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Original

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(Article begins on next page)

1 **CONVENTIONAL AND ULTRASOUND-ASSISTED EXTRACTION OF RICE**
2 **BRAN OIL WITH ISOPROPANOL AS SOLVENT**

3

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16

17 **Abstract**

18 After cereal harvesting, rice is subjected to several milling processes to remove hull,
19 germ, and bran and produce the final white rice. The bran represents around 10% of
20 total grain weight and is usually considered as waste material. One of the most common
21 rice bran applications is the extraction of rice bran oil, rich in γ -oryzanol, which has
22 shown many health benefits including antioxidant, anti-inflammatory, and anti-
23 hypercholesterolemic properties. Rice bran oil is usually extracted by organic solvents,
24 which are toxic for health and the environment. In this work, rice bran oil was extracted

25 through isopropanol extraction, and the best-operating temperature and bran to solvent
26 ratio have been identified. After that, an ultrasound-assisted extraction was conducted
27 at room temperature and with the same rice bran to solvent ratio of the isopropanol
28 extraction.

29 The kinetics evaluation through Peleg's model showed that the solvent extraction
30 reaches the steady-state after 15 minutes while the ultrasound-assisted extraction
31 reaches the steady-state after only 1 minute producing very similar yields in rice bran oil
32 and γ -oryzanol. Comparing these two green extraction techniques through a life cycle
33 assessment, it has emerged that with the same amount of rice bran oil produced, the
34 ultrasound-assisted extraction is the less environmentally impacting process. The room
35 temperature ultrasound-assisted extraction allows minimizing-the energy and time
36 consumption demonstrating to be a sustainable process in line with the principles of
37 green chemistry.

38

39 **Keywords:** Rice bran oil, green extraction, ultrasound-assisted extraction, life cycle
40 assessment.

41

42

43 **1. Introduction**

44 Rice is one of the most important food crops in the world, representing a huge
45 contribution to the dietary need of the global population [1]. According to FAO, world
46 rice production exceeded 755 million tons in 2019 [2]. After harvesting, the rice grain
47 undergoes a milling process to remove all the external layers making the edible white
48 rice kernel [3]. During these milling operations, around 40% of the total grain is lost
49 due to discarding the byproducts, including the husk, the bran, the germ, and the broken

50 rice [4]. Usually, these are burned or used as animal feed, but they may represent an
51 excellent source of bioactive compounds, making them suitable for nutraceutical,
52 cosmetic and pharmaceutical applications [2]. In particular, rice bran, which represents
53 around 10% of the grain weight, contains proteins, fibers, and oil, this latter rich in
54 bioactive and antioxidant compounds [5]. This rice bran oil (RBO) presents a balanced
55 fatty acid composition and high levels of functional molecules, including phytosterols,
56 tocopherols, tocotrienols, and other nutrients. Thanks to its exceptional properties,
57 RBO is commonly used in Asian countries where it is considered as a "healthy oil".
58 Indeed, it has been demonstrated that RBO has antihypertensive, antidiabetic, anti-
59 obesity, and anticarcinogenic properties due to its significant antioxidant and anti-
60 inflammatory activity [6–8]. Several studies confirmed that one of the main responsible
61 for these beneficial effects is γ -oryzanol, an antioxidant mixture of ferulic acid esters of
62 phytosterols, present at high levels in RBO [9]. The conventional method used for the
63 commercial extraction of RBO is solvent extraction (SE) using hexane, a petroleum-
64 derived, flammable, and toxic solvent, dangerous for human health and the
65 environment. The disadvantages of this type of extraction led most researchers to look
66 for alternative approaches, focusing on non-conventional and non-thermal extraction
67 techniques, for RBO extractions [10]. These innovative techniques employ less
68 dangerous solvents, often combined with one or more process intensification steps to
69 reduce time and energy waste, obtaining high-quality extracts devoid of toxic residues
70 [2, 11]. Some studies demonstrated that short-chain alcohols might represent an
71 excellent alternative to hexane as a solvent in RBO extraction. In particular, the use of
72 isopropanol allowed to obtain a high yield in oil and γ -oryzanol [12].

73 Moreover, in recent years, ultrasound-assisted extraction (UAE), thanks to the
74 phenomenon of "acoustic cavitation," has become an effective green method for oil
75 extraction. In their review, Mushtaq et al. [13] concluded that the UAE represents a
76 good alternative for extracting edible oil. This technique allows operating at low
77 temperatures and times, reducing solvent consumption, avoiding thermal damage and
78 preserving their structural and bioactive properties. Indeed, UAE was successfully
79 applied to extract bioactive compounds from several natural matrices, but only a few
80 studies on RBO extraction focus on this green technique [14].
81 The present work aims to show the suitability of isopropanol as non-conventional
82 solvent for RBO extraction and then demonstrate that the substitution of high
83 temperature with room-temperature ultrasound in the extraction with isopropanol as the
84 solvent allows minimizing the extraction time and energy consumption, obtaining
85 comparable yields.
86 Isopropanol SE and room temperature UAE were compared in terms of oil yield and γ -
87 oryzanol content. The extraction kinetics were determined using Peleg's model to
88 identify the best extraction time. Moreover, a life cycle assessment (LCA) study was
89 performed to compare the environmental sustainability of the two processes and to
90 choose the most environmental-friendly extraction process.

91

92 **2. Materials and methods**

93 **2.1 Material and chemicals**

94 Cryo-milled rice bran sample, with a particle diameter of 500 μm , were supplied by
95 Agrindustria Tecco S.R.L. and stored at $-20.0\text{ }^{\circ}\text{C}$ until extraction. HPLC grade hexane,
96 methanol, acetonitrile, acid acetic and isopropanol used for extraction and high-

97 performance liquid chromatography (HPLC) analysis and γ -oryzanol standard for
98 quantification were purchased from Merck (Darmstadt, Germany).

