EXTRACTION OF RICE BRAN OIL USING SUPERCRITICAL CO_2 COMBINED WITH ULTRASOUND

Juliana F. Soares[1], Valéria Dal Prá[2], Francisco Manuel Barrales[c], Philipe dos Santos[3], Raquel C. Kuhn[1], Camila A. Rezende[4], Julian Martínez[3] and Marcio A. Mazutti[1*]

[1] Department of Chemical Engineering, Federal University of Santa Maria, Av. Roraima, 1000, Santa Maria, 97105-900, Brazil
[2] Department of Pharmaceutical Sciences, Federal University of Santa Maria, Av. Roraima, 1000, Santa Maria, 97105-900, Brazil
[3] College of Food Engineering, University of Campinas, Rua Monteiro Lobato, 80, 13083-862 Campinas, SP, Brazil
[4] Institute of Chemistry, University of Campinas, 13083-862 Campinas, SP, Brazil

Abstract - Rice bran oil (RBO) contains oryzanol and tocopherols. Its recovery was performed using conventional techniques with toxic solvents that leave residues in the final product. Supercritical fluid extraction (SFE) has been used, obtaining high global yields without residual solvent. This work proposes to use ultrasound to enhance the kinetics of the RBO extraction using supercritical CO_2. The factors considered were ultrasound power (160 to 320 W) and sonication time (40 to 120 min), at 40 °C and 25 MPa. The best condition (160 W / 40 min) resulted in a 12.65 wt% extraction yield. When ultrasound was not used, the global yield dropped to 9.94 wt%, representing an increase of 27% of global yield due to ultrasound application. This increase can be assigned to the vibration effect promoted by the ultrasonic waves at the interfaces between the solid matrix and solvent. The extracts showed antioxidant activity towards the DPPH radical achieving values around 70% of inhibition. Precursors of oryzanol (campesterol, β-sitosterol, stigmasterol and 4-methylenecycloartanol) were identified in the SC-CO_2 + US extracts. The results presented herein showed that SC-CO_2 + US is a promising technology to be employed for the extraction of bioactive compounds.

Keywords: Oryzanol, Antioxidant activity, Scanning electron microscopy, Byproduct.

INTRODUCTION

Rice bran is the main residue in the milling process of rice (*Oryza sativa* L.) (5 to 8 wt% of the total grain mass). Commonly, rice bran is discarded, used by the industry for oil extraction, animal feed, or as an organic fertilizer (Laokuldilok et al., 2011; Silva et al., 2006). The rice bran oil (RBO) corresponds to 20-25 wt%, and its extraction is an important process to recover high-value compounds present in the rice bran (Embrapa; Kim et al., 1999). RBO has become

a by-product of great interest, since it is a rich source of bioactive compounds, most of them with nutritional, pharmaceutical and cosmetic applications, and contains a balanced fatty acid composition (Jesus et al., 2010).

The extraction of RBO has been accomplished mainly by conventional techniques, using toxic solvents. However, alternative techniques that intend to diminish the environmental deterioration effects, as well as providing products without toxic solvent residues have been recently studied. The conventional extraction procedure that uses *n*-hexane as solvent requires a refining step of degumming and solvent

*Corresponding author: mazutti@ufsm.br (M.A. Mazutti); Tel.: +55-55-3220-9592.

elimination after extraction to obtain a final product. However, complete solvent removal is not attained (Herrero et al., 2010). Furthermore, the extraction with organic solvents must be carried out for a long time at high temperatures, which may lead to thermal degradation of the target compounds (Gil-Chávez et al., 2013).

Alternative extraction techniques, like supercritical fluid extraction (SFE), have been proposed to avoid the excesses of processing of the product and to obtain high purity products, rich in specific active compounds without residual solvent. The use of supercritical CO_2 presents several advantages like the moderate temperature and pressure needed to attain supercritical conditions (around 32 ºC and 7.3 MPa, respectively) that allows one to perform extractions under mild conditions, thus avoiding bioactive compound degradation. Furthermore, CO_2 is a non-toxic solvent and after the extraction is almost completely separated by decompression. As solubility changes with pressure and temperature, the extraction is selective. Another advantage is the intermediate properties between gasses and liquids, which results in high solubility power and the ability to easily penetrate into pores (Brunner, 2005; Sahena et al., 2009).

Supercritical CO_2 has been widely applied for the extraction of oils from vegetable matrixes, such as chia oil (Uribe et al., 2011) and sunflower seed oil (Rai et al., 2016), among others. Furthermore, SFE has been used to extract high-value compounds present in several industrial byproducts, such as wheat germ oil with high concentration of tocopherol and low concentration of phospholipids, thus avoiding a degumming process (Eisenmenger e Dunford, 2008), passion fruit seed oil, with high concentration of tocopherol and carotenoids (Viganó et al., 2016) and rice bran oil, revealing the presence of oryzanols and tocopherol in the extracted oil (Tomita et al., 2014; Wang et al, 2008; Xu e Godber, 2000; Imsanguan et al, 2008; Soares et al., 2016), among others.

Meanwhile, the main limitation of SFE processes is the slow kinetics of the extraction (Riera et al., 2010). To overcome this problem, an alternative is the application of ultrasonic energy (US) to improve the mass transfer and hence accelerate the kinetics of the process and increase the final extraction yield (Riera et al., 2004).

