

## Article

# Supercritical Carbon Dioxide Extraction, Antioxidant Activity, and Fatty Acid Composition of Bran Oil from Rice Varieties Cultivated in Portugal

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**Abstract:** Bran of different rice cultivars produced in Portugal were used to study supercritical carbon dioxide extraction conditions of rice bran oil (RBO) and evaluate and compare antioxidant activity and fatty acid composition of the different rice bran varieties. The effect of plant loading (10–20 g), CO<sub>2</sub> flow rate (0.5–1.5 L/min), pressure (20–60 MPa), and temperature (40–80 °C) was studied. The amount of oil extracted ranged from 11.72%, for Ariete cultivar, to 15.60%, for Sirio cultivar. The main fatty acids components obtained were palmitic (13.37%–16.32%), oleic (44.60%–52.56%), and linoleic (29.90%–38.51%). Excellent parameters of the susceptibility to oxidation of the oils were obtained and compare. RBO of *Ariete* and *Gladio* varieties presented superior DPPH and ABTS radical scavenging activities, whereas, *Minima*, *Ellebi*, and *Sirio* varieties had the lowest scavenging activities. Moreover, the oil obtained towards the final stages of extraction presented increased antioxidant activity.

**Keywords:** rice bran oil; supercritical CO<sub>2</sub>; extraction; fatty acids; antioxidant activity

## 1. Introduction

Rice (*Oryza sativa* L.) is an important staple food and the fourth most produced crop in the world, with a production of almost 770,000 million tons (Mt) (paddy-rice, as of the year 2017) [1]. After paddy rice processing, the rice milling industries produce white rice (endosperm) as the major product along with large quantities of two main by-products generated from the removal of the outer layers of the kernel, consisting of rice bran (about 10% of grain weight, 77,000 Mt) and rice husk (about 20%, 154,000 Mt). There is not a comprehensive utilization of the rice milling by-products in many producing countries. For instance, the bran fraction has a typical low value application in cattle and poultry feed [2,3]. However, rice bran retains much of the nutritional value of the whole grain [4] and is a good source of naturally occurring vitamins, antioxidants, and minerals, being a promising resource for nutraceutical applications [4–7]. To take advantage of its full potential rice bran needs to be processed soon after milling and/or stabilized to avoid hydrolytic and oxidative rancidity (particularly due to the enzymatic activity of naturally

occurring/endogenous lipases, that causes hydrolysis of triglycerides into glycerol and free fatty acids), which can be achieved through stabilization techniques of low temperature refrigeration, heat treatment, humidity control, a combination of heat, water, and pressure treatment, among others [8–10].

Rice bran oil (RBO) is known for its balanced fatty acid composition (rich in unsaturated linoleic and oleic fatty acids) and for being a rich source of bioactive phytochemicals, such as phytosterols, tocopherols, and  $\gamma$ -oryzanol. RBO contains about 43.7% monounsaturated fatty acids, 32.7% polyunsaturated, and 23.6% saturated [11]. The major bioactive phytochemicals in rice bran oil (unsaponifiable constituents of RBO) are  $\gamma$ -oryzanols (ferulic esters of sterols, 0.35%–3.2%), phytosterols (0.78%), and tocopherols and tocotrienols, the family of vitamin E-active substances, 0.025%–0.24%), that are known for having antioxidant activity [6,12–15].

The extraction of RBO is usually accomplished with solvent extraction techniques using hexane, whereas short chain alcohols such as ethanol and isopropanol have also been proposed for their greater safety (lower volatility, lower toxicity, lower risk of explosion) [12–18]. Solvent extraction assisted by microwaves and ultrasounds has also been employed [18–22] as well as hot or cold press extraction [22–25]. Following bran extraction, two products are obtained: crude bran oil as the main product, that is, typically, further refined (degumming, dewaxing, deodorizing) [26,27], and defatted rice bran as a by-product, that can be used as a source of dietary fiber [28] and protein [29,30].

Other techniques for RBO extraction include supercritical fluid extraction (SFE) and the use of compressed gases [21,23,31–35]. Supercritical carbon dioxide (SC-CO<sub>2</sub>) extraction is an eco-friendly process able to overcome the toxicity drawbacks associated with conventional solvent extraction. Carbon dioxide is non-toxic, non-flammable, and non-corrosive, is easily removed completely from products, and is relatively inexpensive and available with high purities. CO<sub>2</sub> has a relatively low critical point (31 °C, 7.38 MPa) which is an important economic factor in supercritical fluid extraction designs, contributing as well to minimize thermal degradation of valuable components from rice bran. Furthermore, the control of temperature and pressure in SC-CO<sub>2</sub> enables this technique to modify solvent properties in a manner that improved extraction efficiency/selectivity of specific compounds is reachable.

Previous works have shown that for appropriate pressure/temperature conditions SC-CO<sub>2</sub> RBO extraction reaches an oil yield comparable to that of conventional hexane extraction [24,36]. Additionally, there was a decrease in phosphate and wax levels in the bran oil obtained with SC-CO<sub>2</sub>, while there was an improvement in color and greater preservation of phytochemicals, compared to the oil extracted by hexane [36]. Better thermal stability was also verified [37]. Consequently, SC-CO<sub>2</sub> fluid extraction is a promising alternative to conventional solvent extraction methods and has been exploited in the present work to extract and compare bran oil of eight Portuguese rice cultivars, located in Alcácer do Sal at Sado River, Portugal.

In a first set of experiments, the influence of different SFE process parameters (plant loading, CO<sub>2</sub> flow rate, temperature, and pressure) on the extraction yield of rice bran oil was evaluated to determine the optimum operating conditions. For this set of experiments, bran from the japonica cultivar Ariete was used. In the second set of experiments, bran samples from three long-grain and five short-grain rice varieties were extracted by SFE, using determined best operating conditions, to evaluate total oil content, fatty acid composition, and the antioxidant activity of the rice bran.

This study focuses on providing new data in supercritical carbon dioxide extraction and composition of rice bran oil obtained from eight rice varieties (three short-grain Japonica and five long-grain Indica) cultivated specifically in Sado River in Portugal. To the best of our knowledge, this is the first study with these eight rice varieties, and their characterization in fatty acid composition, as well as the characterization concerning the antioxidant activity to the different fractions collected with the extraction time and the discussion of the consequent increase of the antioxidant activity in the last collected fractions.

## 2. Materials and Methods

### 2.1. Plant Material

Bran samples of different rice varieties were used in the present study, comprising three short-grain Japonica varieties—*Ariete*, *Euro*, and *Opale*, and five long-grain Indica varieties—*Minima*, *Ellebi*, *Sprint*, *Gladio*, and *Sirio*. Paddy-rice samples of each cultivar were supplied by the rice company Atlantic Meals—Indústria e Comércio Agro Alimentar, S.A., and were collected from Sado estuary growth site region, in Alcácer do Sal, Portugal. The rice grains were dehusked, polished to the same whitening degree ( $40 \pm 1$ ), and the bran fraction was vacuum-packed and stored at  $-20\text{ }^{\circ}\text{C}$  until further processing.

### 2.2. Reagents

Carbon dioxide ( $\text{CO}_2$ ), 99.995%, supplied by Air Liquide (Lisbon, Portugal) was used for supercritical fluid extractions. For antioxidant activity measurements, hexane (HPLC grade, 98.24%, ca. 95% n-hexane, Fisher Chemical), methanol (MeOH; HPLC grade, 99.99%, Fisher Chemical), ultra-pure water (Mili-Q system, Milipore Corporation, Darmstadt, Germany), and dimethyl sulfoxide (DMSO) (for analysis,  $\geq 99.9\%$ , Merck EMSURE ACS) solvents were used and reagents Folin & Ciocalteu's phenol reagent (2 M with respect to acid, Sigma, Darmstadt, Germany), sodium carbonate ( $\text{Na}_2\text{CO}_3$ ) (ACS reagent, anhydrous, 99.95%–100.0% dry basis, Sigma-Aldrich), 2,2-Diphenyl-1-picrylhydrazyl (DPPH) (Aldrich), 2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) ( $\geq 99.0\%$ , Sigma), gallic acid (GA) (98%, Acros organics), and ( $\pm$ )-6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox) (97%, Aldrich) were used as received.

