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

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Abstract

After cereal harvesting, rice is subjected to several milling processes to remove hull, germ, and bran and produce the final white rice. The bran represents around 10% of total grain weight and is usually considered as waste material. One of the most common rice bran applications is the extraction of rice bran oil, rich in γ-oryzanol, which has shown many health benefits including antioxidant, anti-inflammatory, and anti-hypercholesterolemic properties. Rice bran oil is usually extracted by organic solvents, which are toxic for health and the environment. In this work, rice bran oil was extracted through isopropanol extraction, and the best-operating temperature and bran to solvent ratio have been identified. After that, an ultrasound-assisted extraction was conducted at room temperature and with the same rice bran to solvent ratio of the isopropanol extraction.

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

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

1. Introduction

Rice is one of the most important food crops in the world, representing a huge contribution to the dietary need of the global population [1]. According to FAO, world rice production exceeded 755 million tons in 2019 [2]. After harvesting, the rice grain undergoes a milling process to remove all the external layers making the edible white rice kernel [3]. During these milling operations, around 40% of the total grain is lost due to discarding the byproducts, including the husk, the bran, the germ, and the broken rice [4]. Usually, these are burned or used as animal feed, but they may represent an excellent source of bioactive compounds, making them suitable for nutraceutical, cosmetic and pharmaceutical applications [2]. In particular, rice bran, which represents around 10% of the grain weight, contains proteins, fibers, and oil, this latter rich in bioactive and antioxidant compounds [5]. This rice bran oil (RBO) presents a balanced fatty acid composition and high levels of functional molecules, including phytosterols, tocopherols, tocotrienols, and other nutrients. Thanks to its exceptional proprieties, RBO is commonly used in Asian countries where it is considered as a "healthy oil". Indeed, it has been demonstrated that RBO has antihypertensive, antidiabetic, anti-obesity, and anticarcinogenic properties due to its significant antioxidant and anti-inflammatory activity [6–8]. Several studies confirmed that one of the main responsible for these beneficial effects is γ-oryzanol, an antioxidant mixture of ferulic acid esters of phytosterols, present at high levels in RBO [9]. The conventional method used for the commercial extraction of RBO is solvent extraction (SE) using hexane, a petroleum-derived, flammable, and toxic solvent, dangerous for human health and the environment. The disadvantages of this type of extraction led most researchers to look for alternative approaches, focusing on non-conventional and non-thermal extraction techniques, for RBO extractions [10]. These innovative techniques employ less dangerous solvents, often combined with one or more process intensification steps to reduce time and energy waste, obtaining high-quality extracts devoid of toxic residues [2, 11]. Some studies demonstrated that short-chain alcohols might represent an excellent alternative to hexane as a solvent in RBO extraction. In particular, the use of isopropanol allowed to obtain a high yield in oil and γ-oryzanol [12].

Moreover, in recent years, ultrasound-assisted extraction (UAE), thanks to the phenomenon of "acoustic cavitation," has become an effective green method for oil extraction. In their review, Mushtaq et al. [13] concluded that the UAE represents a good alternative for extracting edible oil. This technique allows operating at low temperatures and times, reducing solvent consumption, avoiding thermal damage and preserving their structural and bioactive properties. Indeed, UAE was successfully applied to extract bioactive compounds from several natural matrices, but only a few studies on RBO extraction focus on this green technique [14].

The present work aims to show the suitability of isopropanol as non-conventional solvent for RBO extraction and then demonstrate that the substitution of high temperature with room-temperature ultrasound in the extraction with isopropanol as the solvent allows minimizing the extraction time and energy consumption, obtaining comparable yields.

Isopropanol SE and room temperature UAE were compared in terms of oil yield and γ-oryzanol content. The extraction kinetics were determined using Peleg's model to identify the best extraction time. Moreover, a life cycle assessment (LCA) study was performed to compare the environmental sustainability of-the two processes and and to choose the most environmental-friendly extraction process.

