infinite M1000 microplate reader, Tecan, Gröedig, Austria). A standard
180 curve was prepared with cumene hydroperoxide. Analyses were performed in triplicate and
181 results were expressed as meq O_2/kg oil.

182 For TBARS, briefly, 200 mg of nanoemulsions were mixed with 400 μL of TBARS solution
183 (15% trichloroacetic acid, w/v; 1:2 (v:v) of 0.375% w/v of thiobarbituric acid and 0.25 M
184 HCl) and then heated (90 $^\circ\text{C}$, 15 min). Then, samples were cooled in an ice bath and
185 centrifuged. The absorbance of the collected supernatant was measured at 532 nm. The
186 TBARS content was determined according to a calibration curve obtained using 1,1,3,3-
187 tetramethoxypropane as external standard and expressed as mmol equivalent malondialdehyde
188 (eq MDA)/kg oil.

189 **2.10. Statistical analysis**

190 The XLSTAT software (Addinsoft, France) was used to perform the statistical analysis
191 (Tukey's test, $p < 0.05$, and one-way ANOVA). The graphical representation of the results was
192 created using the GraphPad Prism (5.0) software.

193 **3. Results and discussion**

194 **3.1. Evaluation of the NADES extraction capacity of phenolic compounds from rice bran**

195 Initially, NaDES1 (ChCl:1,2-propanediol:water, 1:1:1), NaDES2(ChCl:lactic acid, 1:10) and
196 NaDES3 (ChCl:xylitol:water, 1:1:1), were only tested for their capacity to extract PCs from
197 RB. NaDES2 showed the highest capacity with 39.97 ± 2.30 and 303.88 ± 16.28 mg GA
198 eq/100 g of RB at 280 and 330 nm, respectively (Fig. 1). As already highlighted in the
199 literature [14, 34], acidity of NaDES is an important attribute to promote the extraction of
200 PCs. To confirm this hypothesis, NaDES3 was acidified (acidic-NaDES3) and tested for the

201 RB extraction, a strategy that has already proven to be effective to release bound PCs. As
202 expected, acidic-NaDES3 significantly increased the phenolic content, by a factor of ten and
203 two, at 280 and 330 nm, respectively. Indeed, acidic-NaDES3 appeared as the best solvent of
204 PCs extraction in this study, reaching 404.23 ± 41.17 and 715.05 ± 102.93 mg GA eq/100 g of
205 RB at 280 and 330 nm, respectively.

206 **3.2. Evaluation of the lipase-catalyzed reaction in the selected NaDES**

207 Some NaDES formulations may present limitations for enzymatic reactions, decreasing or
208 inhibiting the lipase activity [22, 24]. To determine whether NaDES could be adapted for
209 iCALB lipase-catalyzed reactions, a model reaction based on the alcoholysis of vinyl laurate
210 (VL) with octanol was employed. The use of vinyl ester as acyl donors is a fast and effective
211 way to evaluate lipase initial activities [8], and has been successfully applied for estimating
212 the iCALB activity and stability in DES [7]. Conversion was calculated based on the VL
213 decay, while the selectivity for octanol was assessed based on the octyl laurate (C8L)
214 formation (Fig. 2). NaDES1 and NaDES3 allowed for a complete conversion of the VL
215 substrate. Conversely, NaDES2 and acidic-NaDES3 did not provide suitable environment for
216 lipase activity, with a poor selectivity for transesterification, ~5 % and ~50 %, respectively.
217 Low pH values in NaDES2 and acidic-NaDES3 seemed to inhibit the lipase activity and to
218 promote the hydrolysis competitive reaction instead of the ester bond formation. Considering
219 the high extraction efficiency of acidic-NaDES3, we have investigated its capacity to promote
220 the lipase-catalyzed reaction after re-increasing pH to obtain the NaDES3-KCl. This shiftable
221 NaDES-based strategy could be seen as a new and efficient solution to move toward a one-pot
222 NaDES-assisted extraction and biotransformation of natural extracts. Interestingly, NaDES3-
223 KCl allowed for both a very high activity and high yield of conversion of VL into C8L (>90
224 %) (Fig. 2). Regarding the stability of iCALB, NaDES1, NaDES3 and NaDES3_KCl seemed

225 to preserve, at least on the short term, the protein conformation since the lipase catalysis was
226 hold over 24 hours, which confirms that media could be used for long-term lipase reactions.

227 **3.2. One-pot NaDES assisted extraction and biotransformation of rice bran**

228 Two distinct strategies were developed (S1 and S2) (Fig. 3): 1) with NaDES1, and 2) with the
229 shiftable NaDES3 (obtained from the acidic-NaDES3 to the NaDES3_KCl, noted
230 shiftNaDES3). NaDES1 was chosen because it offered a suitable environment for lipase-
231 catalyzed reaction with moderate extraction capacity. While shiftNaDES3 showed the highest
232 yield of PCs extraction (acidic-NaDES3) and represented a potential, assessed by VL
233 substrate, media for biotransformation activity with iCALB (NaDES_KCl). Thus, NaDES1
234 and shiftNaDES3 were evaluated according to their capacity to promote both, the extraction
235 of bioactive molecules and subsequent biotransformation with the iCALB (Fig. 3).

236 **3.2.1. PC and lipid profiles after extraction and biotransformation of rice bran**

237 NADES1 and shiftNaDES3 extracts showed a slight variation in PC profile after potential
238 biotransformation (Sup. Fig. 1). Especially, shiftNaDES3 presented a difference in
239 chromatographic profile and in the total phenolic content ($p < 0.05$) after the enzymatic
240 reaction (Fig. 4). Although hydrophilic NaDES have a great capacity to extract polar
241 molecules, studies have revealed that they may also extract non-water-soluble molecules [26].
242 For instance, hydrophilic glycerol-based NaDES have been already shown to extract FFA
243 from microalgae [35]. In the current study, we performed a qualitative analysis of lipids,
244 extracted from the RB with the NaDES1 and the shiftNaDES3 (Sup Fig. 2). Assessment was
245 performed after 1h extraction, and after 25h (1h + 24h) with or without the addition of
246 iCALB.