### 2.3. Supercritical Fluid Extraction Apparatus and Procedure

Supercritical fluid extraction (SFE) was carried out in a laboratory apparatus from Applied Separations (model Spe-ed SFE-NP), which allows operation at pressures up to 60.0 MPa and temperatures up to  $120\text{ }^{\circ}\text{C}$ , equipped with a  $50\text{ cm}^3$  internal capacity extractor. A detailed description of the SFE apparatus is given elsewhere [38–40]. Briefly, liquid  $\text{CO}_2$  flowing from a cylinder is compressed to the desired pressure using a circulating pump into the extractor, which is preheated to the desired temperature. After equilibrium at the working pressure and temperature, supercritical fluid with solubilized plant material flows out of the extractor vessel expands to atmospheric pressure through a heated metering valve and the extract precipitates into in connected glass U-tube, cooled with ice.  $\text{CO}_2$  flow rate and the total volume were measured at the outlet of the system (mass flow meter and totalizer).

The first set of SFE experiments were conducted in bran of japonica cultivar *Ariete* to evaluate the effect of the following process parameters in the extraction yield: bran loading (10 and 20 g),  $\text{CO}_2$  flow rate from 0.5, 1.0 and 1.5 L/min (0.87, 1.80, and  $2.81 \times 10^{-3}$  kg/min), temperature (40, 60, and  $80\text{ }^{\circ}\text{C}$ ), and pressure (20.0, 30.0, 40.0, and 60.0 MPa). Considering the results from the first set of experiments, all further extractions of the different bran varieties were conducted using 20 g of rice bran loading, 40.0 MPa,  $40\text{ }^{\circ}\text{C}$  and a  $\text{CO}_2$  flow rate of  $1.8 \times 10^{-3}$  kg/min.

For the first set of experiments, the extract was collected directly into glass tubes and the weight of the extract was determined gravimetrically at different intervals over extraction time to evaluate the extraction kinetics. In the second set of experiments, a total of 6 to 7 fractions of the extract was collected over time in separate glass tubes and the quantity of each fraction was determined gravimetrically, and hereafter referred to as fractions T1 to T7. The extraction lasted until no significant increase in the mass of extract was observed. The supercritical extracts were kept at  $-20\text{ }^{\circ}\text{C}$  until further analysis.

The extraction yield of each fraction was calculated as  $\text{Yield}_i = 100 \times w_i/w_{\text{bran}}$ , where  $w_i$  is the weight of fraction  $i$ , with  $i = \text{T1, T2, ..., T7}$ , and  $w_{\text{bran}}$  is the weight of the bran feed. The overall RBO yield is the cumulative weight of all collected fractions (sum of all  $\text{Yield}_i$ ).

#### 2.4. Antioxidant Activities of Bran Oil Fractions

The antioxidant properties of extracted rice bran oil fractions (second set of experiments) were assessed with three spectrophotometric in vitro methods: (i) Total phenolic content (TPC), (ii) DPPH free radical (DPPH<sup>•</sup>) scavenging activity, and (iii) ABTS free radical cation (ABTS<sup>•+</sup>) scavenging activity.

The analyses were performed in RBO fractions T3 and T4 and combined fractions T1 + T2 and T5 + T6 (or T5 + T6 + T7 for *Ellebi* cultivar). Each bran oil sample was dissolved in hexane (1:1, *w/v*) and extracted three times with MeOH:water (60:40 *v/v*) at 1:2 *w/v*. This procedure was used to separate the more polar part (hereafter referred as MeOH-RBO) from the oil (being the remaining oily part referred hereafter as Hex-RBO). The three collected methanolic phases were combined and used directly for measurement of TPC and DPPH<sup>•</sup> and ABTS<sup>•+</sup> scavenging activities. The hexane in the oily phase was evaporated (at 40–50 °C under nitrogen flow) and the oil was redissolved in DMSO and used for DPPH<sup>•</sup> and ABTS<sup>•+</sup> scavenging activity analysis.

##### 2.4.1. Total Phenolic Content

The total phenolic content (TPC) was determined using the Folin–Ciocalteu reagent. Only the methanolic phase of each RBO fraction (polar part) was analyzed for phenolic content. The methodology used was based on the method described elsewhere [41] with some modifications and fully adapted to a 96-well microplate format assay. Sample solution (20 µL) was mixed with diluted Folin–Ciocalteu reagent (10% *v/v*, Mili-Q H<sub>2</sub>O, 100 µL), left to equilibrate for 5 min and neutralized with Na<sub>2</sub>CO<sub>3</sub> (7.5% *w/v*, Mili-Q H<sub>2</sub>O, 80 µL). After a 1-h reaction time in the dark at room temperature, an absorbance at 750 nm was measured (Infinite F50 absorbance microplate reader, TECAN Trading AG). Gallic acid solutions at various concentrations (in MeOH:water 60:40 *v/v*) were made react using the same procedure, in the same microplate, to build a calibration curve. Each microplate assay includes quadruplicates of each sample, gallic acid standard solutions, blanks (20 µL of MeOH:water 60:40 *v/v* and 180 µL Mili-Q H<sub>2</sub>O), and negative controls (20 µL of MeOH:water 60:40 *v/v*, 100 µL of 10% *v/v* Folin reagent and 80 µL of 7.5% *w/v* Na<sub>2</sub>CO<sub>3</sub>). Gallic acid stock solution was prepared daily. Folin–Ciocalteu and Na<sub>2</sub>CO<sub>3</sub> reagents preparation was done before each plate assay.

The sample/gallic acid solutions absorbance was subtracted by the negative control mean absorbance (normalized absorbance). Calibration curve of the standard was determined by fitting a linear regression to the normalized absorbance against gallic acid concentration data (expressed in µg of the antioxidant/mL of the reaction mixture, µg/mL RM) and used to calculate the concentration of polyphenols of each sample. Total phenolic content was expressed in gallic acid equivalents, GAE (mg of GAE/g of RBO fraction).

##### 2.4.2. DPPH<sup>•</sup> Scavenging Activity

The radical scavenging activity of bran oil fractions towards DPPH radicals was used to evaluate the antioxidant activity according to the methods described elsewhere [42,43] with some adaptations and fully adapted to a 96-well plate microplate format assay. Both methanolic and hexane phases of RBO fractions were analyzed. Each 35 µL of the sample was mixed with 140 µL of a 100 µM methanolic solution of DPPH and absorbance at 520 nm was measured (Infinite F50 absorbance microplate reader, Tecan AG) after a 1-h reaction time in the dark at room temperature. Solutions of gallic acid at different concentrations were also tested (expressed in µg of the antioxidant/µmol of initial DPPH<sup>•</sup> in the reaction mixture, µg/µmol DPPH<sup>•</sup>) to build a standard curve.

The reactions were made in 96-well microplates and each microplate assay included quadruplicates of samples, gallic acid standard solutions (standard curve), blanks (35 µL of sample solvent and 140 µL MeOH), and negative controls (35 µL of sample solvent and 140 µL of 0.1 mM DPPH<sup>•</sup>). Gallic acid stock solution was prepared daily. Fresh DPPH radical solution was prepared for each plate assay and used after 30 min stirring under dark.

When analyzing the MeOH-RBO fractions, only the direct solution (combined methanolic phases of RBO) was analyzed. For the assays of Hex-RBO fractions each sample extract was analyzed at various concentrations (expressed in mg/ $\mu$ mol DPPH $\bullet_{in}$ ). Furthermore, the solvent used to prepare the gallic acid solutions and used in blanks and negative controls was MeOH:water 60:40 *v/v* for MeOH-RBO assays and DMSO for Hex-RBO assays.

The percentage of consumed/inhibited DPPH radicals (Inhibition%) due to the antioxidants activity was calculated with the sample/gallic acid solutions mean absorbance (AS) and with reference to the negative control mean absorbance (ANC) according to the equation:

$$\text{Inhibition\%} = 100(A_{NC} - A_S)/A_{NC}. \quad (1)$$

The Inhibition% against gallic acid concentration data was fitted by a five parameters logitlog regression using the Levenberg–Marquardt method and used to calculate the gallic acid equivalent concentration to have the same radical *Inhibition%* obtained for each MeOH-RBO sample. The DPPH $\bullet$  scavenging activity of MeOH-RBO was expressed in GAE (mg GAE/g RBO fraction).

For Hex-RBO samples the different concentrations analyzed and the corresponding DPPH $\bullet$  Inhibition% were also fitted by five parameters logitlog regression that was used to calculate the sample concentration required to inhibit 50% of the radicals ( $IC_{50}^{Sample}$ ).  $IC_{50}$  value for gallic acid was also calculated ( $IC_{50}^{GA}$ ,  $13.9 \pm 0.5 \mu\text{g}/\mu\text{mol DPPH}\bullet_{in}$ ). The ratio  $IC_{50}^{GA}/IC_{50}^{Sample}$  was used to express the DPPH $\bullet$  scavenging activity of Hex-RBO in GAE (mg GAE/g RBO fraction) as suggested elsewhere [44].