99 **2.2 Isopropanol extraction**

100 Rice bran was mixed with isopropanol in a Pyrex reaction flask connected with a Liebig
101 reflux condenser. The flask was put into a water bath with a magnetic stirrer. To choose
102 the best condition, 1:3, 1:6, and 1:9 solid-to-solvent ratio (w/v) was used at different
103 temperatures (30, 45, 60 °C). The extraction time was fixed for 1 h. After these
104 preliminary studies, the best solid-to-solvent ratio was chosen. The temperature range
105 was extended to determine the effect of temperature on the yield of extracted
106 components. The temperature investigated were 30, 60, 90, and 120 °C. At the best
107 temperature, a kinetic study was performed examining the yield in oil and γ -oryzanol
108 against time from 1 minute to 120 min.

109 **2.3 Ultrasound-assisted extraction**

110 For the UAE experiments, a VCX750 Ultrasonic Processors (Sonic and Materials Inc.),
111 with a frequency of 20 kHz and 40 % of amplitude, equipped with a 13 mm probe was
112 used. Rice bran (5 g) was mixed with 45 mL of isopropanol (1:9 w/v) in a Pyrex
113 reaction flask put into a water bath with a magnetic stirrer. The temperature was
114 maintained at room temperature (25 °C) and controlled with a thermocouple. A kinetic
115 study was performed investigating the yield in oil and γ -oryzanol against time from 10 s
116 to 30 min.

117 **2.4 Hexane extraction**

118 Isopropanol and UAE were compared with a conventional hexane extraction following
119 the method of Pengkumsri et al. [15]. Rice bran was mixed with hexane in a 1:10 (w/v)

120 ratio in a Pyrex reaction flask connected with a Liebig reflux condenser. The flask was
121 put into a water bath with a magnetic stirrer at 40 °C for 30 min.

122 **2.5 Determination of RBO Yield**

123 After the extractions, all the samples were filtered two times using a paper filter
124 Whatman grade 1 to separate the liquid phase from the exhausted rice bran. Then the
125 solvent was evaporated using a Heidolph Rotary Evaporator, Laborota 4000. The RBO
126 yield was calculated on the base of Eq. 1:

127

$$RBO\ Yield\ (\%) = \frac{RBO\ (g)}{Rice\ bran\ (g)} \times 100 \quad (1)$$

128 **2.6 Determination of γ -oryzanol content**

129 After the evaporation of the solvent and the determination of the oil yield, the RBO
130 samples were resuspended in 15 mL of isopropanol, and γ -oryzanol content was
131 determined by reversed-phase HPLC. The HPLC system (Shimadzu 20A Prominence)
132 was equipped with a Kinetex C18 column (5 μ m, 150 x 4.6 mm) by Phenomenex and
133 photodiode array (PDA) detector using an isocratic elution. The mobile phase was
134 composed by methanol, acetonitrile and 0.03 % acid acetic at a ratio 52:45:3 (v/v/v) [16,
135 17]. The flow rate was maintained at 0.8 mL/min, and the column oven was
136 thermostated at 30 °C. γ -oryzanol content was determined through a calibration curve
137 prepared using 8 different concentrations of γ -oryzanol standard (0.01-0.8 mg/mL) in
138 isopropanol. The limit of detection (LOD) and the limit of quantification (LOQ) were
139 calculated with the following equation, where σ is the standard deviation of the response

140 and S is the slope of the calibration curve [18]. LOD and LOQ were respectively 0.01
141 and 0.04 mg/mL.

142

143

$$LOD = \frac{3.3 \times \sigma}{S} \quad (2)$$

$$LOQ = \frac{10 \times \sigma}{S} \quad (3)$$

144 The γ -oryzanol yield was calculated using the formula:

$$\gamma - \text{oryzanol yield} = \frac{\gamma - \text{oryzanol (mg)}}{\text{Rice bran (g)}} \quad (4)$$

145

146 **2.7 Statistical analysis**

147 All the extraction experiments were performed in triplicate and analyzed by one-way
148 ANOVA (analysis of variance) with a Tukey's posthoc test ($P \leq 0.05$), after the
149 assessment of the fundamental assumptions of ANOVA: the normality of distributions
150 (Shapiro-Wilk test, p-value $N 0.05$) and the homogeneity of the variances of the
151 residuals (Levene's test with $P(NF) N 0.05$). The statistical software R (version 4.0.4 -
152 Feather Spray - 2021) was used for all.

153

154

155 **2.8 Extraction kinetics**

156 To describe the SE and UAE kinetics of RBO and γ -oryzanol from rice bran was used
157 the model proposed by Peleg [19], a two-parameters, non-exponential empirical
158 equation, originally proposed to describe sorption curves and adapted for the extraction
159 process in the form:

$$C(t) = C_0 + \frac{t}{K_1 + K_2 \times t} \quad (5)$$

160

161 where $C(t)$ is the RBO or γ -oryzanol yield [(g RBO / g bran)·100] or [mg γ -oryzanol / g
162 bran], respectively, at time t ; t is the extraction time [min], C_0 is the yield at time $t = 0$,
163 K_1 is Peleg's rate constant [min· (g bran / g RBO) ·100] or [min· (g bran / mg γ -
164 oryzanol)], and K_2 is Peleg's capacity constant [g bran · 100 / g RBO] or [g bran / mg γ -
165 oryzanol]. Since, C_0 is considered zero, and this term can be omitted from Peleg's
166 equation, the final form of the equation used is:

$$C(t) = \frac{t}{K_1 + K_2 \times t} \quad (6)$$

167

168 It should be noted that a lower K_1 value means a faster rate of extraction, and a lower K_2
169 value suggests maximum yield [20]. The Peleg's rate constant K_1 relates to the
170 extraction rate (B_0) at the start ($t = t_0$).