Although the ultrasound-assisted extraction (UAE) of bioactive compounds at ambient pressure has been investigated using different solvents (ethanol, hexane, etc.) (Zhang et al., 2008; Boonkird et al., 2008; Sun et al., 2011; Carrera et al., 2012; Wang et al., 2013), the processes combining supercritical fluid technology

with ultrasound are relatively recent. Riera et al. (2004), Balachandran et al. (2006), Hu et al. (2007), Reátegui et al. (2014), Santos et al. (2015), Barrales et al. (2015) and Dias et al. (2016) evaluated the use of SFE with and without application of ultrasound, and achieved an increased overall yield of the extracts ranging from 14 to 30% with the use of US.

Therefore, the main objective of this work was to investigate the influence of the output ultrasound power and its application time on the extraction of rice bran oil using supercritical CO_2 as solvent. All the experiments were carried out at 25 MPa and 40 ºC. The kinetics of the process were determined in all experimental runs, and the effect of ultrasound on the physical structure of rice bran was also evaluated using a scanning electron microscope equipped with a field emission gun.

MATERIALS AND METHODS

Materials

The rice bran used in this work was 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 (2,2-diphenyl-1-picrylhydrazyl; 95% purity) and hexane (95% purity) were obtained from Sigma-Aldrich.

Sample characterization

The rice bran was characterized regarding total oil content, moisture, particle size and density, according to the following descriptions. To determine the total oil content, 1 g of rice bran was extracted using 200 mL of hexane as solvent in a Soxhlet apparatus (Marconi, Model MA491/6, Piracicaba, SP, Brazil) during two hours. Moisture content was determined by the gravimetric method, where 10 g of rice bran were placed in a stove (Sterilifer, SX 1.3 DTME, Diadema, SP, Brazil) at 105 ºC during two hours. The final mass was quantified using an analytical balance (Marte, Shimadzu AY220, Kyoto, Japan). Particle size was determined by Sauter Mean Diameter using Tyler series sieves and the Density by Helium Pycnometry (Quantachrome Ultrapyc, 1200e, Boynton Beach, FL, USA). The samples were maintained at -12 ºC before experiments to avoid degradation.

Ultrasound-assisted Supercritical Fluid Extraction

The SFE experiments were performed in a laboratory scale unit located in the Laboratory of High Pressure in Food Engineering (LAPEA-DEA/

FEA-UNICAMP). The unit consists of a solvent reservoir (CO_2), two thermostatic baths (Marconi, Model MA-184 and Marconi, Model MA-126, Campinas, SP, Brazil), a pneumatic pump (PP 111-VE MBR, Maximator, Nordhausen, Germany), a 295 mL jacketed extraction vessel, an ultrasound probe coupled to an output power control, a micrometer valve, coupled to a temperature controller (Marconi, MA 152, Campinas, SP, Brazil) and a collector flask. The ultrasound system (Unique Group, DES500, Campinas, Brazil) consists of a 13 mm diameter (D) titanium probe coupled to a transducer operating from 20 to 99% of its total power (800 W), at 20 kHz frequency. Figure 1 presents a schematic diagram of the experimental unit used in this work.

Approximately 20 g of rice bran were charged into the extraction vessel between two layers of glass spheres (to avoid bed compression), as shown in Figure 1. Supercritical CO_2 was pumped into the bed, and the ultrasound was turned on, at the selected power, during the corresponding time, according to the experimental design described in Table 1. Afterward, the extract was collected after decompression in the micrometer valve, and the solvent mass flow rate was determined by a flowmeter located at the end of collection vessel. The experiments were performed at 40 ºC and 25 MPa. This condition showed higher yield of rice bran extract in previous experiments (Soares et al., 2016), where the pressure (15-25 MPa) and temperature (40-80 ºC) effects were evaluated. Solvent flow rate was maintained constant at 14.82 g CO_2/min during 2 hours of extraction.

The variables investigated in this work were output ultrasound power (160-320 W) and time of ultrasound treatment (40-120 min) using a 2^2 factorial design with central point. Extraction kinetics curves were plotted for all experimental conditions, expressing the global yield as a function of time. All the extractions were carried out in triplicate. The global yield and total oil recovery were calculated according to Equations (1) and (2), respectively.

$$\text{Global yield}\,(\%) = \frac{\text{mass of oil extracted}\,(g)}{\text{mass of initial rice bran}\,(g)} \times 100 \qquad (1)$$