247 NaDES1 and shiftNaDES3 were able to extract TAG and FFA in different concentrations. A
248 decrease in FFA and the appearance of new compounds (~ same retention factor as DAG and
249 TAG) were observed after the biotransformation step (Sup. Fig. 2A). The mono- and di-

250 acylation with the 1,2 propanediol (present in the NaDES1 formulation) with lipids could
251 explain the formation of new compounds. Regarding the lipid profile with shiftNaDES3, an
252 important decrease in TAG with a concomitant increase in FFA were observed (Sup. Fig. 2B).
253 In addition, small spots corresponding to new compounds with higher polarity were observed
254 after the lipase-catalyzed reaction. We can hypothesize that these new faint spots could be
255 phenolic esters formed by esterification with the newly released fatty acids with the alcohol
256 moiety of a flavonoid, lignin, stilbene or between a fatty alcohol with a phenolic acid. This
257 biotransformation effect on the lipids profile, was confirmed by the control experiment
258 (without iCALB), where the lipolysis was much less pronounced with a slight increase in
259 FFA content after KOH addition (Sup. Fig. 2B). In addition, in this control extract, no new
260 compound with higher polarity was observed.

261 In conclusion, slight variations in the lipid profile after biotransformation were observed, such
262 as the increase in new compounds with same Rf of DAG and FFA and the appearance of more
263 polar lipids. The hypothesis that these lipids with higher polarity are newly formed
264 compounds from bonds with PC corroborates the HPLC results, where was observed the
265 decrease of some peaks and the decrease of the phenolic content in the biotransformed
266 extracts. Nevertheless, mass spectrometry analyses should be done to point out the enzymatic
267 biotransformation and elucidate the structural rearrangement between PC and lipids.

268 **3.3. Antioxidant activity of rice bran extracts in oil-in-water emulsion**

269 Extracts obtained with NaDES1 and shiftNaDES3, with or without *in-situ* biotransformation
270 with iCALB, were tested and compared for their ability to prevent lipid oxidation in an O/W
271 emulsion system, stored for 20 days at 40 °C, represented by PV and TBARS values (Fig. 5).
272 Pure NaDES1 and shiftNaDES3, along with the powder of RB were also tested.
273 Contrary to expectations, NaDES showed a strong pro-oxidative effect. Indeed, very high PV
274 (>100 meq O₂/kg) and TBARS (> 6 mmol eq MDA/kg oil) values after 20 days were

275 observed in comparison to the raw emulsion (PV = 79 meq O₂/kg, TBARS = 1.77 mmol eq
276 MDA/kg oil). However, NaDES extracts of RB presented a good antioxidant activity,
277 meaning that the compounds extracted from RB, or the leftover powder, provided antioxidant
278 action and/or reduction of pro-oxidative effects of NaDES. With NaDES extracts, along with
279 the powder of RB, the secondary products of oxidation appeared only after day 6, while they
280 appeared after day 3 for others (Fig. 5B and 5D). Defatted RB showed a protective effect over
281 fish oil and could be used as a natural additive [10]. RB extracts have also shown the capacity
282 to decrease PV in food product susceptible to oxidation (mayonnaise-type emulsion) and thus
283 to extend food shelf life [11]. Such extract can be a sustainable alternative as a food additive
284 to guarantee food quality and safety. In addition, and interestingly, the biotransformation step
285 in NaDES seems to promote a protective activity. For example, the biotransformed extract
286 NaDES1 showed from day 3 (Fig. 5A) PV that stands out from the others ($p < 0.05$). This
287 tendency could be attributed to the molecular restructuring of PCs with lipids, a mechanism
288 known to favor their antioxidant power compared to parent molecules [15, 36]. In addition,
289 the change in the pool of molecules after enzymatic action could also change the physical
290 properties of extracts, improving the stability of lipid emulsion droplets.

291 **4. Conclusion**

292 This is the first study to enzymatically biotransform compounds present in RB based on a
293 green approach to produce enriched antioxidant extracts to be applied in emulsion media.
294 These results confirmed the efficiency of NaDES to extract PCs, but also in minor part of
295 lipids. Acidic NaDES appear to promote higher extraction yields in PCs, but on the other
296 hand, they do not offer lipase friendly environment. Thus, two strategies for the sustainable
297 development of ready-to-use extracts from NaDES were implemented: one with NaDES
298 allowing a moderate extraction and a good enzymatic activity in a model reaction (NaDES1),
299 and other with a shiftable NADES (shiftNaDES3), starting from an acidic medium to

300 optimize the extraction step, before moving to a non-acid environment to recover an
301 enzymatic activity. Although a strong pro-oxidant activity of NaDES could be observed in
302 O/W emulsion, RB extracts in NaDES demonstrated an antioxidant capacity. In addition, the
303 biotransformation of extracts with iCALB seems to promote this antioxidant efficiency, but
304 the nature of the potentially biotransformed products remains to be elucidated. There are still
305 several challenges in developing sustainable analytical methodologies and application with
306 NaDES, but this innovative preliminary work brings valuable results for the scientific
307 community. To sum up, these biotransformed extracts should be characterized by omics tools
308 to elucidate the structural rearrangement, along with to study the physical stability of
309 emulsions in the presence of NaDES.

