

INSTITUTO FEDERAL GOIANO – CAMPUS RIO VERDE
PRÓ-REITORIA DE PESQUISA, PÓS-GRADUAÇÃO E INOVAÇÃO
PÓS-GRADUAÇÃO EM CIÊNCIAS AGRÁRIAS - AGRONOMIA

EXTRAÇÃO DE COMPOSTOS BIOATIVOS UTILIZANDO
CO₂ SUPERCRÍTICO DE ESPÉCIES DO CERRADO

Autor: Rogério Favareto
Orientador: Dr. Marconi Batista Teixeira
Coorientador: Dr. Lúcio Cardozo Filho

Rio verde - GO
Dezembro – 2016

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AGRÁRIAS-AGRONOMIA**

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TITULAÇÃO: Doutor(a) em Ciências Agrárias-Agronomia - Área de
Concentração em Produção Vegetal Sustentável no Cerrado

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LISTA DE SÍMBOLOS, SIGLAS, ABREVIACÕES E UNIDADES

Símbolo/Sigla	Significado	Unidade de Medida
a	parâmetro de energia	
AA	Atividade antioxidante	%
ABTS	2,2-azino-bis-(3-etilbenzotiazolina-6-ácido sulfônico)	
b	parâmetros de correção do volume	Mol m ⁻³
CER	taxa de extração constante	
CO ₂	dióxido de carbono	
DPPH	2,2-difenil-1-picril-hidrazil-hidrato	
Δg	parâmetro de interação binária da equação PR-SW	K
ε	porosidade do leito	
FER	taxa de extração decrescente	
FSC	Fluido supercrítico	
GC-MS	cromatografia gasosa acoplado a um espectrofotômetro de massas	
G^E	energia molar de Gibbs em excesso	J mol ⁻¹
K	relação entre a constante de equilíbrio de adsorção do soluto na primeira monocamada e nas camadas subsequentes	
K_{I2}	parâmetro de interação binária	
K_{Fa}	coeficientes de transferência de massa da fase fluida	s ⁻¹
K_{Sa}	coeficientes de transferência de massa da fase sólida	s ⁻¹
L	Comprimento do extrator	m
LER	baixa taxa de extração	
m	massa	kg
m_0	massa inicial extraível do soluto no leito empacotado	kg
m_s	massa de sólido na base livre de extrato	kg
m_j^{calc}	massa calculada pelo modelo de Sovová	kg
m_j^{exp}	massa experimental	kg
\dot{m}_F	vazão mássica do solvente	kg s ⁻¹
N	Número de dados experimentais	
NRTL	non-random two-liquid	
OF	função objetivo	

P	pressão	Pa
PB	ponto de bolha	
P_c	Pressão crítica	Pa
PO	ponto de orvalho	
PR-WS	Peng-Robinson com a regra da mistura quadrática de Wong-Sandler	
P_i^{calc}	pressão calculada	Pa
P_i^{exp}	pressão experimental	Pa
Q	vazão volumétrica de CO ₂	m ³ s ⁻¹
q_0	fração inicial de extrato na matriz sólida	kg _{extrato} kg _{sólido} ⁻¹
r	parâmetro adimensional ajustável do modelo de Sovová	
R	constante universal dos gases	J mol ⁻¹ K ⁻¹
rmsd	desvio padrão	
ρ_F	densidade do fluido	
ρ_S	densidade do sólido	
S_b	solubilidade do extrato no solvente	kg _{extrato} kg _{CO2} ⁻¹
scCO ₂	dióxido de carbono supercrítico	
t	tempo	s
T	temperatura	K
T_c	temperatura crítica	K
$t_{t,cal}$	Tempo calculado pelo modelo de Pardo-Castaño	s
$t_{t,exp}$	tempo experimental	s
u	velocidade do CO ₂	m s ⁻¹
V	volume molar	Mol m ⁻³
x	fração molar na fase líquida	
x_m	razão entre a massa de soluto presente na primeira monocamada e a massa inicial de soluto que pode ser extraída	
y	Fração molar na fase vapor	
Y	rendimento	
y^*	solubilidade do extrato no FSC	kg _{extrato} kg _{CO2} ⁻¹
w	Fração mássica	
W	parâmetro adimensional ajustável do modelo de Sovová	
Z	parâmetro adimensional ajustável do modelo de Sovová	

RESUMO

FAVARETO, ROGÉRIO. Instituto Federal Goiano – Campus Rio Verde – GO, dezembro de 2016. **Extração de compostos bioativos utilizando CO₂ supercrítico de espécies do cerrado.** Orientador: Dr. Marconi Batista Teixeira. Coorientador: Dr. Lúcio Cardozo Filho.

Pterodon spp. (Fabaceae) e *Duguetia furfuracea* (Annonaceae) conhecidos popularmente como sucupira e araticum-do-cerrado, respectivamente, são plantas nativas do cerrado e são utilizadas na medicina popular por suas propriedades farmacológicas. Estudos indicam que seus extratos possuem compostos com altas atividades biológicas de interesse humano. Entre os métodos de extração de compostos bioativos destaca-se a extração supercrítica por apresentar como vantagens ser um processo rápido, seletivo, não degradativo e isento de resíduos tóxicos. Assim, este trabalho teve como objetivo a extração de frutos de sucupira e de folhas de *D. furfuracea* utilizando dióxido de carbono no estado supercrítico como solvente e avaliar o rendimento do processo, o perfil químico e a atividade antioxidante dos extratos obtidos. Adicionalmente foram realizadas extrações por hidrodestilação dos frutos da sucupira e por Soxhlet das folhas de *D. furfuracea* para efeito de comparação dos resultados. Medidas de transição de fases para o sistema CO₂ (1) + extrato de sucupira (2) foram conduzidas para definir as condições operacionais de temperatura e de pressão dos experimentos de extração da sucupira. As extrações foram conduzidas nas temperaturas de 313–333 K e pressões de 10–22 MPa. Para a extração supercrítica de folhas de *D. furfuracea* um planejamento experimental 2³ com triplicata do ponto central foi utilizado para avaliar os efeitos da temperatura (313-333 K), pressão (15-23 MPa) e vazão volumétrica (3-6 mL min⁻¹). Para a sucupira as extrações supercríticas obtiveram melhores resultados, sendo o maior rendimento mássico (0,212) e o maior

teor de diterpenos vouacapanos (35,66 %) obtidos na extração supercrítica nas condições de 313 K e 22 MPa e a maior atividade antioxidante (77,59 %) obtida nas condições de 323 K e 16 MPa. Os efeitos das variáveis no rendimento de extração de folhas de *D. furfuracea* foram positivos sendo a pressão que teve maior influência. Entretanto os maiores rendimentos, teores de fenóis totais e atividades antioxidantes foram obtidas pelo método Soxhlet. A equação de estado de Peng-Robinson com a regra de mistura quadrática de Wong-Sandler e os modelos matemáticos de Sovová e de Pardo-Castaño et al. correlacionaram de forma satisfatória os dados de equilíbrio de fases do sistema CO₂ (1) + extrato de sucupira (2) e das cinéticas de extrações de frutos de sucupira e de folhas de *D. furfuracea*, respectivamente.

PALAVRAS-CHAVE: *Pterodon* spp., *Duguetia furfuracea*, extração supercrítica, compostos bioativos.

ABSTRACT

FAVARETO, ROGÉRIO. Instituto Federal Goiano – Campus Rio Verde – GO, december, 2016. **Extraction of bioactive compounds using supercritical CO₂ from cerrado species.** Advisor: Marconi Batista Teixeira. Co-advisor: Lúcio Cardozo Filho.

Pterodon spp. (Fabaceae) and *Duguetia furfuracea* (Annonaceae), popularly known as sucupira and araticum-do-cerrado, respectively, are native plants of the Cerrado and are used in popular medicine by their pharmacological properties. Studies indicate that their extracts contain compounds with high biological activities of human interest. Among the methods of extraction of bioactive compounds the supercritical extraction has the advantages of being a fast, selective, non-degradative process and the extract is free of toxic residues. The objective of this work was to extract fruits of sucupira and leaves of *D. furfuracea* using carbon dioxide in the supercritical state as solvent and to evaluate the yield of the process, the chemical profile and the antioxidant activity of the extracts obtained. In addition, extractions by hydrodistillation of sucupira fruits and by Soxhlet of *D. furfuracea* leaves were carried out to compare the results. Phase transition measures for the CO₂ (1) + sucupira extract (2) were conducted to define the operating conditions of temperature and pressure of the sucupira extraction experiments. The extractions were conducted at temperatures of 313-333 K and pressures of 10-22 MPa. For supercritical extraction of *D. furfuracea* leaves an experimental design 2³ with triplicate of the central point was used to evaluate the effects of temperature (313-333 K), pressure (15-23 MPa) and volumetric flow (3-6 mL min⁻¹). For the sucupira, the supercritical extractions obtained better results, being the greater mass yield (0.212) and the higher content of vouacapan diterpenes (35.66 %) obtained in the supercritical extraction in the conditions of 313 K and 22 MPa and the greater antioxidant activity (77.59 %) obtained under the conditions of 323 K and 16 MPa. The effects of the

variables on the yield of *D. furfuracea* leaves were positive and the pressure had the greatest influence. However, the highest yields, total phenol contents and antioxidant activities were obtained by the Soxhlet method. The Peng-Robinson equation of state with the Wong-Sandler quadratic mixing rule and the mathematical models of Sovová and Pardo-Castano et al. satisfactorily correlated system phase equilibrium data CO₂ (1) + sucupira extract (2) and kinetics of extractions of sucupira fruits and of *D. furfuracea* leaves, respectively.

KEY WORDS: *Pterodon* spp., *Duguetia furfuracea*, supercritical extraction, bioactive compounds.

1. INTRODUÇÃO

A natureza é responsável pela produção da maioria das substâncias orgânicas conhecidas, sendo no reino vegetal a maior diversidade química. Produtos naturais são compostos químicos ou substâncias produzidas na natureza e que, geralmente, têm atividade biológica ou farmacológica [1]. Segundo a ANVISA plantas são denominadas como medicinais quando possuem substâncias ou classes de substâncias com ação terapêutica [2]. As ações medicinais e terapêuticas são dependentes da presença de compostos bioativos conhecidos como metabólitos secundários, compostos orgânicos que não estão diretamente envolvidos nos processos de crescimento, desenvolvimento e reprodução dos organismos. A variedade e a complexidade dos metabólitos secundários são consequentes de milhões de anos de evolução, atingindo uma ampla gama de formas de proteção e resistência a fatores bióticos e abióticos [3].

As classificações dos compostos bioativos podem diferir entre si dependendo da intenção de cada classificação. De acordo com Croteau et al. [4] a classificação dos compostos bioativos é dividida em três categorias; terpenos (aproximadamente 25.000 tipos), alcaloides (aproximadamente 12.000 tipos) e compostos fenólicos (aproximadamente 8.000 tipos). As estruturas gerais das diferentes categorias são apresentadas na Fig. 1.1.

Compostos bioativos encontrados em uma variedade de matrizes vegetais, tais como sementes, folhas, frutos, raízes, flores, rizomas, casca entre outros, são atualmente usados nas indústrias farmacêuticas, de cosméticos e de alimentos [5]. Muitos compostos extraídos de fontes naturais apresentam várias aplicações bioativas, tais como antimicrobial, antibacteriano, antifúngico, antiviral, anti-inflamatório, antitumoral, antiobesidade, anticolinesterase, fagocitóticos, inseticidas e antioxidantes [6]. Tais compostos, se purificados, podem custar centenas de euros, como o caso do

eugenol, cumarina e timol, até centenas de milhares de euros como ácidos betulínico e ursolínico como pode ser observado na Fig. 1.2 [7].