$$B_0 = \frac{1}{K_1} \quad (7)$$

171

172 The Peleg capacity constant K_2 relates to a maximum of extraction yield, C_e at
173 equilibrium when $t = \infty$.

$$C_{t \rightarrow \infty} = C_e = \frac{1}{K_2} \quad (8)$$

174

175 The accordance of experimental (\hat{y}_i) data and model-predicted results (y_i) were
176 established by correlation coefficient (R^2), and root mean square error (RMSE) as
177 follows, where n represents the number of experiments.

$$RMSE = \sqrt{\frac{\sum_{i=1}^n (\hat{y} - y)^2}{n}} \quad (9)$$

178

179 **2.9 Life cycle assessment**

180 LCA was performed with SimaPro 9.0.48 software, database Ecoinvent 3.0

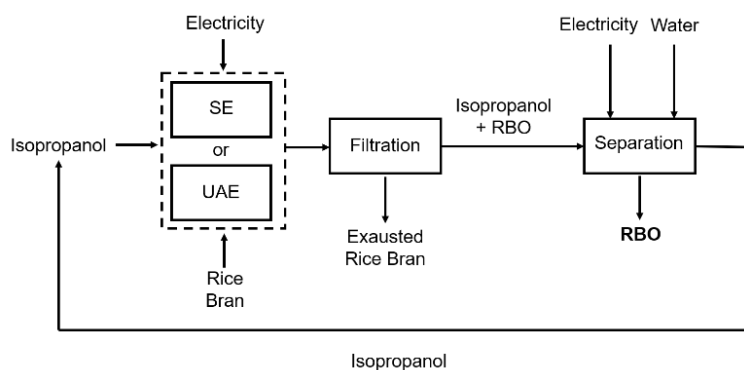
181 **2.9.1 Goal and scope**

182 The goal of LCA was to compare SE vs. UAE of RBO, to choose the best extraction
183 process in terms of environmental sustainability.

184 The functional unit (FU) was 1 g of RBO produced.

185 The boundary conditions are shown in Fig. 1. Briefly, the entire process is divided in
186 three steps: extraction (SE or UAE), filtration and separation. In the extraction step,
187 the entered flows of matter and energy are isopropanol, rice bran and electricity. The
188 rice bran and the isopropanol come out of the extraction together and enter the second
189 step, where filtration occurs. Here the exhausted rice bran, which is discarded, and the

190 isopropanol containing the RBO are separated. Isopropanol and RBO enter the last step
191 where they are separated by a rotavapor which needs cooling water and electricity. In
192 this step, the isopropanol is removed from the RBO and recirculated for a new
193 extraction.



194

195 **Fig. 1** LCA boundary conditions.

196

197 **2.9.2 Life cycle inventory**

198 The life cycle inventory (LCI) defined all inputs and outputs involved in the processes.

199 The primary data came from the present study, the produced emissions, the consumed
200 material, and the required energy were referred to this FU.

201 Expansion system methodology was applied to the recovery of isopropanol in the
202 separation step. The secondary data were taken from Ecoinvent 3.01 and reported in
203 Table 1.

204

205

206

207

208

209

210 **Table 1** Secondary data from Ecoinvent 3.01

Electricity	Electricity, medium voltage {Europe without Switzerland market} Alloc Def Unit
Isopropanol	Isopropanol {GLO} market for Alloc Def Unit
Rice bran	Rice bran from dry milling at plant CN mass
Water	Tap Water from natural resource

211

212

213 **2.9.3 Life cycle impact assessment**

214 Life cycle impact assessment (LCIA) was performed with the ReCIPE Midpoint (H)

215 method. In the present study, the analyzed impact category were: Climate change (kg

216 CO₂ eq), Ozone depletion (kg CFC-11 eq), Human toxicity (kg 1,4-DB eq) and

217 Freshwater eutrophication (kg P eq).

218

219 **3. Results and discussion**

220 **3.1 Solvent extraction**

221 The solvent chosen for all the extractions experiments was isopropanol. Traditionally,

222 RBO was extracted using hexane as the solvent because it presents a low corrosiveness,

223 high stability, and a good capacity for dissolving oil and relevant compounds such as γ -

224 oryzanol [12, 21–23]. Despite these advantages, hexane presents health and

225 environmental risks, therefore researchers and oil industries are focusing on reliable
226 alternative solvents. Moreover, hexane derives from a non-renewable source and
227 presents high flammability and toxicity for the environment and human health [22]. A
228 few types of solvents have been tested to substitute hexane as an extractant of vegetable
229 oil. In particular, short-chain alcohols, including isopropanol, are particularly promising
230 due to their low toxicity [23]. Isopropanol is labeled as "recommended" or "preferred"
231 in several green-solvent selection guidelines, such as the GlaxoSmithKline (GSK),
232 Pfizer, Sanofi, AstraZeneca, and Green Chemistry Institute-Pharmaceutical Roundtable
233 (GCI-PR) [2]. Furthermore, all the components of γ -oryzanol present an alcohol group
234 deriving from the ferulic acid; this increases the polarity, making them more soluble in
235 polar solvents such as short-chain alcohols, including isopropanol [21].