2. Materials and methods

2.1 Material and chemicals

Cryo-milled rice bran sample, with a particle diameter of 500 µm, were supplied by Agrindustria Tecco S.R.L. and stored at -20.0 °C until extraction. HPLC grade hexane, methanol, acetonitrile, acid acetic and isopropanol used for extraction and highperformance liquid chromatography (HPLC) analysis and γ-oryzanol standard for quantification were purchased from Merck (Darmstadt, Germany).

2.2 Isopropanol extraction

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

2.3 Ultrasound-assisted extraction

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

2.4 Hexane extraction

Isopropanol and UAE were compared with a conventional hexane extraction following the method of Pengkumsri et al. [15]. Rice bran was mixed with hexane in a 1:10 (w/v) ratio in a Pyrex reaction flask connected with a Liebig reflux condenser. The flask was put into a water bath with a magnetic stirrer at 40 °C for 30 min.

2.5 Determination of RBO Yield

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

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

2.6 Determination of γ-oryzanol content

After the evaporation of the solvent and the determination of the oil yield, the RBO samples were resuspended in 15 mL of isopropanol, and γ-oryzanol content was determined by reversed-phase HPLC. The HPLC system (Shimadzu 20A Prominence) was equipped with a Kinetex C18 column (5 µm, 150 x 4.6 mm) by Phenomenex and photodiode array (PDA) detector using an isocratic elution. The mobile phase was composed by methanol, acetonitrile and 0.03 % acid acetic at a ratio 52:45:3 (v/v/v) [16, 17]. The flow rate was maintained at 0.8 mL/min, and the column oven was thermostated at 30 °C. γ-oryzanol content was determined through a calibration curve prepared using 8 different concentrations of γ-oryzanol standard (0.01-0.8 mg/mL) in isopropanol. The limit of detection (LOD) and the limit of quantification (LOQ) were calculated with the following equation, where σ is the standard deviation of the response and S is the slope of the calibration curve [18]. LOD and LOQ were respectively 0.01 and 0.04 mg/mL.

$$LOD = \frac{3.3 \times \sigma}{S} \tag{2}$$

$$LOQ = \frac{10 \times \sigma}{S} \tag{3}$$

The γ-oryzanol yield was calculated using the formula:

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

2.7 Statistical analysis

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

2.8 Extraction kinetics

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

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

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

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

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

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

The Peleg capacity constant K_2 relates to a maximum of extraction yield, C_e at equilibrium when t = ∞.

$$C_{t\to\infty} = C_e = \frac{1}{K_2} \tag{8}$$

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

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

2.9 Life cycle assessment

LCA was performed with SimaPro 9.0.48 software, database Ecoinvent 3.0

2.9.1 Goal and scope

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

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

The boundary conditions are shown in Fig. 1. Briefly, the entire process is divided in three steps: extraction (SE or UAE), filtration and separation. In the extraction step, the entered flows of matter and energy are isopropanol, rice bran and electricity. The rice bran and the isopropanol come out of the extraction together and enter the second step, where filtration occurs. Here the exhausted rice bran, which is discarded, and the isopropanol containing the RBO are separated. Isopropanol and RBO enter the last step where they are separated by a rotavapor which needs cooling water and electricity. In this step, the isopropanol is removed from the RBO and recirculated for a new extraction.

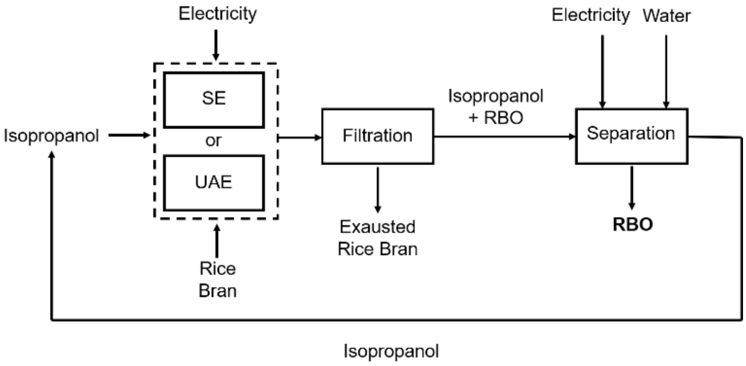

Fig. 1 LCA boundary conditions.

