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

SARMENTO, C.M.P.[1], HENSE, H.[2,*], FERREIRA, S.R.S.[2]

[1]CEFET/PR, Av. Brasil 4232 CP 319, CEP 85840-000, Medianeira, Paraná, Brasil.
LATESC/EQA/CTC – UFSC, CP 476 - 88040-900, Florianópolis, SC – BRAZIL
[*] Corresponding author: h_hense@enq.ufsc.br ; FAX: 55 48 331.9687

Parboiled rice bran oil was obtained using supercritical CO_2 under different conditions of temperature (2°C to 40°C) and pressure (25 to 200 bar). In this study the experiments were carried out with two separators coupled to the extractor. The temperature levels in the first separator were 25°C and 40°C and in the second it was maintained constant at 2°C. The pressures in the first separator were 100 and 150 bar and for the second it was maintained constant at 25 bar. These procedure leads to the precipitation of rice bran oil with different tocol concentrations in the first and in the second separators. The extracts obtained were analysed by HPLC to verify the presence of tocopherols and tocotrienols. Contrary to the majority of vegetable oils, rice bran oil contains higher quantities of tocotrienols, in particular γ-tocotrienol, in relation to tocopherols. Through supercritical fluid extraction (SFE) it was possible to separate fractions enriched in tocopherol/tocotrienol. Rice bran oil was also obtained by conventional extraction with hexane.

Key words: Tocotrienol, SFE, rice bran, tocopherols, natural antioxidants.

INTRODUCTION

Oryza sativa L. (rice) grows in tropical and semitropical climates. Rice cultivation makes available for consumption the polished rice or the parboiled variety. In addition the bran (8%), used as animal feed, as food supplement and for edible oil production. Brazil is the ninth world-wide rice producer and the state of Santa Catarina (south of Brazil) is the highest producer of parboiled rice [1, 2].

Rice bran oil contains 3 to 5% unsaponifiable lipids, which contains a complex of naturally occurring antioxidant components, tocopherol, tocotrienol and oryzanol [3]. Whilst tocopherols are present in nuts and vegetable oil tocotrienols are concentrated in cereal grains and vegetable oils such as palm and rice bran [4]. Most vegetable oils are produced by distillation and solvent extraction. In the case of solvent extraction a problem is separating the solvent from the extracted oil, although the method has the advantage of producing large quantities of oil. When supercritical fluid extraction (SFE) is used there is no risk of solvent contamination and thermolability and chemical modifications, problems that may occur in conventional extraction [5]. The extraction and fractionation with supercritical fluids can be performed in two ways: selective extraction and/or selective separation. The first involves the solvating capacity of the solvent by the control of the extracting temperature and pressure and/or the addition of a co-solvent. In the second method, selective separation is achieved through gradual

depressurisation or gradual heating or cooling of the extract, which allow a controlled fractionation of the extract. Shen et al (1996) assessed the effects of temperature, pressure and extraction time of the rice bran oil in the yield and solute components, extracted with subcritical and supercritical CO_2 [6]. The results indicate an increase in the solubility of the rice bran oil by increasing the temperature up to 40ºC, before subsequently decreasing. The highest solubility was observed at 310 bar and 40ºC.

Due to the importance of the antioxidant components in the rice bran oil, the objective of the present study is to select the optimum conditions of temperature and pressure for the oil fractionation, after the extraction with supercritical CO_2.

I - MATERIALS AND METHODS

The SFE extraction unit used in this study is available at LATESC (Laboratório de Termodinâmica e Extração Supercrítica) – UFSC, SC, Brazil.

The equipment allow two different configurations:

a-Extractor followed by collector resulting in a single separation stage;
b-Extractor coupled with two separators in series and a collector adapted downstream the second separator.

The extractor consisted of a 316L stainless steel encased cylinder, with 140cm^3 (Suprilab, SP, Brazil), with the temperature controlled by a thermostatic bath (Model MQBTZ 99-20, Microquímica, precision ±0.1ºC). The extraction pressure was maintained by a high pressure pump (Model 3200 P/F, Thermo Separation Products). An analogue manometer (Header, 400 bar, precision ±5bar) and a micrometric valve (Model SS31RS4 Swagelok, 5000 psi, 100ºF) were used for pressure monitoring and control respectively. The encased separators, in 316L stainless steel (Gringo), with 100cm^3 each, were connected in series and the temperatures controlled by a thermostatic bath (Model MQBTZ 99-20, Microquímica, precision ±0.1ºC). Analogue manometers (Header, 400 bar precision ±5bar) and a micrometric valve (model 1315GLY, Hoke, 5000psi) were connected to the inlet and the outlet from each separator, for pressure monitoring and control, respectively.

Experimental Procedure

The raw material used in the experiments was parboiled rice bran, variety EPAGRI 108. This bran, an industrial waste product from the production of parboiled rice, was donated by *Empresa Campeiro-Produtos Alimentícios Ind. e Com. Ltda* (SC, Brazil). The samples of parboiled rice bran were placed in plastic bags and stored at -18ºC in a domestic freezer (Brastemp, 250L). 40g of rice bran were used in all experiments with no additional treatment.

