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SUPERCRITICAL FLUID EXTRACTION (SFE) OF RICE BRAN OIL TO OBTAIN FRACTIONS ENRICHED WITH TOCOPHEROLS AND TOCOTRIENOLS

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Abstract - Parboiled rice bran oil was obtained with supercritical CO_2 at temperatures and pressures varying from 25 to 60ºC and from 150 to 250 bar, respectively. This study was divided into two different parts: initially, the experiments were carried out with one separation step. In the second part, experiments were performed with two separators in series. The temperatures and the pressures of the first separator were 25 and 40ºC, and 100 and 150 bar. The second separator was maintained at 2ºC and 25bar. This procedure results in the precipitation of rice bran oil with different concentrations of tocols in the first and second separators. The extracts obtained were analyzed by HPLC to verify the presence of tocopherols and tocotrienols. Unlike other vegetable oils, rice bran oil contains a larger amount of tocotrienols, specially γ-tocotrienol, than of tocopherols. Finally the extraction curves were modeled by Sovová's method.
*Keywords***:** Tocotrienol; Tocopherols; SFE; Rice bran.

INTRODUCTION

Rice (*Oryza sativa L.)* is a semiaquatic species that grows in tropical and semitropical climates. Rice processing produces polished rice or the parboiled variety, in addition to two residues: husks (13%), used as fuel, and bran (8%), used as animal feed and a food supplement and for edible oil production. Brazil is the ninth largest producer of rice worldwide and the state of Santa Catarina (south of Brazil) is the third largest Brazilian producer of rice and the largest producer of parboiled rice. Recent studies revealing the potential of rice bran oil as a nutraceutical/functional food may increase its consumption. Rice bran oil contains 3 to 5% unsaponifiable lipids, which contain a unique complex of naturally occurring antioxidant components, tocopherols, tocotrienols and oryzanol (Lloyd et al., 2000). In their pure form tocopherol and tocotrienol are viscous yellow liquids that readily decompose when exposed to light, oxygen, an alkaline pH, and traces of transition metals; they are insoluble in water but highly soluble in organic solvents (Bramley et al., 2000). While tocopherols are found in nuts and vegetable oil, tocotrienols are concentrated in cereal grains and vegetable oils such as palm oil and rice bran (Theriaut et al., 1999). In supercritical fluid extraction (SFE), there

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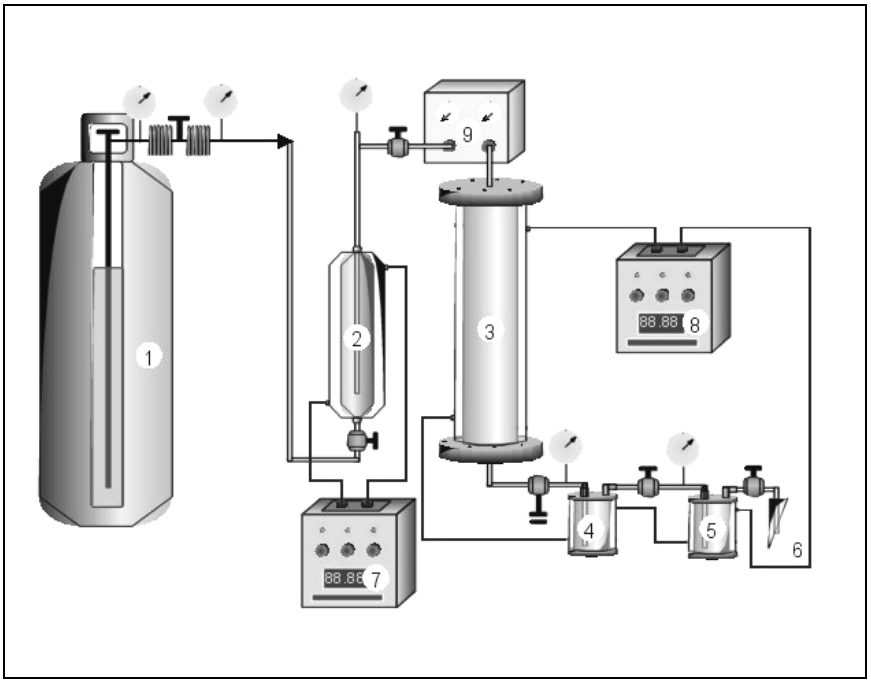
is no risk of solvent contamination and chemical modification, problems which may occur in conventional extraction. Extraction and fractionation with supercritical fluids can be done in two ways: selective extraction and/or selective separation. The first involves the solvation capacity of the solvent by control of the extraction temperature and pressure and/or the addition of a cosolvent. In the second method, selective separation is achieved through gradual depressurization or gradual heating or cooling of the extract, which allows a controlled fractionation of the extract. Shen et al. (1996) assessed the effects of temperature, pressures and extraction time of the rice bran oil on the yield and solute components extracted with subcritical and supercritical CO_2. The results indicate an increase in the solubility of the rice bran oil by increasing the temperature up to 40ºC and then decreasing it. The highest solubility was observed at 310bar and 40ºC. Due to the importance of the antioxidant components in the rice bran oil, the objectives of the present study are to evaluate the ideal temperature and pressure for the SFE of rice bran oil, enriched with tocopherols and/or tocotrienols and also to select the optimum temperature and pressure for oil fractionation.