310 **Author contributions**

311 Conceptualization, M.C.B.S., C.B.-L., M.S.L.F., E.D.; data curation, M.C.B.S., N.B., B.B.,
312 C.B.-L., E.D.; formal analysis, M.C.B.S., B.B., N.B. and E.D.; funding acquisition, M.S.L.F.
313 and C.B.-L.; investigation, M.C.B.S., N.B., B.B. and E.D.; methodology, M.C.B.S., B.B., and
314 E.D.; project administration, P.V., C.B.-L., M.S.L.F. and E.D.; resources, P.V., C.B.L.,
315 M.S.L.F. and E.D; supervision, P.V., C.B.-L., M.S.L.F. and E.D.; validation, M.C.B.S., N.B.,
316 B.B., P.V., C.B.-L., M.S.L.F. and E.D.; visualization, M.C.B.S., N.B., C.B.-L., M.S.L.F. and
317 E.D.; writing—original draft, M.C.B.S.; writing—review and editing, M.C.B.S., C.B.-L.,
318 M.S.L.F and E.D. All authors have read and agreed to the published version of the
319 manuscript.

320 **Acknowledgments:** The authors are grateful to Dr Youna Hemery (Institute of Research for
321 Development (IRD); UMR 204 Food and Nutrition Research in the Global South
322 (NUTRIPASS), IRD/University of Montpellier/SupAgro, Montpellier, France) for providing
323 the red rice bran for analysis and Patrice Autfray (UPR AIDA; CIRAD) for the collection,
324 processing, and transportation of samples.

325 **Funding:** This research was financed by the Foundation for Research Support of State of Rio
326 de Janeiro (FAPERJ) (26/010.100.988/2018; 26/202.709/2018; 26/201.848/2019), National
327 Council for Scientific and Technological Development (CNPq) (427.116/2018; 310.34/2019-
328 4) and supported by UNIRIO, Coordination for Improvement of Personnel with Higher
329 Education (CAPES) (financial code 001).

330 **References**

- 331 [1] V. Huber, K. Häckl, D. Touraud, W. Kunz, Chapter Two - Natural deep eutectic solvents: From
332 simple systems to complex colloidal mixtures, in: R. Verpoorte, G.-J. Witkamp, Y.H. Choi (Eds.),
333 *Advances in Botanical Research*, Academic Press 2021, pp. 17-40.
- 334 [2] K. Radošević, N. Čurko, V. Gaurina Srček, M. Cvjetko Bubalo, M. Tomašević, K. Kovačević Ganić, I.
335 Radojčić Redovniković, Natural deep eutectic solvents as beneficial extractants for enhancement of
336 plant extracts bioactivity, *LWT* 73 (2016) 45-51.
- 337 [3] Y. Dai, J. van Spronsen, G.-J. Witkamp, R. Verpoorte, Y.H. Choi, Natural deep eutectic solvents as
338 new potential media for green technology, *Analytica Chimica Acta* 766 (2013) 61-68.
- 339 [4] L. Duan, L.-L. Dou, L. Guo, P. Li, E.H. Liu, Comprehensive Evaluation of Deep Eutectic Solvents in
340 Extraction of Bioactive Natural Products, *ACS Sustainable Chemistry & Engineering* 4(4) (2016) 2405-
341 2411.
- 342 [5] D.T. da Silva, F.A. Smaniotto, I.F. Costa, J. Baranzelli, A. Muller, S. Somacal, C.S.A. Monteiro, M.
343 Vizzotto, E. Rodrigues, M.T. Barcia, T. Emanuelli, Natural deep eutectic solvent (NADES): A strategy to
344 improve the bioavailability of blueberry phenolic compounds in a ready-to-use extract, *Food*
345 *chemistry* 364 (2021) 130370.
- 346 [6] M. Panić, M. Radić Stojković, K. Kraljić, D. Škevin, I. Radojčić Redovniković, V. Gaurina Srček, K.
347 Radošević, Ready-to-use green polyphenolic extracts from food by-products, *Food chemistry* 283
348 (2019) 628-636.
- 349 [7] E. Durand, J. Lecomte, B. Baréa, G. Piombo, E. Dubreucq, P. Villeneuve, Evaluation of deep
350 eutectic solvents as new media for *Candida antarctica* B lipase catalyzed reactions, *Process*
351 *Biochemistry* 47(12) (2012) 2081-2089.
- 352 [8] E. Durand, J. Lecomte, P. Villeneuve, Deep eutectic solvents: Synthesis, application, and focus on
353 lipase-catalyzed reactions, *European Journal of Lipid Science and Technology* 115(4) (2013) 379-385.
- 354 [9] O.L. Vidal, M.C.B. Santos, A.P. Batista, F.F. Andriago, B. Baréa, J. Lecomte, M.C. Figueroa-Espinoza,
355 N. Gontard, P. Villeneuve, V. Guillard, C.M. Rezende, C. Bourlieu-Lacanal, M.S.L. Ferreira, Active
356 packaging films containing antioxidant extracts from green coffee oil by-products to prevent lipid
357 oxidation, *Journal of Food Engineering* 312 (2022) 110744.
- 358 [10] Z. Rohfritsch, G. Canelli, P. Pollien, R. Bel-Rhliid, Wheat and Rice Bran as Natural Additives for the
359 Protection of Fish Oil from Oxidation, *ACS Food Science & Technology* 1(7) (2021) 1160-1168.
- 360 [11] S. Martillanes, J. Rocha-Pimienta, M.V. Gil, M.C. Ayuso-Yuste, J. Delgado-Adámez, Antioxidant
361 and antimicrobial evaluation of rice bran (*Oryza sativa* L.) extracts in a mayonnaise-type emulsion,
362 *Food chemistry* 308 (2020) 125633.
- 363 [12] Z. Pan, R. Zhang, S. Zicari, *Integrated Processing Technologies for Food and Agricultural By-*
364 *Products*, Academic Press 2019.
- 365 [13] Y. Pang, S. Ahmed, Y. Xu, T. Beta, Z. Zhu, Y. Shao, J. Bao, Bound phenolic compounds and
366 antioxidant properties of whole grain and bran of white, red and black rice, *Food chemistry* 240
367 (2018) 212-221.
- 368 [14] M.C.B. Santos, N. Barouh, E. Durand, B. Baréa, M. Robert, V. Micard, V. Lullien-Pellerin, P.
369 Villeneuve, L.C. Cameron, E.P. Ryan, M.S.L. Ferreira, C. Bourlieu-Lacanal, *Metabolomics of Pigmented*