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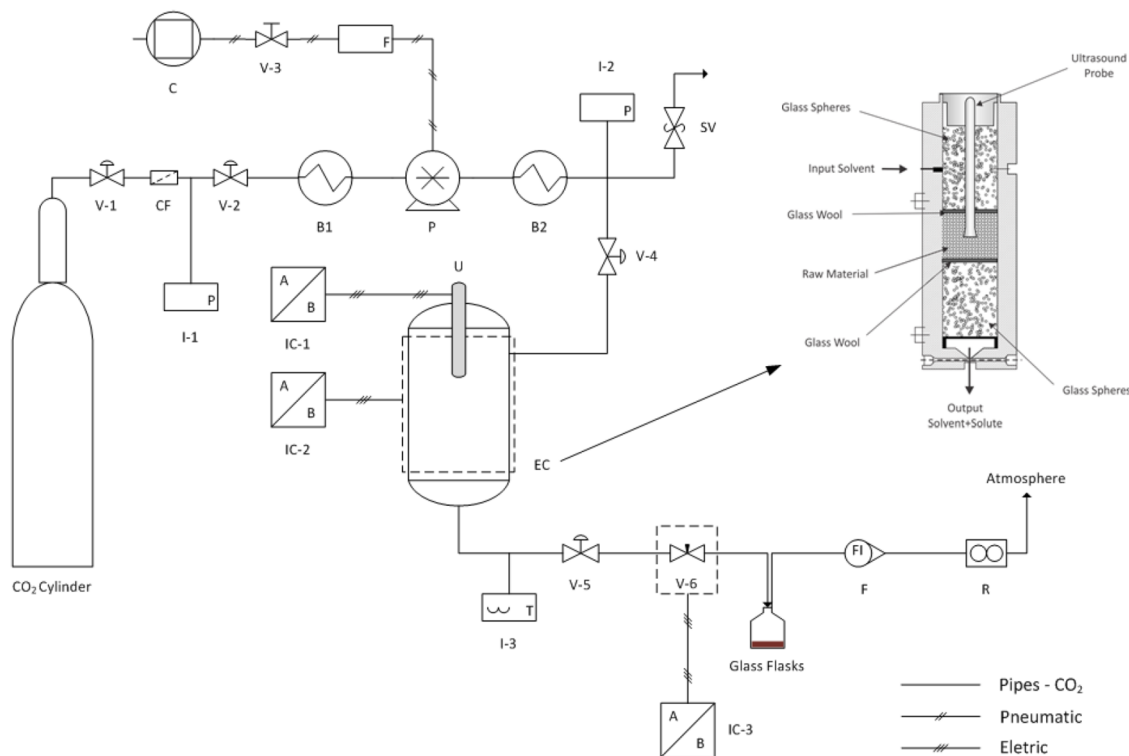

Figure 1. Schematic diagram of the SFE + US experimental unit. V-1, V-2, V-3, V-4 and V-5 – Control valves; V-6 – Micrometer valve; SV – Safety valve; C- Compressor; F- Compressed air filter; CF – CO_2 Filter; B1 –Cooling bath; P - Pump; B2 – Heating bath; I-1 and I-2 – Pressure indicators; I-3 – Temperature indicator; IC-1, IC-2 and IC-3 – Indicators and controllers of ultrasound power, temperature of extraction column and temperature of micrometer valve, respectively; U – Ultrasound probe; R – Flow totalizer; F – Flow meter; EC – Extraction column and internal configuration of the extraction bed of 295 mL for SFE+US used in the kinetic experiments.

Table 1. Global yield and total oil recovery of extracts obtained in the 22 factorial design.

US Power / Treatment time (W/min)	Global yield (wt%)	Total oil recovery (%)
160/40	12.65	81.93
160/120	12.58	81.48
320/40	11.13	72.08
320/120	11.72	75.91
240/80	12.10 (± 0.96)	78.37 (± 6.24)
Without US	9.94 (± 0.88)	64.38 (± 5.74)

Mathematical Modeling

The mathematical model of Sovová (1994) was applied to the experimental curves to further understand the effect of ultrasound on the SFE of rice bran oil. This model assumes that part of the soluble material is easily available to the solvent due to the breaking of cells by milling. The remaining solute is kept inside intact solid-phase particles. Temperature and pressure are regarded as constants. The analytical solution of Sovova's model is given by three different equations corresponding to the mass transfer control mechanism in certain moments of the extraction process:

For $t \leq t_{CER}$:

$$m_{ext(t)} = \dot{m}_F \cdot Y_s \cdot t \cdot \left[1 - \exp\left(-Z \right) \right] \qquad (3)$$

For $T_{CER} < t \leq t_{FER}$:

$$m_{ext(t)} = \dot{m}_F \cdot Y_s \cdot \left\{ t - t_{CER} \cdot \exp\left[\frac{Z \cdot Y_S}{W \cdot X_0} \cdot \right. \right.$$
$$\left. \left. \ln\left(\frac{1}{1-r} \cdot \left(\exp\left(\frac{W \cdot \dot{m}_F}{m_S} \cdot \left(t_{CER} - t \right) \right) - r \right) \right) - Z \right] \right\} \qquad (4)$$

For $t > t_{FER}$:

$$m_{ext(t)} = m_S \cdot \left\{ X_0 - \frac{Y_S}{W} \cdot \ln\left[1 + \left(\exp\left(\frac{W \cdot X_0}{Y_S} \right) - 1 \right) \cdot \right. \right.$$
$$\left. \left. \exp\left(\frac{W \cdot \dot{m}_F}{m_S} \right) \cdot \left(t_{CER} - t \right) \cdot r \right] \right\} \qquad (5)$$

The Z and W parameters are:

$$Z = \frac{k_{Fa} \cdot m_S \cdot \rho_F}{\dot{m}_F \cdot \rho_S \cdot \left(1 - \varepsilon \right)} \qquad (6)$$

$$W = \frac{k_{Sa} \cdot m_S}{\dot{m}_F \cdot \left(1 - \varepsilon \right)} \qquad (7)$$

where: t is the extraction time (min); t_{CER} is the end of the CER period (min); t_{FER} is the end of the falling

extraction rate (FER) period (min); $\dot{m}_F$ is the solvent mass flow rate (g/min); Y_S is the oil solubility in the solvent (g of oil/g of solvent); X_0 is the global yield of oil in the solid matrix (g of oil/g of solid); m_S is the solid mass on an oil-free basis (g); r is the easily accessible oil fraction (X_p/X_0, dimensionless); k_{Fa} is the mass transfer coefficient for the solvent phase (1/min); ε is the bed porosity (dimensionless); k_{Sa} is the mass transfer coefficient for the solid phase (1/min); ρ_F is the fluid density (g/cm³); ρ_S is the solid density (g/cm³); Z and W are dimensionless parameters.