2.9.2 Life cycle inventory

The life cycle inventory (LCI) defined all inputs and outputs involved in the processes. The primary data came from the present study, the produced emissions, the consumed material, and the required energy were referred to this FU.

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

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

2.9.3 Life cycle impact assessment

Life cycle impact assessment (LCIA) was performed with the ReCIPE Midpoint (H) method. In the present study, the analyzed impact category were: Climate change (kg CO_2 eq), Ozone depletion (kg CFC-11 eq), Human toxicity (kg 1,4-DB eq) and Freshwater eutrophication (kg P eq).

3. Results and discussion

3.1 Solvent extraction

The solvent chosen for all the extractions experiments was isopropanol. Traditionally, RBO was extracted using hexane as the solvent because it presents a low corrosiveness, high stability, and a good capacity for dissolving oil and relevant compounds such as γ-oryzanol [12, 21–23]. Despite these advantages, hexane presents health and environmental risks, therefore researchers and oil industries are focusing on reliable alternative solvents. Moreover, hexane derives from a non-renewable source and presents high flammability and toxicity for the environment and human health [22]. A few types of solvents have been tested to substitute hexane as an extractant of vegetable oil. In particular, short-chain alcohols, including isopropanol, are particularly promising due to their low toxicity [23]. Isopropanol is labeled as "recommended" or "preferred" in several green-solvent selection guidelines, such as the GlaxoSmithKline (GSK), Pfizer, Sanofi, AstraZeneca, and Green Chemistry Institute-Pharmaceutical Roundtable (GCI-PR) [2]. Furthermore, all the components of γ-oryzanol present an alcohol group deriving from the ferulic acid; this increases the polarity, making them more soluble in polar solvents such as short-chain alcohols, including isopropanol [21].

At the beginning of this work, a preliminary study on the effects of different solid-to-solvent ratios at various temperatures was performed. After that, using the best solvent-to-solid ratio condition, the temperature range was extended to determine the effect of temperature on the yield of extracted components. At this step, all the extractions were conducted for 1 hour. Figure 2 a) shows the oil yield using 1:3, 1:6, and 1:9 (w/v) solid-to-solvent ratios at three different temperatures (30, 45, and 60 °C). Remarkably, the yield of RBO grows with the increase of the solvent volume at any temperatures tested. This result is in line with Ruen-Ngam et al. [24], which investigated the use of different solvents with an increasing solid-to-solvent ratio and stated that a low volume of solvent does not allow its complete penetration into the rice bran material. The same trend was noted by Hu et al. [12], which obtained a higher yield of RBO and γ-oryzanol, increasing the solvent-to-bran ratio, using both hexane and isopropanol. Indeed the solvent extraction of RBO is mainly considered as a mass transfer process between the solid and liquid phases, where the oil passes from the bran powder to the solvent through a diffusion mechanism [25]. The effect of the solid-to-solvent ratio on the extraction yield is coherent with the mass transfer process. The concentration gradient between the solid and the liquid represents the driving force of this phenomena [26], and it is more significant when the solid is in contact with a large volume of solvent. The high quantity of isopropanol reduces the saturation level of the solvent, increasing oil extraction yields [26]. The same results have been found for the alcoholic SE of antioxidants compounds from different natural sources such as grape pomace, olive leaves or stonebreaker [27–29].