370 Rice Coproducts Applying Conventional or Deep Eutectic Extraction Solvents Reveal a Potential
371 Antioxidant Source for Human Nutrition, *Metabolites* 11(2) (2021) 110.

372 [15] L.J. Lopez Giraldo, Lipophilisation enzymatique de composés phénoliques et évaluation de leurs
373 propriétés antioxydantes, *OCL* 16(4-5-6) (2009) 273-275.

374 [16] L.J. Lopez Giraldo, M. Laguerre, J. Lecomte, M.-C. Figueroa-Espinoza, M. Pina, P. Villeneuve,
375 Lipophilisation de composés phénoliques par voie enzymatique et propriétés antioxydantes des
376 molécules lipophilisées, *OCL. Oléagineux Corps gras Lipides* 14(1) (2007) 51-59.

377 [17] M. Laguerre, L.J. López Giraldo, J. Lecomte, M.-C. Figueroa-Espinoza, B. Baréa, J. Weiss, E.A.
378 Decker, P. Villeneuve, Chain Length Affects Antioxidant Properties of Chlorogenate Esters in
379 Emulsion: The Cutoff Theory Behind the Polar Paradox, *Journal of Agricultural and Food Chemistry*
380 57(23) (2009) 11335-11342.

381 [18] L.J. López Giraldo, M. Laguerre, J. Lecomte, M.-C. Figueroa-Espinoza, N. Barouh, B. Baréa, P.
382 Villeneuve, Lipase-catalyzed synthesis of chlorogenate fatty esters in solvent-free medium, *Enzyme
383 and Microbial Technology* 41(6) (2007) 721-726.

384 [19] N. Phonsatta, P. Deetae, P. Luangpituksa, C. Grajeda-Iglesias, M.C. Figueroa-Espinoza, J. Le
385 Comte, P. Villeneuve, E.A. Decker, W. Visessanguan, A. Panya, Comparison of Antioxidant Evaluation
386 Assays for Investigating Antioxidative Activity of Gallic Acid and Its Alkyl Esters in Different Food
387 Matrices, *Journal of Agricultural and Food Chemistry* 65(34) (2017) 7509-7518.

388 [20] X. Qiu, C. Jacobsen, P. Villeneuve, E. Durand, A.-D.M. Sørensen, Effects of Different Lipophilized
389 Ferulate Esters in Fish Oil-Enriched Milk: Partitioning, Interaction, Protein, and Lipid Oxidation,
390 *Journal of Agricultural and Food Chemistry* 65(43) (2017) 9496-9505.

391 [21] O. Laguna, E. Durand, B. Baréa, S. Dauguet, F. Fine, P. Villeneuve, J. Lecomte, Synthesis and
392 Evaluation of Antioxidant Activities of Novel Hydroxyalkyl Esters and Bis-Aryl Esters Based on Sinapic
393 and Caffeic Acids, *Journal of Agricultural and Food Chemistry* 68(35) (2020) 9308-9318.

394 [22] E. Durand, J. Lecomte, B. Baréa, E. Dubreucq, R. Lortie, P. Villeneuve, Evaluation of deep eutectic
395 solvent–water binary mixtures for lipase-catalyzed lipophilization of phenolic acids, *Green Chemistry*
396 15(8) (2013) 2275-2282.

397 [23] D. Kahveci, M. Laguerre, P. Villeneuve, 7 - Phenolipids as New Antioxidants: Production, Activity,
398 and Potential Applications, in: M.U. Ahmad, X. Xu (Eds.), *Polar Lipids*, Elsevier 2015, pp. 185-214.

399 [24] P. Villeneuve, Lipases in lipophilization reactions, *Biotechnology Advances* 25(6) (2007) 515-536.

400 [25] M. Jablonský, A. Škulcová, A. Malvis, J. Šima, Extraction of value-added components from food
401 industry based and agro-forest biowastes by deep eutectic solvents, *Journal of Biotechnology* 282
402 (2018) 46-66.

403 [26] A. Mišan, J. Nađpal, A. Stupar, M. Pojić, A. Mandić, R. Verpoorte, Y.H. Choi, The perspectives of
404 natural deep eutectic solvents in agri-food sector, *Critical Reviews in Food Science and Nutrition*
405 60(15) (2020) 2564-2592.

406 [27] M. Ruesgas-Ramón, M.C. Figueroa-Espinoza, E. Durand, Application of Deep Eutectic Solvents
407 (DES) for Phenolic Compounds Extraction: Overview, Challenges, and Opportunities, *Journal of
408 Agricultural and Food Chemistry* 65(18) (2017) 3591-3601.

409 [28] A. Delavault, O. OPOCHENSKA, L. Laneque, H. Soergel, C. Muhle-Goll, K. Ochsenreither, C. Sylđatk,
410 Lipase-Catalyzed Production of Sorbitol Laurate in a “2-in-1” Deep Eutectic System: Factors Affecting
411 the Synthesis and Scalability, *Molecules* 26(9) (2021) 2759.