MATERIAL AND METHODS

Equipment

The SFE extraction unit used in this study is represented schematically in Figure 1. The equipment consists of a CO_2 reservoir and the extractor consists of a 316L stainless steel encased cylinder, 140cm^3 (Suprilab, Campinas, SP), with temperatures from 25 and 40°C controlled by a thermostatic bath (Model MQBTZ 99-20, Microquímica, precision ±0.1ºC, São José, SC). The extraction pressure was maintained by a high-pressure pump (Model 3200 P/F, Thermo Separation Products, Riviera Beach, USA). An analogical manometer (Header, 400bar, precision ±5bar) and a micrometric valve (Model SS31RS4 Swagelok, 5000 psi, 100ºF) were used for pressure monitoring and control, respectively. The encased separators (4 and 5) of 316L stainless steel, 100cm^3 each, were connected in series and the temperature was controlled by a thermostatic bath (Model MQBTZ 99-20, Microquímica, precision ±0.1ºC). Analogical manometers (Header, 400 bar precision ±5bar) and micrometric valves (model 1315GLY, Hoke, 5000psi) were connected to the inlet and the outlet of each separator for pressure monitoring and control, respectively.



1. CO_2 cylinder;
2. surge tank;
3. extractor;
4 and 5. separators;
6. collector and gas-measuring device,
7 and 8. thermostatic baths;
9. isocratic pump.

Figure 1: SFE unit

Experimental Procedure

The raw material used in the experiments was parboiled rice bran, EPAGRI-108. This bran, an industrial waste product from the production of parboiled rice, was donated by "Empresa Campeiro, Produtos Alimentícios Indústria e Comércio Ltda" (Tubarão, SC). The samples of parboiled rice bran were placed in plastic bags and stored at -18ºC in a home freezer (Brastemp, 250L). Forty grams of rice bran were used in all experiments to form the raw material fixed bed with no additional treatment. This bran, which had a particle diameter of 0.20 to 0.22 mm, was packed into the extractor. This study was divided into two parts, stages 1 and 2.

Stage1

Experiments were performed in a single step (without separators) in order to select the optimal temperature (T) and pressure (P) for obtaining a high yield of rice bran oil and the highest content of tocols in the oil. The operational conditions for extractions done in a single separation stage were 150, 200, and 250 bar and 25, 40, 50, and 60ºC with an average solvent flow rate of 0.0756 kg h^{-1}. The experiments were conducted for each operational conditions using two different procedures: the first one was used to the yield of a total extraction process of 8 hours. The second procedure was to obtain the extraction curve, with solute collected at one-hour intervals, during an extraction process of 8 hours.

Stage 2

For the solute/solvent separation, two vessels in series were used to obtain the tocopherol- and tocotrienol-enriched fractions. For this group of experiments, the extraction conditions used were 200 bar/40°C and 200 bar/25°C. The separation conditions were set as 2, 25, and 40°C and 100 and 150 bar for vessel 1. In vessel 2, the operational conditions were maintained constant at 2°C and 25bar.

