



Extraction of *Mucuna deeringiana* seed oil using supercritical carbon dioxide

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ABSTRACT. The work aimed to investigate the extraction of *Mucuna deeringiana* seed oil using supercritical carbon dioxide as solvent, and the chemical profile of fatty acid components. The experiments were performed in a laboratory scale unit in a temperature range from 40°C to 60°C and pressure from 176.7 to 250 bar. The results indicated that particle diameter, temperature and pressure were important variables for CO₂ extraction yields. The extracted oils were analyzed qualitatively and quantitatively in terms of their fatty acid compounds, and the results showed the presence of essential fatty acids. The main fatty acids were linoleic acid (about 40%), palmitic acid (about 20%) and oleic acid (about 16%); approximately 5% of linolenic acid is reported. No significant differences were found in the fatty acid analysis for the range of variables investigated.

Keywords: *Mucuna deeringiana*, supercritical fluid extraction, carbon dioxide.

Extração do óleo das sementes de *Mucuna deeringiana* usando dióxido de carbono supercrítico

RESUMO. O trabalho tem como objetivo a investigação a extração de óleo de sementes de *Mucuna deeringiana* usando dióxido de carbono supercrítico como solvente e perfil químico dos ácidos graxos constituintes. Os experimentos foram realizados em uma unidade de escala de laboratório na faixa de temperatura de 40 a 60°C e pressão de 176,4 a 250 bar. Os resultados indicaram que o diâmetro das partículas, temperatura e pressão foram variáveis importantes para o rendimento da extração com CO₂. Os óleos extraídos foram analisados quantitativa e qualitativamente em relação à composição em ácidos graxos e os resultados mostraram a presença de ácidos graxos essenciais. Os principais ácidos graxos foram ácido linoleico (cerca de 40%), ácido palmítico (cerca de 20%) e ácido oleico (cerca de 16%), percentuais de cerca de 5% de ácido linolênico são relatados. Diferenças não-significativas foram encontradas na análise de ácidos graxos para a faixa das variáveis investigada.

Palavras-chave: *Mucuna deeringiana*, extração com fluido supercrítico, dióxido de carbono.

Introduction

Mucuna deeringiana is a legume found in tropical regions, native to Asia (MISRA; WAGNER, 2004; HUISDEN et al., 2010). This plant contains high levels of proteins, lipids, minerals and other nutrients, compared to soybean (CHIKAGWA-MALUNGA et al., 2009a and b; SIDDHURAJU; BECKER, 2005). The greatest impediment to the promotion of *Mucuna* as food or feed is the presence of antinutrients including: phenolics, tannins, lectins, phytic acid and trypsin inhibitors (ADEBOWALE et al., 2005; BHAT et al., 2007; CHIKAGWA-MALUNGA et al., 2009a; GUERRANTI et al., 2004; SIDDHURAJU; BECKER, 2005; VIJAYAKUMARI et al., 2007).

The oil from *Mucuna deeringiana* seeds is rich in essential fatty acids, which comprise the omega-3 and omega-6 family (AJAYI et al., 2006;

EZEAGU et al., 2005). In humans, linoleic acid (C18:2) and alpha-linolenic acid (C18:3) are required to maintain cell membranes, brain function and nerve impulse transmission under normal conditions. These fatty acids also participate in the transfer of atmospheric oxygen to blood plasma, hemoglobin synthesis and cell division, and are called essential because they are not synthesized by the body (MARTIN et al., 2006). The incorporation of essential fatty acids into food products and in the pharmaceutical industry has been reported (ÁLVAREZ et al., 2011; KASSIS et al., 2010; KOUSSOROPLIS et al., 2011; QUISPE-CONDORI et al., 2011).

Conventional methods for extracting vegetable oils using organic solvents have the drawback of thermal degradation and the need for further steps to remove the solvent (GARCÍA-RISCO et al.,

2011). Supercritical fluid extraction (SFE) is described in the literature as an alternative to classic extraction methods (ARIAS et al., 2009; BERNARDO-GIL et al., 2009; LANG; WAI, 2001). SFE from obtaining vegetable oils rich in essential fatty acids has been recently reported in the literature (CORSO et al., 2010; FREITAS et al., 2008; MHEMDI et al., 2011; NIMET et al., 2011; PEDERSSETTI et al., 2010; SOUZA et al., 2008).