#### 2.4.3. ABTS $\bullet^+$ Scavenging Activity

The scavenging ability of RBO fractions towards the long-life radical cation ABTS $\bullet^+$  was determined in both MeOH and hexane phases of RBO fractions according to a modified version of the method described by Re and colleagues [45] and fully adapted to a 96-well microplate assay format. ABTS radical cation was produced by the chemical reaction of ABTS aq. solution (14 mM) with potassium persulfate aq. solution (4.9 mM) at 1:1 *v/v* ratio for 12–16 h in the dark at room temperature. The ABTS $\bullet^+$  radical was then diluted in MeOH to exhibit an absorbance near 1 unit in the reaction media (a dilution factor of 1:29 *v/v* was used). The diluted ABTS $\bullet^+$  solution (150  $\mu$ L) was then used to react with sample solution (50  $\mu$ L). After a 1-h reaction time in the dark at room temperature absorbance at 750 nm was measured (Infinite F50 absorbance microplate reader, TECAN Trading AG). The vitamin E analogue Trolox was used as the standard and different concentrations of it (expressed in  $\mu\text{g}/\text{mL RM}$ ) were made to react with diluted ABTS radical for calibration curve construction.

Each microplate assay included quadruplicates of samples, trolox standard solutions, blanks (50  $\mu$ L of solvent and 150  $\mu$ L of MeOH), and negative controls (50  $\mu$ L of solvent and 150  $\mu$ L of diluted ABTS $\bullet^+$ ). Trolox stock solution was prepared daily. ABTS radical solution was used within 2–3 days, being stored in the fridge. Dilution of ABTS radical solution was made before each plate assay.

The calculation steps employed in the ABTS assay were the same as described for the DPPH assay with the exception that the standard calibration curve of Trolox (relating the Inhibition% of ABTS $\bullet^+$  with trolox concentration) was obtained by linear regression. The ABTS $\bullet^+$  scavenging activity of MeOH-RBO was expressed in Trolox equivalents, TOXE (mg TOXE/g RBO fraction).  $IC_{50}$  values for Trolox ( $IC_{50}^{TOX}$ ,  $7.7 \pm 0.3 \mu\text{g}/\text{mL}$ ) and Hex-RBO extracts ( $IC_{50}^{Sample}$ ) were calculated and the ABTS $\bullet^+$  scavenging activity of Hex-RBO was expressed through the ratio  $IC_{50}^{TOX}/IC_{50}^{Sample}$ , in mg TOXE/g RBO fraction.

#### 2.5. Determination of the Fatty Acids Profile

$\text{BF}_3/\text{MeOH}$  (10%, *w/w*) was used for preparing the carboxylic acids methyl esters. As a general procedure, an aliquot of lipid extract and 5 mL of  $\text{BF}_3/\text{MeOH}$  was added in a round bottom flask and heat in a sand bath at 100  $^\circ\text{C}$  for 5 min. After cooling, 5 mL n-hexane and 20 mL water saturated with NaCl were added. After liquid–liquid extraction, the organic

phase was collected, and 1 g of magnesium sulphate was added. After filtration, the n-hexane was evaporated, and the residue was immediately dissolved in 500  $\mu\text{L}$  n-hexane.

GC-MS analyses were performed on an Agilent 6890 Series gas chromatograph coupled to an Agilent 5973N mass selective detector (Agilent Technologies, Little Falls, DE, USA). A programmed temperature vaporization injector with a baffled liner was used, operating in the solvent vent mode with compressed air for inlet cooling. The large volume injection was performed (vent time, 0.35 min; flow, 40 mL/min; pressure, 0 psi; purge, 60 mL/min at 2 min), for which the inlet temperature was programmed from 60  $^{\circ}\text{C}$  (0.4 min) to 300  $^{\circ}\text{C}$  at a rate of 600  $^{\circ}\text{C}/\text{min}$ . The injection volume was set at 1  $\mu\text{L}$ . GC analysis was performed on a Zebron ZB-5 (30 m  $\times$  0.25 mm I.D., 0.25  $\mu\text{m}$  df; Phenomenex, Torrance, CA, USA) capillary column (5% phenyl, 95% polydimethylsiloxane), using helium as carrier gas maintained in a constant inlet pressure mode of 7.81 psi. The oven temperature was programmed from 160  $^{\circ}\text{C}$  (1 min) at 10  $^{\circ}\text{C}/\text{min}$  to 280  $^{\circ}\text{C}$  and hold for 10 min. The transfer line, ion source, and quadrupole analyzer temperatures were maintained at 280  $^{\circ}\text{C}$ , 230  $^{\circ}\text{C}$ , and 150  $^{\circ}\text{C}$ , respectively, and a solvent delay of 4 min was selected. In the full-scan mode, electron ionization mass spectra in the range 35–550 Da were recorded at 70 eV electron energy with an ionization current of 34.6  $\mu\text{A}$ . Data recording and instrument control were performed by the MSD ChemStation software (G1701CA; version C.00.00; Agilent Technologies, Little Falls, DE, USA).

## 2.6. Oxidation Stability Index Determination

The fatty acids composition from the oils allows comparing their oxidation stability index (OSI), from the American Oil Chemist's Society (AOCS). OSI can be used to relate various oils to anticipate their respective shelf lives and subsequently, the evaluation can be used to assess the efficiency of antioxidants or determine how much longer the oil lasts. To compare the susceptibility to oxidation of the oils, the following parameters were determined [46,47]:

$$\text{OX} = (0.02C_{18:1} + C_{18:2} + 2C_{18:3})/100, \quad (2)$$

$$\text{APE} = (2(C_{18:1} + C_{18:2} + C_{18:3}))/100, \quad (3)$$

$$\text{BAPE} = (C_{18:2} + 2C_{18:3})/100, \quad (4)$$

where, OX is the oxidizability, APE is the allylic position equivalent, and BAPE the Bis-allylic position equivalent indexes. Finally, OSI analysis can for instance be used to compare different oils to predict their respective shelf-life, and to evaluate the effectiveness of antioxidants. The predictive value of the oil stability index OSI can be evaluated from Equation (5) [48]:

$$\text{OSI} = 3.91 - 0.045\text{BAPE}. \quad (5)$$

## 2.7. Statistical Analysis

Five parameters logit log regression using the Levenberg–Marquardt algorithm and the respective  $\text{IC}_{50}$  calculations were done in the Magellan Software (V 7.2, Tecan AG). Linear regressions were calculated using the MS Excel software as well as  $\text{IC}_{50}$  values of linear regression curves. Extraction yield in duplicated, antioxidant activity and TPC values are reported as the mean  $\pm$  standard deviation of at least triplicated analysis. The One-way ANOVA with post-hoc Tukey HSD was applied and would likely identify which of the pairs of the treatments are significantly different from each other.

## 3. Results

### 3.1. Influence of SFE Conditions on the Extraction Yield of Bran Oil

In the present work rice bran extraction with  $\text{SC-CO}_2$  was first assessed using different operating conditions to evaluate the influence of plant loading,  $\text{CO}_2$  flow rate, temperature, and pressure on the oil extraction yield. The specific conditions of the experiments and the overall oil yield (total mass of extracted oil to the mass of rice bran feed) are given in Table 1.

**Table 1.** Operating conditions and overall oil yield ( $\pm$ SD) of supercritical CO<sub>2</sub> extraction of rice bran (cultivar Ariete).

