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# Extraction of rice bran oil using supercritical CO<sub>2</sub> and compressed liquefied petroleum gas



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## ABSTRACT

This work focused on the extraction of rice bran oil using supercritical carbon dioxide (SC-CO<sub>2</sub>) and compressed liquefied petroleum gas (LPG). For the supercritical extractions, the influence of pressure and temperature on the extraction yield was evaluated from 150 to 250 bar and from 40 to 80 °C, whereas for compressed LPG extractions were performed at 5–25 bar and 20–40 °C. The antioxidant activity of the extracts was assayed by DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging method and the chemical composition by gas chromatography-mass spectrometry (GC–MS). The highest yields were 12.68 and 12.07 wt%, whereas the maximum antioxidant activities were 71.67 and 67.49% for extraction using SC-CO<sub>2</sub> and compressed LPG, respectively. The chemical profile of fatty acids was similar for both solvents. The antioxidant compound found in both processes was the  $\beta$ -sitosterol, which is one of the components of  $\gamma$ -oryzanol. From kinetics analysis it was demonstrated that using LPG it is possible to decrease the solvent/feed mass by a factor of approximately 30, and extraction time by a factor of 15. Considering the slight difference in the yield and antioxidant activities of extracts between the solvents, some performed in the solvent than supercritical CO<sub>2</sub> for extraction of rice bran oil, since the extraction period can be considerably reduced while lowering the energy required for solvent recompression.

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## 1. Introduction

Agricultural by-products are often treated as waste, and therefore, their nutraceutical values are lost (Danielski et al., 2005). In this context, rice bran corresponds to 5 to 8 wt% of the total grain mass, is a low value product, and has been used by the industry for extraction of oil as an ingredient in animal feed and as an organic fertilizer (Silva et al., 2006a, b). The extraction of oil from rice bran is an important process for the recovery of value-added compounds present in this by-product (Kim et al., 1999). Global interest in rice bran oil has increased steadily since it contains a balanced fatty acid composition and is a rich natural source of antioxidants and bioactive compounds, most of them with nutritional,

\* Corresponding author. E-mail address: mazutti@ufsm.br (M.A. Mazutti). pharmaceutical and cosmetic applications (Jesus et al., 2010; Chen et al., 2011).

Different techniques have been used to extract the rice bran oul, such as conventional techniques using organic solvents (Amarasinghe and Gangodavilage, 2004; Arab et al., 2011), supercritical extraction with carbon dioxide (SC–CO<sub>2</sub>) (Tomita et al., 2014; Monosroi et al., 2010) and microwave-assisted extraction (Zigoneanu et al., 2008; Terigar et al., 2011). The conventional extraction procedure using organic solvents (n-hexane) requires an additional step for refining the oil before its use (Herrero et al., 2010) and, for this reason, supercritical fluid extraction (SFE) has been preferred for different oleaginous raw materials (Uribe et al., 2011; Eisenmenger and Dunford, 2008; Davarnejad et al., 2008), including rice bran (Tomita et al., 2014; Wang et al., 2008; Xu and Godber, 2000; Imsanguan et al., 2008).

SFE is considered an ideal method for extracting compounds from agricultural by-products. This method offers advantages over

conventional extraction, such as increased selectivity, automaticity, environmental safety, superior quality of extracts and a drastic decreased in the use of organic solvents resulting in extracts without solvent residue (Xynos et al., 2012; Wang et al., 2008). However, one of the main difficulties in the use of supercritical fluids for extraction is the slow kinetics of the process. Generally, the solubility of the compounds of interest in the supercritical fluid is lower than in the solvents used in conventional processes; hence, the mass transfer rate is decreased (Riera et al., 2010, 2007).