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

Indeed, the temperature is one of the most significant factors influencing the mass transfer and the RBO yield [25]. Capellini et al. [23] performed RBO extraction using isopropanol and ethanol at different temperatures (50, 60, 70, and 80 °C). They found that temperature increase resulted in a growth in oil yield, regardless of the solvent. The same results had been found by, Imsanguan et al.[28], Xu and Godber [19], and Oliveira et al.[20]. All these authors [19, 20, 28] agree that the diffusivity and the solubility of the compounds to extract increase with the increase of temperature, and the extraction output is enhanced [30]. At high temperatures, degradation of the sample matrix structure occurs, making it more permeable to the solvent. Moreover, a temperature rise produces a decrease in solvent viscosity, increasing its diffusivity. However, the high temperature may cause the degradation of thermolabile compounds and the evaporation of the solvent. This has been reported for different plant matrices such as tomatoes, grape waste material and muitle [20, 31–33]. Furthermore, all these extractions have been compared to hexane extraction. As can be noted from Fig. 2, the conventional hexane extraction, proposed by Pengkumsri et al. [15], and used as the reference method, produced only 4.1 ± 0.25 g of RBO/100 g of rice bran. This value is lower than the yields obtained with isopropanol in any operating condition, which reaches the maximum of 8.21 ± 0.69 g of RBO/100 g of rice bran at 90°C and bran to solvent ratio of 1:9, demonstrating that isopropanol is a solvent suitable to replace hexane in RBO extractions. These results are confirmed by Ruen-Ngam et al., Oliveira et al. and Capellini et al. [22, 24, 34] which affirm that alcoholic solvents like isopropanol, can extract higher amounts of oil than hexane due to their high polarity. In particular, isopropanol can extract higher quantities of phospholipids and unsaponifiable material from solid matrices and obtain RBO richer in γ-oryzanol. Indeed, the scructure of γ-oryzanol presents both sides of polar and non-polar (ferulic acid and sterol) making this group of molecules particularly soluble in short-chain alcohols which show dielectric constant higher than hexane and lower than water [24].

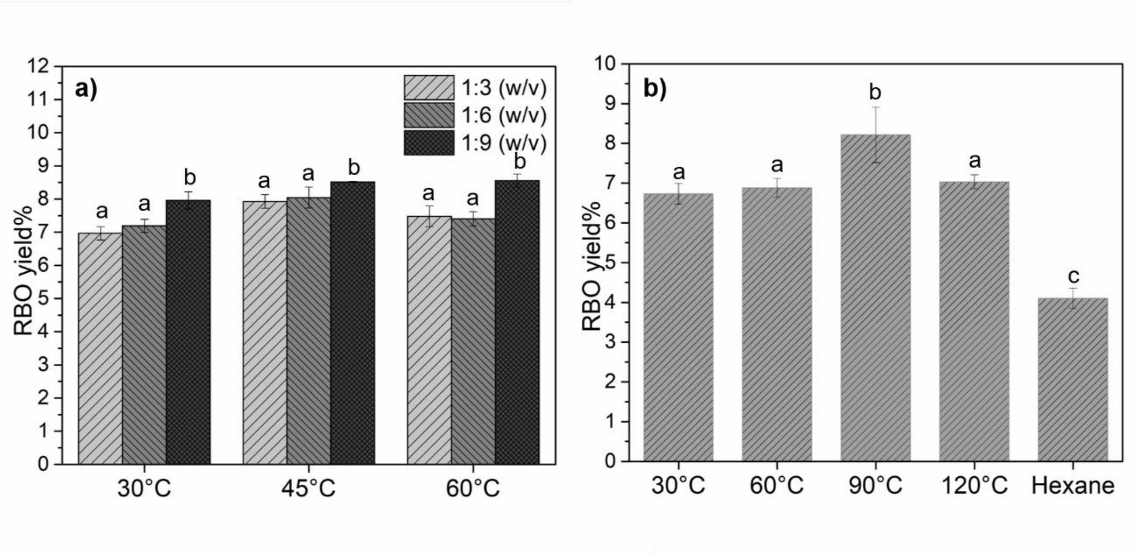

Fig. 2 a) Effect of bran to solvent ratio at different temperatures on RBO yield (%). b) effect of higher temperature on the RBO yield (%) with 1:9 (w/v).