Carbon dioxide is the most commonly used solvent in SFE, mainly due to its physical and chemical properties, such as low critical pressure (73.82 bar) and temperature (31°C) and chemical inertness (DÍAS-REINOSO et al., 2006; REVERCHON; DE MARCO, 2006; SEÑORÁNS; IBAÑEZ, 2002). Carbon dioxide has advantages compared to liquid solvents due to its adjustable solvent power and properties, ranging from gas to liquid. It should also be considered that extracts can be obtained without traces of solvent at the end of the process. In this sense, at selected temperature and pressure conditions, pressurized CO₂ is a natural solvent for the oil extraction process (BRUNNER, 2005; CORSO et al., 2010; HUISDEN et al., 2010).

In this context, this study aims to investigate the extraction of *Mucuna deeringiana* oil using supercritical carbon dioxide as solvent under different conditions of extraction and quantification of the oils obtained in terms of fatty acids. The extraction yields and kinetics are reported here. The characteristics of the extracted oil are determined by quantitative gas chromatography analysis.

Material and methods

Materials

Mucuna deeringiana seed samples were obtained from Pró Sementes (São Paulo, Brazil). The seeds were milled using an electric mill (IKA, model A11 B, Brazil) and classified using a Tyler sieve (Bertel, ASTM) to produce particles with average diameters of 1.18 mm and 0.6 mm.

All solvents and reagents used in this study were of analytical grade. The standard methyl heptadecanoate (> 99% purity) and catalyst boron trifluoride methanol were obtained from Sigma-Aldrich Chemical Co. Carbon dioxide (CO₂) was technical grade and obtained from Air Liquide (Brazil) with 99.5% purity.

Extraction apparatus and procedure

The experiments were performed in the 'home-made' equipment described in Figure 1. The

experimental unit consists basically of a solvent reservoir, two thermostatic baths, two syringe pumps (Teledyne ISCO 500), a 166.5 cm³ jacketed extraction vessel, an absolute pressure transducer (Smar, LD301) equipped with a portable programmer (Smar, HT 201) with a precision of 0.12 bar and a collector vessel with a glass tube. About 60 g of finely comminuted dried seeds were charged into the extraction vessel. The solvent was pumped at a constant flow rate of 3 mL min.⁻¹ into the bed, which was supported by two 200-mesh wire disks at both ends, and was kept in contact with the herbaceous matrix for at least 30 min to allow system stabilization. Afterward, the extract was collected by opening the metering valve and needle valve, and the solvent mass flow was accounted for by pump recordings. After that, the mass of the extracted oil was weighed, and the glass tube was reconnected to the equipment. This procedure was performed until no significant mass was extracted or, as in some cases, the extraction period exceeded a pre-established limit. The collection of oil took place every ten minutes, and every 20 minutes after two hours of extraction.

The experiments were accomplished isothermally at constant pressure. The investigated experimental range was 40 to 60°C and 60 to 254 bar (Table 1). Solvent density was calculated according to Angus et al. (1976). The yield of extraction was calculated as the ratio between the mass of extracted oil and raw material used.

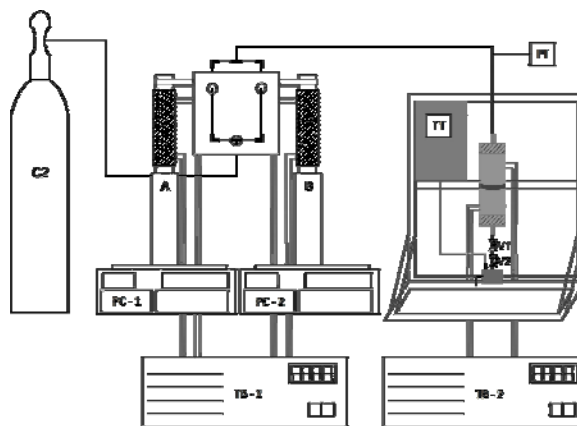


Figure 1. Experimental apparatus: C2: solvent reservoir; V1: metering valve; V2: needle valve; A and B: Syringe pump; PC-1 and PC-2: Pump control; PI: Pressure transmitter; TI: Temperature transmitter; TB-1 and TB-2: Thermostatic baths.