412 [29] A. Delavault, K. Ochs, O. Gorte, C. Sylđatk, E. Durand, K. Ochsenreither, Microwave-Assisted One-
413 Pot Lipid Extraction and Glycolipid Production from Oleaginous Yeast *Saitozyma podzolica* in Sugar
414 Alcohol-Based Media, *Molecules* 26(2) (2021) 470.

415 [30] M.d.S. Oliveira, V. Feddern, L. Kupski, E.P. Cipolatti, E. Badiale-Furlong, L.A. de Souza-Soares,
416 Changes in lipid, fatty acids and phospholipids composition of whole rice bran after solid-state fungal
417 fermentation, *Bioresource Technology* 102(17) (2011) 8335-8338.

418 [31] J. Folch, M. Lees, G.H.S. Stanley, A SIMPLE METHOD FOR THE ISOLATION AND PURIFICATION OF
419 TOTAL LIPIDES FROM ANIMAL TISSUES, *Journal of Biological Chemistry* 226(1) (1957) 497-509.

- 420 [32] M. Ruesgas-Ramón, M.L. Suárez-Quiroz, O. González-Ríos, B. Baréa, G. Cazals, M.C. Figueroa-
421 Espinoza, E. Durand, Biomolecules extraction from coffee and cocoa by- and co-products using deep
422 eutectic solvents, *Journal of the Science of Food and Agriculture* 100(1) (2020) 81-91.
- 423 [33] T.F. Ferreira da Silveira, M. Laguerre, C. Bourlieu-Lacanal, J. Lecomte, E. Durand, M.C. Figueroa-
424 Espinoza, B. Baréa, N. Barouh, I.A. Castro, P. Villeneuve, Impact of surfactant concentration and
425 antioxidant mode of incorporation on the oxidative stability of oil-in-water nanoemulsions, *LWT* 141
426 (2021) 110892.
- 427 [34] P. Loypimai, A. Moongngarm, P. Chottanom, Extraction solvents affecting phytochemicals in
428 food colorant prepared from purple glutinous rice bran, *Applied Biological Chemistry* 60(2) (2017)
429 181-189.
- 430 [35] S. Mehariya, F. Fratini, R. Lavecchia, A. Zorro, Green extraction of value-added compounds
431 from microalgae: A short review on natural deep eutectic solvents (NaDES) and related pre-
432 treatments, *Journal of Environmental Chemical Engineering* 9(5) (2021) 105989.
- 433 [36] M.-C. Figueroa-Espinoza, P. Villeneuve, Phenolic Acids Enzymatic Lipophilization, *Journal of*
434 *Agricultural and Food Chemistry* 53(8) (2005) 2779-2787.

435

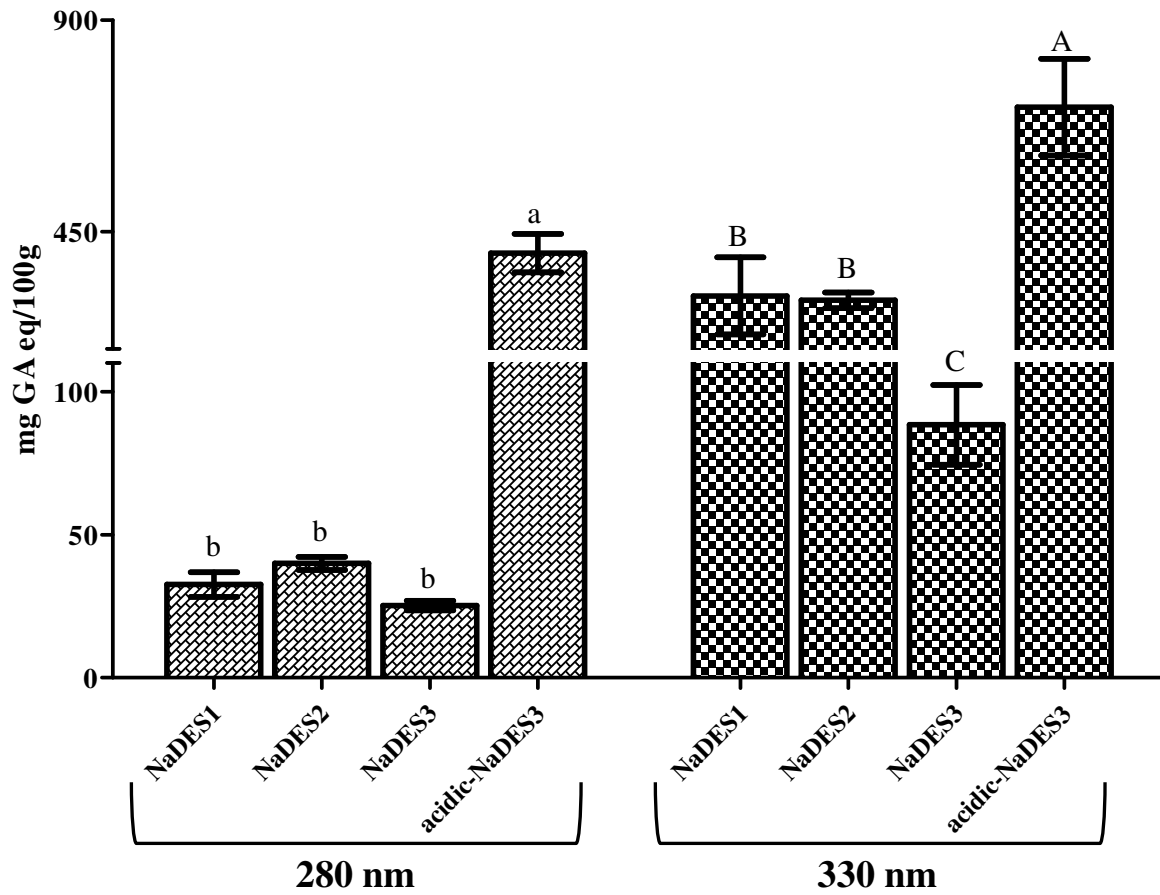


Figure 1 - Total content of phenolic in mg GA eq/100 g of RB. Lowercase letters are significantly different at $p < 0.05$ according to Tukey test between the different extractions for each NaDES at 280 nm and uppercase at 330 nm.