3.2 SE kinetic

The kinetic study was performed measuring the yields in RBO and γ-oryzanol against the time, starting from 1 min up to 2 h. RBO yield % and γ-oryzanol were plotted in function of time (Fig. 3). The shapes of the graphs indicate that the extraction yields are significantly time-dependent. The yields in RBO and γ-oryzanol rise rapidly with time initially, and then, after the knee at about 15 min, start to keep constant producing 6.89 ± 0.089 g of RBO/ 100 g of rice bran and 0.41 ± 0.038 mg of γ-oryzanol/g of rice bran. These profiles fit with the typical SE curve composed of a fast extraction step called "washing phase", followed by a slower extraction step called "diffusion step" [35]. Indeed, the mass transfer rate into the solvent is exceptionally high at the beginning of extraction, when the solvents penetrate the rice bran, thanks to the elevated concentration gradients. Progressing with the extraction, the solutes diffuse from the interior of the bran to the solvent. The mass transfer of solutes becomes more difficult because the concentration gradient between the solid and the liquid phase decreases and the extraction rate becomes slower [20, 35]. The obtained constants of the model (rate constant K_1, constant capacity K_2) and the calculated parameters (initial extraction rate (B_0) and the maximum yield extraction (C_e), regression coefficient (R^2), and the root mean square error (RMSE) are reported in Table 2. The model fitted well with the experimental data, with reasonable accuracy, as evidenced by high R^2 and low RMSE. In particular, experimental data fit slightly worse in the case of γ-oryzanol yield, but this could be due to the multiple treatments of the sample before the HPLC analysis, causing uncertainty in measurement and high standard deviation. In both cases, there was concordance between experimental and predicted yield values. Yields are lower than those reported in the literature [5, 36, 37], but this is certainly due to different experimental conditions and, above all, to the type of rice bran used for the extraction. Indeed the composition of rice bran changes based on the rice variety, the growing conditions, the milling system employed and the stabilization process [2]. Peleg's equation is one of the most suitable models to describe the SE from plant matrix as demonstrated by several studies. Karacabey et al. [38] compared the first-order kinetic model, Peleg's model, two-site kinetic model and modified Gompertz equation to describe solid–liquid extraction kinetics of trans-resveratrol from grape cane. Jurinjak Tusek at al. [39] compared Peleg's, Page's, and Logarithmic model for total polyphenols, antioxidants extraction yield from Asteraceae plants. Poojary and Passamonti [20] the first-order kinetic model, the mass transfer model, and Peleg's model for understanding the behavior of lycopene extraction from tomato processing waste. All these authors agree that Peleg's model showed a better fit to the experimental data than other models investigated in their studies.

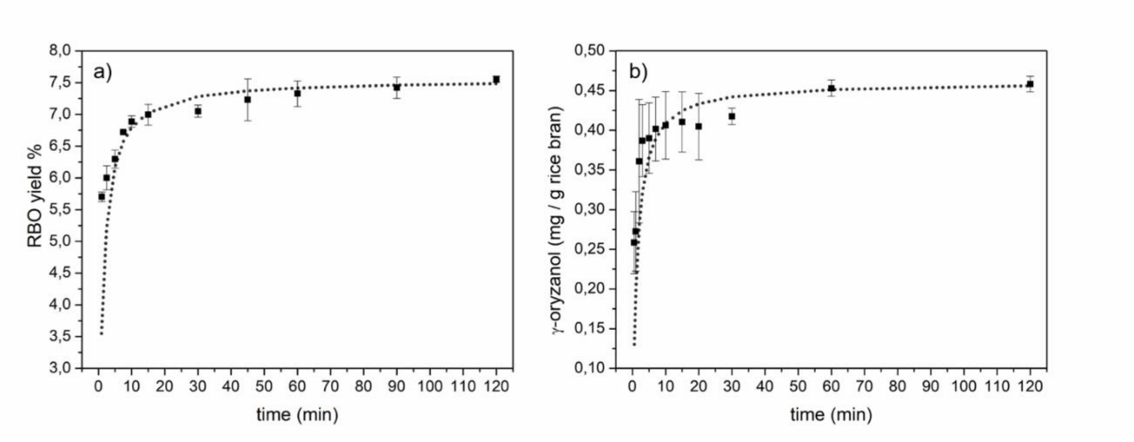

Fig. 3 Isopropanol extraction (SE) kinetics of a) RBO and b) γ-oryzanol. Black squares represent the experimental values. Each experiment was conducted in triplicate, and the error bars correspond to standard error. Dotted lines represent the modeled values by Peleg's equation.