The extractions with propane have shown important benefits when compared with supercritical CO<sub>2</sub>, e.g., higher yield and selectivity, shorter extraction time and less solvent (Illés et al., 1999; Hamdan et al., 2008; Freitas et al., 2008; Corso et al., 2010; Ribas et al., 2014). However, there are no studies reporting the extraction of bioactive compounds using liquefied petroleum gas (LPG), which contains propane and n-butane as the main constituents. The low cost and the fact that it is readily available make LPG an attractive alternative to other costly fluids such as propane, nbutane and CO<sub>2</sub> (Silva et al., 2013a, b). LPG has been reported in the high-pressure treatment of some enzymes to increase their catalytic power (Silva et al., 2014, 2013a, b).

In this sense, the main objective of this work was to obtain rice bran oil using compressed LPG and supercritical  $CO_2$  (SC- $CO_2$ ). The extracts obtained in each process were chemically characterized and used for determination of antioxidant activity against DPPH radical.

# 2. Material and methods

# 2.1. Materials

The rice bran used in this work is from harvest 2013 and was provided by Primo Berleze & Cia Ltda. (Santa Maria, RS, Brazil). Carbon dioxide (99.9% purity) was purchased from White Martins. DPPH (1,1-diphenyl- 2-picrylhydrazyl) was obtained from Sigma-–Aldrich, whereas the LPG was purchased from Liquigas (Santa Maria, RS, Brazil) and is composed of a mixture of propane (50.3 wt %), n-butane (28.4 wt%), isobutane (13.7 wt%), ethane (4.8 wt%) and other minor constituents (methane, pentane, isopentane).

## 2.2. Samples

Samples were previously characterized in terms of total oil, moisture content and mean particle diameter. Total oil content was determined by hexane Soxhlet extraction. A sample of approximately 1 g of rice bran was extracted with 200 mL of hexane as a solvent in a Soxhlet apparatus (Marconi, Model MA491/6) for 2 h. Moisture content was determined by the gravimetric method, where 10 g of sample was placed in a stove (Sterilifer, SX 1.3 DTME) at 105 °C for 2 h, and the final mass quantified on an analytical balance (Marte, AY220). Particle size was investigated by Sauter Mean Diameter using Tyler series and density by Helium Pycnometry (Quantachrome Ultrapyc, 1200e). The samples were maintained at -12 °C until the moment of experiments to avoid degradation.

#### 2.3. Experimental apparatus and procedure for the extractions

The experiments were performed in a laboratory scale unit consisting of a solvent reservoir, two thermostatic baths, a syringe pump (ISCO 500D), a 100 cm<sup>3</sup> jacketed extraction vessel, an absolute pressure transducer (Smar, LD301) equipped with a portable programmer (Smar, HT 201) with a precision of 0.12 bar, a collector vessel with a glass tube, and a cold trap.

In each run, approximately 10 g of sample was charged into the

extraction vessel. The solvent (CO<sub>2</sub> or LPG) was pumped into the bed, which was supported by two 300-mesh wire disks at both ends, and was kept in contact with the vegetable matrix for at least 30 min to allow for the system to stabilize. Afterwards, the extract was collected by opening the micrometer valve and the solvent mass flow rate was accounted by the pump recordings. The experiments were accomplished at constant pressures and temperatures and a solvent flow rate of 4 g min<sup>-1</sup>. For the experiments carried out with CO<sub>2</sub> as solvent, extractions were performed at 40–80 °C and 150–250 bar, whereas for LPG at 20–40 °C and 5–25 bar. Extraction kinetics curves were determined for all experimental conditions. Kinetics curves consisted of determining the extract yield as a function of time or solvent/feed mass (S/F, ml<sub>solvent</sub>/gbran) ratio. The extract yield and recovery were calculated according to the following equations.

$$Yield(\%) = \frac{mass of oil extracted(g)}{mass of initial rice bran(g)} \times 100$$
 (1)

$$Recovery(\%) = \frac{\text{mass of oil extracted}(g)}{\text{mass of total oil content}(g)} \times 100$$
(2)

#### 2.4. Statistical analysis

The influence of process variables (pressure and temperature) on runs were evaluated by means of two central composite design (one for each solvent). Statistical analysis of experimental data was carried out using the software Statistica 7.0 (Statsoft Inc., USA). A significance level of 5% was used for all analyzes.