The comparison between the model parameters obtained in the different conditions indicates the influence of ultrasound power and application time on the SFE kinetics. The model was adjusted to each experimental SFE curve individually aiming to obtain the mass transfer coefficients in the solid (k_s) and fluid (k_f) phases and the solid ratio inside the intact cells (X_k). The Powell (2009) free routine was used to adjust the model to the experimental curves, as shown by Carvalho et al. (2015). This routine is an iterative adjustment method that works with a range of values of the parameters defined by the user in a limited number of iterations. Within this range, the routine searches the parameter values that minimize the objective function (f), which was defined as the sum of squared error.

The process data needed to apply the model were: extraction global yield (X_0), mass of solid feed (F - 0.02 kg), temperature (T - 40º C) and pressure (P - 25 MPa) of extraction, solvent density (ρ_s - 889.08 kg/m³) and mass flow rate (Q_{CO2} - 14.82 kg/s), particle diameter (d_p - 32 x 10⁻⁵ m), solid feed density (ρ_f - 1380 kg/m³), extract solubility in the solvent (Y^* - 0.0053 kg/kg), height (H - 0.005 m) and diameter (d - 0.05 m) of the extraction bed.

Chemical characterization of the extracts

The extracts were characterized by gas chromatography (HP 6890) interfaced with a mass spectral detector-GC/MS (HP 5973) with an automatic injection system (HP 6890), using a capillary column HP-5MS (30 m x 0.32 mm x 0.25 µm). Helium was used as the carrier gas with a flow rate of 0.002 L/min

at a pressure of 34820 Pa. The mass spectrometer was operated in electron impact mode at 70 eV. Samples of 1 µL were injected at a 250 ºC interface temperature, with the following column temperature gradient programming: 70 ºC (1 min); 12 ºC/min up to 280 ºC. Compound identification was achieved by matching the mass spectra against the NIST 8.0 MS library (National Institute of Standards and Technology, Gaithersburg, MD).

Antioxidant Activities of Extracts

The antioxidant activities of the obtained extracts were evaluated against DPPH radical following the methodology of Al Fatimi et al. (2007) with some modifications. The method consists of the addition of 1500 µL of extract to 1480 µL of a DPPH solution plus 20 µL of ethanolic solution. A blank assay was performed using 1500 µL of an ethanolic solution instead of the extract. The resulting solution was maintained at rest for 30 minutes. 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_{DPPH}) was calculated according to Equation 8, where A_{DPPH}, A, and A_B are the absorbance of DPPH solution, sample, and blank, respectively.

$$AA_{DPPH} = \left(\frac{A_{DPPH-}(A - A_B)}{A_{DPPH}} \right) \times 100 \qquad (8)$$

Field Emission Scanning Electron Microscopy (FESEM)

The rice bran structure was analyzed before and after the supercritical extractions without and with ultrasound, using a scanning electron microscope equipped with a field emission gun (FESEM) (Quanta 650, FEI, Hillsboro, Oregon, USA). Prior to analysis, the samples were coated with gold in a SCD 050 sputter coater (Oerlikon-Balzers, Balzers, Liechtenstein). Both equipments were available at the National Laboratory of Nanotechnology (LNNano, Campinas-SP/Brazil). The sample structure analyses were performed under vacuum, using a 5 kV acceleration voltage and a large number of images were obtained on different areas of the material to ensure the reproducibility of the results.

RESULTS AND DISCUSSION

Sample Characterization

The raw material presented total oil and moisture content of 15.44 and 11.22 wt%, respectively. These values were similar to those obtained by Gunawan et al. (2006), which presented 16.71% of oil and 10.51% of moisture content in rice bran. Mean particle diameter was 320.12 µm and density 1.38 g/cm³.

The Effect of Ultrasound on Extraction Yield

Table 1 presents the global yield and the total oil recovery of extracts obtained in the experimental design, as well as of an additional run carried out without the use of ultrasound. The highest yield and total oil recovery (12.65 wt% and 81.93%, respectively) were obtained at 160 W/40 min, whereas the lowest yield and total oil recovery (11.13 wt% and 72.08%) were obtained at 320 W/40 min. The highest oil recovery obtained in the SFE with US makes this process an attractive technology when compared to conventional methods since it does not use toxic solvent and does not require another step to remove solvent from the oil.

The global yield obtained in run 160 W/40 min was about 27% higher than that obtained without the application of ultrasound for the same extraction time (2 hours). This result is in good agreement with other studies previously published in the literature. Riera et al. (2004) achieved a 20% higher yield using ultrasound for the extraction of almond oil, whereas Balachandran et al. (2006) and Santos et al. (2015) obtained yields about 30% higher in the extraction of ginger and pepper, respectively. The highest yields obtained with ultrasound may be assigned to the vibration effect promoted by the ultrasonic waves in the extraction process at the interfaces between solid matrix and solvent and also by the fact that ultrasound provide a greater solvent penetration into cellular materials and improve mass transfer due to the effects of microstreaming (Gogate and Kabadi, 2009)

Figure 2 presents the experimental and modeled SFE curves of each of the investigated conditions. In the first few minutes, an increase in the extraction rate of SFE curves with US is observed when compared to the SFE curve without US. This result is similar to that presented by Balachandran et al. (2006) and Santos et al. (2015). The mathematical model of Sovová (1994) was applied in order to further understand the effect of ultrasound on the mass transfer rates. The values of the adjusted parameters, such as mass transfer coefficients in the solid phase (k_s), fluid phase (k_f), concentration of solute inside the unbroken cells (X_k), and the objective function (f) for the SFE conditions with and without US are shown on Table 2.