3.3 UAE kinetic

After determining the best isopropanol extraction conditions in terms of temperature and bran to solvent ratio, an intensification process step was performed to work at room temperature and reduce the reaction time. The extraction was conducted with the help of ultrasound as described in Paragraph 2.3, maintaining the bran to solvent ratio of 1:9, as in the previous experiments. The extraction yields in RBO and γ-oryzanol in the function of time were plotted to find the best extraction time and to understand the process kinetic (Fig. 4). These two yields were initially measured every 10 s up to 1 min and then less frequently up to 30 min. As shown in Fig.4 the UAE reaches the steady-state after only 1 minute producing 6.33 ± 0.22 g of RBO/100 g of rice bran and 0.32 ± 0.016 mg of γ-oryzanol/g of rice bran. Peleg's model was adapted to experimental conditions and used for data approximation (see paragraph 3.2) and the results are reported in Tab. 2. The initial extraction rate (B_0) is much higher than the B_0 found for the isopropanol extraction. Indeed, the knee of the curve occurs at about 1 min, where yields reach the maximum values and then stabilize. The maximum yield extraction (C_e) is slightly lower than SE and the R^2, but this may be due to the difficulty of manually measuring the yield every 10 s. This difficulty caused a high uncertainty and an elevated standard deviation producing the worst fit with the model. Until now, to the best of the authors' knowledge, previous studies on the combination of isopropanol and UAE do not exist. Cravotto et al. [40] and Khoei et al. [41] studied the RBO extraction using UAE and water as the solvent, demonstrating that the ultrasound is suitable for aqueous extracting rice bran oil. Other authors studied the effect of UAE of RBO combined with short-chain alcohols like methanol and ethanol [42, 43], demonstrating the feasibility of this kind of extraction. Still, no one managed to complete the extraction in such a short time. Kumari et al. [41] and Galvan et al. [42] showed in their works that Peleg's model efficiently describes the kinetic of UAE for other plant matrixes such as potato peels and black chokeberry wastes.

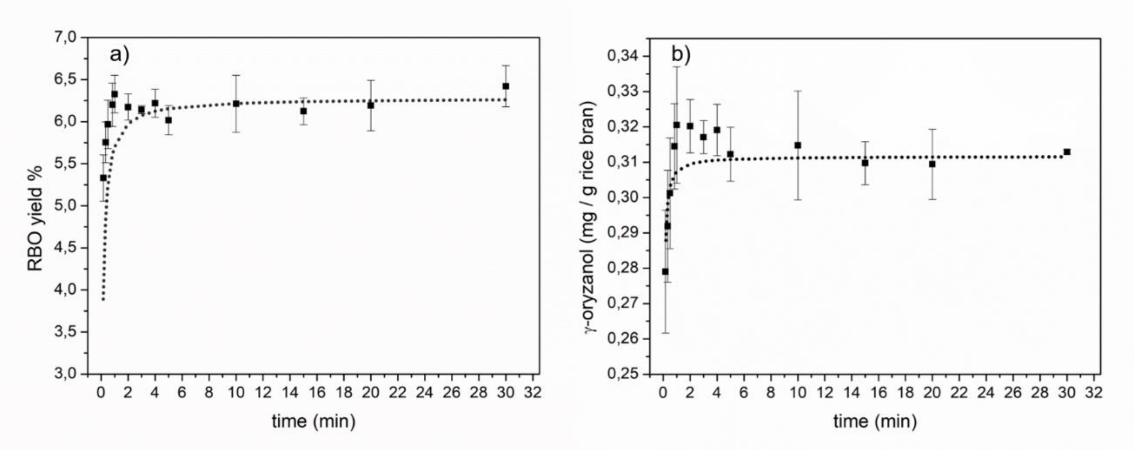

Fig. 4 UAE kinetics of a) RBO and b) γ-oryzanol. Black squares represent the experimental values. Each experiment was conducted in triplicate, and the error bars