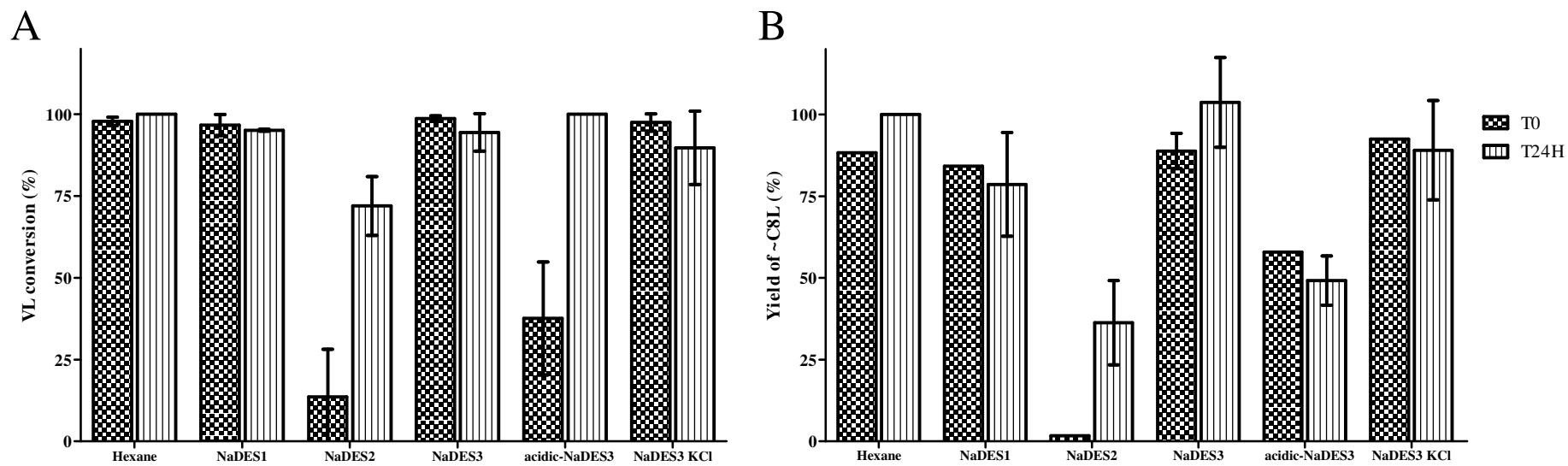


Figure 2 – A) Conversion of vinyl laurate (VL) and B) yield of octyl laurate (C8L) in each NaDES, and in hexane as a control.

Phase 1: Extraction

1h

S1

NaDES
(ChCl:1,2-propanediol:water,
1:1:1)
pH ~ 5,42

*S2

NaDES
(ChCl:xylitol:water, 1:1:1)
+ HCl
pH ~ 0,00

*shiftableNaDES

25 mg rice bran, 1 mL NaDES, 40°C, 200 rpm

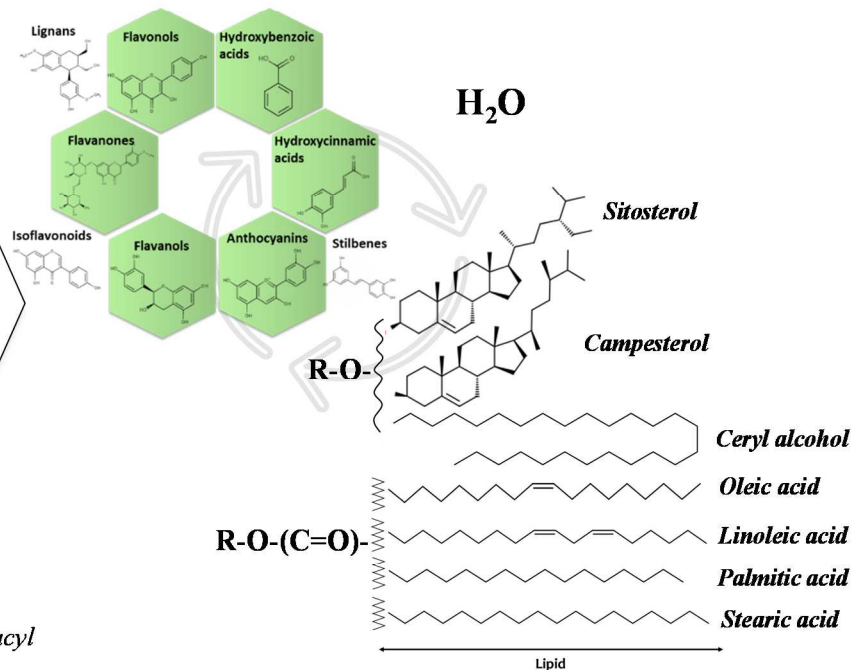
+KOH
↑pH

+ iCALB lipase

R=H or acyl

Phase 2: Biotransformation

24h



10 mg of iCALB to 1 mL of NaDES, 50°C, 200 rpm

Figure 3 - Global strategy to develop a sustainable one-pot extraction and biotransformation environment. S1: strategy 1 and S2: strategy 2 based on shiftable NaDES.