236 At the beginning of this work, a preliminary study on the effects of different solid-to-
237 solvent ratios at various temperatures was performed. After that, using the best solvent-
238 to-solid ratio condition, the temperature range was extended to determine the effect of
239 temperature on the yield of extracted components. At this step, all the extractions were
240 conducted for 1 hour. Figure 2 a) shows the oil yield using 1:3, 1:6, and 1:9 (w/v) solid-
241 to-solvent ratios at three different temperatures (30, 45, and 60 °C). Remarkably, the
242 yield of RBO grows with the increase of the solvent volume at any temperatures tested.
243 This result is in line with Ruen-Ngam et al. [24], which investigated the use of different
244 solvents with an increasing solid-to-solvent ratio and stated that a low volume of solvent
245 does not allow its complete penetration into the rice bran material. The same trend was
246 noted by Hu et al. [12], which obtained a higher yield of RBO and γ -oryzanol,
247 increasing the solvent-to-bran ratio, using both hexane and isopropanol. Indeed the
248 solvent extraction of RBO is mainly considered as a mass transfer process between the

249 solid and liquid phases, where the oil passes from the bran powder to the solvent
250 through a diffusion mechanism [25]. The effect of the solid-to-solvent ratio on the
251 extraction yield is coherent with the mass transfer process. The concentration gradient
252 between the solid and the liquid represents the driving force of this phenomena [26],
253 and it is more significant when the solid is in contact with a large volume of solvent.
254 The high quantity of isopropanol reduces the saturation level of the solvent, increasing
255 oil extraction yields [26]. The same results have been found for the alcoholic SE of
256 antioxidants compounds from different natural sources such as grape pomace, olive
257 leaves or stonebreaker [27–29].

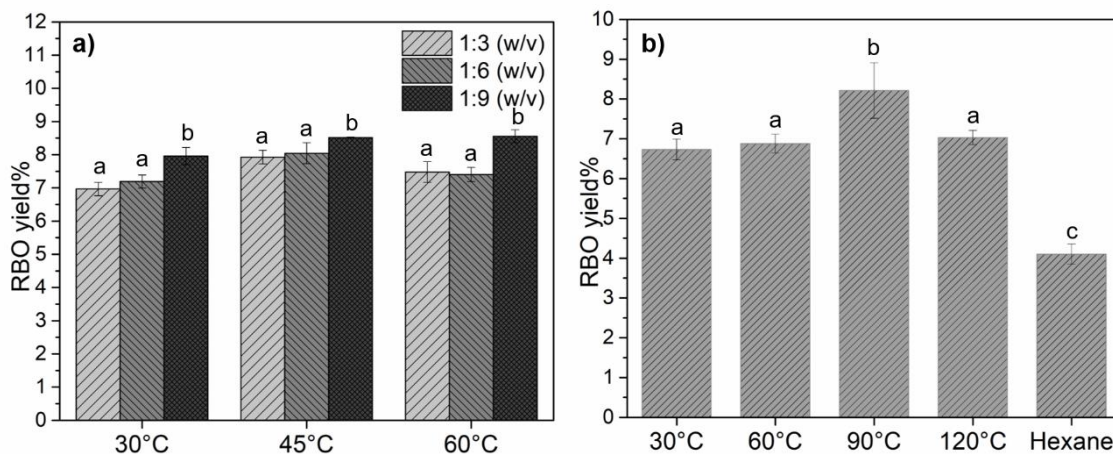
258 Because no striking differences were noted between yields at the previously mentioned
259 temperatures, it was chosen to test the 1:9 solid-to-solvent ratio at 30, 60, 90, and 120
260 °C to understand if the high temperatures can affect the extraction. As reported in Fig.
261 2b, a temperature of 90 °C ($p < 0.05$) gave the best results in terms of RBO extraction
262 yield.

263 Indeed, the temperature is one of the most significant factors influencing the mass
264 transfer and the RBO yield [25]. Capellini et al. [23] performed RBO extraction using
265 isopropanol and ethanol at different temperatures (50, 60, 70, and 80 °C). They found
266 that temperature increase resulted in a growth in oil yield, regardless of the solvent. The
267 same results had been found by, Imsanguan et al.[28], Xu and Godber [19], and Oliveira
268 et al.[20]. All these authors [19, 20, 28] agree that the diffusivity and the solubility of
269 the compounds to extract increase with the increase of temperature, and the extraction
270 output is enhanced [30]. At high temperatures, degradation of the sample matrix
271 structure occurs, making it more permeable to the solvent. Moreover, a temperature rise
272 produces a decrease in solvent viscosity, increasing its diffusivity. However, the high

273 temperature may cause the degradation of thermolabile compounds and the evaporation
274 of the solvent. This has been reported for different plant matrices such as tomatoes,
275 grape waste material and muiltle [20, 31–33]. Furthermore, all these extractions have
276 been compared to hexane extraction. As can be noted from Fig. 2, the conventional
277 hexane extraction, proposed by Pengkumsri et al. [15], and used as the reference
278 method, produced only 4.1 ± 0.25 g of RBO/100 g of rice bran. This value is lower than
279 the yields obtained with isopropanol in any operating condition, which reaches the
280 maximum of 8.21 ± 0.69 g of RBO/100 g of rice bran at 90°C and bran to solvent ratio
281 of 1:9, demonstrating that isopropanol is a solvent suitable to replace hexane in RBO
282 extractions. These results are confirmed by Ruen-Ngam et al., Oliveira et al. and
283 Capellini et al. [22, 24, 34] which affirm that alcoholic solvents like isopropanol, can
284 extract higher amounts of oil than hexane due to their high polarity. In particular,
285 isopropanol can extract higher quantities of phospholipids and unsaponifiable material
286 from solid matrices and obtain RBO richer in γ -oryzanol. Indeed, the structure of γ -
287 oryzanol presents both sides of polar and non-polar (ferulic acid and sterol) making this
288 group of molecules particularly soluble in short-chain alcohols which show dielectric
289 constant higher than hexane and lower than water [24].