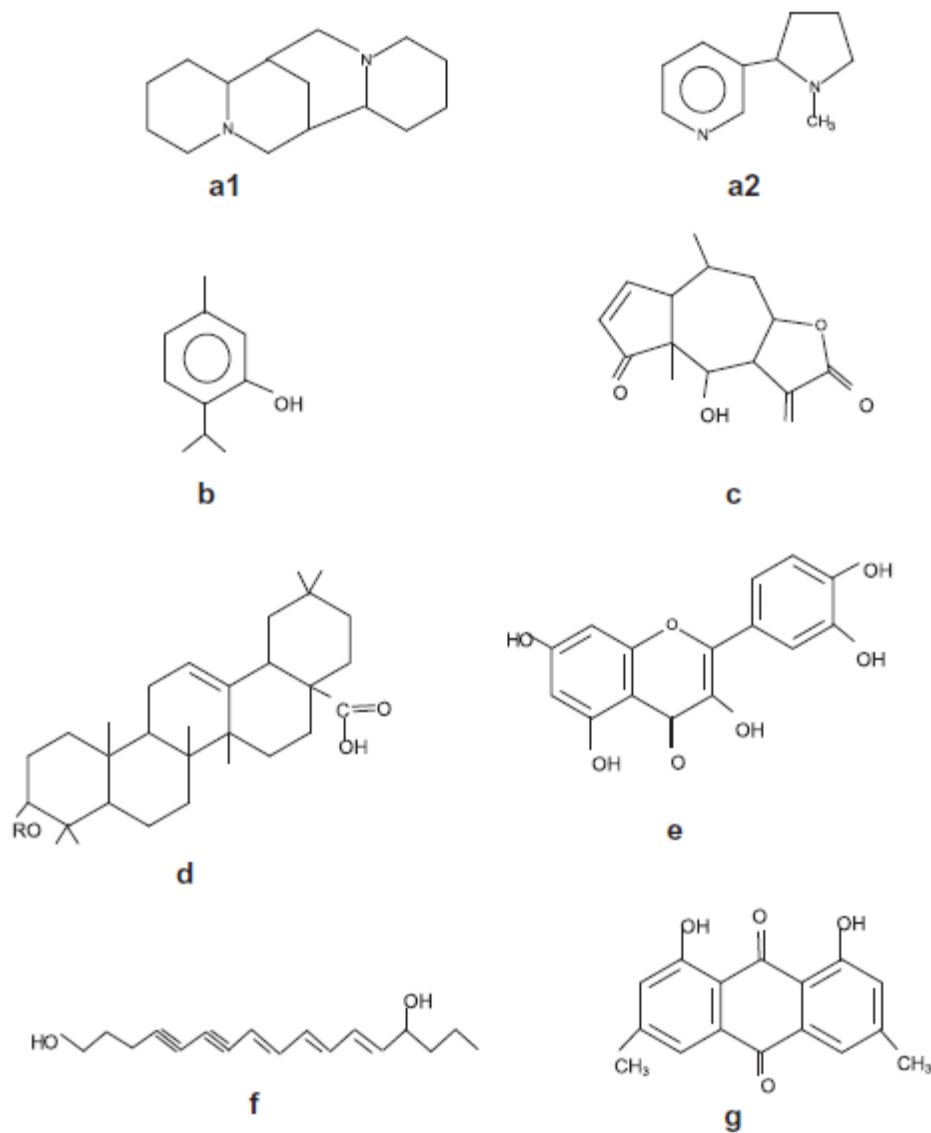


Fig. 1.1. Estruturas gerais de compostos bioativos: alcaloides (a1 e a2), monoterpenos (b), sesquiterpenos (c), triterpenos, saponinas e esteroides (d), flavonoides (e), poliacetilenos (f) e policetídeos (g).

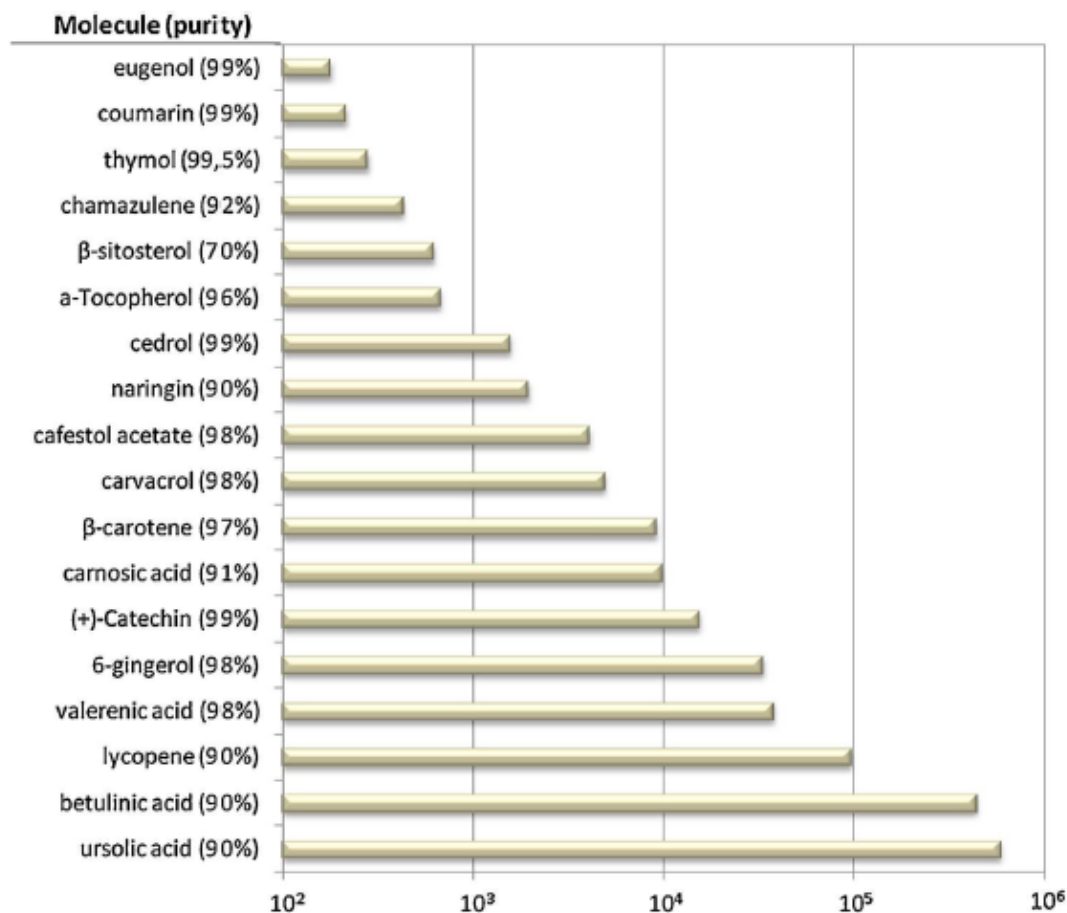


Fig.1.2. Preço comercial de compostos bioativos em Euros/kg[7].

Para se obter compostos bioativos, a extração e a recuperação da matriz sólida podem ser divididas em três etapas: dessorção da matriz e sua solubilização, difusão interna do soluto através da fase sólida até a superfície e difusão externa do soluto através do filme. A quantidade e a composição do extrato dependem do tipo, do tempo e da temperatura de extração, assim como do solvente utilizado e da relação solvente-amostra [8]. Um bom solvente deve apresentar baixa toxicidade, baixo ponto de ebulição, alta transferência de massa do soluto, facilidade na recuperação do soluto e preservar as características dos compostos bioativos [9].

1.1 Técnicas de extração

1.1.1 Destilação por arraste a vapor

Destilação por arraste a vapor é um processo de destilação modificada que utiliza vapor para extrair componentes voláteis de uma matriz. Os extratos obtidos são classificados como óleos voláteis ou essenciais. Óleos voláteis são uma mistura de terpenoides voláteis que são produzidos pelo metabolismo secundário das plantas e tem componentes da família dos terpenos como majoritários, principalmente monoterpenos, monoterpenos oxigenados, sesquiterpenos e sesquiterpenos oxigenados. Óleos essenciais consistem em substâncias voláteis lipofílicas que são principalmente hidrocarbonetos ou componentes monofuncionais derivados do metabolismo de mono e sesquiterpenos, fenilpropanoides, aminoácidos e ácidos graxos [10].

O processo de destilação por vapor pode ter três variações dependendo do contato do vapor e da matéria-prima; destilação por vapor direto, hidrodestilação e destilação por vapor seco. Um fluxograma geral dos processos de destilação por arraste a vapor é apresentado na Fig. 1.3.

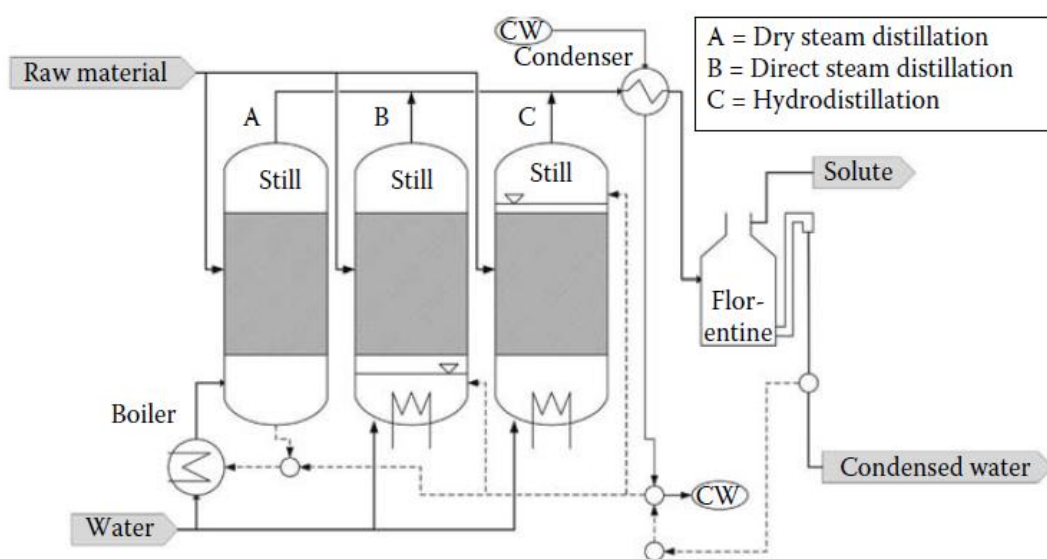


Fig.1.3. Fluxograma geral de processos de destilação por arraste de vapor [10].

O processo se baseia no contato da matéria-prima com água em ebulição (hidrodestilação) ou com vapor de água, que pode ser produzido dentro (destilação por vapor direto) ou fora (destilação por vapor seco) da câmara com a matéria-prima, fazendo com que os solutos voláteis vaporizem e sejam arrastados com o vapor. Suas vantagens são obtenção de produtos livres de solventes, alta capacidade de processamento, baixo custo de investimento e ser uma tecnologia bem conhecida. Como

desvantagens pode ocorrer a degradação térmica de produtos e alto consumo de energia [10].

Na Fig. 1.4, é apresentado o valor comercial de alguns óleos essenciais, sendo a faixa de valores de 20 euros para o óleo essencial de eucalipto até 450 euros para o de camomila.

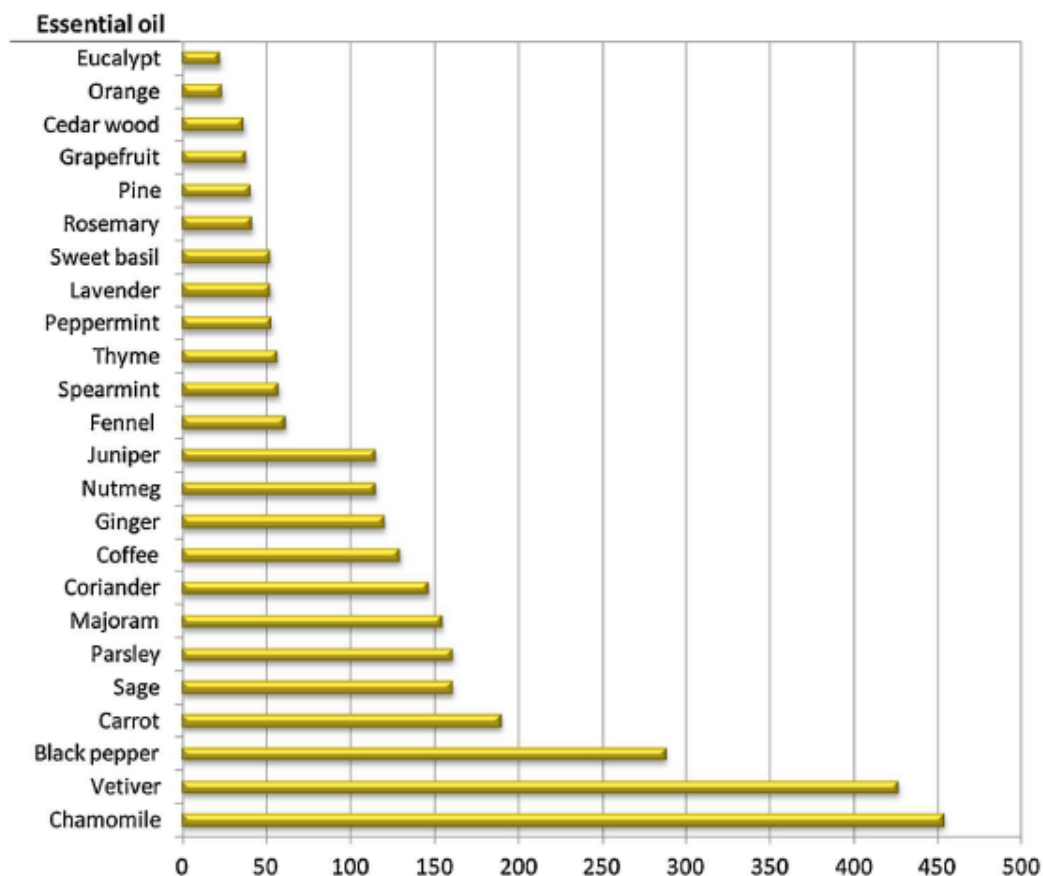


Fig.1.4. Preço comercial de oleos essenciais em Euros/kg [7].

1.1.2 Extração por solventes

Extração sólido-líquido, ou extração por solventes, possui numerosas aplicações industriais, sendo a produção de óleos fixos, conhecidos como óleos vegetais, seja o exemplo mais conhecido. Estes processos recebem o nome de lixiviação, decocção ou elução dependendo do objetivo e das condições, embora a extração sempre ocorra com a dissolução seletiva de um ou mais solutos da matriz sólida pelo líquido solvente. A escolha do solvente é baseada em fatores tais como propriedades físico-químicas, custo e toxicidade [10]

1.1.3 Extração com líquidos pressurizados

O método de extração com líquidos pressurizados consiste na aplicação de altas pressões para manter o solvente no estado líquido em temperaturas superiores ao seu ponto normal de ebulição acelerando assim a cinética de extração pela pressão forçar a entrada do solvente na matriz porosa e a temperatura diminuir a viscosidade do solvente. É uma técnica desenvolvida como uma alternativa aos métodos de Soxhlet, maceração, percolação ou refluxo, tendo como vantagem o tempo de extração, o consumo de solvente, rendimento e reprodutibilidade [11].