The values of the solid phase mass transfer coefficient (k_s) were lower than those of the fluid
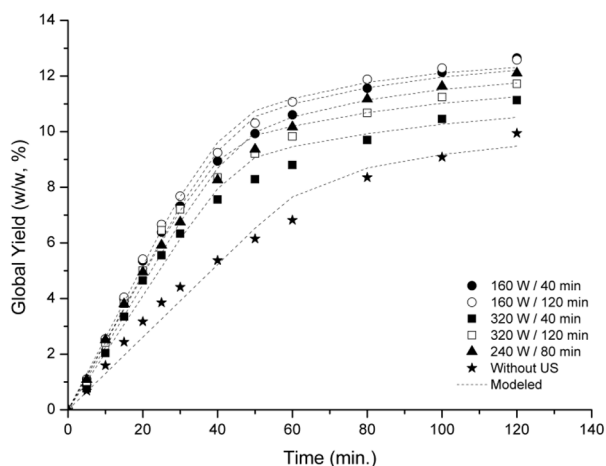
Figure 2. Experimental and modeled SFE curves from rice bran at 250 bar and 40 ºC.

phase mass transfer coefficient (k_f) for the evaluated experiments. This behavior can be explained due to the difficulty to remove the solute located internally in the particles, which results in a long time for the solute cross the interface between the fluid and the solid solute, located on the surface of the particles. Moreover, the smaller values obtained for mass transfer coefficients in the solid phase (k_s) indicate that the mechanism of diffusion is slower than convection in the SFE process (Santos et al., 2015; Weinhold et al., 2008).

The mass transfer coefficient in the fluid phase (k_f) increased and the time of the constant extraction rate period (CER) decreased when US was applied. This indicates that ultrasonic vibrations influenced the convective mechanism of extraction, i.e., intensified the mass transfer at the beginning of the process, since the ultrasonic waves could have damaged the cell walls, enhancing the removal of extractable compounds from the inner rice bran particles, and thus resulting in higher extraction yields. Other possible explanation was proposed by Santos et al. (2015), who affirmed that the interactions between solute and solid matrix and the desorption phenomena of solutes absorbed on a vegetal matrix could have been affected by the

energy supplied to the solid in the form of ultrasonic waves. Others authors suggest that the increase in the global yield of extraction can be related to cavitation, mechanical and thermal phenomena (Shirsath et al., 2010; Sutkar and Gogate, 2009). The mass transfer coefficient in the solid phase (k_s) and the concentration of solute inside the unbroken cells (X_k) showed no significant changes with and without US application, suggesting that ultrasound effects are restricted to the particle's surface.

One can also observe in Figure 2 that, under the condition that resulted in the highest yield (160 W/40 min), the extraction time was reduced by approximately 60% when compared to SFE without US. Riera et al. (2004) obtained a reduction of approximately 30% in the extraction time of oil from particulate almonds, using a 28 MPa pressure, a 20 kHz frequency, and a 50 W power at 55 ºC. Analyzing the kinetics presented in Figure 2 it can be seen that the increase in ultrasound power from 160 to 320 W decreased the global yield, whereas the time of application of ultrasound did not result in a significant difference. This result may be associated with the temperature rise in bulk fluid, which is a function of rate of power dissipation, altering gas solubility and vapor pressure affecting the ease of generation of cavitational events as well as final collapse intensity (Gogate et al. 2011). The alteration in temperature decreases the fatty acid solubility, especially oleic acid (C18:1). According to Maheshwari et al. (1992), a reduction of about 190% in the solubility of oleic acid occurs when the temperature of SC-CO_2 increase from 40ºC to 50 ºC.

Hu et al. (2007) reported that, for a given shape and fixed radiation area, the vibration is proportional to the power of the ultrasound, with consequent increase in the extraction yield, as previously evidenced in the works of Balachandran et al. (2006), Hu et al. (2007) and Gao et al. (2009). However, in the present work, the extraction yields obtained at a 160 W power were greater than those obtained at 320 W. This result can be explained by the fact that US may cause an excessive bed compaction, forming preferential paths

Table 2. Adjusted parameters, objective function (f) and constant rate period (tcer) calculated with Sovová's model (Sovová, 1994) applied to SFE with and without US from rice bran.

Parameters	Without US	160 W/ 40 min	160 W/ 120 min	320 W/ 40 min	320 W/ 120 min	240 W/ 80 min
k_f(s^{-1}) × 10^3	2.75	6.28	6.86	4.86	6.14	5.54
k_s(s^{-1}) × 10^4	2.40	2.15	2.66	1.61	1.91	2.43
X_k(kg$_{solute}$/kg$_{bran}$)	0.0199	0.0253	0.0252	0.0223	0.0234	0.0242
f × 10^8	9.69	5.48	2.51	9.32	5.89	5.47
t_{cer}(s)	3127.66	1742.36	1584.70	1979.99	1649.94	1886.05

for the solvent, which therefore does not reach all the extractable material. This compaction was noted when the extraction vessel was opened after SC-CO_2 + US runs at the mentioned conditions. Another possible reason for the yield reduction at higher ultrasound power is that temperature can increase locally when US is applied, which would decrease the CO_2 density and reduce its solvation power.