Plant Loading (g)	CO <sub>2</sub> Flow Rate (L/min)	Temperature (°C)	Pressure (MPa)	Oil Yield (% wt.)
Influence of plant feed				
10	1.0	40	40.0	11.83 $\pm$ 0.49
20				11.72 $\pm$ 0.49
Influence of CO <sub>2</sub> flow rate				
20	0.5	40	40.0	11.42 $\pm$ 0.43
	1.0			11.72 $\pm$ 0.48
	1.5			11.14 $\pm$ 0.45
Influence of temperature				
20	1.0	40	40.0	11.72 $\pm$ 0.46
		60		11.70 $\pm$ 0.50
		80		11.79 $\pm$ 0.48
Influence of pressure				
20	1.0	40	20.0	10.68 $\pm$ 0.42
			30.0	11.46 $\pm$ 0.44
			40.0	11.72 $\pm$ 0.48
			60.0	11.80 $\pm$ 0.47

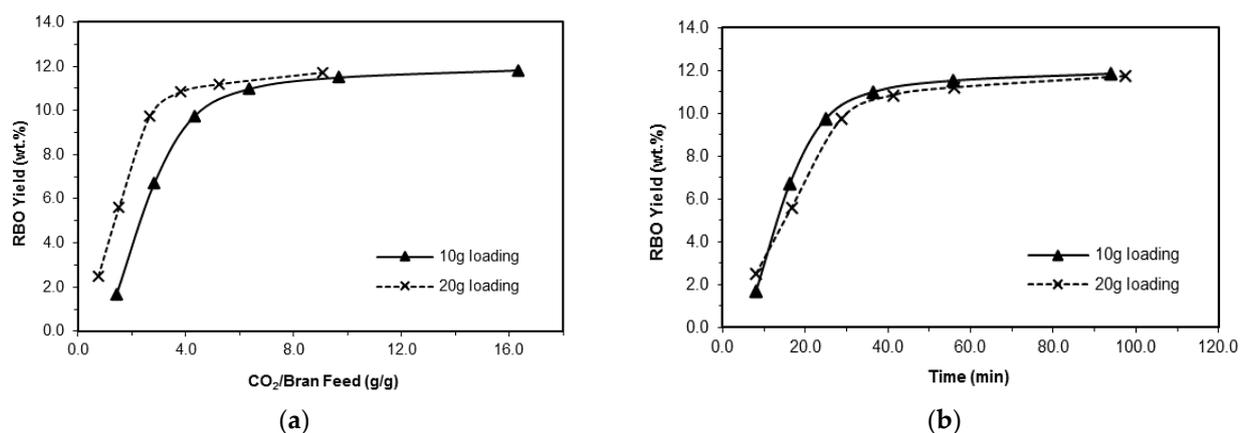
Under the studied conditions the extracting capability of supercritical CO<sub>2</sub> over the rice bran cultivar used, japonica Ariete, was found to lie between 10.7% and 11.8% of oil yield, suggesting a low impact in the overall yield or that this range is near the total oil content of the rice bran used. In fact, the One-way ANOVA with post-hoc Tukey HSD was applied to the analysis of the results and identify which of the pairs of treatments are significantly different from each other. All the results to the maximum yield obtained presents differences not statistically significant, in Table 1.

Nevertheless, data in Table 1 shows that the lowest yield was obtained at the lowest pressure and temperature (20.0 MPa and 40 °C), whereas the higher yields were obtained for extractions at higher temperature (80 °C) and higher pressure (60.0 MPa).

RBO content is dependent on rice genotype/rice variety and growing environment conditions. In addition, the milling process and the degree of milling also affect oil yield extraction [6]. Oil content of rice bran of 12%–22% [6]; 15%–21% [49]; 15%–22% [36]; 10%–23% [35]; 15%–20% [50] have been reported, which are in agreement with our results.

### 3.1.1. Influence of Plant Loading

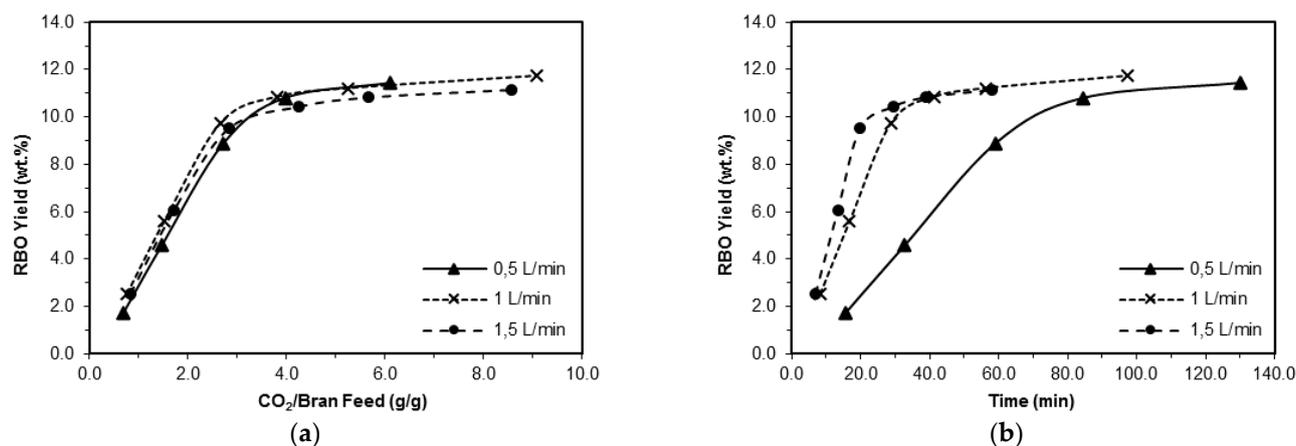
The supercritical extraction efficiency of rice bran oil using a reactor loading of 10 g and 20 g of plant material was compared performing the experiments at 40.0 MPa, 40 °C, and a CO<sub>2</sub> flow rate of 1 L/min. The overall oil yield was similar in both extractions: 11.7% for 10 g loading and 11.8% for 20 g loading. Comparing the curves of extracted oil as a function of consumed CO<sub>2</sub> (expressed as solvent/feed ratio) (Figure 1a) and as a function of extraction time (Figure 1b), using the higher bran load of 20 g is beneficial over 10 g of bran since the oil is extracted using a smaller amount of CO<sub>2</sub> solvent per bran feed and the required extraction time is similar, which is advantageous to lower operating costs and increase production capacity. Looking at the fourth data point in each extraction curve, 10 g loading yielded 10.99% of oil with solvent/feed ratio of 6.35 g/g whereas 20 g loading yielded 10.84% by using only 3.82 g/g of solvent/feed ratio. Therefore, a 20 g rice bran loading was used in further experiments.



**Figure 1.** Supercritical fluid extraction yield of rice bran oil as a function of (a) consumed CO<sub>2</sub> and (b) time using 10 g and 20 g of rice bran loading (at 40 MPa, 313 K, and CO<sub>2</sub> flow rate of 1 L/min).

### 3.1.2. Influence of the Solvent Flow Rate

The effect of three different CO<sub>2</sub> flow rates (0.5, 1.0, and 1.5 L/min) was tested conducting the supercritical extraction experiments at 40.0 MPa, 40 °C, and using 20 g of rice bran loading. The increase of the flow rate significantly increased the rate of extraction and reduced the required extraction time to reach equilibrium, as shown in Figure 2b.



**Figure 2.** Supercritical fluid extraction yield of rice bran oil as a function of (a) consumed CO<sub>2</sub> and (b) time for different CO<sub>2</sub> flow rates (at 40 MPa, 313 K, and using 20 g of rice bran loading).

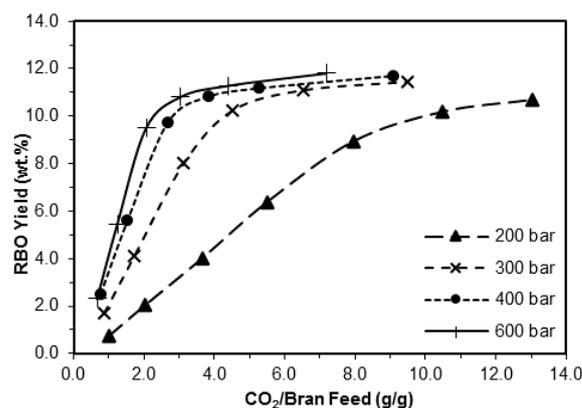
However, the overall yield became slightly lower at the higher flow rate, which can be explained by the shortening of residence time and contact time between the solvent, SC-CO<sub>2</sub>, and solutes in bran to an extent that dissolution of all material cannot be accomplished remains below the saturation concentration.

At the lowest CO<sub>2</sub> flow rate (0.5 L/min) the rate of extraction, before reaching the equilibrium, is considerably lower, resembling the behavior of a solubility limited extraction curve in contrast to the steepest extraction curves at the highest flow rates (1 and 1.5 L/min), characteristic of diffusion-controlled extraction. In terms of consumed CO<sub>2</sub>, the extraction curves were very similar for the three flow rates tested, as can be seen in Figure 2a.

Since the lowest flow rate would considerably increase extraction time and a higher flow rate increase the operating costs the middle solvent flow rate of 1 L/min was chosen for the next experiments.