1.1.4 Extração com fluidos supercríticos

Os processos de extração sólido-fluido são operações de separação de um ou mais solutos presentes em uma matriz sólida, podendo o solvente estar na fase líquida ou no estado supercrítico. Um fluido é dito supercrítico quando sua temperatura e pressão estão acima do ponto crítico conforme apresentado na Fig. 1.5.

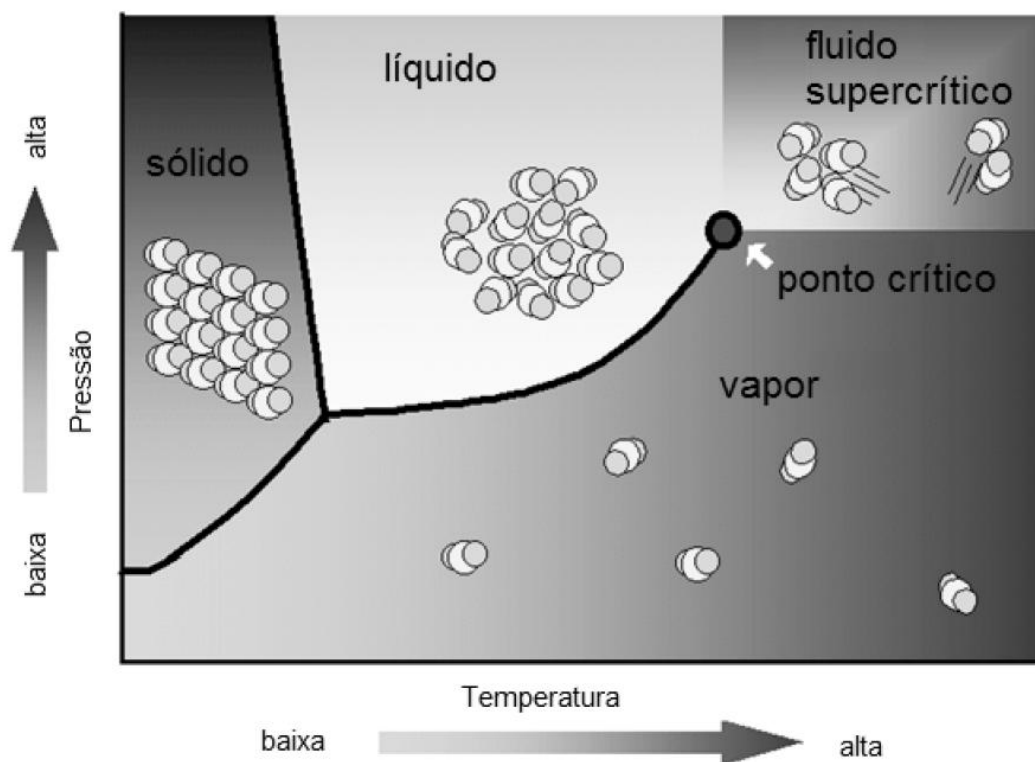


Fig.1.5. Diagrama P x T para um componente puro [12]

Uma das principais características dos fluidos supercríticos é a possibilidade de alterar sua densidade pela manipulação da temperatura e/ou pressão. Como a solubilidade esta relacionada com a densidade do fluido, com aumento da pressão de extração pode-se alterar o poder de solvatação. Por causa da baixa viscosidade e alta difusividade do fluido supercrítico, tem sido utilizado como solvente em processos de extração por aumentar as taxas de extração [13].

O fluido supercrítico mais utilizado é o dióxido de carbono devido a sua baixa temperatura crítica (304,2 K) e moderada pressão crítica (73,8 bar), além de não ser tóxico, não inflamável, relativamente barato e facilmente encontrado. O dióxido de carbono é um bom solvente para componentes hidrofóbicos ou levemente hidrofílicos [14].

A curva de extração geralmente é dada em termos da massa cumulativa extraída ou rendimento de extração em função do tempo conforme pode ser observado na Fig. 1.6. A curva geral de extração supercrítica é dividida em três períodos que são controlados por diferentes mecanismos de transferência de massa.

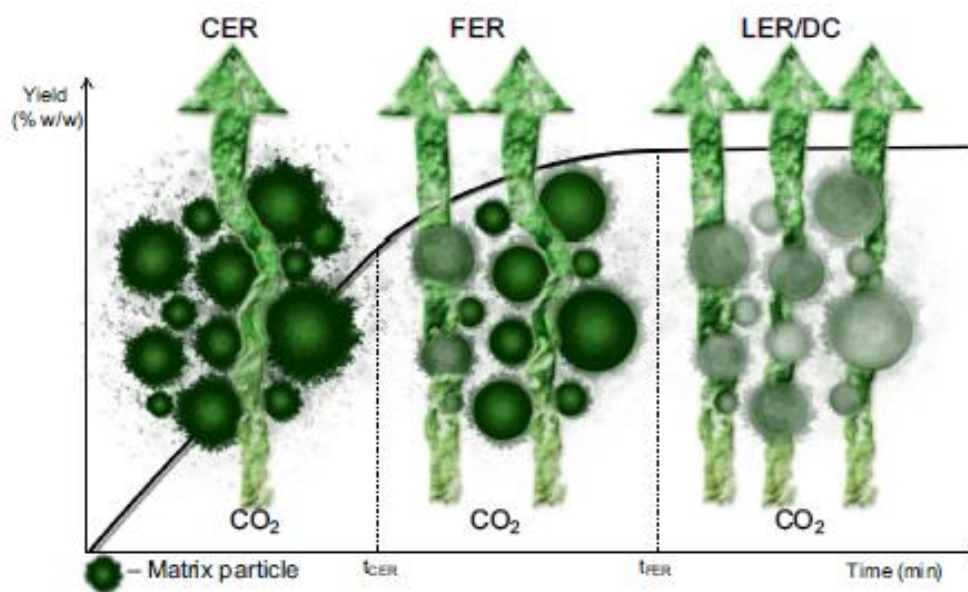


Fig.1.6. Os três períodos de uma curva geral de extração supercrítica [6].

- CER; taxa de extração constante, em que é extraído o soluto facilmente acessível na superfície da matriz sólida e a convecção domina o mecanismo de transporte de massa;

- FER; taxa de extração decrescente, em que é extraído o soluto de fissuras da matriz sólida e começa o mecanismo de difusão em adição a convecção.
- LER; baixa taxa de extração, em que é extraído o soluto das partes internas da matriz sólida e a difusão controla o mecanismo de transferência de massa.

Tendo em vista a vasta diversidade de moléculas encontradas em matrizes naturais, os extratos de extrações supercríticas são tipicamente misturas das seguintes famílias de componentes conforme apresentado na Fig. 1.7.

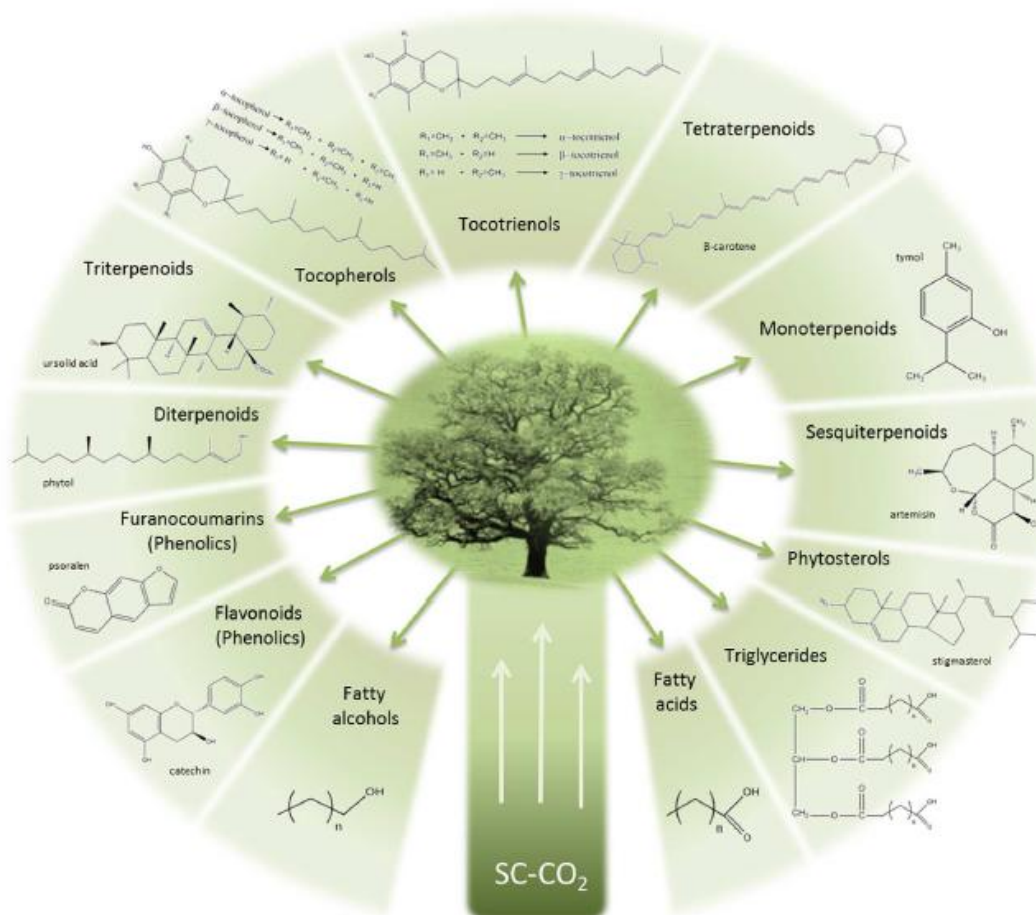


Fig.1.7. Principais famílias de componentes encontrados em extratos suérricos [7].

A otimização do processo de extração supercrítica requer o conhecimento de dados termodinâmicos (solubilidade e seletividade) e de valores cinéticos (coeficientes de transferência de massa) que são descritos abaixo.

1.2 Técnicas de medidas de transição de fases

O conhecimento do comportamento de fases entre o soluto e o fluido supercrítico é um dos fatores mais importantes para o projeto de uma coluna de extração, sendo o soluto considerado como uma mistura de diferentes componentes como mencionado anteriormente.

Os dados experimentais de equilíbrio podem ser determinados utilizando várias metodologias. Segundo Dohrn et al. [15] há duas classes principais de métodos: analíticos e sintéticos conforme esquematizado na Fig. 1.8.

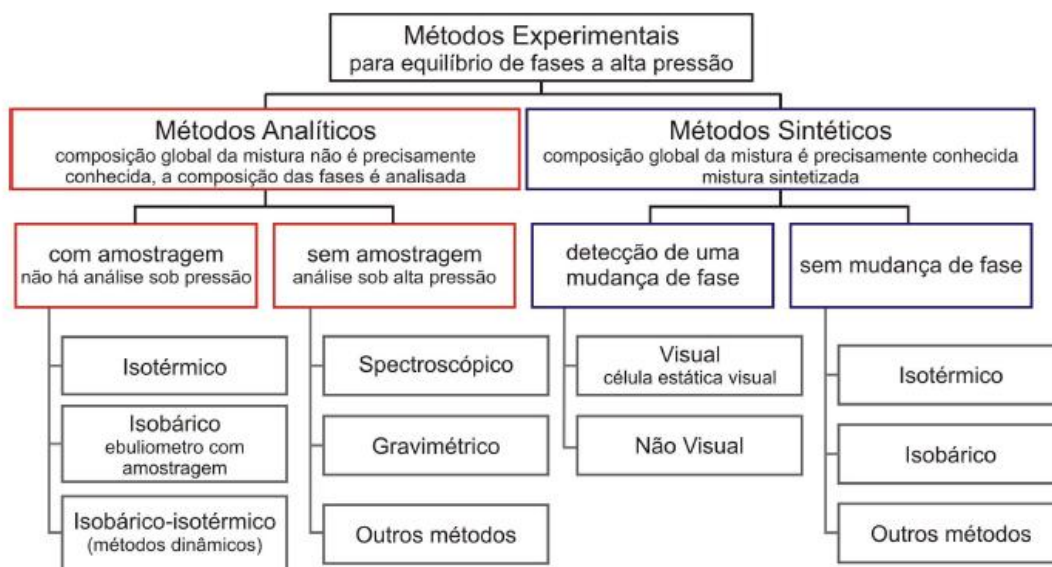


Fig.1.8. Classificação dos métodos de determinação do equilíbrio de fases [16].

A principal diferença entre os métodos analíticos e sintéticos é o conhecimento da composição global da mistura no início do experimento. Os métodos analíticos não necessitam conhecimento global de mistura e requerem equipamentos de análise físico-química das fases no sistema, que podem ser realizadas através de amostragem ou sem amostragem. Nos métodos sintéticos, é necessário o conhecimento da composição global do sistema e a determinação do equilíbrio de fases podem ser determinados com ou sem mudança de fases[15].

1.3 Modelagem Matemática

1.3.1 Equilíbrio de fases

O sistema de extração é bastante complexo compreendendo o solvente supercrítico, uma mistura de componentes que formam o soluto e uma matriz sólida em que se encontra o soluto. Para simplificação, pode-se considerar o soluto como um pseudocomponente, em que as propriedades termofísicas do soluto podem ser estimadas pela regra de Kay [17].

Para modelagem dos dados experimentais utiliza-se o critério de isofugacidade das fases em equilíbrio.