Antioxidant Activity and Chemical Composition of Extracts

Table 3 shows the antioxidant activities determined by the DPPH method and the chemical composition of the extracts identified by GC-MS. The antioxidant activities ranged from 69.3% at 320 W/40 min to 72.4% at 160 W/40 min and 240 W/80 min. The extract obtained by SFE without application of US presented antioxidant activity of 71.7%, which is similar to the other conditions with US. Oleic acid (C18:1), linoleic acid (C18:2), palmitic acid (C16:0), campesterol, β-sitosterol, 4-methylenecycloartanol, and stigmasterol were identified in the extracts. Run 160 W/40 min, which presented the highest overall yield, was the only condition where four sterols, precursors of oryzanol (campesterol, β-sitosterol, stigmasterol and 4-methylenecycloartanol), were identified. In the extract obtained without ultrasound application, only β-sitosterol was found. In run 320 W/40 min no compounds with antioxidant potential were identified.

Field Emission Scanning Electron Microscopy (FESEM)

To analyze the effect of the extraction process on the physical structure of the rice bran particles, scanning electron microscopy (SEM) images were obtained on the surface of rice bran samples before extraction, after SFE and after SFE assisted by ultrasound. Samples

that underwent extraction at 160 W for 40 min were chosen to be analyzed here. At least 20 images were obtained per sample, and representative micrographs are shown in Figure 3. The rice bran samples after SFE (Figure 3b) and SFE-US (Figure 3c) present a greater amount of particles deposited on their surfaces when compared to the unextracted rice bran particles (Figure 3a). This effect is more pronounced on SFE-US treated samples, as shown in Figure 3c. Particle deposition in extracted samples results from the supercritical fluid flow and, mainly, to the ultrasonic waves, which damage the cell walls, leading to the release of extractable material from the inner region of the matrix to the surface (Santos et al., 2015). This process enhances the removal of the inner rice bran extracts.
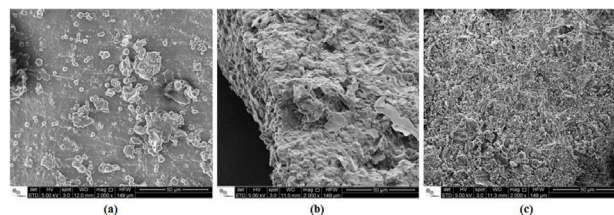

Figure 3. Images obtained by scanning electron microscopy (FESEM) on the surface of rice bran particles before extraction (a), after supercritical extraction without US (b) and after supercritical extraction with ultrasound at 160 W for 40 min (c).

Balachandran et al. (2006) and Santos et al. (2015) also verified, using SEM images, that the ultrasonic vibrations damage the cell walls, enhancing the removal of the extracts. The FESEM analysis of the extracted samples showed that the increase in RBO yields and extraction rates can be explained by physical effects on the particle surface. According to Balachandran et al. (2006), these effects are probably caused by ultrasonic vibrations on the particle surface or simply by a rapid change in the fluid density induced by the pressure of the ultrasonic waves.

Table 3. Antioxidant activity and c hemical composition of rice bran extracts.

US Power/ Treatment time (W/min)	Antioxidant activity (%)	Chemical composition (%)						
		16:0	**18:2**	**18:1**	**1**	**2**	**3**	**4**
160/40	72.4 ± 0.4	0.59	4.12	16.35	50.15	3.21	13.72	11.86
160/120	69.4 ± 1.2	n.d.	11.48	29.38	47.97	11.17	n.d.	n.d.
320/40	69.3 ± 0.3	n.d.	62.35	37.65	n.d.	n.d.	n.d.	n.d.
320/120	70.0 ± 1.2	4.24	21.69	28.85	45.22	n.d.	n.d.	n.d.
240/80	72.4 ± 0.4	0.58	n.d.	24.88	59.11	n.d.	15.43	n.d.
Without US	71.7 ± 2.5	3.89	26.73	39.10	30.28	n.d.	n.d.	n.d.

Palmitic acid (16:0); Linoleic acid (18:2); Oleic acid (18:1); β-Sitosterol (1); 24-Methylenecycloartanol (2); Stigmasterol (3); Campesterol (4). n.d. – not detected.

CONCLUSIONS

The extraction of bioactive compounds from rice bran using supercritical CO_2 combined with ultrasound (SC-CO_2 + US) was studied. The highest yield obtained was 12.65 wt% for SC-CO_2 + US with a power of 160 W applied during 40 minutes. At the same temperature and pressure, the yield of SC-CO_2 extraction without US was 9.94 wt%, thus 27% lower than the one obtained with SC-CO_2 + US. Furthermore, the extraction time was reduced by approximately 60% with US application. Kinetic analysis demonstrated that the increase in ultrasound power from 160 to 320 W decreased the global yield, whereas the time of application of ultrasound did not result in a significant difference, so ultrasound can be applied for the shortest time to reduce energy demand. The antioxidant activity towards DPPH radical of extracts obtained by SC extraction with and without US presented values around 70% of inhibition. The precursors of oryzanol (campesterol, β-sitosterol, stigmasterol, and 4-methylenecycloartanol) were identified in the SC-CO_2 + US. The results presented in this work show that SC-CO_2 + US is a promising technology to be employed for the extraction of bioactive compounds from rice bran.