### 3.1.3. Influence of the Pressure

To evaluate the effect of pressure on rice bran oil SC-CO<sub>2</sub> extractions, experiments were carried using CO<sub>2</sub> pressures between 20.0 and 60.0 MPa, at constant temperature (40 °C), constant CO<sub>2</sub> flow rate (1 L/min), and using a 20 g of rice bran loading. The results presented in Table 1 and Figure 3 show that the extracted oil (maximum yield) slightly increases with increasing pressure (from 10.7% up to 11.8%), whereas the extraction rate markedly increases (curves becoming steeper). The required amount of CO<sub>2</sub> to reach equilibrium becomes lower with pressure, passing from a CO<sub>2</sub>/feed consumption of 13 g/g for a yield of 10.68% at 20.0 MPa to only 3.82 g/g for a yield of 10.84% at 40.0 MPa.



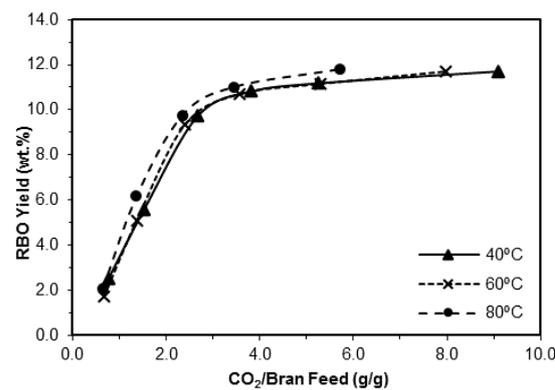
**Figure 3.** Supercritical fluid extraction yield of rice bran oil as a function of consumed CO<sub>2</sub> at different pressures (at 313 K, CO<sub>2</sub> flow rate of 1 L/min and using 20 g of rice bran loading).

The positive effect of pressure is attributed to the consequent increase of CO<sub>2</sub> density and thus its solvating power, resulting in a higher solubility of RBO in the SC-CO<sub>2</sub> and higher oil yields [31,51,52]. From the studied operating conditions, the CO<sub>2</sub> pressure seems to have the greatest impact on the extraction process. The improvement is less pronounced when increasing pressure from 40.0 to 60.0 MPa. Therefore, to avoid more demanding operational conditions 40.0 MPa was set for further extractions.

### 3.1.4. Influence of the Temperature

The influence of temperature on supercritical extractions was assessed by performing experiments at three different temperatures (40, 60, and 80 °C) keeping constant pressure (40.0 MPa), CO<sub>2</sub> flow rate (1 L/min), and using a 20 g rice bran loading. Extraction curves at 40 °C and 60 °C are very similar, as shown in Figure 4, and a slight improvement in extraction rate and final oil yield is noticeable at the highest temperature, 80 °C. For sufficiently high pressures, above the critical point, the solubility of bran oil in CO<sub>2</sub> can increase with temperature due to the increase in vapor pressure of solutes, overcoming the competing effect of lowering CO<sub>2</sub> density with temperature.

For instance, temperature had a negative impact on RBO extraction at pressures between 10–25 MPa [31,33,34,53], whereas favored RBO extraction when using pressures above ca. 30 MPa [31,34,36]. Wang et al. determined a cross over region of 27 MPa [34]. The study of Perretti et al. [51] (studied conditions: 34.5–51.7–68.9 MPa and 40–60–80 °C) determined a crossover pressure region of 34.5–41.4 MPa, and that oil solubility at 80 °C is already above solubility at 40 °C and 60 °C for the pressure of 40 MPa, but oil solubility at 60 °C only crossover solubility at 40 °C for pressures little above 40 MPa, in agreement with the results from the present study: oil yield at 60 °C was close to that at 40 °C, 11.70%, and 11.72%, respectively, and was increased to 12.06% at 80 °C, for extractions conducted at 40.0 MPa (Table 1). Since the extraction only slightly improves with temperature, the lower temperature of 40 °C was chosen for the second set of experiments on RBO extraction of different rice bran varieties.

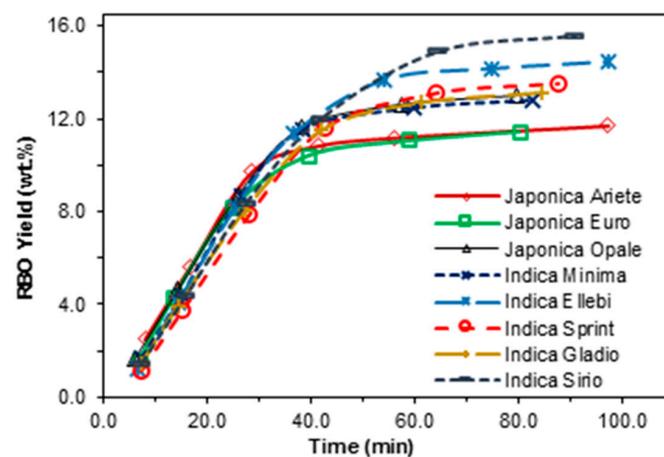


**Figure 4.** Supercritical fluid extraction yield of rice bran oil as a function of consumed CO<sub>2</sub> at different temperatures (at 40 MPa, CO<sub>2</sub> flow rate of 1 L/min, and using 20 g of rice bran loading).

### 3.2. SFE and Antioxidant Activity of Bran Oil Varieties

#### 3.2.1. Extraction Yields

The fractional extraction of the rice bran varieties with SC-CO<sub>2</sub> produced oily extracts that ranged in color from light yellow (early stage of extraction, first fractions) to green (final stage of extraction, last fractions). Extractions were carried at 40.0 MPa, 40 °C, and a CO<sub>2</sub> flow rate of 1 L/min and, on average, 8.7 ± 0.6 g of CO<sub>2</sub>/g of bran loading were used to complete the extractions. Optimizing the extraction conditions for one variety of rice bran, in the previous sections, and extend it to all others was assumed because we are extracting the oil, which is mainly composed of triacylglycerols that in turn reflected in the fatty acid composition. Considering the composition of the oil and knowing the behavior of either the initial solubility (initial phase of extraction) or at a later stage of extraction, where diffusion phenomena control the extraction of the oil from the matrix, there is no reason why similar rice bran matrices of the same type and region may behave differently in supercritical CO<sub>2</sub>. Since the solubility of the compounds is characterized, another factor that controls the extraction is the interaction with the matrix that, in this case, is similar. The extraction yield of RBO for the different rice varieties as a function of the extraction time is shown in Figure 5. The rate of extraction was similar for all varieties and the overall RBO yield ranged from 11.72% to 15.60%. It has been reported in literature oil contents in the bran of 12%–25% and that is dependent on rice genotype/variety and growing conditions, as well as the degree of milling [6,24,54,55]. Indica Sirio and Indica Ellebi varieties exhibited the highest yields whereas Japonica Euro and Japonica Ariete exhibited the lowest (Table 2).



**Figure 5.** Rice bran oil yield as a function of extraction time for the different rice varieties obtained by supercritical CO<sub>2</sub> extraction.

**Table 2.** Overall extraction yield of RBO obtained by supercritical CO<sub>2</sub> extraction for the different varieties and antioxidant activities of MeOH- and Hex-RBO. TPC-Total phenolic content; DPPH-DPPH• scavenging activity; ABTS<sup>-</sup> ABTS•<sup>+</sup> scavenging activity, with (±SD).