#### 2.5. Gas chromatography-mass spectrometry analysis

The extracts were analyzed with a gas-chromatograph (HP 6890) interfaced with a mass selective detector — GC/MS (HP 5973) with automatic injection system (HP 6890), using a capillary column HP-5MS ( $30 \text{ m} \times 0.32 \text{ mm} \times 0.25 \mu \text{m}$ ); helium was the carrier gas with a flow rate of 2 mL min<sup>-1</sup> at a pressure of 5.05 psi; electronic impact mode of 70 eV; samples of 1  $\mu$ L were injected at 250 °C interface temperature, with the following column temperature gradient programming; 70 °C (1 min); 12 °C/min up to 280 °C.

#### 2.6. Antioxidant activities of extracts

The antioxidant activities were evaluated towards DPPH radical following the methodology of Al Fatimi et al. (2007) with some modifications. The method consists of the addition of 1500  $\mu$ L of extract to 1480  $\mu$ L of a DPPH solution plus 20  $\mu$ L of ethanolic solution. A blank assay was performed using 1500  $\mu$ L of an ethanolic solution instead of the extract. The resulting solution was maintained at rest for 30 min. The absorbance of the samples was determined at 522 nm in a UV–Vis 2600 spectrophotometer (Shimadzu, Kyoto, Japan). The antiradical activity towards DPPH (AA<sub>DPPH</sub>) was calculated according Equation (1), where A<sub>DPPH</sub>, A and A<sub>B</sub> are the absorbance of DPPH solution, sample and blank, respectively.

$$AA_{DPPH}(\%) = \left(\frac{A_{DPPH-}(A - A_B)}{A_{DPPH}}\right) \times 100$$
(3)

#### 60

#### Table 1

Extraction yields and antioxidant activities of extracts from rice bran obtained with supercritical CO2 and compressed LPG as solvents.

Temperature/Pressure (°C/bar)	$CO_2$ density (kg m <sup>-3</sup> )	Yield (wt%)	Oil recovery (%)	Antioxidant activity (%)
Supercritical CO <sub>2</sub>				
40/150	792.33	9.89	64.05	56.0 ± 2.0
80/150	432.19	0.70	4.53	Nd
40/250	892.86	12.68	82.12	55.0 ± 2.0
80/250	691.82	12.24	79.27	$68.0 \pm 3.0$
60/200	732.43	10.19	66.00	72.0 ± 2.0
60/200	732.43	9.88	63.99	72.0 ± 2.0
60/200	732.43	10.14	67.62	72.0 ± 2.0
Compressed LPG				
20/5	Nd	9.99	64.70	$65.3 \pm 0.8$
40/5	Nd	<0.01	Nd	Nd
20/25	Nd	11.45	74.16	64.0 ± 3.0
40/25	Nd	12.07	78.17	67.0 ± 2.0
30/15	Nd	11.75	76.10	$65.0 \pm 3.0$
30/15	Nd	12.66	81.99	65.0 ± 3.0
30/15	Nd	11.79	76.36	65.0 ± 3.0

Nd – not determined.

# 3. Results and discussion

The raw material presented total oil and moisture content of  $15.44 \pm 0.11$  and  $11.22 \pm 0.31$  wt%, respectively. These values were similar to those obtained by Gunawan et al. (2006), who presented  $16.71 \pm 0.86\%$  of oil and  $10.51 \pm 0.89$  wt% of moisture content. Mean particle diameter was  $320.12 \pm 51.4$  µm and density  $1.38 \pm 0.01$  g cm<sup>-3</sup>.