Characterization of the Oil Composition

The analyses were carried out to characterize the parboiled rice bran and parboiled rice bran oil. For the rice bran, the AOAC (1999) methods for moisture, ashes, lipids, and proteins were used. For the parboiled rice bran oil, HPLC (High Performance Liquid Chromatography) was used. The HPLC analysis was conducted according to AOCS (1989) (Ce 8-89) for determination of tocopherols and tocotrienols in oils and vegetable fats. The equipment consisted of a Fluorescence Detector RF - 10Axl, Shimadzu, Kyoto, Japan, and a Lichrosorb Si 60, 5µm column.

Modeling of Extraction Curves

The model described by Sovová (1994) is based on material balances assuming the solvent flows axially through a cylindrical fixed bed with surface velocity U. The solvent is assumed to be free of solute at the entrance of the extractor vessel, and it is assumed that temperature and pressures are constant during the process. The particle size and the distribution of solute inside the cells were assumed to be homogeneous and protected by a cell wall. The solids milling damaged the cell walls partially exposing the solute to the solvent. The convection in the fluid phase term in the mass balance equations is most representative and the dispersion and diffusion terms in the solid phase are neglected. Sovová divided the extraction process into three parts: a constant extraction rate period (CER), where the solute is easily transferred from solid to fluid phase; followed by a decreasing period (FER), where there is little easily accessible solute on the solid surface; and finally, a period controlled by the diffusion process for extraction of the solute from inside the solid phase (Martinez et al., 2003; Ferreira and Meireles, 2002). The Sovová model is represented by the following equations for the extraction curve:

for the CER period: $t < t_{CER}$

for the FER period: $t_{CER} \le t < t_{FER}$

$$m_{extr} = Y^*[1-\exp(-Z)]Q_{CO_2}t \quad (1)$$

$$m_{extr} = Y^*\left[t - t_{CER} \quad \exp(z_W - Z)\right]Q_{CO_2} \quad (2)$$

for the diffusion-controlled period: $t \ge t_{FER}$

$$m_{extr} = N\left\langle X_O - \frac{Y^*}{W}\ln\left\{\begin{array}{l} 1+\left[\exp\left(\frac{WX_O}{Y^*}\right)-1\right] \\ \exp\left[\left(\frac{W\ Q_{CO_2}}{N}\right)(t_{CER}-t)\right]\frac{X_k}{X_O}\end{array}\right\}\right\rangle \quad (3)$$

The fluid-phase mass transfer coefficient (k_{Ya}) and the solid-phase mass transfer coefficient (k_{Xa}) were calculated from Eq. 4 and 5 (Povh et al., 2001).

$$k_{Ya} = \frac{M_{CER}}{\rho_{CO_2} \; S \; H \; \Delta\overline{Y}} \tag{4}$$

$$k_{Xa} = \frac{k_{Ya} \; \rho_{CO_2} \; \Delta Y}{\rho_S \; \Delta X} \tag{5}$$

where:

$$\Delta\overline{Y} = \frac{Y_{CER}}{\ln[Y^*/(Y^* - Y_{CER})]} \tag{6}$$

$$\Delta Y = \frac{M_{CER}}{\rho_{CO_2} \; S \; H \; K_{Ya}} \tag{7}$$

$$\Delta X = \frac{(X_p + X_K)}{2} \tag{8}$$

RESULTS AND DISCUSSION

The composition of the parboiled rice bran determined by the AOAC methods (1999), is represented by 7.27 % w/w for water content, 8.21 % w/w for ashes, 10.87 % w/w for proteins, 26.28 % w/w for lipids and 47.12 % w/w for carbohydrates. This result shows the importance of rice bran as a lipid source. In the experiments in stage 1, where a single separation step was used, the operational conditions (temperature and pressure) were evaluated in order to obtain the highest oil yield and the highest level of tocols in the oil. Figure 2 shows the yield isotherms for an extraction time of 8 hours as a function of operating pressure.