Rice Variety	RBO Yield wt. %	MeOH-RBO			Hex-RBO	
		TPC mg/kg	DPPH• mg/kg	ABTS <sup>-</sup> mg/kg	DPPH mg/kg	ABTS• <sup>+</sup> mg/kg
<i>J. Ariete</i>	11.72 ± 0.48 <sup>a</sup>	5.71 ± 0.11 <sup>a</sup>	1.30 ± 0.06 <sup>a</sup>	13.99 ± 0.20 <sup>a</sup>	77.36 ± 2.39 <sup>a</sup>	795.6 ± 6.6 <sup>a</sup>
<i>J. Euro</i>	11.48 ± 0.46 <sup>ab</sup>	3.24 ± 0.17 <sup>b</sup>	0.84 ± 0.05 <sup>b</sup>	9.41 ± 0.11 <sup>b</sup>	59.83 ± 0.85 <sup>b</sup>	484.2 ± 3.2 <sup>b</sup>
<i>J. Opale</i>	13.00 ± 0.48 <sup>ac</sup>	5.83 ± 0.19 <sup>ac</sup>	1.18 ± 0.04 <sup>ac</sup>	14.71 ± 0.10 <sup>c</sup>	72.67 ± 1.11 <sup>ac</sup>	659.7 ± 5.3 <sup>c</sup>
<i>I. Minima</i>	12.79 ± 0.51 <sup>abcd</sup>	2.91 ± 0.18 <sup>b</sup>	0.73 ± 0.05 <sup>b</sup>	8.53 ± 0.15 <sup>d</sup>	51.44 ± 0.58 <sup>d</sup>	442.7 ± 12.3 <sup>b</sup>
<i>I. Ellebi</i>	14.46 ± 0.55 <sup>e</sup>	7.48 ± 0.21 <sup>e</sup>	1.77 ± 0.04 <sup>d</sup>	18.89 ± 0.15 <sup>e</sup>	54.30 ± 0.43 <sup>bd</sup>	500.6 ± 9.8 <sup>bd</sup>
<i>I. Sprint</i>	13.55 ± 0.56 <sup>cdef</sup>	5.84 ± 0.18 <sup>acd</sup>	1.47 ± 0.07 <sup>a</sup>	15.26 ± 0.12 <sup>f</sup>	74.41 ± 3.67 <sup>ac</sup>	704.5 ± 20.4 <sup>c</sup>
<i>I. Gladio</i>	13.11 ± 0.52 <sup>acdef</sup>	5.10 ± 0.23 <sup>d</sup>	1.14 ± 0.03 <sup>ac</sup>	12.72 ± 0.19 <sup>g</sup>	86.87 ± 4.03 <sup>e</sup>	767.1 ± 46.8 <sup>a</sup>
<i>I. Sirio</i>	15.60 ± 0.55 <sup>e</sup>	8.90 ± 0.22 <sup>f</sup>	1.77 ± 0.14 <sup>d</sup>	21.34 ± 0.17 <sup>h</sup>	62.51 ± 2.59 <sup>b</sup>	538.8 ± 11.7 <sup>bd</sup>
<b>p (ANOVA)</b>	(p < 0.05)	(p < 0.01)	(p < 0.01)	(p < 0.01)	(p < 0.01)	(p < 0.01)

Within the same column, different superscript lowercase letters denote statistically significant differences between the results. ANOVA with post-hoc Tukey HSD.

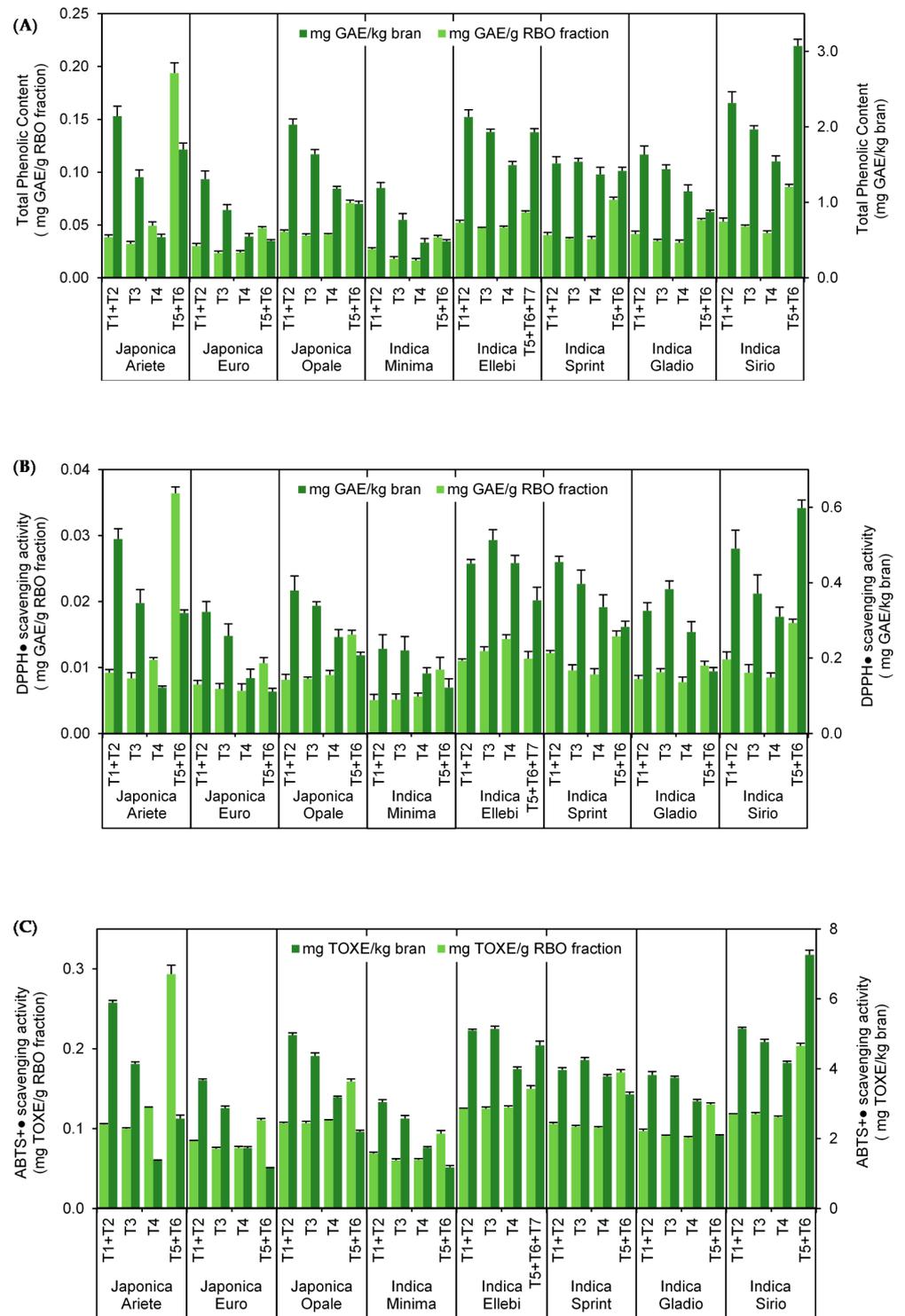
In the present study, long-grain cultivars (Indica) were slightly superior to short-grain cultivars (*Japonica-J.*) in terms of amount of extracted oil (average yield of 12.1 ± 0.8 for Japonica samples and 13.9 ± 1.1 for Indica-I., samples).

### 3.2.2. Antioxidant Activities of Rice Bran Oil Fractions

The antioxidant properties of RBO fractions were assessed using three different in vitro assays, namely, the Total Phenolic Content using Folin–Ciocalteu reagent method, DPPH• scavenging activity, and ABTS•<sup>+</sup> scavenging activity. The RBO fractions were firstly extracted with a MeOH-water mixture, to separate the polar portion from the bran oil, with the specific purpose of extracting existing phenolic compounds from the oil for TPC analysis [56]. Therefore, the polar portion (MeOH-RBO) was tested with TPC assay as well as with DPPH and ABTS assays, whereas the oily part (Hex-RBO) was tested only with DPPH and ABTS assays.