Para equilíbrio L-V tem-se:

$$\hat{f}_i^V = \hat{f}_i^L \quad (1)$$

em que \hat{f}_i^V é a fugacidade do componente i na fase vapor e \hat{f}_i^L é a fugacidade do componente i na fase líquida são dadas por:

$$\hat{f}_i^\alpha = \hat{\phi}_i^\alpha x_i P \quad (2)$$

em que, α pode representar tanto a fase líquida como a fase vapor, $\hat{\phi}_i^\alpha$ é o coeficiente de fugacidade do componente i na fase α , x_i é a fração molar e P a pressão.

O coeficiente de fugacidade para um componente i presente em uma mistura de componentes pode ser calculado por:

$$\ln \hat{\phi}_i = \frac{1}{RT} \int_{V \rightarrow \infty}^{V=RT/p} \left[\frac{RT}{V} - N \left(\frac{\partial P}{\partial N_i} \right)_{T, NV, N_{j \neq i}} \right] dV - \ln Z, \quad (3)$$

Em que R é a constante universal dos gases, V o volume molar, Z é o coeficiente de compressibilidade, N é o número total de mols N_i é o número de mols do componente i .

Para o cálculo do coeficiente de fugacidade geralmente utiliza-se de equações de estado, como por exemplo, se for utilizado a equação de estado de Peng-Robinson [18] dada pela Equação (4):

$$P = \frac{RT}{V - b_p} - \frac{a_p}{V(V + b_p) + bp(V - b_p)} \quad (4)$$

os coeficientes de fugacidade das fases líquida e vapor podem ser determinadas por:

$$\ln \bar{\phi}_i^V = \frac{b_{pi}}{b_p} (Z^V - 1) - \ln \left(Z^V - \frac{b_p P}{RT} \right) - \frac{a_p}{2\sqrt{2}RT} \left(\frac{2 \sum_j y_j a_{pji}}{a_p} - \frac{b_{pi}}{b_p} \right) \ln \left(\frac{Z^V + (1 + \sqrt{2}) \frac{b_p P}{RT}}{Z^V + (1 - \sqrt{2}) \frac{b_p P}{RT}} \right) \quad (5)$$

E

$$\ln \bar{\phi}_i^L = \frac{b_{pi}}{b_p} (Z^L - 1) - \ln \left(Z^L - \frac{b_p P}{RT} \right) - \frac{a_p}{2\sqrt{2}RT} \left(\frac{2 \sum_j y_j a_{pji}}{a_p} - \frac{b_{pi}}{b_p} \right) \ln \left(\frac{Z^L + (1 + \sqrt{2}) \frac{b_p P}{RT}}{Z^L + (1 - \sqrt{2}) \frac{b_p P}{RT}} \right) \quad (6)$$

Em que a_p e b_p são os parâmetros de Peng-Robinson, .

Os parâmetros de Peng-Robinson podem ser calculados utilizando regras de mistura. Para a regra de mistura de Wong-Sandler [19] estes parâmetros são calculados pelas seguintes equações:

$$a_p = \sum_i \sum_j z_i z_j a_{pji} \quad (7)$$

$$b_p = \sum_i \sum_j z_i z_j b_{pji} \quad (8)$$

1.3.2 Extração supercrítica

A modelagem de extração supercrítica envolvem geralmente a análise das curvas de extração no qual aspectos cinéticos e termodinâmicos são relacionados quantitativamente para ampliações de escala e otimização do processo. Os modelos de extração supercrítica podem ser divididos em três classes: empíricos, simplificados e fenomenológicos [7]. Os modelos empíricos, tais como Tan e Liou [20], que considera a cinética de extração como sendo de primeira ordem em relação às concentrações do soluto e negligencia a dispersão axial na coluna do extrator, apesar de suas simplicidades não permitem determinar os coeficientes de transferência de massa e assim, são pouco utilizados quando o objetivo é ampliação de escala.

Os modelos fenomenológicos incluem equações de taxa, balanços de massa e requerem dados da cinética e do equilíbrio de fases, além de algumas variáveis da estrutura da matriz porosa. O modelo proposto por Sovová [21] é um exemplo de modelo fenomenológico. Este modelo leva em consideração três períodos de extração, taxa de extração constante (CER), taxa de extração decrescente (FER) e baixa taxa de extração (LER) que são controlados pelos mecanismos de transferência de massa por difusão e convecção. A solução analítica é dada pelas Equações (15-17):

Para $t < t_{CER}$:

$$m(t) = \dot{m}_F S_b t [1 - \exp(-Z)] \quad (9)$$

Para $t_{CER} \leq t < t_{FER}$:

$$m(t) = \dot{m}_F S_b \left\{ t - t_{CER} \exp \left[\frac{Z S_b}{W q_0} \ln \left[\frac{1}{1-r} \left(\exp \frac{W \dot{m}_F}{m_S} (t - t_{CER}) - r \right) \right] - Z \right] \right\} \quad (10)$$

Para $t \geq t_{FER}$:

$$m(t) = m_S \left\{ q_0 - \frac{S_b}{W} \ln \left[1 + \left(\exp \left(\frac{W q_0}{S_b} \right) - 1 \right) \exp \left(\frac{W \dot{m}_F}{m_S} (t_{FER} - t) \right) r \right] \right\} \quad (11)$$

$$Z = \frac{K_{Fa} m_S \rho_F}{\dot{m}_F \rho_S} \quad (12)$$

$$W = \frac{m_S K_{Sa}}{\dot{m}_F (1 - \varepsilon)} \quad (13)$$

$$t_{CER} = \frac{(1-r) m_S q_0}{S_b Z \dot{m}_F} \quad (14)$$

$$t_{FER} = t_{CER} + \frac{m_S}{W \dot{m}_F} \ln \left[r + (1-r) \exp \left(\frac{W q_0}{S_b} \right) \right] \quad (15)$$

Em que $m(t)$ é a massa extraída em função do tempo t , \dot{m}_F a vazão mássica do solvente, S_b a solubilidade do extrato no solvente; Z e W são os parâmetros adimensionais ajustáveis do modelo, q_0 é a fração inicial de extrato na matriz sólida, m_S é a massa de sólido na base livre de extrato, ρ_F e ρ_S são as densidades do fluido e do sólido, respectivamente, K_{Fa} e K_{Sa} são os coeficientes de transferência de massa da fase fluida e sólida, respectivamente, ε a porosidade do leite, r é a fração facilmente acessível de extrato, um parâmetro ajustável do modelo e t_{CER} e t_{FER} são os finais do primeiro e do segundo período, , respectivamente.

Outro modelo fenomenológico foi descrito por Pardo-Castaño et al. [22]. O modelo se baseia na teoria de absorção de Brunauer–Emmett–Teller e expressa o

rendimento de extração (Y) como função do tempo (t) com três parâmetros ajustáveis; y^* , solubilidade do extrato no FSC, K , a relação entre a constante de equilíbrio de adsorção do soluto na primeira monocamada e nas camadas subsequentes e x_m , a razão entre a massa de soluto presente na primeira monocamada e a massa inicial de soluto que pode ser extraída, conforme Equação (4.2):

$$t = \frac{m_0}{2\dot{m}_F y^*} \left\{ x'_0 - x' + (2 - K) \left[Y - x_m \ln \left(\frac{\alpha}{\beta} \right) \right] + K x_m \ln \left[\frac{\alpha'}{\beta' (1 - Y)^2} \right] \right\} \quad (16)$$

Em que:

$$x'_0 = \sqrt{a + b + c} \quad (17)$$

$$x' = \sqrt{a(1 - Y)^2 + b(1 - Y) + c} \quad (18)$$

$$a = K^2 \quad (19)$$

$$b = 2(2 - K)Kx_m \quad (20)$$

$$c = (Kx_m)^2 \quad (21)$$

$$\alpha = x' + (2 - K)(1 + Y) + Kx_m \quad (22)$$

$$\alpha' = x' + (2 - K)(1 - Y) + Kx_m \quad (23)$$

$$\beta = x'_0 + K + (2 - K)x_m \quad (24)$$

$$\beta' = x'_0 + (2 - K) + Kx_m \quad (25)$$

$$y^* = y_{sat} \left[1 - \exp \left(-\frac{KL}{u\varepsilon} \right) \right] \quad (26)$$

Em que m_0 é a massa inicial extraível do soluto no leito empacotado, \dot{m}_F é a vazão mássica de CO_2 , L é o comprimento do extrator, u é a velocidade do CO_2 e ε a porosidade do leito.

1.4 Considerações finais

O Cerrado é o segundo maior bioma brasileiro abrangendo 21 % do território nacional, com 2 milhões de km^2 . Este bioma ocupa os estados de Goiás, Mato Grosso, Mato Grosso do Sul, Tocantins, Bahia, Minas Gerais e Distrito Federal, além de ocupar parte dos estados do Maranhão, Piauí, Rondônia e São Paulo [23]. O termo Cerrado é comumente utilizado para designar o conjunto de ecossistemas que ocorrem no Brasil Central destacando que sua vegetação não possui uma fitofisionomia única em toda a sua extensão. As variações fitofisionômicas do Cerrado produzem um gradiente, em

densidade e altura, definido por formações campestres (Campo Limpo) a florestais (Cerradão), e entre estes, existe três fitofisionomias intermediárias, Campo Sujo, Campo Cerrado e Cerrado stricto sensu, descritas a partir do aumento da densidade de árvores [24].

A biodiversidade do Cerrado apresenta 12.669 espécies sendo 4215 espécies endêmicas e, nesse sentido, o Cerrado é confirmado como a mais diversificada savana tropical do mundo [25]. Apesar desta biodiversidade cerca de metade dos 2 milhões de km² originais do Cerrado foram transformados em pastagens, plantações de culturas anuais e outros tipos de uso [26] levando com que muitas espécies sejam extintas [27] e incluindo o Cerrado a um dos 34 *hotspots* mundiais de biodiversidade [28,29]. Em razão ao alto desmatamento, grande diversidade taxonômica e a sua flora ainda pouco estudada necessita-se de mais pesquisas com as espécies nativas em relação a conservação dos recursos naturais e recursos fitoterápicos oferecidos pelas plantas medicinais [30].

Árvores nativas do Brasil, típicas do Cerrado brasileiro, o gênero *Pterodon* spp. pertence à família Fabaceae e compreende quatro espécies: *Pterodon abruptus* Vogel, *Pterodon apparicioi* Pederdoli, *Pterodon pubescens* Benth, *Pterodon emarginatus* Vogel (sinônimo *Pterodon polygalaeiflorus* Benth) [31]. Conhecidas popularmente como sucupira, sucupira-branca ou faveira [32] suas sementes, cascas e o óleo da sucupira são utilizados na medicina popular por apresentarem potencial farmacológico com propriedades antirreumática, anti-inflamatórias e antinociceptiva [33].

A espécie *Duguetia furfuracea* (A. St.-Hil.) Benth e Hook. f. pertencente a família Annonaceae é um arbusto encontrado no Cerrado e considerado uma planta daninha pelos pecuaristas por invadir as pastagens [34]. Conhecido popularmente como sofre-do-rim-quem-quer, araticum-bravo, araticum-do-campo, araticum-do-cerrado, atabrava e ata de lobo [35,36] é utilizado na medicina popular como antirreumática, cicatrizante de feridas, no tratamento de cólica nos rins e contra pediculose [37].

Estudos sobre as propriedades farmacológicas da sucupira mostram que os extratos etanólico das sementes [38] e hexânico e metanólico dos frutos de *P. polygalaeiflorus* apresentam significantes atividades larvicidas contra o mosquito *Aedes aegypti* [39] e atividades antinociceptivas [40]. O óleo-resina tem efeitos antiespasmódico e de relaxante vascular [41]. O extrato hidroetanólico das folhas de *P. emarginatus* [42] e o extrato oleaginoso [43] e etanólico [44] dos frutos de *P. pubescens*

exibem atividades antinociceptivas em modelos animais. O óleo essencial de sementes de *P. emarginatus* exerce alta citotoxicidade em células cancerígenas, demonstrando atividade antiproliferativa [45], reduz e limita a severidade e o desenvolvimento de doenças autoimunes como a esclerose múltipla [46].

Estudos fitoquímicos mostram que o óleo essencial de *P. emarginatus* é composto por hidrocarbonetos sesquiterpênicos e sesquiterpenos oxigenados, sendo os componentes majoritários o β -cariofileno, o β -elemeno, o espatulenol, o α -humuleno e o γ -muuroleno [47,48]. Os diterpenos vouacapanos presentes nos extratos das espécies de *Pterodon* obtidos por solventes orgânicos estão diretamente relacionados com as atividades biológicas [49].

A análise fitoquímica do óleo essencial das folhas e ramos de *D. furfuracea* revelou a presença de sesquiterpenos [50], flavonoides e vários alcaloides [51,32]. O extrato alcaloídico, obtido das cascas do caule subterrâneo, apresentam atividades antitumoral, tripanocida e leishmanicida [53]. O extrato etanólico apresenta atividade citoprotetora à bactéria *E. coli* frente ao metal pesado cloreto de mercúrio indicando que a espécie é uma fonte promissora no combate a metais pesados [34]. Os extratos hexânicos e etanólico da *D. furfuracea* apresentam altos valores de atividade larvicida contra larvas de *Aedes aegypti* [55].