Fatty acid analysis

The quantification of fatty acid in *Mucuna* oil was performed using an Agilent GC 7890 gas chromatograph coupled with a mass detector MS 8990, fitted with a capillary column (ZBWAX, 30m x 0.25mm x 0.25 µm). Column temperature

was programmed starting at 120°C, heating to 180 °C at 15°C min.⁻¹ and to 240°C at 5°C min.⁻¹, holding 5 min. The carrier gas was set at 40 psi with a flow rate of 1.5 mL min.⁻¹. The analyses were performed with the injector and detector both 250 °C, and the injection volume was 1.0 µL in the 1:50 split mode. In order to perform the determination of total fatty acids content by gas chromatography, a derivatization of the oil with BF₃/methanol was conducted following the AOAC standard method Ce 2-66 (WALKER, 1990).

The identification of compounds present in *Mucuna deeringiana* seed oil was conducted by comparing the spectrum data to those presented in the Wiley library. For the quantification of fatty acids, methyl heptadecanoate were used as internal standard. The analysis of variance of data was performed using software SAS 9.1.3 (SAS Institute).

Results and discussion

Extraction yields

Table 1 presents the experimental conditions and extraction yields obtained from the extraction of *Mucuna deeringiana* seed oil using pressurized carbon dioxide as solvent. Extraction yield was defined in this work as 100 times the mass of oil extracted by the mass of *Mucuna deeringiana* seed raw material after a certain period of extraction. The calculation of the extraction yield was performed after a fixed extraction period (330 min.) in order to permit a direct comparison between the results obtained under different experimental conditions. The total time of extraction varied according to the experimental conditions, aiming at an exhaustive extraction with the compressed solvent.

From Table 1, comparing runs 1 and 4, it could be noted that diameter particle influences the extraction yield. Particles with smaller diameters allow greater contact of the solvent, facilitating the extraction of oil; thus, at 40°C and 250 bar, 3.05 and 4.57% of extraction yield were obtained for particles with diameter of 1.2 and 0.6, respectively.

Figure 2 presents the kinetics of *Mucuna deeringiana* seed oil extractions using carbon dioxide as solvent. From this figure, it is clearly noted that pressure and temperature exert a pronounced effect on the extraction. Extraction pressure and extraction temperature are two main factors affecting supercritical fluid extraction (SFE). Increasing pressure at constant temperature will increase the density and dissolving capacity of supercritical CO₂.

Increasing pressure in extractions with carbon dioxide as solvent had a positive effect on the

extraction yield. This effect can be observed through a comparison of runs 3 and 4 at 40°C, or runs 5 and 6 at 60°C. An increase in pressure facilitates penetration in the pores of the matrix, facilitating the contact of the solvent with the compounds that will be extracted. Figure 2 represents the kinetic curves for the extraction of *Mucuna* seed oil with supercritical CO₂ as solvent; one can observe that the increase in pressure favors the initial rates of extraction.

This effect was also presented by Yamini et al. (2002), who reported that with increasing pressure the rate of extraction is increased at all stages of the process due to the increased density (and solvation power) in supercritical CO₂. Thus, for high pressure have the highest yields in oil. The positive effect of pressure in supercritical extraction with CO₂ of grape seed oil (FREITAS et al., 2008) and canola seed oil (PEDERSSETTI et al., 2010) is reported. However, increasing temperature had a negative effect on the extraction yield at constant density (runs 4 and 5).