Table 1 presents the results in terms of global yield, total oil recovery and antioxidant activity of the extracts of rice bran obtained with SC-CO<sub>2</sub> and compressed LPG. The yield was calculated as the ratio between the mass of extracted oil and the mass of initial rice bran, and the recovery was calculated as the ratio between the mass of extracted oil content determined by the Soxhlet method (0.1544  $g_{oil}$ ,  $g_{Dran}^{-1}$ ). For extractions accomplished

with Supercritical CO<sub>2</sub>, the highest yield and total oil recovery (12.68 wt% and 82.12%) were obtained in the run 40 °C/250 bar, whereas the lowest yield and total oil recovery (0.70 wt% and 4.53%) were obtained in run 80 °C/150 bar. The highest oil recovery achieved in this study is in good agreement with that reported by Wang et al. (2008), who obtained a maximum rice bran oil recovery of 87.5% by SC-CO<sub>2</sub>. By other hand, Balachandran et al. (2008) reported the highest yield using hexane as solvent (conventional method) when compared with SC-CO<sub>2</sub>, what could be attributed to undesirable materials extracted with hexane, as phosphatides, wax and trace metals. The antioxidant activities ranged from 54.55% at 40 °C/250 bar to 71.67% at 60 °C/200 bar.

Using compressed LPG, the highest yield and total oil recovery (12.07 wt% and 78.17%) were obtained in the run 40  $^{\circ}$ C/25 bar, whereas the lowest yield and total oil recovery (9.99 wt% and

Table 2

Effect of process parameters on extraction yield obtained with supercritical CO<sub>2</sub> and compressed LPG.

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	Effect	Standard error	t (5)	p-value	-95.% Cnf.Limt	+95.% Cnf.Limt
Supercritical CO <sub>2</sub>						
Mean/Intercept	9.39	0.34	27.25	0.0001	8.29	10.49
Temperature (L)	-4.82	0.91	-5.28	0.0133	-7.72	-1.91
Pressure (L)	7.17	0.91	7.86	0.0043	4.26	10.07
Temperature × Pressure	4.38	0.91	4.80	0.0172	1.47	7.28
Compressed LPG						
Mean/Intercept	9.96	1.07	9.35	0.0026	9.96	1.07
Temperature (L)	-4.68	2.82	-1.66	0.1954	-4.68	2.82
Pressure (L)	6.76	2.82	2.40	0.0960	6.76	2.82
Temperature × Pressure	5.30	2.82	1.88	0.1566	5.30	2.82
Temperature × Pressure	5.30	2.82	1.88	0.1566	5.30	2.82

L – Linear effect.

# Table 3

Chemical composition of rice bran extracts obtained with compressed LPG and supercritical CO2.

Run	Chemical composition (%)					
	Palmitic acid (16:0)	Oleic acid (18:1)	Linoleic acid (18:2)	β-Sitosterol		
LPG — 5 bar/20 °C	22.24	47.30	30.46	Nd		
LPG — 25 bar/20 °C	19.84	44.62	35.54	Nd		
LPG — 25 bar/40 °C	20.52	35.82	43.66	4.94		
LPG — 15 bar/30 °C	22.14	42.20	30.72	Nd		
CO <sub>2</sub> - 150 bar/40 °C	20.98	41.14	37.88	Nd		
CO <sub>2</sub> - 250 bar/40 °C	20.90	51.03	28.07	Nd		
CO <sub>2</sub> - 250 bar/80 °C	20.91	38.38	35.27	5.44		
CO <sub>2</sub> – 200 bar/60 °C	21.50	39.01	31.36	8.13		

Nd - not detected.

64.70%) were obtained at run 20 °C/5 bar. The antioxidant activities ranged from 63.55% at 20 °C/25 bar to 67.49% at 40 °C/25 bar, without a significant difference. In a general way, the yield and antioxidant activity obtained with LPG as solvent were slightly lower than with CO<sub>2</sub>.