Os trabalhos presentes na literatura concentram-se na obtenção de óleos (arraste a vapor) e/ou de extratos de sucupira e de araticum-do-cerrado obtidos por solventes orgânicos (maceração, percolação e Soxhlet). No entanto, estudos envolvendo o uso de tecnologia supercrítica para a obtenção de seus extratos são escassos. A aplicação da tecnologia supercrítica para a obtenção de extratos a partir de fontes vegetais com potenciais farmacológicos apresenta como vantagens ser um processo rápido, seletivo, não degradativo e não necessita de processos posteriores de separação [56]. Uma das principais características do fluido supercrítico (FSC) é a manipulação de suas propriedades físico-químicas pela mudança da temperatura e da pressão. A baixa viscosidade do fluido aumenta sua difusividade na matriz sólida e diminui o tempo de extração. A manipulação da densidade do fluido influencia no poder de solvatação e conseqüentemente no rendimento e seletividade da extração [57]. Dentre os FSC utilizados o dióxido de carbono supercrítico (scCO₂) é o mais utilizado por ser ambientalmente seguro, possuir moderada temperatura crítica e preservar o extrato de reações de oxidação [58].

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2. OBJETIVOS

2.1 Objetivo Geral

Avaliar o rendimento do processo, a composição química e a atividade antioxidante dos extratos obtidos de frutos de sucupira e de folhas de *D. furfuracea* utilizando dióxido de carbono no estado supercrítico como solvente.

2.1 Objetivos Específicos

- Determinar as melhores condições de temperatura de extração.
- Determinar as melhores condições de pressão de extração.
- Avaliar a influência do comportamento de fases no processo de extração supercrítica de frutos de sucupira.
- Determinar a melhor vazão volumétrica da extração de folhas de *D. furfuracea*.
- Comparar os extratos de sucupira obtidos utilizando scCO₂ com o óleo essencial obtido por hidrodestilação.
- Comparar os extratos de *D. furfuracea* obtidos utilizando scCO₂ com os extratos obtidos por Soxhlet utilizando etanol e hexano.
- Ajustar modelos termodinâmicos aos dados de equilíbrio de fases e a cinética de extração.

3. CAPÍTULO I

Study of the Supercritical Extraction of *Pterodon* Fruits (Fabaceae): Phase diagram, Mass transfer and Antioxidant activity

(The Journal of Supercritical Fluids)

Abstract

In this study, the extraction of sucupira fruits (*Pterodon* spp.) was conducted by using the supercritical carbon dioxide as solvent and the mass percent yield, chemical profile and the antioxidant activity of the extracts obtained were evaluated. The extractions were conducted in 313–333 K and 10–22 MPa. Extractions conditions were defined by phase transition measurements for the system CO₂ (1) + sucupira extract (2). The phase equilibrium data were modeled using the Peng-Robinson equation with Wong-Sandler quadratic mixing rule. Sovová model was applied to fit the kinetic extraction curves. The extractions were conducted in 313–333 K and 10–22 MPa. The highest mass percent yield (21.2 %) and the highest content of vouacapan diterpenes (35.66 %) were obtained from the supercritical extraction on the conditions of 313 K and 22 MPa. The highest antioxidant activity (77.59 %) was obtained at 323 K and 16 MPa.

Key words: *Pterodon* spp.; VLE data; Supercritical extraction; Mass transfer coefficients; Antioxidant activity; Mathematical modeling.

3.1 Introduction

Pterodon spp. is a tree species of Fabaceae family which includes four species: *Pterodon abruptus* Benth, *Pterodon apparicioi* Pederdoli, *Pterodon polygalaeflorus*

Benth and *Pterodon emarginatus* Vogel (synonym *Pterodon pubescens* Benth) [1]. They are native Brazilian trees, typically found in the Cerrado which are popularly known as sucupira, sucupira-branca and faveira [2]. The seeds, peels and the oil of the sucupira plant have been used in popular medicine by presenting potential pharmacological effect with antirheumatic, anti-inflammatory, and antinociceptive properties [3].

Studies about the pharmacological properties of the sucupira show that the ethanolic extract of the seeds [4] and hexane and methanoloic extract of the fruits of *P. polygalaeflorus* show considerable larvicide activities against the *Aedes aegypti* mosquito [5] and antinociceptive activities [6]. The resin-oil has antispasmodic and vascular relaxing effects [7]. The hydroethanolic extract of the leaves of *P. emarginatus* [8] and the oilseed extract [9] and ethanolic [10] of the fruits of *P. pubescens* have antinociceptive activities in animals. The essential oil of seeds of *P. emarginatus* shows high citotoxicity in cancer cells, demonstrating antiproliferative activity [11], reduces and limits the severity and the development of autoimmune diseases such as multiple sclerosis [12].

Phytochemical studies show that the essential oil of *P. emarginatus* is composed of sesquiterpene hydrocarbons and oxygenated sesquiterpenes, whose major components are β -caryophyllene, β -elemene, spathulenol, α -humulene and γ -muurolene [13,14]. The vouacapan diterpenes existing in the extracts of the *Pterodon* species obtained by organic solvents are directly related to the biological activities [15].

The searches found in the literature focus on the production of the oils (steam-distillation) and/or the extracts obtained from organic solvents (maceration, percolation and Soxhlet) extracted from the fruits, from the leaves and from the stems of the *Pterodon* plants to evaluate its pharmacological potential, phytochemical profile, biological activities and antioxidant activities [16]. However, studies involving the use of supercritical technology to obtain the extract from the fruit of *Pterodon* plants are limited. Santos et al. [17] produced nanoemulsions of extract of *Pterodon* fruits obtained via supercritical carbon dioxide (scCO₂) extraction to evaluate the Antileishmanial activity. The application of the supercritical technology to obtain the extracts through plant sources with pharmacological potentials is preferred because the extraction by applying the carbon dioxide in supercritical state is characterized by a fast and selective process, and it does not need any further separation processes [18].

The knowledge of the phase system behavior of carbon dioxide and the plant extract is important for the project and for the definition of the operating conditions of temperature and pressure on the extraction process with scCO₂. However, there are few studies which use this information to carry out experiments of extraction using pressurized fluids [19].

In this context, the objective of this work was the study of the extraction of bioactive compounds from sucupira fruits using scCO₂ as solvent. The performance of the extraction with scCO₂ was assigned considering the mass percent yield and the kinetic extraction. The chemical profiles of the extracts obtained were determined by applying gas chromatography coupled to mass spectrometer (GC-MS). The antioxidant activities of the extracts were evaluated by DPPH method. The conditions of the supercritical conditions were defined by experimental data of phase transition system CO₂ (1) + sucupira extract (2). In addition, sucupira fruit oil was obtained by hydrodistillation and the results of mass percent yield, chemical profile and antioxidant activities were compared to the extract obtained from the extraction with scCO₂. Finally, the experimental data of equilibrium phases were modeled by Peng-Robinson equation of state combined with the Wong-Sandler quadratic mixing rule. The kinetic of extraction with scCO₂ was represented by Sovová model.

3.2 Material and Methods

3.2.1 Sample preparation

The sucupira fruits were acquired in a local Market in Maringá, Brazil. The fruits were kiln-dried with closed circulation (Nova Ética 400/4ND) at a temperature of 323 K until constant mass is reached. The fruits were crushed with an average size of 2 mm and stored at temperature 277 K. The moisture content of the fruits determined by the gravimetric method was 3 %.

3.2.2 Phase equilibrium measurements

The phase equilibrium measurements of CO₂ (1) + sucupira extract (2) system were performed by applying the synthetic-static method [20-22]. The experimental apparatus consists basically of a high pressure cell with a variable volume with two sapphire windows. This cell is connected to a syringe pump (Isco 260D) and attached to

a heating jacket, in which the pressure and the temperature are monitored by a pressure transmitter (Smar LD301) and a thermocouple (PT-100), respectively.

Initially, a certain quantity of mass of the extract is introduced in the cell with variable volume by glass syringe. The mass of extract is determined using an analytical balance (Denver Instrument APX-200). After that, a pre-established amount of CO₂ is added by using the syringe pump. Since the composition is known and the temperature is fixed, the pressure of the system is increased through a piston in the inside of the cell with variable volume until a single phase is reached. After a stabilization period, about 30 min, depressurization starts (0.1 to 0.3 MPa min⁻¹) until a new phase is formed. With a visual observation of the phase transition formed, through the sapphire window, it is possible to classify it as bubble point (BP) or dew point (DP). The experiments were conducted at temperatures of 313, 323 and 333 K. The pressures of phase transitions were calculated in triplicate.

3.2.3 Extraction methods

- Hydrodistillation (HDE)

The essential oil of sucupira fruits was obtained by hydrodistillation using the Clevenger apparatus. Forty grams of sucupira crushed fruits are added in 500 mL of distilled water at normal boiling temperature. The process of extraction of hydrodistillation lasted 2 h [23]. The experiments of extraction were made in triplicate and the samples of the essential oil were dried with anhydrous sodium sulfate and stored at 255 K. The mass percent yield was expressed as a mass percent of the extracted oil in relation to the initial mass of the fruits used for extraction.

- Extraction with supercritical fluid (SFE)

The experiments of supercritical extraction were conducted in a laboratory scale unit reported in previous works [24-26]. Briefly, the unit consists in a solvent reservoir (CO₂, White Martins S.A. with purity of 99.9 %), a syringe pump (Isco 500D), two thermostatic baths (Julabo F25-ME and Quimis Q214M2) and a stainless steel extractor with 28 cm length and 2.85 cm internal diameter. The experimental procedure starts with the introduction of 20 g of sucupira fruits in the extractor. Then, CO₂ is added and the expected conditions of temperature and pressure are adjusted for one hour. The

experiments were conducted at a constant mass flow of 3 g min^{-1} . The samples of extracts were collected in five minute intervals on the first 30 minutes, and ten minutes intervals until the extraction process finished.

The extraction temperature range (303 to 333 K) were defined, initially, through values used in the literature for the extraction of bioactive compounds [27]. The conditions of extraction pressure were defined through the knowledge of phase diagram of CO_2 (1) + sucupira extract (2).

3.2.4 Antioxidant activity

The analyses of the antioxidant activities were performed through the free radical method DPPH (2, 2-diphenyl-1-picryl-hydrazyl-hydrate) described by Mensor et al. [28]. The results were expressed in inhibition percentage of free radical based on the decrease of the absorbance measured at 516 nm. The analyses were performed in triplicate.

3.2.5 Gas chromatography

The chemical profiles of the extracts were performed by GC-MS in a chromatograph (Agilent Technologies 7890A) coupled to a mass detector (Agilent Technologies 5975 C) using a column DB-5 ($30 \text{ m} \times 0.25 \text{ mm} \times 0.25 \text{ mm}$) with helium as carrier gas (1 mL min^{-1}). The temperature of the injector was 493.15 K and the of detector was 553.15 K. The Split reason was 1:5. The temperature of the oven increased from 343.15 to 553.13 K at a rate of 3 K min^{-1} and maintained constantly for 5 min. The injected volume was 1 μL in triplicate. The identification of the components was made by comparing the mass spectra of the databank of the equipment, by the Kovat index [29] and by comparing the chromatograms with the results obtained from the oilseed extract [9] and ethanolic fruit extract [10] of *Pterodon pubescens* Benth.

3.2.6 Mathematical modelling

- Phase Equilibrium

The measurements of phase transitions were conducted by using the sucupira extract obtained at 333 K and 22 MPa. The sucupira extract was considered as a pseudo-component. The extract composition was calculated by converting the percent in area of

the components with values higher than 1 % of the GC-MS analysis into mass percent. The thermophysical properties of the extracts were calculated by Kay rule [30]. The critical temperature and pressure of the pure components were calculated by Marrero-Gani method [31] and the acentric factor by the method of Constantinou et al. [32].

The thermodynamic modelling of the experimental data of vapor-liquid equilibrium was performed by using Peng-Robinson equation of state [33] combined with the Wong-Sandler mixing rule [34]. The parameters a and b are given by the Eqs. (1) and (2):

$$a = RT \frac{QD}{1-D} \quad (1)$$

$$b = \frac{Q}{1-D} \quad (2)$$

where R is the universal constant of the gases and:

$$Q = \sum_i \sum_j x_i x_j \left(b_{ij} - \frac{a_{ij}}{RT} \right) \quad (3)$$

$$D = \sum_i x_i \frac{a_i}{b_i RT} + \frac{G^E}{CRT} \quad (4)$$

where x is the molar fraction and:

$$C = -\frac{1}{\sqrt{2}} \ln(1 + \sqrt{2}) \quad (5)$$

As presented by Orbey and Sandler [35], the combination in the mixing rule was used:

$$b_{ij} - \frac{a_{ij}}{RT} = \frac{(b_i + b_j)}{2} - \frac{\sqrt{a_i a_j} (1 - K_{ij})}{RT} \quad (6)$$

The calculation of Molar excess Gibbs energy, G^E , was made using the NRTL model [36]. In this work α_{ji} was considered equal to 0.2. The parameters of binary interaction, Δg_{12} , Δg_{21} and K_{12} were optimized by the minimization of the objective function given by the Eq. (7):

$$OF = \sum_{i=1}^N (P_i^{exp} - P_i^{calc})^2 \quad (7)$$

where N is the number of experimental data and P_i^{exp} and P_i^{calc} are the experimental pressures and calculated, respectively. The stochastic simulated annealing method was used refining with the simplex method applied to the calculations of bubble point [37].