290

291



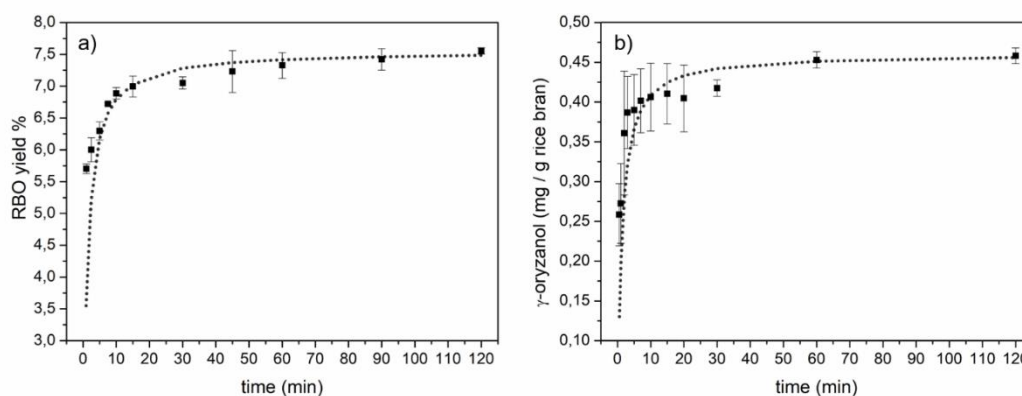
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293 **Fig. 2** a) Effect of bran to solvent ratio at different temperatures on RBO yield (%). b)
 294 effect of higher temperature on the RBO yield (%) with 1:9 (w/v).

295 3.2 SE kinetic

296 The kinetic study was performed measuring the yields in RBO and γ -oryzanol against
 297 the time, starting from 1 min up to 2 h. RBO yield % and γ -oryzanol were plotted in
 298 function of time (Fig. 3). The shapes of the graphs indicate that the extraction yields are
 299 significantly time-dependent. The yields in RBO and γ -oryzanol rise rapidly with time
 300 initially, and then, after the knee at about 15 min, start to keep constant producing 6.89
 301 ± 0.089 g of RBO/ 100 g of rice bran and 0.41 ± 0.038 mg of γ -oryzanol/g of rice bran.
 302 These profiles fit with the typical SE curve composed of a fast extraction step called
 303 "washing phase", followed by a slower extraction step called "diffusion step" [35].
 304 Indeed, the mass transfer rate into the solvent is exceptionally high at the beginning of
 305 extraction, when the solvents penetrate the rice bran, thanks to the elevated
 306 concentration gradients. Progressing with the extraction, the solutes diffuse from the
 307 interior of the bran to the solvent. The mass transfer of solutes becomes more difficult

308 because the concentration gradient between the solid and the liquid phase decreases and
309 the extraction rate becomes slower [20, 35]. The obtained constants of the model (rate
310 constant K_1 , constant capacity K_2) and the calculated parameters (initial extraction rate
311 (B_0) and the maximum yield extraction (C_e), regression coefficient (R^2), and the root
312 mean square error (RMSE) are reported in Table 2. The model fitted well with the
313 experimental data, with reasonable accuracy, as evidenced by high R^2 and low RMSE.
314 In particular, experimental data fit slightly worse in the case of γ -oryzanol yield, but this
315 could be due to the multiple treatments of the sample before the HPLC analysis, causing
316 uncertainty in measurement and high standard deviation. In both cases, there was
317 concordance between experimental and predicted yield values. Yields are lower than
318 those reported in the literature [5, 36, 37], but this is certainly due to different
319 experimental conditions and, above all, to the type of rice bran used for the extraction.
320 Indeed the composition of rice bran changes based on the rice variety, the growing
321 conditions, the milling system employed and the stabilization process [2]. Peleg's
322 equation is one of the most suitable models to describe the SE from plant matrix as
323 demonstrated by several studies. Karacabey et al. [38] compared the first-order kinetic
324 model, Peleg's model, two-site kinetic model and modified Gompertz equation to
325 describe solid–liquid extraction kinetics of trans-resveratrol from grape cane. Jurinjak
326 Tusek et al. [39] compared Peleg's, Page's, and Logarithmic model for total
327 polyphenols, antioxidants extraction yield from Asteraceae plants. Poojary and
328 Passamonti [20] the first-order kinetic model, the mass transfer model, and Peleg's
329 model for understanding the behavior of lycopene extraction from tomato processing
330 waste. All these authors agree that Peleg's model showed a better fit to the experimental
331 data than other models investigated in their studies.