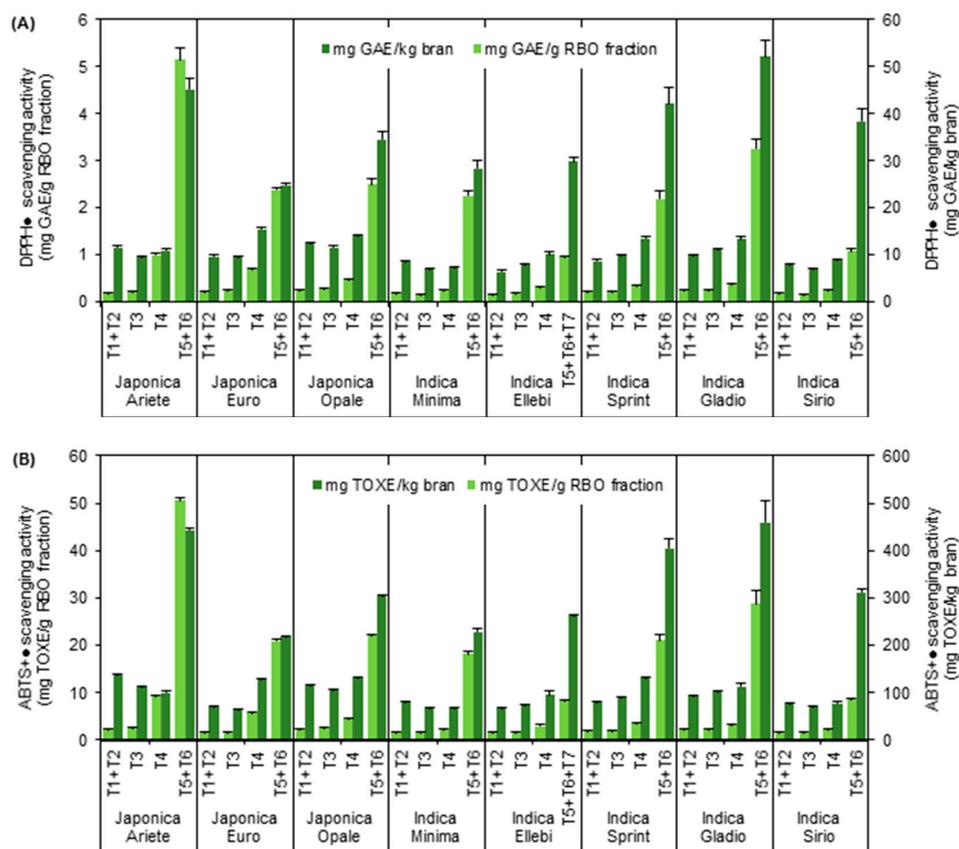
The total phenolic content of MeOH-RBO fractions for the eight varieties of rice (Figure 6A) ranged from 0.016 mg of GAE/g of RBO fraction (fraction T4 of Indica Minima variety) to 0.194 (Japonica Ariete, fraction T5 + T6). The last fractions T5 + T6 exhibited slightly higher values of TPC compared to fractions T1 + T2, T3, and T4 for each variety. Considering the extraction yield of each fraction, TPC values ranged from 0.47 mg of GAE/kg of bran (Indica Minima, fraction T4) to 3.07 (Indica Sirio, fraction T5 + T6). Comparing the sum of TPC values (expressed per bran quantity) of all fractions for each variety (Table 2), Indica Minima and Japonica Euro cultivars showed the lowest phenolic content, whereas Indica Sirio and Indica Ellebi the highest.

The DPPH radical scavenging activity of MeOH-RBO fractions varied from 0.0051 mg of GAE/g of RBO fraction (Indica Minima, T1 + T2) to 0.0364 (Japonica Ariete, T5 + T6) and from 0.111 mg of GAE/kg of bran (Japonica Euro, T5 + T6) to 0.598 (Indica Sirio, T5 + T6) (Figure 6B). The ABTS•<sup>+</sup> scavenging activities of MeOH-RBO fractions ranged from 0.0602 (Indica Minima, T3) to 0.294 (Japonica Ariete, T5 + T6) mg of TOXE/g of RBO fraction and from 1.151 (Japonica Euro, T5 + T6) to 7.26 (Indica Sirio, T5 + T6) mg of TOXE/kg of bran (Figure 6C). The sum of the scavenging activities of all fractions revealed again the lowest activities for Indica Minima and Japonica Euro varieties and the highest for Indica Sirio and Indica Ellebi varieties, both for DPPH and ABTS assay (Table 2).



**Figure 6.** Antioxidant activity of MeOH-RBO fractions: (A) total phenolic content, (B) DPPH radical scavenging, and (C) ABTS radical cation scavenging activity.

The DPPH radical scavenging activity (in GAE units) for each Hex-RBO fraction is presented in Figure 7A.



**Figure 7.** Antioxidant activity of Hex-RBO fractions: (A) DPPH radical scavenging and (B) ABTS radical cation scavenging activity.

The results show a scavenging activity between 0.16 and 0.27 mg GAE/g RBO fraction for T1 + T2 fractions, 0.16 and 0.29 for T3 fraction, 0.24 and 1.00 for T4 fraction, and 0.96 and 5.15 for T5 + T6 fractions. Considering the extraction yield of each fraction the results were between 6.48 and 12.47 (T1 + T2), 7.03 and 11.64 (T3), 7.36 and 15.54 (T4), and 24.84 and 52.09 (T5 + T6) mg GAE/kg of bran. Figure 7B presents the ABTS radical scavenging activity (in TOXE units) for each Hex-RBO fraction.

Moreover, the scavenging ability, as a function of concentration, of fractions T1 + T2 and T3 were similar, fraction T4 was superior, whereas the last fraction T5 + T6 presented a markedly higher inhibition capacity and for lower concentrations, which was noticed for all rice varieties and corroborated by both DPPH and ABTS assays. The radical scavenging activities of Hex-RBO fractions were much higher compared to those observed for the MeOH-RBO fractions (Figure 6B,C), revealing that most of the antioxidant ability remained in the oily phase. In fact, scavenging activities of MeOH-RBO were only 1%–3% and 2%–4% (towards DPPH• and ABTS•+, respectively) of the activities exhibited by Hex-RBO extracts.

DPPH and ABTS assays revealed that the antioxidant activity of RBO changes from the beginning to the end of the supercritical extraction and that more antioxidant compounds (able to scavenge DPPH and ABTS radicals) are extracted towards the later stage of extraction. According to previous work [49],  $\gamma$ -orizanol is preferentially extracted from the rice bran during the late stages of the extraction, suggesting that the high antioxidant activity for the last fractions T5 + T6 in the present study may be due to the presence of  $\gamma$ -orizanol in high concentration. Other studies also found increased concentrations of  $\gamma$ -orizanol compounds in later stages of SC-CO<sub>2</sub> extraction of rice bran [36,57].

The dependence of DPPH and ABTS radicals' inhibition with RBO dosage were described with logitlog regressions and the concentration of each RBO fraction required to inhibit 50% of the radicals (IC<sub>50</sub>) determined and used to express the final scavenging

activity of Hex-RBO fractions in gallic acid equivalents (DPPH assay) or Trolox equivalents (ABTS assay) with the ratios  $IC_{50}^{GA}/IC_{50}^{Sample}$  and  $IC_{50}^{TOX}/IC_{50}^{Sample}$ , respectively. A high linear correlation was found between the  $IC_{50}$  values obtained in the DPPH and ABTS assays ( $R^2 = 0.9753$ ) and between the GAE and TOXE ( $R^2 = 0.9934$ ) for Hex-RBO fractions, as shown in Figure 8.

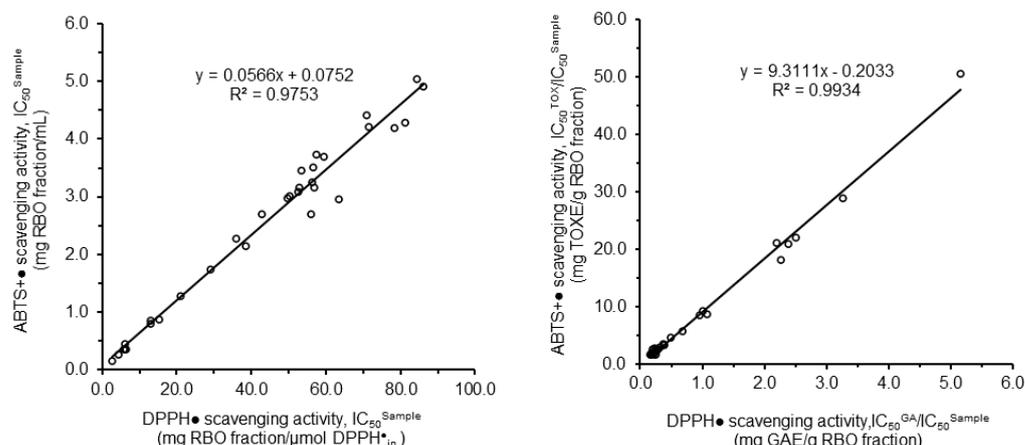


Figure 8. Correlation between ABTS and DPPH assays for Hex-RBO analysis.

### 3.3. Fatty Acid Composition and Oxidation Stability Index Determination

The fatty acid profile of the oil extracted from rice bran using best conditions of SC-CO<sub>2</sub> were determined and are shown in Table 3.

Table 3. Fatty acid composition (% ± SD of total fatty acids) from FAME GC-FID analysis of bran oils obtained from 8 rice varieties japonica and indica, from Portugal, obtained at 40 MPa, 313 K, and flow rate of 1.0 L/min.