- Kinect extraction

The modeling of the experimental data of sucupira extraction was performed by applying Sovová model [38]. This model takes three periods of extraction into consideration, constant extraction rate (CER), falling extraction rate (FER) and low extraction rate (LER) which are controlled by mechanisms of diffusion and convection mass transfer. The analytical solution is given by Eqs (8-10):

For $t < t_{CER}$:

$$m(t) = \dot{m}_F S_b t [1 - \exp(-Z)] \quad (8)$$

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

$$m(t) = \dot{m}_F S_b \left\{ t - t_{CER} \exp \left[\frac{Z S_b}{W q_0} \ln \left[\frac{1}{1-r} \left(\exp \frac{W \dot{m}_F}{m_S} (t - t_{CER}) - r \right) \right] - Z \right] \right\} \quad (9)$$

For $t \geq t_{FER}$:

$$m(t) = m_S \left\{ q_0 - \frac{S_b}{W} \ln \left[1 + \left(\exp \left(\frac{W q_0}{S_b} \right) - 1 \right) \exp \left(\frac{W \dot{m}_F}{m_S} (t_{FER} - t) \right) r \right] \right\} \quad (10)$$

where

$$Z = \frac{K_{Fa} m_S \rho_F}{\dot{m}_F \rho_S} \quad (11)$$

$$W = \frac{m_S K_{Sa}}{\dot{m}_F (1 - \varepsilon)} \quad (12)$$

where $m(t)$ is the extracted mass in function with the time t , \dot{m}_F is the solvent mass flow rate, S_b is the extract solubility in the solvent, Z and W are dimensionless model parameters, q_0 is the initial oil concentration in the solid matrix, m_S is the solid mass on an oil-free basis, ρ_F and ρ_S are the fluid and solid densities respectively, K_{Fa} and K_{Sa} are the mass transfer coefficients of fluid and solid phases, respectively, ε the soil porosity and r is the easily accessible oil fraction, an adjustable parameter of the model. The ends of the first and second period, t_{CER} and t_{FER} , respectively, are given by the Eqs (13) and (14):

$$t_{CER} = \frac{(1-r)m_S q_0}{S_b Z \dot{m}_F} \quad (13)$$

$$t_{FER} = t_{CER} + \frac{m_S}{W \dot{m}_F} \ln \left[r + (1-r) \exp \left(\frac{W q_0}{S_b} \right) \right] \quad (14)$$

The parameters Z and W were calculated by minimization of the objective function given by the Equation (15) using the Downhill Simplex method:

$$OF = \sum_{j=1}^N (m_j^{calc} - m_j^{exp})^2 \quad (15)$$

where N is the number of experimental data and m_j^{calc} and m_j^{exp} are the mass calculated by Sovová method and experimental, respectively.

3.3 Results and Discussion

3.3.1 Phase Equilibrium

Table 3.1 shows the chemical components identified in the sucupira extract obtained by scCO₂ at 22 MPa and 333 K used in the phase equilibrium experiments. Only the components identified by GC-MS with a percentage above 1 % in area were considered. The mass and molar standard fractions, critical properties, acentric factor of the components identified and the pseudo-component are represented in Table 3.1.

Table 3.1. Chemical composition of the components, critical properties and acentric factor of the sucupira extract used in the measurements of phase transition.

Compound	Molar Fraction	Molar mass	T _c ^a (K)	P _c ^a (MPa)	ω ^b
α-copaene	0.027	204.35	722.19	2.04	0.29
β-cubebene	0.018	204.35	724.69	2.04	0.22
β-elemene	0.066	204.35	707.46	1.85	0.34
β-caryophyllane	0.281	204.35	753.19	2.05	0.48
α-humulene	0.034	204.35	747.52	2.04	0.58
Alloaromadendrene	0.013	204.35	744.39	2.03	0.33
γ-murolene	0.204	204.35	748.13	2.06	0.38
bicyclogermacrene	0.102	204.35	681.22	2.05	0.51
Sphatulenol	0.036	220.35	760.00	2.20	0.76
6α-acetoxycouacapan	0.053	344.49	826.35	1.54	0.41
6α,7β-dimethoxycouacapan-17-ene	0.116	344.49	838.29	1.56	0.35
6α-acetoxy-7β-hydroxycouacapan	0.019	360.49	858.61	1.56	0.79
6α,7β-diacetoxycouacapan	0.030	402.52	857.80	1.39	0.34
Sucupira extract		237.51	759.23	1.93	0.44

^aCalculated by Marrero-Gani method[31]; ^bCalculated by Constantinou et al. method [32].

Table 3.2 shows the experimental data of phase transition system CO₂ (1) + sucupira extract (2). The mass fractions of CO₂ measured ranged from 0.309 to 0.960 at temperatures of 313, 323 and 333 K. Two kinds of transition of vapor-liquid phases were observed. For mass fractions until 0.700 bubble points were observed and for mass fractions over 0.900 dew points were observed. The values of the pressures are average values of triplicate, and ranged from 8.26 to 28.72 MPa.

Table 3.3 shows the adjusted values of the parameters of binary interaction of Peng-Robinson equation with Wong-Sandler mixing rule (PR-WS) using the Eq. (7). The parameters were adjusted by using the experimental values of all the bubble points to the temperature rate measured. The value of the standard deviation calculated represents a satisfactory adjustment of the experimental data by the proposed model.

Table 3.2. Experimental values for the phase equilibrium of the system CO₂ (1) + sucupira extract (2).

w ₁	x ₁ , y ₁	Equilibrium Type	P (MPa) and T (K)		
			313 K	323 K	333 K
0.309	0.707	BP	8.26±0.04	9.70±0.01	11.26±0.04
0.400	0.783	BP	13.42±0.03	15.06±0.12	17.38±0.05
0.500	0.844	BP	18.92±0.04	21.71±0.08	23.1±0.07
0.600	0.890	BP	23.19±0.19	24.78±0.06	26.59±0.02
0.700	0.926	BP	26.33±0.05	27.51±0.10	28.72±0.06
0.900	0.980	DP	23.98±0.15	25.97±0.18	28.52±0.06
0.950	0.990	DP	14.33±0.01	17.05±0.09	19.50±0.07
0.960	0.992	DP	12.97±0.04	16.06±0.06	18.48±0.09

Table 3.3. Parameters of interaction adjusted of PR-WS model of phase equilibrium CO₂ (1) + sucupira extract (2).

T (K)	Δg_{12} (K)	Δg_{21} (K)	K_{12}	rmsd (MPa)*
313–333	1899.75	-663.19	0.2593	0.72

*root mean square deviation:
$$rmsd = \sqrt{\frac{\sum_{i=1}^N (P_i^{exp} - P_i^{calc})^2}{N}}$$

The composition-diagram of the system CO₂ (1) + sucupira extract (2) is represented in Fig. 3.1. The symbols which are not filled represent the data of phase transition. The rise in temperature results in a higher pressure of phase transition classifying the system as type I according to the classification of Scott and Konynenburg [39].

3.3.2 Yield and kinetic extraction

The temperatures of 313, 323 and 333 K are recommended by the literature [40] for the extraction of bioactive compounds. The range of pressure of extraction (10 – 22 MPa) was defined to evaluate the performance of mass percent yield and the kinetic extraction in the regions of different phases. According to Figure 3.1, at pressure 10 MPa the system is found on the biphasic region, at 22 MPa on monophasic region and at

16 MPa next to vapor-liquid transition region. The symbols filled in Figure 3.1 represent the extraction conditions.

Table 3.4 show the mass percent yield and antioxidant activities of the supercritical extractions and hydrodistillation of the sucupira fruits. The yields obtained by supercritical extraction, particularly, for the Exp. 2 (21.2 %) and 4 (20.4 %) were higher than the hydrodistillation (2.6 %). These results demonstrate the adaptation of the supercritical technology on the extraction of biocompounds in relation to the conventional technologies.

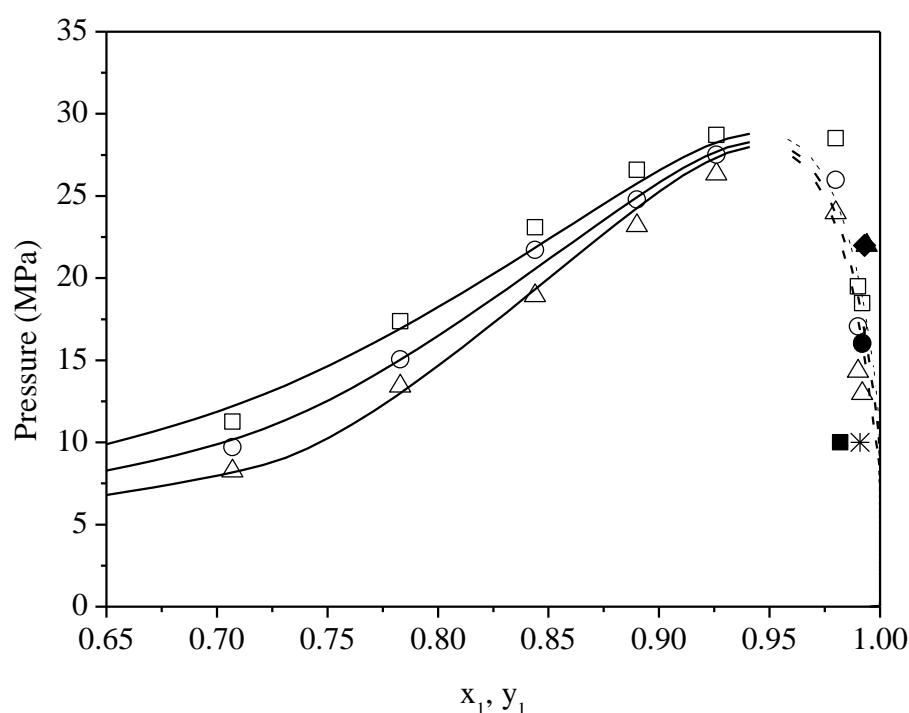


Figure 3.1. Pressure-composition diagram for CO₂ (1) + sucupira extract (2) at 313 K (Δ), 323 K (○), 333 K (□). Solid and dashed lines denote BP and DP, respectively, fitted by using by PR-WS model. Extraction conditions: Exp 1 (*); Exp 2 (▲); Exp 3 (■); Exp 4 (◆); Exp 5 (●).

Table 3.4. Mass percent yield and antioxidant activity of the supercritical extractions and by hydrodistillation of sucupira fruits.

Exp.	P (MPa)	T (K)	ρ_F (g cm ⁻³)	Y(%)	AA (%)
1 ^{br}	10	313	0.630	11.0	74.03
2 ^{mr}	22	313	0.858	21.2	77.13
3 ^{br}	10	333	0.230	1.0	75.71
4 ^{mr}	22	333	0.754	20.4	77.39
5 ^{tr}	16	323	0.721	17.6±0.7	77.59±0.12
HDE				2.6±0.8	50.26±0.44

^{br}biphasic region; ^{mr}monophasic region; ^{tr}VL transition region; Y(%): mass percent yield; AA(%):antioxidant activity; HDE; hydrodestillation.

The values of mass percent yield were higher in the extraction conditions of higher density of carbon dioxide and in the monophasic region. The solvation of scCO₂ is favored by the increase of the density [41] and decrease of the resistance of the mass transfer due to the presence of a single phase [19]. The values of the mass percent yield of the extractions performed in the biphasic are compatible to Santos et al. research [17].

The kinetic curves of supercritical extractions of sucupira were modeled by Sovová. To calculate the parameters of Sovová model, showed in Table 5, the following parameters were used: $\dot{m}_F = 3 \text{ g min}^{-1}$, $q_0 = 0.21 \text{ g}_{\text{ext}} \text{ g}_{\text{sólid}}^{-1}$, $m_s = 15.76 \text{ g}$ and $\varepsilon = 0.8125$.

Table 3.5. Adjusted parameters of Sovová model of the supercritical extractions of sucupira fruits.

Exp.	Z	W	r	t_{CER} (min)	t_{FER} (min)	K_{Fa} (min ⁻¹)	K_{Sa} (min ⁻¹)
1 ^{br}	2.21	0.03	0.59	17.7	63.4	0.097	1.1×10^{-3}
2 ^{mr}	2.80	0.73	0.59	4.3	21.2	0.092	2.6×10^{-2}
3 ^{br}	0.68	0.19	0.5	497.7	1520.0	0.063	6.9×10^{-3}
4 ^{mr}	2.80	0.29	0.59	5.8	32.4	0.107	1.1×10^{-2}
5 ^{tr}	1.04	0.13	0.59	13.9	32.2	0.041	4.6×10^{-3}

^{br}biphasic region; ^{mr}monophasic region; ^{tr}VL transition region

The mass transfer coefficients of solid-liquid phase, K_{Fa} and K_{Sa} , were calculated based on the adjustable non-dimensional parameters Z and W, respectively. The values of mass transfer coefficients, K_{Fa} and K_{Sa} , indicate predominance of the convective stage compared to the diffusive stage.

In the monophasic region the values of K_{Sa} and the extraction periods (t_{CER} , and t_{FER}) were higher and lower, respectively, in relation to the values of biphasic region. In the monophasic region the resistance of mass transfer is lower than the biphasic region.

Figure 3.2 shows the experimental and calculated data of kinetic extraction curves. The kinetic extraction curves were represented satisfactorily by Sovová model.

3.3.3 Gas chromatography

Table 3.6 shows the chemical composition of sucupira extracts obtained by supercritical extraction and by hydrodistillation. A total of 19 components were identified, divided in three classes of terpenes: sesquiterpene hydrocarbons, oxygen sesquiterpenes and vouacapanos diterpenes.