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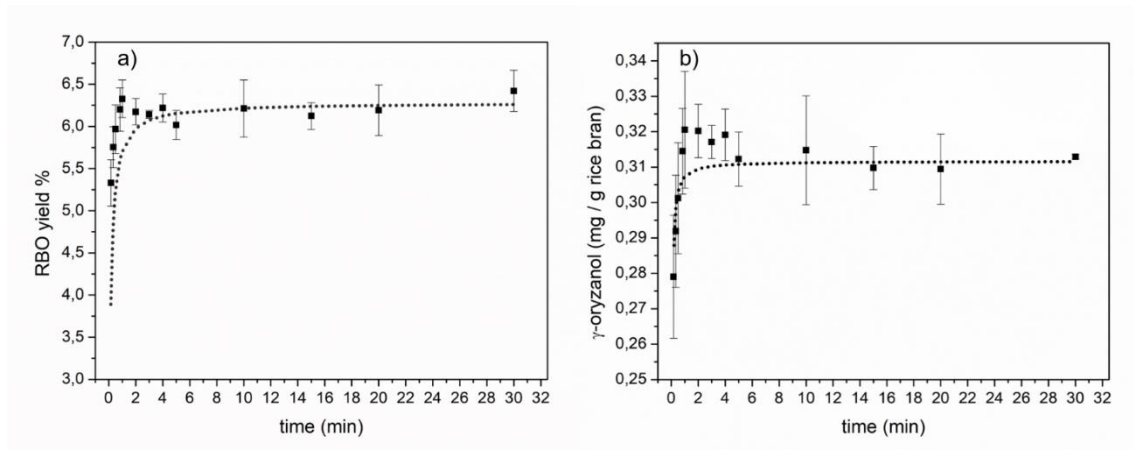
333 **Fig. 3** Isopropanol extraction (SE) kinetics of a) RBO and b) γ -oryzanol. Black squares
 334 represent the experimental values. Each experiment was conducted in triplicate, and the
 335 error bars correspond to standard error. Dotted lines represent the modeled values by
 336 Peleg's equation.

337

338 3.3 UAE kinetic

339 After determining the best isopropanol extraction conditions in terms of temperature
 340 and bran to solvent ratio, an intensification process step was performed to work at room
 341 temperature and reduce the reaction time. The extraction was conducted with the help of
 342 ultrasound as described in Paragraph 2.3, maintaining the bran to solvent ratio of 1:9, as
 343 in the previous experiments. The extraction yields in RBO and γ -oryzanol in the
 344 function of time were plotted to find the best extraction time and to understand the
 345 process kinetic (Fig. 4). These two yields were initially measured every 10 s up to 1 min
 346 and then less frequently up to 30 min. As shown in Fig.4 the UAE reaches the steady-
 347 state after only 1 minute producing 6.33 ± 0.22 g of RBO/100 g of rice bran and $0.32 \pm$
 348 0.016 mg of γ -oryzanol/g of rice bran. Peleg's model was adapted to experimental
 349 conditions and used for data approximation (see paragraph 3.2) and the results are

350 reported in Tab. 2. The initial extraction rate (B_0) is much higher than the B_0 found for
351 the isopropanol extraction. Indeed, the knee of the curve occurs at about 1 min, where
352 yields reach the maximum values and then stabilize. The maximum yield extraction (C_e)
353 is slightly lower than SE and the R^2 , but this may be due to the difficulty of manually
354 measuring the yield every 10 s. This difficulty caused a high uncertainty and an elevated
355 standard deviation producing the worst fit with the model. Until now, to the best of the
356 authors' knowledge, previous studies on the combination of isopropanol and UAE do
357 not exist. Cravotto et al. [40] and Khoei et al. [41] studied the RBO extraction using
358 UAE and water as the solvent, demonstrating that the ultrasound is suitable for aqueous
359 extracting rice bran oil. Other authors studied the effect of UAE of RBO combined with
360 short-chain alcohols like methanol and ethanol [42, 43], demonstrating the feasibility of
361 this kind of extraction. Still, no one managed to complete the extraction in such a short
362 time. Kumari et al. [41] and Galvan et al. [42] showed in their works that Peleg's model
363 efficiently describes the kinetic of UAE for other plant matrixes such as potato peels
364 and black chokeberry wastes.



365

366 **Fig. 4** UAE kinetics of a) RBO and b) γ -oryzanol. Black squares represent the
367 experimental values. Each experiment was conducted in triplicate, and the error bars

368 correspond to standard error. Dotted lines represent the modeled values by Peleg's
369 equation.

370 **3.4 SE and UAE kinetics comparison**

371 The obtained constants of the model (rate constant K_1 , constant capacity K_2) and the
372 calculated parameters, initial extraction rate (B_0) and the maximum yield extraction
373 (C_e), regression coefficient (R^2), and the root mean square error (RMSE), for the two
374 kinds of extractions, are reported in Table 2. As can be noted, the initial extraction rate
375 is higher for the UAE than SE, demonstrating that UAE can reach a steady state in a
376 shorter time. Assuming infinite extraction time, the maximum yield obtained is 7.55 g
377 RBO/100 g bran and 0.46 mg γ -oryzanol/g bran for the SE and 6.34 g RBO/100 g bran
378 and 0.31 mg γ -oryzanol/g bran for the UAE. SE allows obtaining slightly higher
379 quantities of RBO and γ -oryzanol at 90°C, but UAE allows to reach the maximum yield
380 in just one minute and therefore in much shorter times, operating at room temperature.
381 In their work, Mohammed Danlami et al. [43] and Zhang et al. [44] compared
382 traditional SE with that of other extraction techniques to extract valuable components
383 from plants. They affirmed that ultrasound facilitates the extraction of thermally
384 sensitive compounds enhancing the extraction rate and reducing the extraction
385 temperature. Khoei and Chekin [44], in their works, extracted RBO using aqueous
386 extraction and compared the conventional SE with UAE. The two extraction techniques
387 allowed to obtain very similar RBO yield and they demonstrated that the application of
388 ultrasound permitted to work at room temperature in a shortened extraction time. The
389 global production of RBO exceeds 1.7 million tons per year [11]. Although the use of
390 ultrasound at room temperature leads to a slightly lower oil yield, on such a large annual
391 production this decrease may not be relevant. Using UAE instead of SE would allow to

392 increase the global production of RBO as the extraction has a shorter duration and it is
 393 therefore possible to increase the number of annual extractions. The results showed in
 394 the present work are in accordance with the literature studies cited, the yields obtained
 395 with the two extraction techniques are very similar, but the UAE seems to be the most
 396 promising as it allows to reduce the time and energy costs derived from the use of high
 397 temperatures. An LCA study will be described in the next paragraph to verify if the
 398 UAE is the most sustainable extraction, even environmentally.