At 150 bar for all temperatures and at 25, 50, and 60°C for all pressures, the process yield increased with decreasing process temperature due to the reduction in solvent density. Between 150 and 200 bar the isotherms at 25°C and 40°C intersect because at 200 bar and 250 bar the increase in temperature from 25°C to 40°C indicates an increase in process yield. This is justified by the increase in solute vapor pressure with the increase in temperature. These results agree with the results of Shen et al. (1996) that the apparent solubility of rice bran oil increases with pressure at 40°C and decreases at higher temperatures. He concludes that rice bran oil is more highly soluble at 310 bar and 40°C and that lower solubilitie's of carbon dioxide favor the separation of free fatty acids and triglicerides.

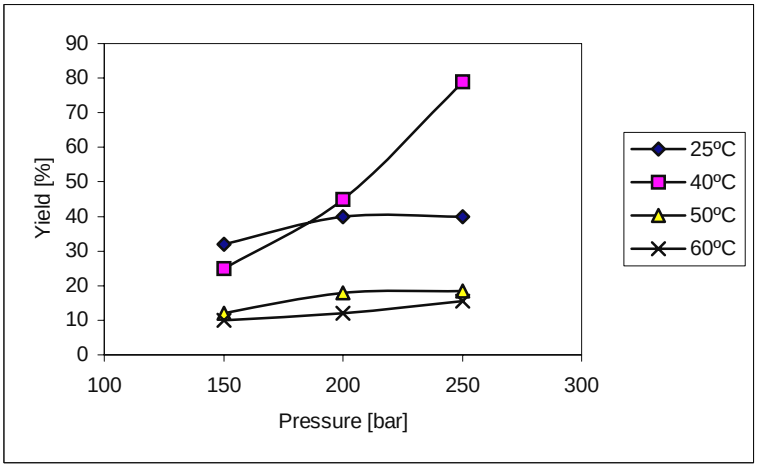


Figure 2: Percentage of oil accumulated as a function of temperature.

Therefore the experiments at 40°C were selected to evaluate the tocol composition by HPLC analysis.

In Table 1 the amount of tocopherols and tocotrienols in the extracts obtained in the experiments with a single separation stage, stage 1, for an eight-hour extraction at a flow rate of 1.5 $mL.min^{-1}$ are presented. Muñoz (1999) extracted tocopherols from wheat germ at a pressure of 200 bar and a temperature of 40°C in three hours and concluded that the yield increases up to CO_2 flows of 0.0756 $kg.h^{-1}$.

As shown in Table 1, the experiments were carried out at 25ºC and 40ºC and at 150, 200, and 250bar. It is possible to observe from the results that the rate of tocol extraction increased with temperature for the range evaluated. It can also be seen that larger quantities of total tocotrienol isomers than of total tocopherol isomers were detected under

all conditions. At a constant pressure, larger quantities of tocotrienols were found under the supercritical condition.

Rice bran oil was also obtained by conventional extraction with hexane to compare it with SFE. The hexane extract had a total yield of 52.19mg of tocols/100g of oil detected by HPLC. Furthermore, larger quantities of tocotrienol isomers were found. In the HPLC analysis of the hexane extracts, the results were not as good as those for the extracts obtained by extraction with supercritical CO2. This may be the result of a higher temperature in the conventional extraction, a longer time of exposure of the oil to air, and solvent/bran contact. The effect of extraction time on oil composition is observed in Table 2, which shows the mass of tocols in the extracts obtained at a one-hour intervals at 200bar and 40ºC.

Table 1: Mass of tocols in Stage1, by HPLC.