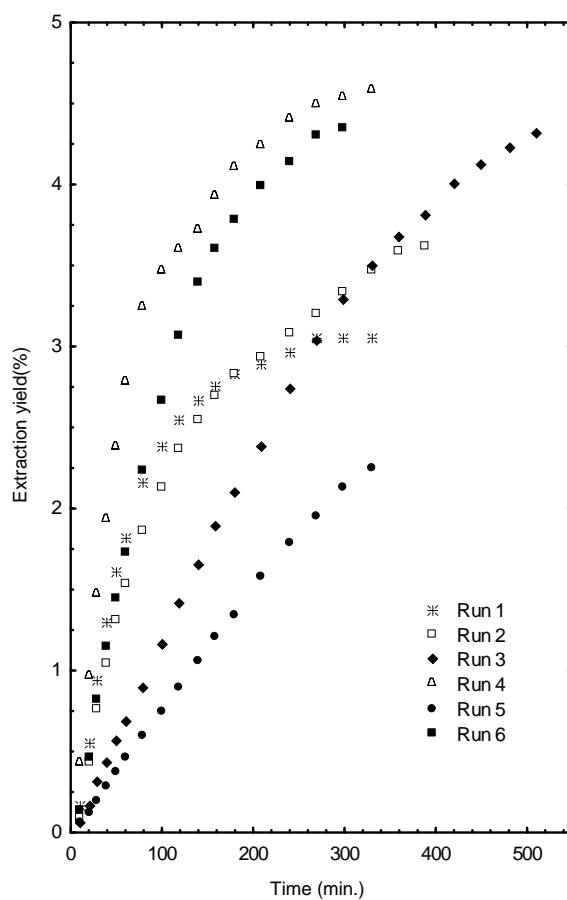


Figure 2. Kinetics of *Mucuna deeringiana* seed oil extraction using pressurized carbon dioxide. Experimental conditions are labeled according to Table 1.

Table 1. Experimental conditions and yield obtained from the extraction of *Mucuna deeringiana* seed using supercritical carbon dioxide.

Experimental condition	Diameter of particle (mm)	Temperature (°C)	Pressure (bar)	Solvent density (Kg cm ⁻³)	Time of extraction (min.)	Extraction yield (%)*
1	1.18	40	250	0.86058	330	3.05
2	1.18	60	244.1	0.68125	330	3.46
3	0.6	40	150	0.78112	330	3.50
4	0.6	40	250	0.86058	330	4.57
5	0.6	60	176.7	0.86056	330	2.24
6	0.6	60	244.1	0.68125	330	4.34

*Results in g 100 g⁻¹ of oil.

Composition of *Mucuna deeringiana* oil

Figure 3 presents the monitored ion chromatogram (MIC) of the typical samples extracted with CO₂ for run 4 (40°C and 250 bar). Table 2 shows the results for the quantification of fatty acids in the *Mucuna* oil extracted using supercritical carbon dioxide as analyzed by gas chromatography. Ezeagu et al. (2005) and Ajayi et al. (2006) determined the fatty acid compositions (%) of oil extracted by petroleum ether (boiling point range 40–60°C) from *Mucuna* seeds, and the results were consistent with those obtained in this study. An analysis of the chemical distributions of the fatty acids of the oil extracted at the different temperatures and pressures investigated in this study indicated no significant differences between the results considering a level of significance of 5% ($p > 0.05$) in the ANOVA test. Pederssetti et al. (2010) studied canola seed oil extraction, Corso et al. (2010) the extraction of sesame seed oil, and Nimet et al. (2011) extraction of the sunflower seed oil (all using carbon dioxide), and also found that this solvent did not significantly influence the fatty acid distribution in the oil extracted.

The main component in oils for different experimental conditions was linoleic acid (omega-6) and about 5% of linolenic acid (omega-3) is reported in *Mucuna deeringiana* oil obtained with supercritical carbon dioxide. These fatty acids are considered essential, used in food, are present in both plant and animal species (COOK et al., 2000; GLEW

et al., 2010; HARGRAVE et al., 2005; KRIS-ETHERTON et al., 2000).

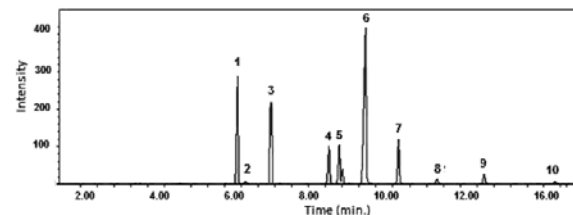


Figure 3. Mass spectra - (1) palmitic acid; (2) palmitoleic acid; (3) Internal standard – methyl heptadecanoate; (4) stearic acid; (5) oleic acid; (6) linoleic acid; (7) linolenic acid; (8) arachidic acid; (9) behenic acid; (10) lignoceric acid.

Due to the high content of omega-6 *Mucuna deeringiana* oil may be acceptable as a substitute for unsaturated oils, or even marketed as a mixture with commonly traded vegetable oils, such as soybean, canola, sunflower and corn.