ACKNOWLEDGEMENTS

REFERENCES

Al Fatimi, M., Wurster, M., Schröder, G., Lindequist, U., Antioxidant, antimicrobial and cytotoxic activities of selected medicinal plants from Yemen. Journal of Ethnopharmacology 111 657-666 (2007).

Balachandran, S., Kentish, S.E., Mawson, R., Ashokkumar, M., Ultrasonic enhancement of the supercritical extraction from ginger. Ultrasonics Sonochemistry 13 471-479 (2006).

Barrales, F.M., Rezende, C.A., Martínez, J., Supercritical CO_2 extraction of passion fruit (Passiflora edulis sp.) seed oil assisted by ultrasound. Journal of Supercritical Fluids 104 183-192 (2015).

Brunner G., Supercritical fluids: technology and application to food processing, Journal of Food Engineering, 67, 1-2, 21-33 (2005).

Boonkird, S., Phisalaphong, C., Phisalaphong, M., Ultrasound-assisted extraction of capsaicinoids from Capsicum frutescens on a lab- and pilot-plant scale. *Ultrasonic Sonochemistry15*, 1075-1079 (2008).

Carrera, C., Ruiz-Rodríguez, A., Palma, M., Barroso, C.G., Ultrasound assisted extraction of phenolic compounds from grapes. Analytica Chimica Acta 732 100-104 (2012).

Carvalho, E.P., Martínez, J., Martínez, J.M., Pisnitchenko, F., On optimization strategies for parameter estimation in models governed by partial differential equations. Mathematics and Computers in Simulation 114 14-24 (2015).

Davarnejad, R., Kassim, K.M., Zainal, A., Sata, S.A.J. Supercritical fluid extraction of β-carotene from crude palm oil using CO_2. Journal of Food Engineering 89 472-478 (2008).

Dias A. L. B., Sergio C. S. A., Santos P., Barbero G. F., Rezende C. A., Martínez J., Effect of ultrasound on the supercritical CO2 extraction of bioactive compounds from dedo de moça pepper (Capsicum baccatum L. var. pendulum), Ultrasonics Sonochemistry, 31 284-294 (2016).

Eisenmenger, M., Dunford, N.T., Bioactive components of commercial and supercritical carbon dioxide processed wheat germ oil. Journal of the American Oil Chemists' Society 85 55-61 (2008).

EMPRESA BRASILEIRA DE PESQUISA AGROPECUÁRIA - EMBRAPA. Agência Embrapa de Informação Tecnológica (Ageitec). Access: <http://www.agencia.cnptia.embrapa.br/gestor/tecnologia_de_alimentos/arvore/CONT000gc8yujq302wx5ok01dx9lcx1g7v3u.html >.

Gao, Y., Nagy, B., Liu, X., Simándi, B., Wang, Q., Supercritical CO_2 extraction of lutein esters from marigold (*Tagetes erecta* L.) enhanced by ultrasound. Journal of Supercritical Fluids 49 345-350 (2009).

Gil-Chávez, G.J., Villa, J.A., Ayala-Zavala, J.F., Heredia, J.B., Sepulveda, D., Yahia, E.M., González-Aguilar, G.A., Extraction and production of bioactive compounds to be used as nutraceuticals and food ingredients: an overview. Comprehensive

Reviews in Food Science and Food Safety 12 5-23 (2013).

Gogate, P.R., Sutkar, V.S., Pandit, A.B., Sonochemical reactors: Important design and scale up considerations with a special emphasis on heterogeneous systems. Chemical Engineering Journal 166 1066-1082 (2011).

Gogate, P.R., Kabadi, A.M., A review of applications of cavitation in biochemical engineering/ biotechnology. Biochemical Engineering Journal 44 60-72 (2009)

Gunawan, S., Vali, S.R., Ju, Y., Purification and Identification of Rice Bran Oil Fatty Acid Steryl and Wax Esters. Journal of the American Oil Chemists' Society 83 (5) 449-456 (2006).

Herrero, M., Mendiola, J.A., Cifuentes, A., Ilbáñes, E., Supercritical fluid extraction: Recent advances and applications. Journal of Chromatography A 1217 2495-2511 (2010).

Hu, A.-J, Zhao, S., Liang, H., Qiu, T.- Q, Chen, G., Ultrasound assisted supercritical fluid extraction of oil and coixenolide from adlay seed. Ultrasonics Sonochemistry 14 219-224 (2007).

Imsanguan, P., Roaysubtawee, A., Borirak, R., Pongamphai, S., Douglas, S., Douglas, P.L., Extraction of α-tocopherol and γ-oryzanol from rice bran. LWT - Food Science and Technology 41 1417-1424 (2008).

Jesus, S.P., Grimaldi, R., Hense, H., Recovery of γ-oryzanol from rice bran oil byproduct using supercritical fluid extraction. Journal of Supercritical Fluids 55 149-155 (2010).

Kim, H.J., Lee, S.B., Park, K.A., Hong, I.K., Characterization of extraction and separation of rice bran oil rich in EFA using SFE process. Separation and Purification Technology 15 1-8 (1999).

Laokuldilok, T., Shoemaker, C.F., Jongkaewwattana, S., Tulyathan, V., Antioxidants and antioxidant activity of several pigmented rice brans. Journal of Agricultural and Food Chemistry 59 193-199 (2011).

Maheshwari, P., Nikolov, Z.L., White, T.M., Hartel, R. Solubility of fatty acids in supercritical carbon dioxide. Journal of the American Oil Chemists' Society, 69(11) 1069-1076 (1992).