Tocols	Mass (mg/100g)					
	Extractions conditions P (bar) / T (°C)					
	150/25	150/40	200/25	200/40	250/25	250/40
α tocoferol	3.32	5.48	5.21	179.18	5.54	2.67
β tocoferol	0.00	0.00	0.00	1.21	0.00	0.00
γ tocoferol	1.32	2.49	1.71	54.67	2.65	0.00
δ tocoferol	0.00	0.00	0.00	0.00	0.00	0.00
Total tocoferol	4.64	7.97	6.92	234.96	8.19	2.67
α tocotrienol	1.94	0.99	2.14	96.16	2.57	6.81
β tocotrienol	0.00	0.00	0.00	0.71	0.00	0.00
γ tocotrienol	26.50	78.40	95.51	587.92	45.82	50.73
δ tocotrienol	0.00	4.23	7.72	19.37	1.87	2.60
Total tocotrienol	31.21	83.62	105.37	704.16	50.26	60.14
Total tocols	35.85	91.59	112.30	939.12	58.45	62.81

From the results presented in Tables 1 and 2 we observe that larger quantities of tocotrienols than of tocols were always found under any extraction condition, and at 200bar and 40ºC larger quantities of all of the tocopherol and tocotrienol isomers were extracted. Therefore, this experiment was selected to evaluate the composition profile during the time of extraction. The results indicate that there is a higher concentration of the □-tocotrienol isomer and it is extracted in a process of up to seven hours. The experiments in Stage 2 were carried out under the conditions shown in Table 3. The extracts collected in separators 1 and 2 from each experiment were analyzed by HPLC for quantification of tocols and the results are also presented in Table 3.

The adjustment of the extraction conditions (T and P) as well as of the separation conditions (T, P) resulted in control of the solubility of the components of rice bran oil. This behavior defines the oil characteristics and the properties and quantities of the fractions deposited in the separators. Thus, the fractions of oil obtained in the two separators had different characteristics. As can be seen from the results shown in Table 3**,** larger quantities of isomers of tocotrienol than of tocopherol isomers were found in all of the experiments carried out. In experiments E1, E2, and E3, a greater tendency for tocols to be deposited in separator 1 was observed while in experiments E2 and E3 the mass of tocols was zero in the oil fraction collected in separator 2. Experiment E3 was the most remarkable, since it showed the highest capacity to deposit or separate a single fraction of tocopherols/tocotrienols in separator 1. Significant quantities were collected in experiment E6, although these were similar in the two separators. Therefore, this experiment was unremarkable as it failed to separate a specific fraction of tocopherol/tocotrienol in one of the separators. The only difference between experiment E3 and E6 was the extraction temperature (T=40°C for E1, E2, E3 and T=25°C for E4, E5, E6), so it can be concluded that the greater difference in temperature from extractor to separator affected the precipitation of solute. In this case total precipitation occurs in the first separator where the temperature change was more abrupt.

Sovová's model applied to the description of the extraction curve (Figure 3) showed a good agreement with experimental data at 40°C and 300 bar (Table 4), even using data for the antioxidant solubility of 0.0159 g_{oil}/g_{CO2} obtained experimentally by using the Proclin procedure from SAS for Windows-Version 8.2 (SAS Institute Inc., Cary, NC, EUA) at a temperature of 40°C and a pressure of 200 bar (CO_2 density of 840.62 g/cm^3).

Table 2: Tocol compositions of the extracts obtained at one-hour intervals, analyzed by HPLC (200bar /40°C).

	Mass (g/100g)							
Tocols/Time	**1h**	**2h**	**3h**	**4h**	**5h**	**6h**	**7h**	**8h**
α tocopherol	22.17	27.18	25.95	26.16	27.22	25.00	5.44	19.94
β tocopherol	0.00	0.00	0.56	0.00	0.00	0.64	0.00	0.00
γ tocopherol	6.80	7.86	7.42	7.34	7.86	7.68	1.24	8.43
δ tocopherol	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
α tocotrienol	12.93	0.00	15.34	15.68	16.30	15.93	3.08	16.86
β tocotrienol	0.00	0.71	0.00	0.00	0.00	0.00	0.00	0.00
γ tocotrienol	106.13	88.03	89.03	92.72	92.79	95.87	23.31	0.00
δ tocotrienol	3.01	3.12	3.04	3.13	3.42	3.31	0.32	0.00
Total	151.04	126.90	141.34	145.04	147.59	148.43	33.39	45.23

Table 3: Fractions collected in both of the separators in each experiment - Stage 2.