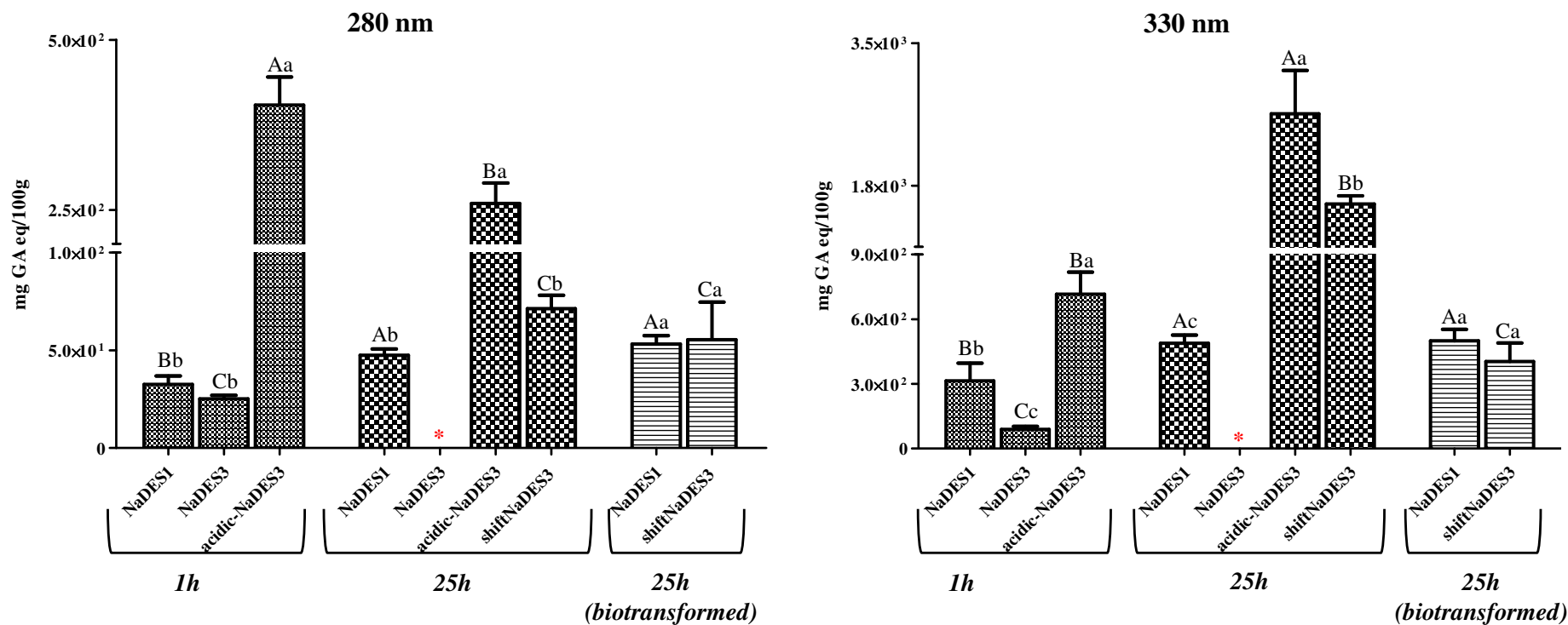


Figure 4 - Total content of phenolic compounds of RB extracts at 280 and 330 nm. Time of extraction are represented below: 1h of extraction; 25h: 1h of extraction and 24h of reaction without enzyme (control) and; 25h biotransformed: 1h of extraction followed by 24h of iCALB lipase reaction. Uppercase letters compare the different time of extraction for the same NaDES extracts, while lowercase letters compare the extraction time for each NaDES ($p < 0,05$ according to Tukey test). *Below the limit of detection.