Powell, M.J.D., Subroutine BOBQYA; Department of Applied Mathematics and Theoretical Physics: Cambridge University (2009).

Rai A., Mohanty B., Bhargava R., Supercritical extraction of sunflower oil: A central composite design for extraction variables, Food Chemistry 192 647-659 (2016).

Reátegui, J.L.P., Machado, A.P.F., Barbero, G.F., Rezende, C.A., Martínez, J. Extraction of antioxidant compounds from blackberry (Rubus sp.) bagasse using supercritical CO_2 assisted by ultrasound. Journal of Supercritical Fluids 94 223-233 (2014).

Riera, E., Blanco, A., Acosta, V.M., Gallego-Juárez, J.A., Blasco, M., Mulet, A., Prototype for the use of ultrasound in supercritical media. In: 19th International Congress on Acoustics, Madrid (2007).

Riera, E., Blanco, A., García, J., Benedito, J., Mulet, A., Gallego-Juárez, J.A., Blasco, M., High-power ultrasonic system for the enhancement of mass transfer in supercritical CO_2 extraction processes. Physics Procedia 3 141-146 (2010).

Riera, E., Golás, Y., Blanco, A., Gallego, J.A., Blasco, M., Mulet, A., Mass transfer enhancement in supercritical fluids extraction by means of power ultrasound. Ultrasonics Sonochemistry 11 241-244 (2004).

Sahena F., Zaidul I.S.M., Jinap S., Karim A.A., Abbas K.A., Norulaini N.A.N., Omar A.K.M., Application of supercritical CO2 in lipid extraction - A review, Journal of Food Engineering, 95, 2 240-253 (2009).

Santos, P., Aguiar, A.C., Barbero, G.F., Rezende, C.A., Martínez, J., Supercritical carbon dioxide extraction of capsaicinoids from malagueta pepper (Capsicum frutescens L.) assisted by ultrasound. Ultrasonics Sonochemistry 22 78-88 (2015).

Shirsath, S.R.; Sonawane, S.H. and Gogate, P.R. Intensification of extraction of natural products using ultrasonic irradiations—A review of current status, Chemical Engineering and Processing: Process Intensification, 53 10-23 (2012).

Silva, M.A. da, Sanches, C., Amante E.R., Prevention of hydrolytic rancidity in rice bran. Journal of Food Engineering 75 487-491 (2006).

Soares, J.F., Dal Prá, V., de Souza, M., Lunelli, F.C., Abaide, E., Silva, J.R.F., Kuhn, J. Martínez, M.A. Mazutti, Journal of Food Engineering 170 58-63 (2016).

Sovová, H., Rate of the vegetable oil extraction with supercritical CO_2 - I. Modelling of extraction curves. Chemical Engineering Science 49 409-414 (1994).

Sun, Y., Liu, D., Chen, J., Ye, X., Yu, D., Effects of different factors of ultrasound treatment on the extraction yield of the all-trans-b-carotene from

citrus peels. Ultrasonics Sonochemistry 18 243-249 (2011).

Sutkar, V.S. and Gogate, P.R. Design aspects of sonochemical reactors: Techniques for understanding cavitational activity distribution and effect of operating parameters, Chemical Engineering Journal, 155, 1-2, 26-36 (2009).

Tomita, K., Machmudah, S., Wahyudiono, Fukuzato, R., Kanda, H., Quitain, A.T., Sasaki, M., Goto, M., Extraction of rice bran oil by supercritical carbon dioxide and solubility consideration. Separation and Purification Technology 125 319-325 (2014).

Uribe, J.A.R., Perez, J.I.N., Kauil, H.C., Rubio, G.R., Alcocer, C.G., Extraction of oil from chia seeds with supercritical CO_2. Journal of Supercritical Fluids 56 174-178 (2011).

Viganó Juliane, Coutinho Janclei P., Souza Danilo S., Baroni Naiara A.F., Godoy Helena T., Macedo Juliana A., Martínez Julian, Exploring the selectivity of supercritical CO2 to obtain nonpolar fractions of passion fruit bagasse extracts, Journal of Supercritical Fluids, 110 1-10 (2016).

Wang, C.-H., Chen, C.-R., Wu, J.-J., Wang, L.-Y., Chang, C.-M.J., Ho, W.-J., Designing supercritical carbon dioxide extraction of rice bran oil that contain oryzanols using response surface methodology. Journal of Separation Science 31 1399-1407 (2008).

Wang, X., Wu, Y., Chen, G., Yue, W., Liang, Q., Wu, Q. Optimisation of ultrasound assisted extraction of phenolic compounds from Sparganii rhizoma with response surface methodology. Ultrasonics Sonochemistry 20 846-854 (2013).

Weinhold, T.D.S.; Bresciani, L.F.V.; Tridapalli, C.W.; Yunes, R.A.; Hense, H.; Ferreira, S.R.S. Polygala cyparissias oleoresin: comparing CO2 and classical organic solvent extractions Chem. Eng. Process., 47 109-117 (2008).

Xu, Z., Godber, J.S. Comparison of Supercritical Fluid and Solvent Extraction Methods in Extracting γ-Oryzanol from Rice Bran. Journal of the American Oil Chemists' Society 77 547-551 (2000).

Zhang, Z-S., Wang, L-J., Li, D., Jiao, S-S., Chen, X.D., Mao, Z., Ultrasound-assisted extraction of oil from flaxseed. Separation and Purification Technology 62 192-198 (2008).