	Separator 1 (mg/100g)				Separator 2 (mg/100g)			
Experiment	**P(bar)**	**T(°C)**	**Tocopherol**	**Tocotrienol**	**P(bar)**	**T(°C)**	**Tocopherol**	**Tocotrienol**
E_1	100	25	5.72	32.32	25	2	0.85	11.63
E_2	100	40	0.00	4.88	25	2	0.00	0.00
E_3	150	25	7.40	24.10	25	2	0.00	0.00
E_4	100	25	0.00	8.66	25	2	10.23	10.73
E_5	100	40	0.00	6.85	25	2	1.08	1.08
E_6	150	25	2.73	23.52	25	2	24.32	29.97

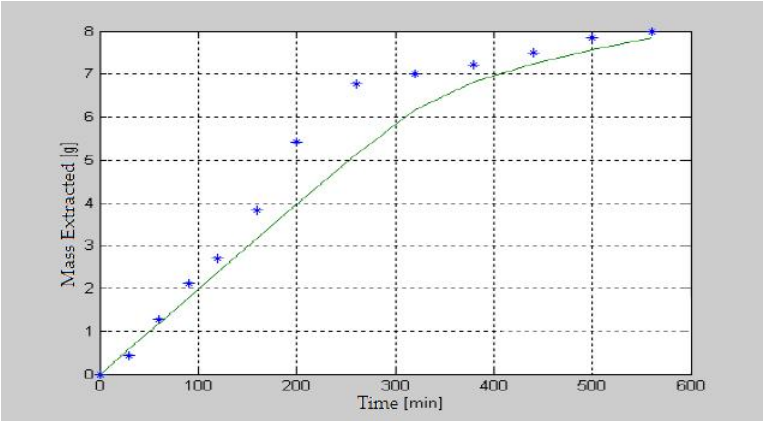


Figure 3: Experimental points and modeled extraction curve at temperatures of 40°C and pressure of 300 bar (CO_2 flow of 5g/min.)

Table 4: Use modeling conditions.

Mean particle diameter (mm)	**Real density (g/cm^3)**	**Apparent density (g/cm^3)**	**Bed porosity (e)**	**Q_{CO2} (g_{CO2}/min)**	**Y^* (g_{oil}/g_{CO2})**	**ρ_{CO2} (Kg/m^3)**
0.21	1.31	0.28	0.78	5	0.0159	910.61

CONCLUSIONS

Parboiled rice bran contains a significant percentage of lipids, approximately 26.28%, which are easily extracted, with high solubility at 200bar and 40°C. For the first stage it can be concluded that

1-The effect of pressure on the solubility of parboiled rice bran oil was more marked than that of temperature.

2-At a constant pressure, the percentage of extracted oil increased with the increase in temperature up to 40°C, decreasing thereafter. At a constant temperature the percentage of oil extracted increased with pressure.

3-At 200bar and 40°C, 939.12mg of tocols per 100g of oil were extracted; these were concluded to be the best conditions for extraction of parboiled rice oil enriched with tocopherol/tocotrienol in this study. Also in this stage it was concluded that tocols are more soluble in supercritical CO_2 than in liquid CO2. Under all operational conditions, larger quantities of tocotrienols, especially the γ-tocotrienol isomer, were always found.

For the second stage it can be concluded that:

1-In experiment E3 the separation of a tocol-enriched fraction was demonstrated in separator 1, with the γ-tocotrienol isomer found in larger quantities. It is possible that this component may be more soluble under the chosen operational conditions.

2-Knowledge of the solubility in the fluid and vapor phases is fundamental for the separation of tocol-enriched fractions.

The extraction curves can be modeled by using easy models, such as Sovová's method, even using mean solubility as the experimental parameter.

Rice bran oil is different from the majority of vegetable oils, since it has a larger quantity of tocotrienols than tocopherols, thereby showing similarities with palm oil. It also contains a low percentage of linoleic acid that provides it with greater stability.

The results demonstrated the viability of the process, enabling the use of parboiled rice as a

Low-cost, abundant raw material, which is regarded as an industrial waste, in order to obtain a product of high aggregate value, using supercritical extraction, a clean technology.

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