The fractionating experiments used two separators in series, in order to obtain tocopherol and tocotrienol enriched fractions [7]. Two extracting operational conditions were selected for the oil obtantion: 200bar/25°C and 200bar/40°C, performed over a period of eight hours, with an average CO_2 flow rate of about 1.5mL.min^{-1} as proposed by MUNÕZ [8]. The fractionation experiments were carried out according to the conditions presented in Table 1. The set experimental design indicate that the conditions for the second separator were maintained constant at 25 bar and 2°C.

Table 1: Conditions of temperature and pressure in the performed experiments.

	Extraction		Separator 1		Separator 2	
Exp.	P(bar)	T(°C)	P(bar)	T(°C)	P(bar)	T(°C)
E1	200	40	100	25	25	02
E2	200	40	100	40	25	02
E3	200	25	100	25	25	02
E4	200	25	100	40	25	02
E5	200	25	150	25	25	02
E6	200	25	150	40	25	02

Oil Composition characterization

The analysis of the parboiled rice bran oil were performed in *Laboratório de Óleos e Gorduras* (UNICAMP-SP, Brazil). The oil samples, obtained at different extraction conditions and from the two separators, and also obtained by hexane extraction were analyzed by high performance liquid chromatography (HPLC), according to the methodology described by AOAC (Ce 8-89) for the 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.

II – RESULTS AND DISCUSSION

It was observed a high lipid content in the bran raw material, obtained with SFE and with conventional extraction, and the yield was up to 71.64mg/100g. For the hexane extraction the oil analyzes indicate 52.19mg tocols/100g oil, detected by HPLC. For the hexane samples it was found higher amount of tocotrienol isomers compared to the tocopherols isomers. Moreover, the analyzes of the SFE samples indicate higher amount of tocols, compared to the conventional extracts. This behavior can be explained by different factors as the higher extraction temperature of the conventional extraction, ore the higher oil/air contact during the Sohxlet extraction and consequently degradation of tocols.

The results for the HPLC analysis are shown in Table 2, for the SFE fractions collected in the two separators for each experimental condition, as shown in Table 1.

The extraction conditions of temperature and pressure, as well as the separating conditions, resulted in different solubility of the components of the rice bran oil, and it was determinant to determine the characteristics, properties and quantities of the fractions deposited in the separators. Thus, fractions of oil with distinct characteristics were obtained in the two separators. As can be seen from the results shown in Table 2, isomers of tocotrienol were present in higher quantities in relation to isomers of tocopherol in all of the experiments performed.

For experiments E1, E2 and E3 it was detected a tendency for higher tocols concentration, deposited in separator 1, while for experiments E2 and E3 the mass of tocols were zero in the oil fraction collected in separator2.

Table 2: Composition of tocols in the oil fractions collected in separators 1 and 2.

	Separator 1 (mg/100g)			Separator 2 (mg/100g)		
Exp.	Tocopherol	Tocotrienol	Total	Tocopherol	Tocotrienol	Total
E1	5.72	32.32	38.04	0.85	11.63	12.48
E2	0.00	4.88	4.88	0.00	0.00	0.00
E3	7.40	24.10	31.50	0.00	0.00	0.00
E4	0.00	8.66	8.66	0.50	10.23	10.73
E5	0.00	6.85	6.85	0.00	1.08	1.08
E6	2.73	23.52	26.26	5.64	24.32	29.97

Experiment E3 was the most remarkable one because it shows the highest fractionation capacity, i.e., indicates the better separation for a single fraction of tocopherols/tocotrienols in separator 1, according to the temperature and pressure conditions.

Experiments E4, E5 and E6, where the extractions were performed in subcritical conditions (temperature below critical temperature), variable quantities of tocols were deposited in the two separators.

Significant amount of tocol was collected in experiment E6, although the results for separators 1 and 2 were very similar. Therefore, this behaviour indicate that for experiment E6 no fractionation of tocopherol/tocotrienol was observed at the conditions used in the present experiment.

The extracts collected in the two separators in experiments E3 and E6 were chosen for the assessment of their tocopherol and tocotrienol isomer composition, and the results for this analysis are presented in Table 3.

Table 3. Tocopherol and Tocotrienol isomer composition for experiments E3 and E6 (mg/100g).

	Experiment 3 – E3		Experiment 6 – E6	
Component	Separator 1	Separator 2	Separator 1	Separator 2
α tocopherol	6.00	0.00	1.96	4.55
β tocopherol	0.00	0.00	0.00	0.00
γ tocopherol	0.00	0.00	0.77	1.09
δ tocopherol	0.00	0.00	0.00	0.00
α tocopherol	2.70	0.00	1.79	2.31
β tocopherol	0.00	0.00	0.00	0.00
γ tocopherol	20.20	0.00	20.44	20.15
δ tocopherol	1.20	0.00	1.28	1.86
Total tocols	31.50	0.00	26.26	29.97

The results presented in Table 3 shown that tocols precipitate preferentially when the solvent changes its state from supercritical to subcritical conditions, in the two separators. There are no selective separation when only temperature and/or pressure conditions are changed. It seams that there is a relation between the solvent state and the process selectivity or fractionated precipitation of tocols.