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

3.4 SE and UAE kinetics comparison

The obtained constants of the model (rate constant K_1, constant capacity K_2) and the calculated parameters, initial extraction rate (B_0) and the maximum yield extraction (C_e), regression coefficient (R^2), and the root mean square error (RMSE), for the two kinds of extractions, are reported in Table 2. As can be noted, the initial extraction rate is higher for the UAE than SE, demonstrating that UAE can reach a steady state in a shorter time. Assuming infinite extraction time, the maximum yield obtained is 7.55 g RBO/100 g bran and 0.46 mg γ-oryzanol/g bran for the SE and 6.34 g RBO/100 g bran and 0.31 mg γ-oryzanol/g bran for the UAE. SE allows obtaining slightly higher quantities of RBO and γ-oryzanol at 90°C, but UAE allows to reach the maximum yield in just one minute and therefore in much shorter times, operating at room temperature. In their work, Mohammed Danlami at al. [43] and Zhang et al. [44] compared traditional SE with that of other extraction techniques to extract valuable components from plants. They affirmed that ultrasound facilitates the extraction of thermally sensitive compounds enhancing the extraction rate and reducing the extraction temperature. Khoei and Chekin [44], in their works, extracted RBO using aqueous extraction and compared the conventional SE with UAE. The two extraction techniques allowed to obtain very similar RBO yield and they demonstrated that the application of ultrasound permitted to work at room temperature in a shortened extraction time. The global production of RBO exceeds 1.7 million tons per year [11]. Although the use of ultrasound at room temperature leads to a slightly lower oil yield, on such a large annual production this decrease may not be relevant. Using UAE instead of SE would allow to increase the global production of RBO as the extraction has a shorter duration and it is therefore possible to increase the number of annual extractions. The results showed in the present work are in accordance with the literature studies cited, the yields obtained with the two extraction techniques are very similar, but the UAE seems to be the most promising as it allows to reduce the time and energy costs derived from the use of high temperatures. An LCA study will be described in the next paragraph to verify if the UAE is the most sustainable extraction, even environmentally.

Table 2 Peleg's parameters for SE and UAE. Rate constant K_1, constant capacity K_2, initial extraction rate (B_0), maximum yield extraction (C_e), regression coefficient (R^2), 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**	**RMSD**
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

3.5 Life cycle assessment

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

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

Fig. 5 shows the results of comparative LCA between SE and UAE in terms of four impact categories: Climate change (kg CO_2 eq), Ozone depletion (kg CFC-11 eq), Human toxicity (kg 1,4-DB eq), and Freshwater eutrophication (kg P eq). In each impact category, the single contribution of the filtration steps, electricity, and isopropanol to the emission and the total emission of all the two extraction procedures are shown.

Regarding the Climate change impact category, to produce 1 gram of RBO, the SE and UAE produce 0.206 and 0.156 kg of CO_2 eq, respectively; hence, the application of UAE allows a reduction of the total impact of 25 %. In this impact category, the most impacting step for both processes is filtration, which produces a considerable amount of exhausted rice bran as waste material.

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

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

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

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

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

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

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

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

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

(OD) reported by the authors were equal to 0.899 kg CO_2 eq and to 0.00015 kg CFC-11 eq, respectively, whereas in the present work the GWP was 0.206 kg CO_2 eq and the OD was $1.43\cdot10^{-8}$ kg CFC-11 eq. Hence, the present study reached potential impacts lower than Amiri et al. [45] ones in a range between 77.00%-99.98 %.