The sesquiterpene hydrocarbons were predominant on the chemical composition of the extracts regardless the extraction technique used. The vouacapan diterpenes, related to the biological activities [15, 16] of the extracts, are present only on the extracts obtained by scCO₂. The values of concentration of diterpenes obtained from the extractions conducted on the biphasic region are compatible to Santos et al. work [17].

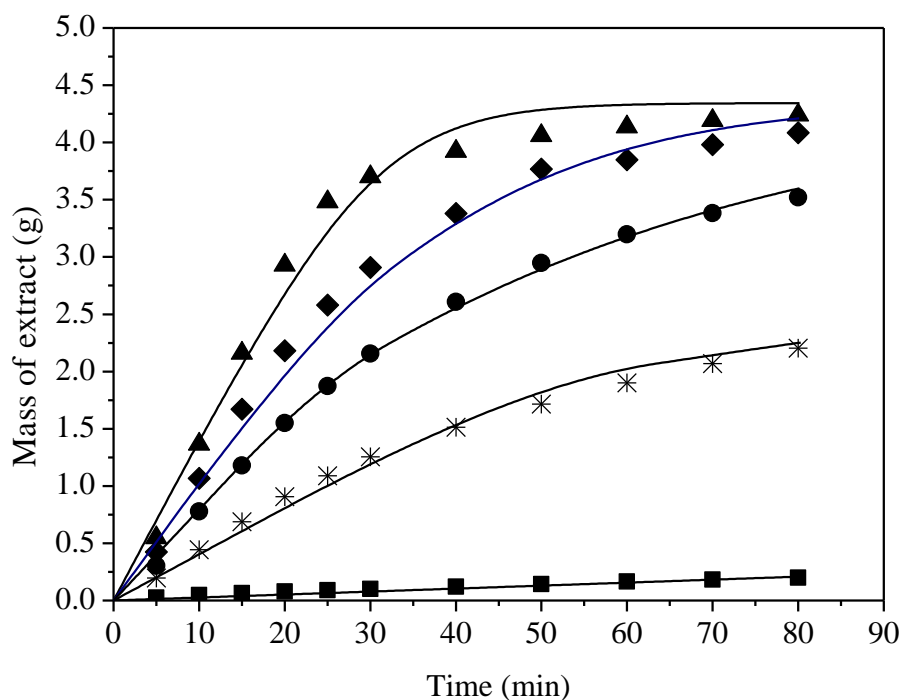


Figure 3.2. Kinetic curves of experimental and fitted extractions of sucupira: (—) Sovová model; (*) Exp 1; (▲) Exp 2; (■) Exp 3; (◆) Exp 4; (●) Exp (5).

3.3.4 Antioxidant activity

Table 3.4 shows the antioxidant activity values of the extracts obtained by scCO₂ and hydrodistillation of sucupira fruits. The extracts which have higher concentrations of vouacapan diterpenes show higher values of antioxidant activities. The hydroxyl groups present in the vouacapan diterpenes favor the capacity of free-radical inhibition

[42]. In the monophasic region and next to the phase transition the extracts obtained show higher concentrations of vouacapan diterpenes favoring the antioxidant activity.

Table 3.6. Chemical composition of extracts obtained by supercritical extractions and by hydrodistillation extractions.

Compound	RI	Experiments (% em Área)					HD
		1 ^a	2 ^b	3 ^a	4 ^b	5 ^c	
γ -elemene	1335	0.51	0.30	0.60	0.45	0.32	
α -copaene	1373	2.77	2.07	3.70	2.16	2.14	5.77
β -cubebene	1388	1.75	1.33	2.25	1.39	1.34	4.32
β -elemene	1389	5.18	3.45	8.48	5.27	4.50	5.73
β -caryophyllane	1417	35.71	25.18	35.49	22.34	26.93	40.24
α -humulene	1451	3.82	2.73	4.36	2.72	2.83	5.45
alloaromadendrene	1458	1.41	1.02	1.64	1.03	1.08	1.92
γ -muurolene	1479	22.24	15.73	24.17	16.24	16.96	15.61
bicyclogermacrene	1494	10.70	7.85	11.13	8.13	8.33	9.31
δ -cadinene	1522	0.37	0.26	0.38	0.32	0.29	0.61
sphatulenol	1573	3.62	2.85	3.03	3.10	3.15	8.85
1-hexadecanol	1874	0.34	0.36		0.40	0.36	
vouacapane	2196	0.30	0.61	0.14	0.68	0.52	
6 α -hidroxyvouacapane-7,17 β -lactone	2308		0.52		0.49	0.43	
18 α -hidroxyvouacapane7,17 β -lactone	2432		0.48		0.39	0.35	
6 α -acetoxylvouacapane	2457	1.99	7.45	0.72	7.05	5.35	
6 α ,7 β -dimetoxylvouacapan-17-ene	2512	5.59	19.78	1.56	15.54	15.86	
6 α -acetoxi-7 β -hidroxyvouacapane	2516	0.22	1.42		2.60	1.43	
6 α ,7 β -diacetoxylvouacapane	2545	1.02	5.39	0.19	4.77	4.32	
sesquiterpene hydrocarbons		84.47	59.92	92.19	60.05	64.73	88.96
Oxygenated sesquiterpenes		3.62	2.85	3.03	3.10	3.15	8.85
vouacapan diterpenos		9.12	35.66	2.62	31.52	28.27	0
Other		0.34	0.36	0.00	0.40	0.36	0
Total identified		97.54	98.79	97.84	95.06	96.50	97.81

^abiphasic region; ^b monophasic region; ^cVL transition region

3.4 Conclusion

The extraction of chemical components from the sucupira fruit of highest biological activities using scCO₂ were higher than hydrodistillation, shown higher effectiveness on the extraction of bioactive compounds. The knowledge of the phase diagram enabled a better definition of the supercritical extraction conditions resulting in higher mass percent yield compared to the available data in the literature. The supercritical extractions conducted in the monophasic region resulted in higher vouacapan diterpenes concentration, and, consequently more antioxidant activities. The values of mass transfer coefficients calculated confirm the information found in the phase diagram generated. The experimental data of phase transition were only bubble and dew point types. The equation of state of Peng-Robinson with the Wong-Sandler quadratic mixing rule and the Sovová model represented satisfactorily the experimental data.

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4. CAPÍTULO II

Extraction of leaves of *Duguetia Furfuracea* (Annonaceae) using Green and Organic Solvents

(Brazilian Journal of Chemical Engineering)

Abstract

Duguetia furfuracea (Annonaceae) is a shrub found in the Brazilian Cerrado and it is used in popular medicine as antirheumatic, wound healing, treatment for renal colic and against pediculosis. The aim of this study was to analyze the extraction of leaves of *Duguetia furfuracea*, using supercritical CO₂, ethanol or hexane as solvents. For the supercritical extraction, an experimental planning 2³ with triplicates of central point was used to evaluate the effects of temperature (313-333 K), pressure (15-23 MPa) and volumetric flow rate (3-6 mL min⁻¹) in the extraction yield, total phenolic content and antioxidant activity and compared to the extracts obtained by Soxhlet extractor. The effects of the variables in the extraction yield were positive and the pressure had greater influence. However, the highest performances, total phenolic content and antioxidant activities were obtained by the Soxhlet method. The mathematical model based on the Brunauer–Emmett–Teller theory of adsorption correlated satisfactorily the experimental values of the supercritical extraction.

Key words: *Duguetia furfuracea*, supercritical CO₂, Total phenols, Antioxidant activity.

4.1 Introduction

The Cerrado, a term commonly used to describe the group of ecosystems (savannas, forests, veredas and gallery forests) of the plateau in the center of Brazil [1,2]. It is the second largest Brazilian biome occupying 23 % of the national territory [3]. The Cerrado is the most diverse tropical savanna in the world, totaling more than 12,600 species (4,215 Brazilian endemics) [4]. Despite this, about half of the 2 million km² of Cerrado were turned into planted pastures, annual crops and other uses [5,6] and then considered one of the world's biodiversity hotspots [7,8]. Due to the high deforestation, large taxonomic diversity and its flora still understudied, it needs more research with native species to conservation of natural resources and phytotherapeutics resources offered by medicinal plants [9].

The *Duguetia furfuracea* (A. St.-Hil.) Benth and Hook. f. species belongs to the Annonaceae family, and it is a shrub found in the Cerrado and considered a weed by farmers for invading pastures [10]. Popularly known as “sofre-do-rim-quem-quer,” “araticum-bravo,” “araticum-do-campo,” “araticum-do-cerrado”, “ata-brava,” e “ata de lobo” [11,12]. It is used in traditional medicine as antirheumatic, wound healing, treatment in pain kidney and against pediculosis [13].

The phytochemical analysis of the essential oil from leaves and branches of *D. furfuracea* revealed the presence of sesquiterpenes [14], flavonoids and several alkaloids [15,16]. The alkaloid extracts obtained from the tree barks of the underground stems have antitumor, trypanocidal and leishmanicide activities [17]. The ethanol extract shows cytoprotective activity to the bacterium *E. coli* against to the mercuric chloride indicating that *D. furfuracea* is a promising source of cytoprotectors to combat heavy metals [18]. Hexane and ethanol extracts of *D. furfuracea* have high larvicidal activity against larvae of *Aedes aegypti* [19].

In this context, the aim of this study was to analyze the extraction of leaves of the *Duguetia furfuracea* using supercritical CO₂, ethanol and hexane as solvents. The effects of temperature, pressure and volumetric flow rate of CO₂ were evaluated in the kinetics and the performance of supercritical extraction. The extracts were analyzed and compared to extracts obtained by Soxhlet using ethanol and hexane as solvents in terms of extraction efficiency, total phenolic content, antioxidant activity, and chemical composition.

4.2 Material and Methods

4.2.1 Raw material

Samples of *Duguetia furfuracea* leaves were collected in the morning in April 2015 at the University of Rio Verde located in Rio Verde in the southwestern state of Goias, Brazil. The leaves were dried in a drying oven with forced circulation (Nova Etica 400 / 4ND) at 40 ° C for 24 hours. Then the sample were crushed in a knife mill (Solab SL-30) and then sieved through a set of sieves W.S Tyler (Mentor, OH, USA). For the extraction it was used the sample with a grain size between 20 and 42 mesh.

4.2.2 Extraction Methods

- Soxhlet extraction

The extraction with organic solvent were conducted according to the AOAC method [20] using Soxhlet extractor with ethanol (Nuclear, minimum purity 99.8 %) and hexane solvents (Nuclear, minimum purity 99.6 %). About 5 g of the crushed leaves of *Duguetia furfuracea* were put on a filter paper cartridge and inserted into the extractor coupled to a flask containing 150 mL of solvent recycling over the sample. The sample was subjected to extraction for a period of 6 hours. The extractions were performed in triplicate and the results were expressed as the average \pm standard deviation.

- Supercritical fluid extraction (SFE)

The extraction experiments with supercritical fluid were conducted on a laboratory scale unit as it may be seen in earlier studies [21,23]. The unit consists of a reservoir of solvent (CO₂, White Martins SA, with 99.9 % purity), one syringe pump (Isco model 500D), two thermostatic baths (Julabo F25-ME and Quimis Q214M2) and a stainless steel extractor 17 cm long and internal diameter of 2.85 cm. The experimental procedure consisted of introducing about 30 g samples of *Duguetia furfuracea* leaves inside the extractor, and then selecting the temperature and pressure desired for the experiment. After 30 min, time to stabilize the system, it is necessary to adjust the flow rate through the micrometric valve at the bottom of the extractor and measured the extract weight every 10 min for a period of 3 hours.

4.2.3 Experimental design

A complete factorial planning 2^3 with triplicate of the central point was used to evaluate the influence of pressure (P), temperature (T) and volumetric flow rate (Q) of CO₂ in the extraction performance (Y). The encoded and uncoded values used are shown in Table 4.1.

Table 4.1. Operating conditions used in the extraction with supercritical CO₂.

Variables	-1	0	1
Pressure (MPa)	15	19	23
Temperature (K)	313	323	333
Flow rate (mL min ⁻¹)	3.0	4.5	6.0

The analysis of variance was performed using the Design-Expert software (Stat-Ease, Inc., Minneapolis, USA) by response surface methodology considering 95% of confidence by F-Test. The general model used for variable performance response is the polynomial given by Eq. (4.1):

$$Y = b_0 + \sum_{i=1}^3 b_i X_i + \sum_{i,j=1;i < j}^3 b_{ij} X_i X_j \quad (4.1)$$

where b_0 is the constant fixing the performance at the central point of the experiment, b_i are the regression coefficients for linear effects, b_{ij} are the coefficients of the effects of interactions, X_i and X_j are the independent variables normalized.

4.2.4 Total phenolic content (TPC)

The determination of total phenolic content of *Duguetia furfuracea* extracts was performed by spectroscopy using the Folin-Ciocalteu method with modifications according to the methodology of Singleton and Rossi [24]. For analysis, the solutions were prepared by dissolving 0.1 g of extract in 10 mL of methanol, and then collected an aliquot of 0.1 mL of this solution and added to 7.4 mL of distilled water and 0.5 mL of Folin-Ciocalteu reagent. After three minutes, 2 ml of 15 % aqueous solution of sodium carbonate was added and the solution left standing for 2 hours away from light, and then measured the absorbance at 760 nm. The total phenolic content was determined through a calibration curve constructed with gallic acid used as standard and expressed in mg of GAE (gallic acid equivalents)/g extract.