Data of Table 1 were used to calculate the effects of temperature and pressure in the global yield of rice bran oil, which are presented

in Table 2 for both solvents. For supercritical CO<sub>2</sub>, pressure and temperature present a positive and negative effect on the global yield, respectively, and the interaction between them presents a positive effect. The increase of pressure from 150 to 250 bar (at 40 and 80 °C) increased the extract yield and the extraction rate. This effect is due to the increase in the density and, consequently, the solvating power of solvent, which increases the solubility of rice



Fig. 1. Overall kinetic curves for the extraction of rice bran oil using supercritical CO<sub>2</sub> (a) and compressed LPG (b) as solvents.

bran oil in CO<sub>2</sub>. This effect was also identified in the work of Kim et al. (1999), Danielski et al. (2005) and Wang et al. (2008). However, the temperature increase from 40 to 80 °C (at 150 and 250 bar) decreased the extract yield, which is attributed to a decrease in the density of the solvent with the temperature increase, which consequently decreases the solubility of rice bran oil in CO<sub>2</sub>. Tomita et al. (2014) and Wang et al. (2008) also observed this effect in the SC-CO<sub>2</sub> of rice bran oil. For extractions using compressed LPG, pressure, temperature and their interaction were not statistically significant, although the increases in pressure and temperature have shown a trend to improve the yield.

The extracts from rice bran obtained with SC-CO<sub>2</sub> and compressed LPG were characterized by GC-MS (Table 3). In all extracts, the presence of oleic, linoleic and palmitic acid were identified, which are the main fatty acids of rice bran oil. The antioxidant compound found in both processes was  $\beta$ -sitosterol, is one of the components of  $\gamma$ -oryzanol and it presents antioxidant activity. This compound was identified in runs performed at 80 °C/250 bar and 60 °C/200 bar using SC-CO<sub>2</sub> and in the run at 40 °C/25 bar using compressed LPG. In these runs, were obtained the highest antioxidant activities, being possible to suppose that  $\beta$ -sitosterol is an antioxidant.

Comparing the extraction yields and antioxidant activities, little difference is noted between the solvents employed in this work. However, the main difference can be seen in the kinetic profiles presented in Fig. 1 for runs performed with SC-CO<sub>2</sub> (Fig. 1a) and compressed LPG (Fig. 1b). The solvent/feed mass using LPG decreased by a factor of approximately 30, and extraction time by a factor of 15. lowering the solvent spent on an industrial plant. This result is corroborated by Ribas et al. (2014), which used propane (the main constituents of LPG) and CO<sub>2</sub> for the extraction of candeia oil, obtaining the best results in terms of oil extracted per mass of solvent consumed in comparison with CO<sub>2</sub>,.

Besides the reduction in the extraction time that is an important factor for the economic feasibility of the processes (Pereira and Meireles, 2010), the energy required for recompression of LPG is lower than SC-CO<sub>2</sub>. For instance, at 65 °C and 25 bar, LPG exhibits a density of 9.27 mol/L, while CO<sub>2</sub> will reach a similar density only at 124 bar. Furthermore, LPG is readily available, cheaper, and it can be used in much lower pressures compared to carbon dioxide. However, while CO<sub>2</sub> is considered as generally recognized as safe (GRAS), LPG is highly flammable. Thus, the process extraction with compressed LPG requires more safety, attention and control. Thus, LPG requires less energy on recompression compared to carbon dioxide. LPG is plenty available, cheaper and it can be used under much lower pressures compared to carbon dioxide.

### 4. Conclusion

In this work, SC-CO<sub>2</sub> and compressed LPG were used as solvents for rice bran oil extractions. The highest yields were 12.68 and 12.07 wt%, whereas the maximum antioxidant activities were 71.67 and 67.49% for extraction using SC-CO<sub>2</sub> and compressed LPG, respectively. The chemical profile of fatty acids was similar for both solvents, and the antioxidant compound found in both processes was  $\beta$ -sitosterol, which is one of the components of  $\gamma$ -oryzanol. From kinetics analysis it was demonstrated that using LPG it is possible to decrease the solvent/feed mass by a factor of approximately 30, and extraction time by a factor of 15. Considering the slight difference in the yield and antioxidant activities of extracts between the solvents, compressed LPG is a more promising solvent than supercritical CO<sub>2</sub> for extraction of rice bran oil, since the extraction period can be considerably reduced while lowering the energy required for solvent recompression.

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