Fatty Acid (%)	J. Ariete	J. Euro	J. Opale	I. Minima	I. Ellebi	I. Sprint	I. Gladio	I. Sirio
C14:0 (Myristic)	0.06 ± 0.01	0.12 ± 0.01	0.06 ± 0.01	0.07 ± 0.01	0.09 ± 0.01	0.11 ± 0.01	0.07 ± 0.01	0.09 ± 0.01
C16:0 (Palmitic)	15.39 ± 0.94 <sup>a</sup>	14.63 ± 0.85 <sup>ab</sup>	16.32 ± 0.95 <sup>abc</sup>	13.37 ± 0.78 <sup>ab</sup>	14.94 ± 0.83 <sup>abc</sup>	14.16 ± 0.81 <sup>abc</sup>	13.94 ± 0.81 <sup>ab</sup>	13.76 ± 0.74 <sup>ab</sup>
C18:0 (Stearic)	1.43 ± 0.08 <sup>a</sup>	1.77 ± 0.10 <sup>b</sup>	1.53 ± 0.09 <sup>abc</sup>	2.01 ± 0.12 <sup>bd</sup>	1.84 ± 0.10 <sup>bd</sup>	2.11 ± 0.12 <sup>de</sup>	1.93 ± 0.11 <sup>bde</sup>	1.75 ± 0.09 <sup>bcd</sup>
C18:1 (Oleic)	52.56 ± 3.15 <sup>ab</sup>	47.47 ± 2.75 <sup>ab</sup>	45.85 ± 2.60 <sup>ab</sup>	49.63 ± 2.86 <sup>ab</sup>	46.50 ± 2.55 <sup>ab</sup>	49.85 ± 2.83 <sup>ab</sup>	44.60 ± 2.54 <sup>b</sup>	48.87 ± 2.61 <sup>ab</sup>
C18:2 (Linoleic)	29.90 ± 1.82 <sup>a</sup>	35.32 ± 2.06 <sup>b</sup>	35.50 ± 2.05 <sup>ab</sup>	34.06 ± 1.99 <sup>ab</sup>	35.83 ± 1.98 <sup>b</sup>	32.86 ± 1.87 <sup>ab</sup>	38.41 ± 2.22 <sup>b</sup>	34.67 ± 1.87 <sup>ab</sup>
C20:0 (Arachidic)	0.24 ± 0.01	0.33 ± 0.02	0.32 ± 0.02	0.39 ± 0.02	0.37 ± 0.02	0.45 ± 0.03	0.47 ± 0.03	0.39 ± 0.02
C20:1 (Gadoleic)	0.11 ± 0.01	0.18 ± 0.01	0.18 ± 0.01	0.18 ± 0.01	0.19 ± 0.01	0.18 ± 0.01	0.22 ± 0.01	0.19 ± 0.01
C22:0 (Behenic)	0.06 ± 0.01	0.06 ± 0.01	0.07 ± 0.01	0.07 ± 0.01	0.07 ± 0.01	0.08 ± 0.01	0.09 ± 0.01	0.04 ± 0.01
C24:0 (Lignoceric)	0.11 ± 0.01	0.11 ± 0.01	0.16 ± 0.01	0.17 ± 0.01	0.15 ± 0.01	0.17 ± 0.01	0.10 ± 0.01	0.16 ± 0.01

Within the same raw, different superscript lowercase letters denote statistically significant differences between the results. ANOVA with post-hoc Tukey HSD. The statistical analysis were performed only to compositions higher than 0.5%.

The main saturated fatty acids identified in the oils were palmitic and stearic acids, C16:0 and C18:0, respectively, monounsaturated fatty acids were oleic (C18:1), and the diunsaturated were linoleic (C18:2), which account for around 99% of the total composition. These results agree with those reported in other studies [58,59]. In general, the fatty acids identified presented similar percentages in the rice bran oils obtained. The presence of approximately 80% of unsaturated fatty acids in all bran oils emphasizes its potential health protective effect. Besides biomedical interest, rice bran oil can also constitute a potential feedstock for biodiesel production. Oxidation stability it is an important pre-requisite to evaluate the quality and stability with/without antioxidants of the oils, as the presence of double bonds in fatty acids offers a high level of reactivity with oxygen. The AOCS defined a methodology to measure the relative resistance of oils to oxidation, where the fatty acids composition of the oils allows to compare their OS).

The allylic position equivalent (APE), bis-allylic position equivalent (BAPE), and oxidability (OX) indices were determined to estimate the degree of unsaturation of the different rice bran oils obtained with SFE extraction technique (Table 4).

**Table 4.** Main fatty acid groups. SFA, MUFA, and DUFA composition of bran oils obtained from 8 rice varieties japonica and indica, from Portugal, obtained at 40 MPa, 313 K, and flow rate of 1.0 L/min. Calculated unsaturation index, UI and OX, APE, BAPE, and OSI.

	<i>J. Ariete</i>	<i>J. Euro</i>	<i>J. Opale</i>	<i>I.Minima</i>	<i>I. Ellebi</i>	<i>I. Sprint</i>	<i>I. Gladio</i>	<i>I. Sirio</i>
SFA	17.29 ± 1.06	17.02 ± 1.00	18.46 ± 1.09	16.11 ± 0.95	17.48 ± 0.98	17.08 ± 0.99	16.63 ± 0.98	16.26 ± 0.88
MUFA	52.67 ± 3.16	47.95 ± 2.76	46.03 ± 2.61	49.81 ± 2.87	46.69 ± 2.56	50.03 ± 2.84	44.82 ± 2.55	49.06 ± 2.62
DUFA	29.9 ± 1.82	35.32 ± 2.06	35.5 ± 2.05	34.06 ± 1.99	35.83 ± 1.98	32.86 ± 1.87	38.51 ± 2.22	34.67 ± 1.87
UI	1.125 ± 0.068	1.186 ± 0.069	1.170 ± 0.067	1.179 ± 0.069	1.184 ± 0.065	1.158 ± 0.066	1.218 ± 0.070	1.184 ± 0.064
APE	1.649 ± 0.099	1.662 ± 0.096	1.627 ± 0.093	1.674 ± 0.097	1.647 ± 0.091	1.654 ± 0.094	1.662 ± 0.095	1.671 ± 0.090
BAPE	0.299 ± 0.018	0.353 ± 0.021	0.355 ± 0.021	0.341 ± 0.020	0.358 ± 0.020	0.329 ± 0.019	0.385 ± 0.022	0.347 ± 0.019
OX	0.310 ± 0.019	0.363 ± 0.021	0.364 ± 0.021	0.351 ± 0.020	0.368 ± 0.020	0.339 ± 0.019	0.394 ± 0.023	0.356 ± 0.019
OSI	3.897 ± 0.001	3.894 ± 0.001	3.894 ± 0.001	3.895 ± 0.001	3.894 ± 0.001	3.895 ± 0.001	3.893 ± 0.001	3.894 ± 0.001

SFA: Saturated fatty acids; MUFA: Monounsaturated fatty acids; DUFA: Diunsaturated fatty acid; UI: Unsaturation index is defined by  $UI = (2 \text{ DUFA\% molar fraction} + \text{MUFA \% molar fraction})/100$ .

Analysis of the FA composition showed similar levels of monounsaturation and diunsaturation and, therefore, the same behavior was observed for the indices evaluated, like the UI. The oils had higher values of APE ( $\approx 1.65$ ) compared to BAPE ( $\approx 0.34$ ). The bis-allylic position is more receptive to self-oxidation than the allylic position, while it is more reactive in the development of free radicals [60].

BAPE and OX had analogous values, varying only in terms of the combination of oleic and linoleic acid considered for the calculation of OX. The results for APE, BAPE, and OX obtained in this study are consistent with results reported for others non-edible oils [47], which is due to the similar amounts of allylic and bis-allylic positions in the oils. Oils with high content of monounsaturated fatty acids are more suitable for application in biodiesel, since they can confer a balance between the physical-chemical properties of the biofuel as kinematic viscosity, cold flow properties, and oxidative stability [61,62]. Finally, the OSI values were obtained and according to the proposed classification to the oils [47], where it can present greater stability with/without antioxidants. All the samples evaluated are in the best category with an  $OSI \geq 3$ .

#### 4. Conclusions

In this work, it has been demonstrated that SC-CO<sub>2</sub> is a good method to extract oil from rice bran, contributing to the valorization of this by-product, therefore enhancing the transition to a circular economy. The increase in pressure up to 40 MPa allows to obtain a higher final yield, but for the temperature, an increase from 40 to 80 °C does not significantly increase this yield. The highest oil yield was obtained by the cultivated *Indica Sirio* with a yield of 15.60% being the lower value of 11.48% to the *Japonica Euro*.

The chemical composition of rice bran oil shows fatty acids with linoleic and oleic acid predominating, representing more than 80% of the oil and allowing to obtain highly rich unsaturated fatty acids with significant antioxidant activity. From an unsaturation index, no significant distinction can be found to the oils. However, from the susceptibility oxidation (OSI), the best result was obtained from the *Jap. Ariete*, and all present had very good stability with an  $OSI \geq 3$  h.

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