Figures 1 and 2 show the chromatograms obtained by HPLC, for the extracts collected in experiment E3 for the first and second separator, respectively, with the operational conditions according to **Table 1**. The peaks identified as "t" represents the tocopherols and the peaks identified with "T" represents tocotrienols. It is evident from these figures that the tocol fractions (tocopherols and tocotrienols) precipiteted completely in separator 1, showing an adequate operational condition for rice bran oil fractionation.

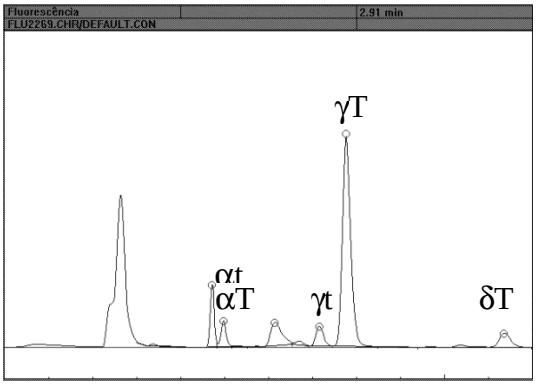

Figure 1: Chromatogram E3 separator 1

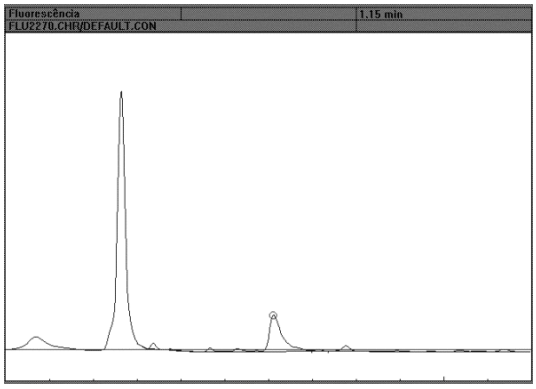

Figure 2: Chromatogram E3 separator 2

III - CONCLUSION

Parboiled rice bran presents a significant quantity of lipids, approximately 26.28%, which are easily extracted, with high solubility at 200 bar and 40°C.

1-In experiment E3 the separation of a tocol enriched fraction was demonstrated in separator 1, with the γ-tocotrienol isomer present in higher quantities. Possibly this component present higher solubility in the operational condition used in experiment 3.

2-Knowledge of the solubility of the solute in solvent phase is fundamental for the separation of tocol enriched fractions.

Rice bran oil is different from the majority of vegetable oils, since it presents a greater quantity of tocotrienols than tocopherols exhibiting, therefore, similarities with palm oil. It also contains a low percentage of linoleic acid that endows 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, that is regarded as industrial waste, in order to obtain a product of high aggregate value, using supercritical extraction, a clean technology.

IV - REFERENCES

[1] ICEPA – Instituto de Planejamento e Economia Agrícola de Santa Catarina 1998-1999, Secretaria Estadual de Desenvolvimento Rural e da Agricultura, Florianópolis, p. 26-29, 2000.

[2] SINDARROZ. O arroz parboilizado de Santa Catarina. Internet. http://www .sindarroz-sc.com.br em 08 de setembro 2001.
[3] LLOYD, B. J.; SIEBENMORGEN, T. J.; BEERS, K. W. Effects of commercial processing on antioxidant in rice bran. Cereal Chemists**,** V. 77-5 p. 551-555, 2000.
[4] THERIAUT, A.; CHAO, J. Z.; WANG, Q.; GAPOR, A. ; ADELI, K. Tocotrienol; A review of its therapeutic potential. Clinical Biochemistry, V. 32, p. 309-319, 1999.
[5] KIM, H. J. S.; LEE, B.; PARK, K. A.; HONG, I. K. Characterization of extraction and separation of rice bran oil rich in EFA process. Separation an Purification Technology V. 15, p. 1-8, 1999.
[6] SHEN, Z.; PALMER, M. V.; TING, S. S. T.; FAIRCLOUGH, R. J. Pilot scale extraction and fractionation of rice bran oil with dense carbon dioxide. Journal of the American oil Chemical Society, V. 44, p. 3033-3039, 1996.
[7] SARMENTO, C. "Extração Supercrítica de Óleo de Farelo de Arroz e Obtenção de Frações Enriquecidas em Tocoferóis e Tocotrienóis", master thesis at UFSC, 2002.
[8] MUÑOZ, M. A. B.; GÓMEZ, A. M.; OSSA, E. M. Optimizacion del proceso de extraccion de tocoferol de germe de trigo com dióxido de carbono líquido y supercrítico. Grasas y Aceites, V. 50-4, p. 275-279, 1999.