399 **Table 2** Peleg's parameters for SE and UAE. Rate constant K_1 , constant capacity K_2 ,
 400 initial extraction rate (B_0), maximum yield extraction (C_e), regression coefficient (R^2),
 401 and the root mean square error (RMSE).

		K_1 [min · (g bran / g RBO) · 100 or min · (g bran / mg γ -oryzanol)]	K_2 [g bran · 100 / g RBO or g bran / mg γ -oryzanol]	B_0 [g RBO · 100 / min g bran or mg γ -oryzanol / min g bran]	C_e [(g RBO / g bran) · 100 or mg γ -oryzanol / g bran]	R^2	RMSE
SE	RBO	0,1506	0,1324	6,6401	7,5529	0,9302	0,0534
	γ -oryzanol	2,7668	2,1712	0,3614	0,4606	0,8919	0,7076
UAE	RBO	0,0175	0,1577	57,1429	6,3412	0,7743	0,6070
	γ -oryzanol	0,0442	3,2091	22,6244	0,3116	0,7947	0,0070

402

403 3.5 Life cycle assessment

404 After evaluating the technical feasibility of oil extraction from rice bran, the
 405 environmental sustainability of the different approaches was analyzed through LCA.

406 This analysis aimed to understand if to produce one gram of RBO, it is more
407 environmentally friendly to heat at 90°C for 15 minutes or to generate ultrasounds
408 (20kHz) for one minute at room temperature.

409 Fig. 5 shows the results of comparative LCA between SE and UAE in terms of four
410 impact categories: Climate change (kg CO₂ eq), Ozone depletion (kg CFC-11 eq),
411 Human toxicity (kg 1,4-DB eq), and Freshwater eutrophication (kg P eq). In each
412 impact category, the single contribution of the filtration steps, electricity, and
413 isopropanol to the emission and the total emission of all the two extraction procedures
414 are shown.

415 Regarding the Climate change impact category, to produce 1 gram of RBO, the SE and
416 UAE produce 0.206 and 0.156 kg of CO₂ eq, respectively; hence, the application of
417 UAE allows a reduction of the total impact of 25 %. In this impact category, the most
418 impacting step for both processes is filtration, which produces a considerable amount of
419 exhausted rice bran as waste material.

420 The SE process emits to the atmosphere $1.95 \cdot 10^{-8}$ kg CFC-11 eq while the UAE process
421 emits only $1.46 \cdot 10^{-8}$ kg CFC-11 eq reducing the contribution to the Ozone depletion of
422 25 %. To evaluate if the difference among the two adopted techniques was statistically
423 significant a test of student was carried out considering $p < 0.05$. In this case, the most
424 significant contribution is due to electricity consumption, and the value is almost the
425 same in the two treatments. SE and UAE differ in the emissions in the filtration step
426 because the amount of RBO + isopropanol is higher in the second one, which produces
427 a minor quantity of exhausted rice bran.

428 Concerning the human toxicity impact category, no significant difference between SE
429 and UAE has been found, because the solvent employed was the same in both the
430 extractions.

431 Regarding the freshwater eutrophication impact category, SE and UAE produce 7.13
432 $\cdot 10^{-5}$ and $1.31 \cdot 10^{-5}$ kg P eq, respectively. Therefore, there is a massive reduction of the
433 impact of applying ultrasound instead of conventional extraction.

434 In all impact categories, the recycling of isopropanol originates avoided emissions
435 represented in Fig. 5 by negative bars.

436 For the best of author's knowledge, in the scientific literature LCA studies on oil
437 extraction from rice bran with SE and UAE techniques were not available.

438 To discuss the achieved results, the comparisons with other studies were performed
439 considering studies with cradle to gate approach, the product extracted as a functional
440 unit and midpoint as method to analyse the data coming from life cycle inventory.