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

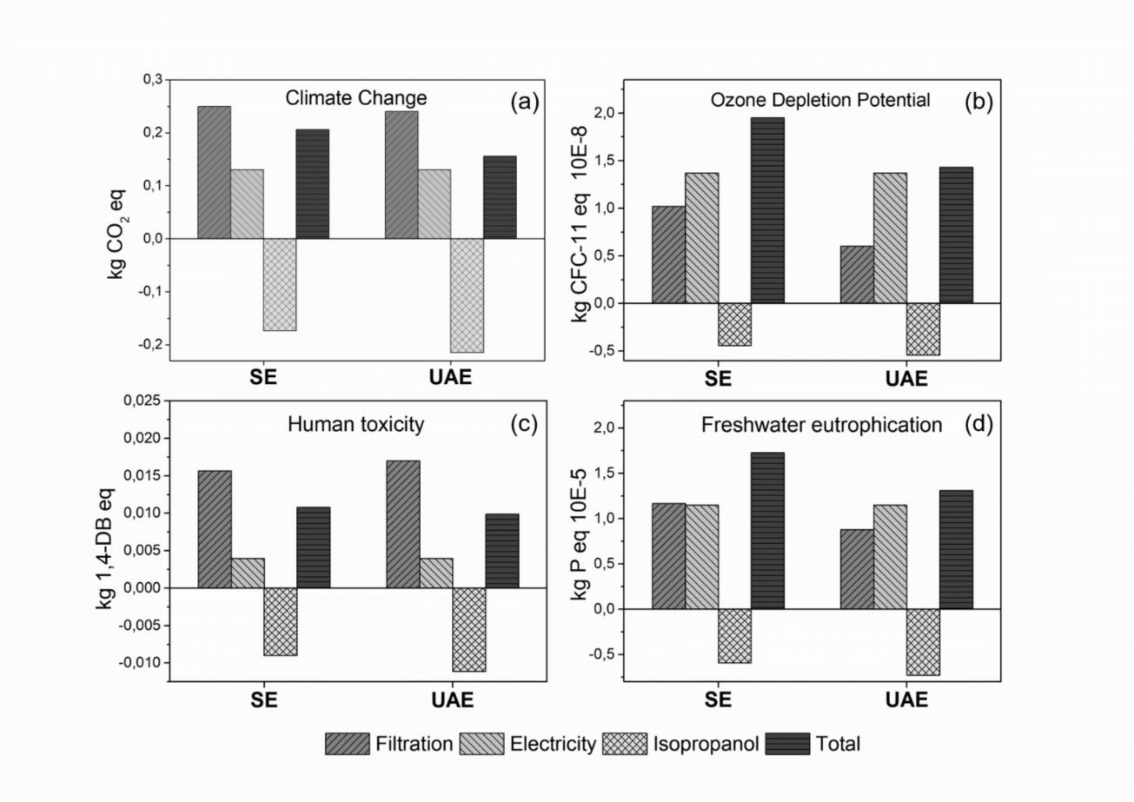

Fig. 5 Comparative LCA results between SE and UAE. Four main impact categories are illustrated: Climate change, Ozone depletion, Human toxicity and Freshwater eutrophication. Each graph reports the result for SE (on the left) and for UAE (on the right); for each extraction process, the contribution of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

4. Conclusions

In the present work, the isopropanol SE of RBO was optimized in terms of temperature and bran to solvent ratio. The best RBO yield was obtained at 90°C and 1:9 bran to solvent ratio. The results were compared with the RBO yield obtained in a standard hexane extraction, demonstrating that isopropanol is suitable to RBO extraction, making the substitution of organic and toxic solvents possible. The kinetics of isopropanol SE at the best-operating conditions was evaluated and compared with a room temperature UAE using a 1:9 bran to solvent ratio. The two extraction techniques produced similar yields in terms of RBO and γ-oryzanol, but UAE reduced remarkably the extraction time. A comparative LCA between the two extraction techniques showed that UAE allows lower the emission contribution to climate change, ozone depletion, and freshwater eutrophication compared to SE, to produce 1 gram of RBO generating high yield, operating at room temperature in a very short time, in line with the principles of green chemistry. To the best of the authors' knowledge, this paper shows for the first time a comparison between the extraction of RBO with isopropanol at 90°C and with isopropanol at room temperature assisted by ultrasounds both from a technical and environmental point of view. The evaluation carried out and the results obtained can be the basis for new experimental campaigns or to design a scale-up RBO production plant.

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