The oil extracted from *Mucuna deeringiana* seed had polyunsaturated fatty acid/saturated fatty acid (PUFA/SFA) ratios above 1.2, considered suitable for food (FRANÇA et al., 2011; MARINO et al., 2008; MATSUSHITA et al., 2006, 2010; RAMOS-FILHO et al., 2008), as values lower than 0.45 are not advisable for health in terms of heart disease. The unsaturated/saturated ratios have low values, which indicates that the shelf life must be long (BERNARDO-GIL; LOPES, 2004; BERNARDO-GIL et al., 2009; ÖZDEMİR et al., 2001; XU; HANNA, 2010).

Table 2. Quantification of fatty acids in the *Mucuna deeringiana* oils extracted with supercritical carbon dioxide CO₂.

Run		1	2	3	4	5	6
T (°C)		40	60	40	40	60	60
P (bar)		250	244.1	150	250	176.7	244.1
Fatty acids ¹							
	Palmitic	20 ^a ± 0.6	20 ^a ± 0.3	21 ^a ± 0.0	20 ^a ± 0.1	21 ^a ± 0.3	20 ^a ± 0.0
	Palmitoleic	0.2 ^a ± 0.0	0.3 ^a ± 0.0	0.3 ^a ± 0.0	0.2 ^a ± 0.0	0.3 ^a ± 0.0	0.2 ^a ± 0.0
	Stearic	11 ^a ± 0.8	11 ^a ± 0.2	11 ^a ± 0.1	11 ^a ± 0.1	11 ^a ± 0.1	11 ^a ± 0.1
	Oleic	15 ^a ± 0.7	16 ^a ± 0.1	16 ^a ± 0.1	16 ^a ± 0.3	16 ^a ± 0.1	15 ^a ± 0.1
	Linoleic	42 ^a ± 1.1	40 ^a ± 0.6	42 ^a ± 0.2	42 ^a ± 0.4	40 ^a ± 0.2	42 ^a ± 0.7
	Linolenic	4.6 ^a ± 0.5	4.6 ^a ± 0.4	4.9 ^a ± 0.2	5.0 ^a ± 0.0	5.0 ^a ± 0.6	5.1 ^a ± 0.1
	Eicosanoic	1.9 ^a ± 0.8	2.4 ^a ± 0.1	1.9 ^a ± 0.2	1.2 ^a ± 1.4	2.0 ^a ± 0.1	2.0 ^a ± 0.2
	Behenic	3.8 ^a ± 0.5	4.6 ^a ± 0.3	3.1 ^a ± 0.1	3.9 ^a ± 0.1	2.9 ^a ± 0.2	3.6 ^a ± 0.3
	Lignoceric	1.1 ^a ± 0.0	1.1 ^a ± 0.2	0.4 ^a ± 0.0	1.2 ^a ± 0.0	1.2 ^a ± 0.1	1.0 ^a ± 0.2
SFA ²		37.8	39.41	37.4	37.3	38.1	37.6
MUFA ³		61.8	60.09	63.2	63.2	61.3	62.3
PUFA ⁴		46.6	44.6	46.9	47.0	45.0	47.1
PUFA/SFA		1.23	1.13	1.25	1.26	1.18	1.25

¹Results in g 100 g⁻¹ of oil. ²SFA - saturated fatty acid. ³MUFA - monounsaturated fatty acids. ⁴PUFA - polyunsaturated fatty acid.

Conclusion

This work reported experimental data for the extraction of *Mucuna deeringiana* seeds using carbon dioxide as solvent. Results showed that particles with smaller diameters increased the extraction yield. For the carbon dioxide extraction, increased pressure had a positive effect and increased temperature had a negative effect on the extraction yield in the range of variables investigated. The results reported a yield of 4.57% at 40°C, 250 bar and extraction time of 330 min. The chemical profiles of the oils extracted with carbon dioxide at different experimental conditions were similar. It was found that the obtained oils showed percentages of linoleic and linolenic acids comprising about 45% of the composition of oil from *Mucuna deeringiana*.

Acknowledgements

The authors thank CNPq, PROCAD/Pro-Engenharias - CAPES and Maringá State University (UEM) for the scholarships.

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Received on June 30, 2011.

Accepted on February 8, 2012.

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