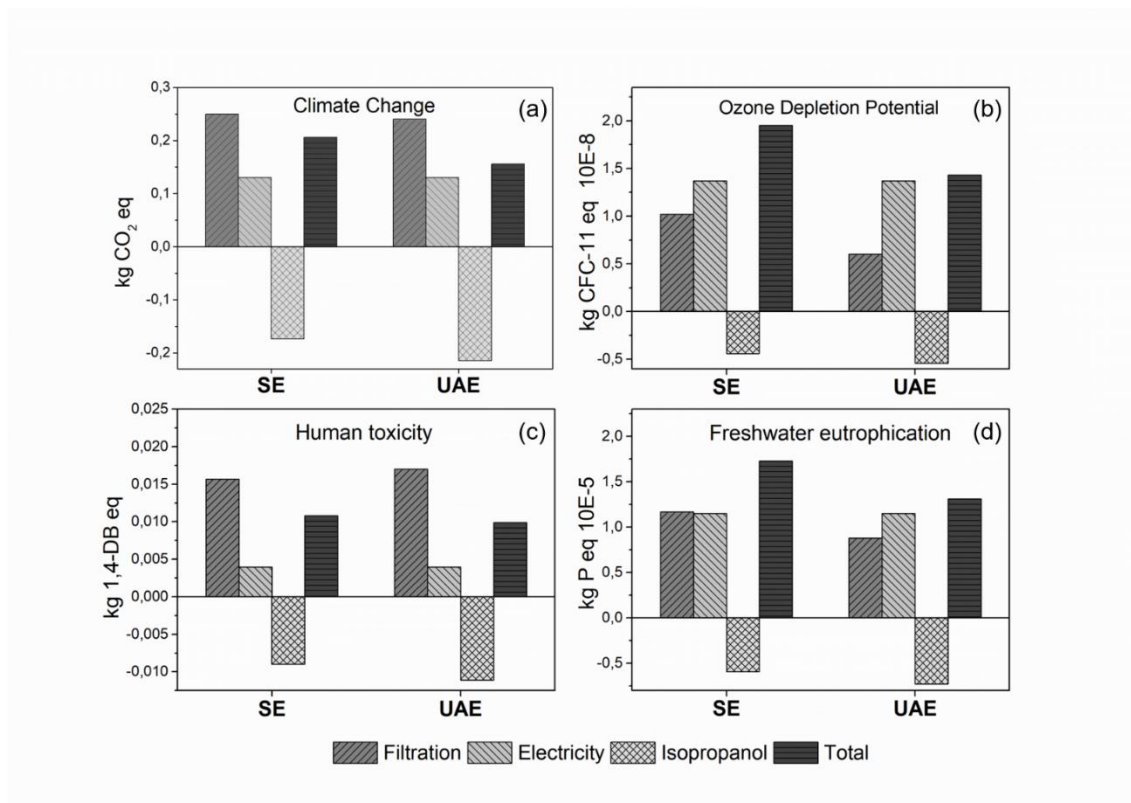
441 Papadaki et al. [45] carried out an LCA study comparing SE, micro-waves, and
442 ultrasounds to recover the bioactive compounds from microalgae. The authors
443 demonstrated that among the three extraction techniques, ultrasound was the most
444 suitable one, since it reached the highest yielding, the lowest economic cost and
445 medium environmental impacts. Such results agree with the outputs of the present work.

446 Castro-Puyana et al., [46] performed bio-compounds extraction from rosemary plant by
447 means of green solvent and pressurized hot water extraction. The SE impacts obtained
448 by the authors were in line with the impacts of SE technique achieved in the present
449 work.

450 Amiri et al. [47] carried out an LCA about alkaloids extracted from the *Atropa*
451 *belladonna* by methanol. The global warming potential (GWP) and ozone depletion

452 (OD) reported by the authors were equal to 0.899 kg CO₂ eq and to 0.00015 kg CFC-11
453 eq, respectively, whereas in the present work the GWP was 0.206 kg CO₂ eq and the
454 OD was $1.43 \cdot 10^{-8}$ kg CFC-11 eq. Hence, the present study reached potential impacts
455 lower than Amiri et al. [45] ones in a range between 77.00%-99.98 %.

456 Barjovenu et al. [48][48] performed an LCA study on polyphenols extraction from
457 spruce bark, by means of SE using ethanol and UAE. The difference between SE using
458 ethanol and UAE was 70.00%, whereas in the present study was 24%. The main
459 difference with the present work is due to the different solvent used. However, the UAE
460 technique resulted in an environmental impact lower than SE. Thus, the present work
461 proved that UAE technique is both technically more efficient and environmentally more
462 sustainable than SE technique. The present study proved the technical feasibility of the
463 two proposed techniques SE and UAE and the feasible scale up, furthermore the LCA
464 study, performed considering the data at laboratory scale, underscored the bottleneck of
465 the processes, which are the filtration steps and the energy consume for both the
466 techniques. Hence, the recommendation and future prospective are the minimisation of
467 waste production at filtration step improving the technique and the optimisation of the
468 energy consume by doing a proper design of the plant.



469

470 **Fig. 5** Comparative LCA results between SE and UAE. Four main impact categories are
 471 illustrated: Climate change, Ozone depletion, Human toxicity and Freshwater
 472 eutrophication. Each graph reports the result for SE (on the left) and for UAE (on the
 473 right); for each extraction process, the contribution of filtration, electricity, isopropanol
 474 and their sum (total) are reported.

475

476 **4. Conclusions**

477 In the present work, the isopropanol SE of RBO was optimized in terms of temperature
 478 and bran to solvent ratio. The best RBO yield was obtained at 90°C and 1:9 bran to
 479 solvent ratio. The results were compared with the RBO yield obtained in a standard
 480 hexane extraction, demonstrating that isopropanol is suitable to RBO extraction, making
 481 the substitution of organic and toxic solvents possible. The kinetics of isopropanol SE at

482 the best-operating conditions was evaluated and compared with a room temperature
483 UAE using a 1:9 bran to solvent ratio. The two extraction techniques produced similar
484 yields in terms of RBO and γ -oryzanol, but UAE reduced remarkably the extraction
485 time. A comparative LCA between the two extraction techniques showed that UAE
486 allows lower the emission contribution to climate change, ozone depletion, and
487 freshwater eutrophication compared to SE, to produce 1 gram of RBO generating high
488 yield, operating at room temperature in a very short time, in line with the principles of
489 green chemistry. To the best of the authors' knowledge, this paper shows for the first
490 time a comparison between the extraction of RBO with isopropanol at 90°C and with
491 isopropanol at room temperature assisted by ultrasounds both from a technical and
492 environmental point of view. The evaluation carried out and the results obtained can be
493 the basis for new experimental campaigns or to design a scale-up RBO production
494 plant.

495

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