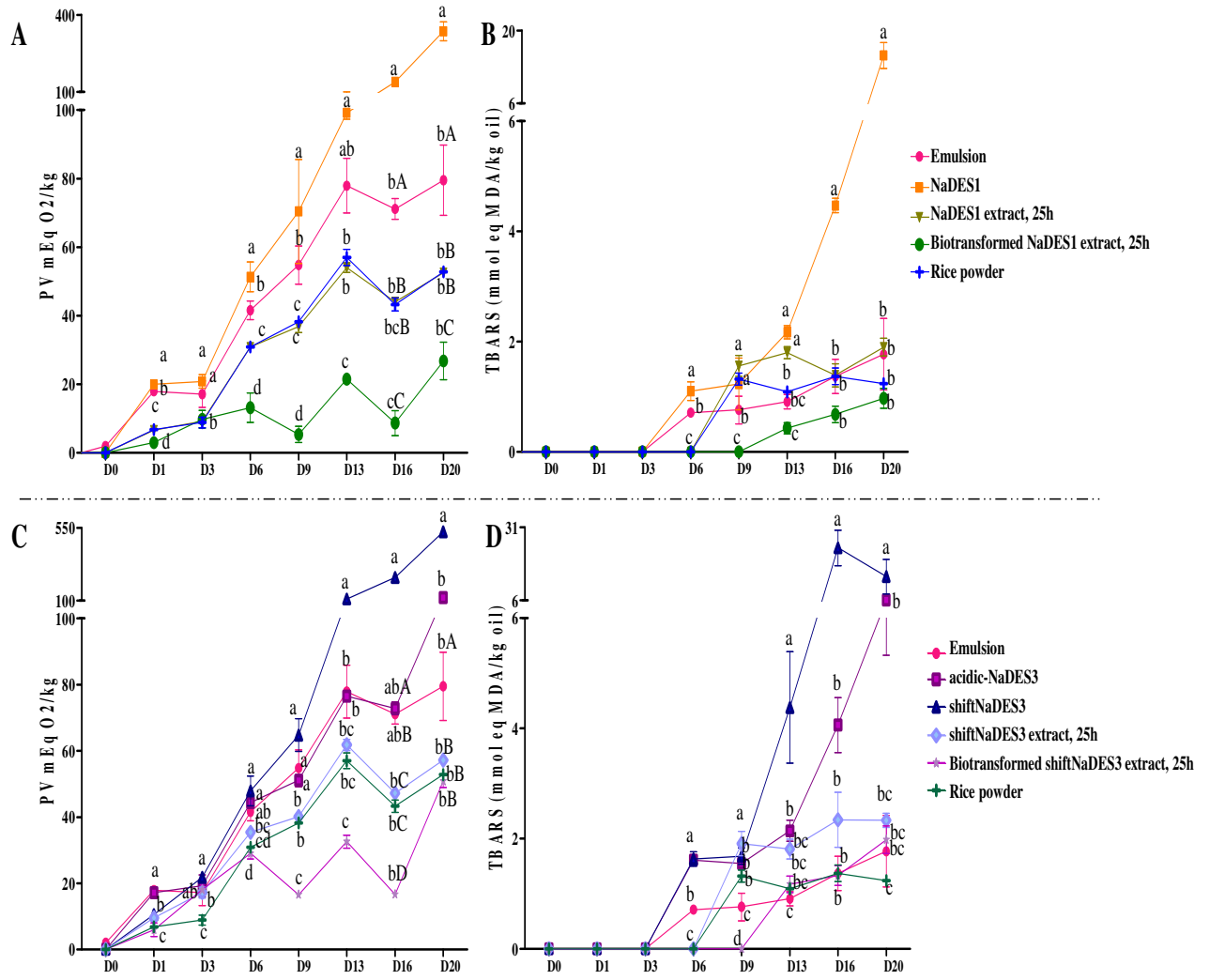


Figure 5 - Oxidative stability of nanoemulsions measured by peroxide value (PV) and 2-thiobarbituric acid reactive substances (TBARS). A) PV results from S1 strategy, with controls and NaDES1 extracts, B) TBARS results from S1 strategy, with controls and NaDES1 extracts, C) PV results from S2 strategy, with controls and shiftNaDES3 extracts. D) TBARS results from S2 strategy, with controls and shiftNaDES3 extracts. Different letters significantly mean difference ($p < 0.05$, Tukey test) between samples at the same incubation time.

Phase 1: Extraction

1h

S1

NaDES
(ChCl:1,2-propanediol:water, 1:1:1)
pH ~ 5,42

*S2

NaDES
(ChCl:xylitol:water, 1:1:1)
+ HCl
pH ~ 0,00

*shiftableNaDES

25 mg rice bran, 1 mL NaDES, 40°C, 200 rpm

+KOH

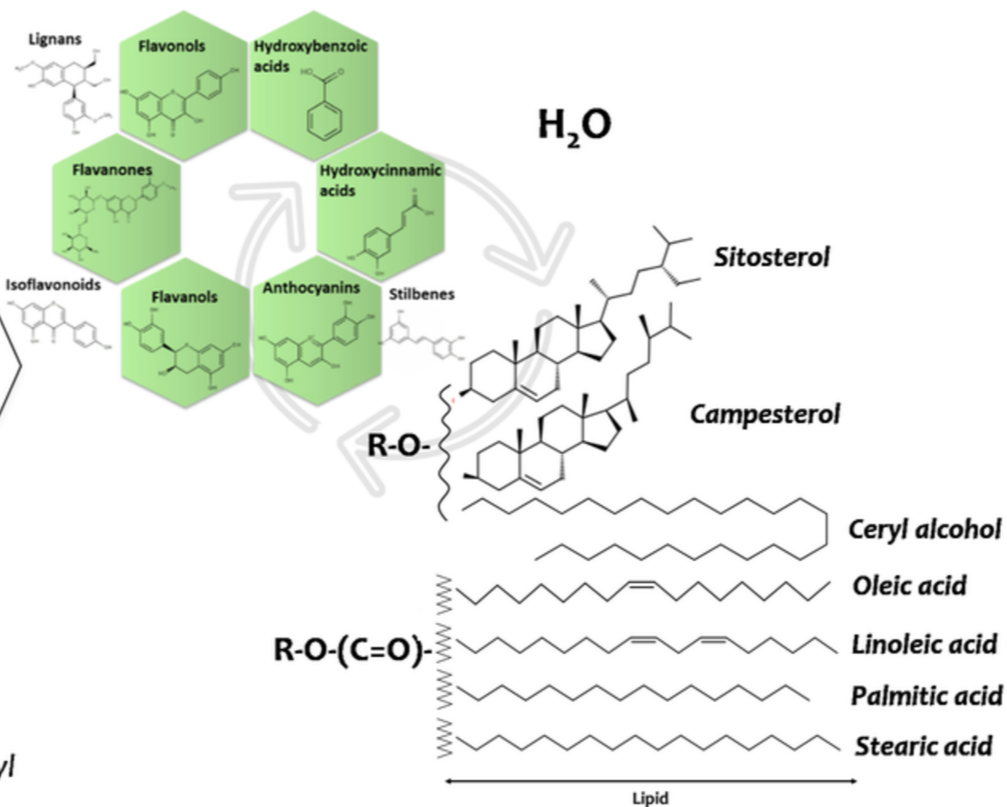
↑pH

+ iCALB lipase

R=H or acyl

Phase 2: Biotransformation

24h



10 mg of iCALB to 1 mL of NaDES, 50°C, 200 rpm

Phase 3: Test of activities

Antioxidant assay

Biotransformed extracts
NaDES formulation -
Comparison of antioxidant
efficiencies in emulsion