4.2.5 Antioxidant activity

The determination of antioxidant activity was performed by ABTS method (2,2-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) with some modifications according to Re et al. [25]. The ABTS⁺ radical is prepared from the reaction of 5 ml of 7 mM ABTS aqueous solution with 88 μ L of 140 mM potassium persulphate solution. The mixture was kept in the dark for 16 hours. Then, it was diluted 1 ml of this mixture in ethyl alcohol until the measurement of absorbance at 734 nm was 0.70 (\pm 0.05). With three different dilutions of the extract, blends a 30 μ L aliquot of these aliquots with 3 ml of ABTS⁺ and its absorbance measured 6 minutes after mixing. The total antioxidant activity was determined by a standard curve of Trolox and its value expressed in μ M TEAC (Trolox equivalent antioxidant activity)/g extract.

4.2.6 Gas chromatography

The analysis of the chemical composition of the extracts was performed on a gas chromatograph (Agilent Technologies, Model 7890A) coupled to a mass detector (Agilent Technologies, Model 5975 C). The capillary column used was a VF-WAXMS 30 m x 0.25 mm x 0.25 μ m. The initial column temperature was 55 $^{\circ}$ C rising 15 $^{\circ}$ C/min to 140 $^{\circ}$ C, then 10 $^{\circ}$ C/min to 180 $^{\circ}$ C maintaining this for 2 min and 5 $^{\circ}$ C/min to 240 $^{\circ}$ C keeping this temperature for 8 min. Helium was used as carrier gas with a flow rate of 1 ml/min. The temperatures were 280 $^{\circ}$ C in the injector, and 280 $^{\circ}$ C and 200 $^{\circ}$ C in the ion source and interface, respectively. The components identification was performed by comparing their mass spectra with those from the National Institute of Standards and Technology and Kovat's indices [26].

4.2.7 Modeling of the experimental data

A model described by Pardo-Castaño et al. [27] was used to correlate the experimental values of the supercritical extraction. The model is based on the absorption theory of Brunauer-Emmett-Teller and it expresses the extraction yield (Y) as a function of time (t) with three parameters; y^* , extract solubility in the SFE, K , the relationship between the solute adsorption equilibrium constant of the first monolayer and the subsequent layers and x_m , the ratio of the mass of solute present in the first monolayer and the initial mass of solute that can be derived according to the Eq. (4.2):

$$t = \frac{m_0}{2\dot{m}_F y^*} \left\{ x'_0 - x' + (2 - K) \left[Y - x_m \ln \left(\frac{\alpha}{\beta} \right) \right] + K x_m \ln \left[\frac{\alpha'}{\beta' (1 - Y)^2} \right] \right\} \quad (4.2)$$

where:

$$x'_0 = \sqrt{a + b + c} \quad (4.3)$$

$$x' = \sqrt{a(1 - Y)^2 + b(1 - Y) + c} \quad (4.4)$$

$$a = K^2 \quad (4.5)$$

$$b = 2(2 - K)Kx_m \quad (4.6)$$

$$c = (Kx_m)^2 \quad (4.7)$$

$$\alpha = x' + (2 - K)(1 + Y) + Kx_m \quad (4.8)$$

$$\alpha' = x' + (2 - K)(1 - Y) + Kx_m \quad (4.9)$$

$$\beta = x'_0 + K + (2 - K)x_m \quad (4.10)$$

$$\beta' = x'_0 + (2 - K) + Kx_m \quad (4.11)$$

$$y^* = y_{sat} \left[1 - \exp \left(-\frac{KL}{u\varepsilon} \right) \right] \quad (4.12)$$

where m_0 is the extractable initial mass of the solute in the packed bed, \dot{m}_f is the mass flow of CO₂, L is the extractor length, u is the speed of CO₂, y_{sat} is the solute mass fraction in a saturated SCF phase and ε the porosity of the bed.

The objective function, defined as the average absolute relative deviation (AARD), used is expressed by the Eq. (4.13):

$$\text{AARD} = \frac{100}{N} \sum_{t=1}^N \left(\frac{t_{t,\text{exp}} - t_{t,\text{cal}}}{t_{t,\text{exp}}} \right) \quad (4.13)$$

where n is the number of experimental values, $t_{t,\text{exp}}$ and $t_{t,\text{cal}}$ are experimental times and calculated by the model, respectively.

4.3 Results and Discussion

4.3.1 Extraction yield

Table 4.2 shows the experimental conditions for the extraction, extraction yield, the antioxidant activities and the total phenolic content of *Duguetia furfuracea* extracts obtained by supercritical CO₂ and Soxhlet extractions with the organic solvents hexane and ethanol.

Table 4.2. Yields, total phenolic compounds and antioxidant activity of supercritical and Soxhlet extractions of leaves of *Duguetia furfuracea*.

Exp.	P (MPa)	T (K)	Q (mL min ⁻¹)	Y (%)	TPC (mg GAE g ⁻¹ extract)	TEAC (μmol Trolox g ⁻¹ extract)
1	15	313	3	0.51	53 ± 3 ^b	166 ± 2 ^b
2	23	313	3	0.89	42 ± 13 ^b	130 ± 1 ^b
3	15	333	3	0.45	30 ± 3 ^b	67 ± 0.5 ^b
4	23	333	3	0.97	63 ± 120 ^b	170 ± 1 ^b
5	15	313	6	0.60	28 ± 4 ^b	98 ± 1 ^b
6	23	313	6	0.92	44 ± 1 ^b	102 ± 0.3 ^b
7	15	333	6	0.61	32 ± 6 ^b	96 ± 0.4 ^b
8	23	333	6	1.21	38 ± 4 ^b	97 ± 0.3 ^b
9*	19	323	4.5	1 ± 0.01 ^c	31 ± 10 ^b	90 ± 18 ^b
10*	Hexane			2 ± 0.2 ^b	210 ± 32 ^a	531 ± 123 ^a
11*	Ethanol			9 ± 0.7 ^a	253 ± 45 ^a	606 ± 168 ^a

Different letters indicate statistically significant difference at 5 % of significance.

The analysis of variance was used to check the influence of the variables temperature, pressure and volume flow rate in the performance of the supercritical extraction at a level of 95 % of confidence and to adjust the polynomial regression model as is shown by Eq. (1). The results of the regression model in terms of encoded levels were significant for the F-Test, which are given by Eq. (4.14):

$$Y = 0.77 + 0.23P + 0.041T + 0.066Q + 0.053PT + 0.035TQ \quad (14)$$

The significant effects ($p < 0.05$) were the pressure, temperature, and volumetric flow rate of CO_2 and the interactions pressure-temperature, and temperature-flow rate. The effect of the pressure interaction with the flow was not significant ($p > 0.05$).

The adjusted regression coefficient was 98.6 % indicating that the proposed model and the experimental values have good agreement. Figure 4.1 shows the response surface of the extraction efficiency as a function of temperature and pressure with a constant volumetric flow rate of 6.0 mL min^{-1} .

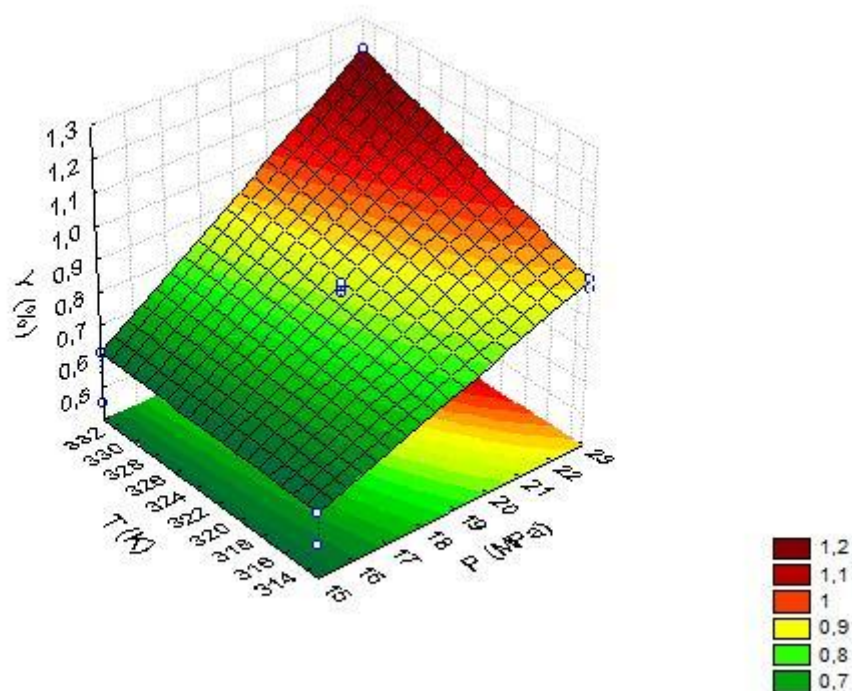


Figure 4.1. Influence of temperature and pressure on the SFE yield at constant solvent flow rate ($Q_{\text{CO}_2} = 6.0 \text{ mL min}^{-1}$)

The positive pressure effect can be seen in both Figure 4.1 and Table 2 for experiments 5 and 6, which the yields were 0.60 and 0.92 %, respectively. This effect

can be explained by an increase of CO₂ density and consequently the solvating power of the solvent [28] promoted by an increasing in the pressure at constant temperature. The positive effect of temperature observed in the experiments 6 and 8 can be explained by the fact that at higher temperatures, the solvent viscosity is lower and greater is the vapor pressure of the extract, and consequently there is an increasing in the solvent diffusivity [29]. While the pressure and temperature affect the thermodynamic properties, such as density and solubility, and transport diffusivity, the flow rate increases the convective mass transfer coefficient and decreases the concentration of the solute in the bulk of the supercritical phase, thus obtaining a positive effect on the extraction yield [30]. The extraction by Soxhlet showed the highest results, with significant difference at 95 % of confidence level. The highest yield was obtained by ethanol, which is a polar solvent. This behavior can be attributed to the higher temperature of the recirculating solvent and solute-solvent interactions [31].

4.3.2. Modeling of the experimental data

Table 3 shows the values of the parameters y^* , K and x_m adjusted to the experimental values. According to Pardo-Castaño et al. [27] a single value of x_m encompasses all the experimental conditions studied due to its weak dependence on these conditions. This occurs because this property is dependent on the solid-solute interaction. The values of y^* are associated with solubility and can be noted that higher values 8.04 and 5.48 for the experiments 6 and 2, respectively, are obtained for the experimental conditions of higher pressure, 23 MPa, and less temperature, 313 K, consequently higher density of CO₂.

Table 4.3. Adjustable parameters for the Pardo-Castaño et al. model.

Exp.	$y^* \times 10^3$	K	x_m
1	1.60	66.7	0.591
2	5.48	31.9	0.591
3	1.85	198	0.591
4	2.09	2.05	0.591
5	1.53	100	0.591
6	8.04	90.1	0.591

Figure 4.2 shows the correlation of experimental values and the adjusted model according to Pardo-Castaño et al. [27].

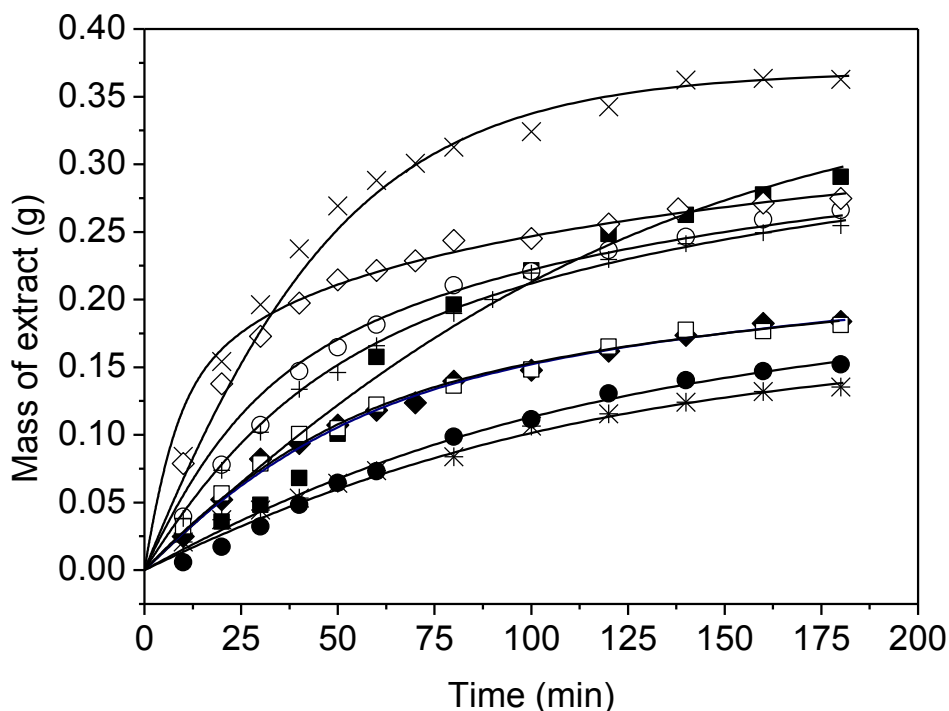


Figure 4.2. Calculated and experimental kinetic curves of extraction from leaves of *Duguetia furfuracea*: (—) model proposed by Pardo-Castaño et al. [27]; (•) Exp 1; (○) Exp 2; (□) Exp 3; (■) Exp 4; (◻) Exp (5); (◇) Exp 6; (◐) Exp 7; (×) Exp 8, (◻) Exp 9.

4.3.3 Gas chromatography

Table 4.4 shows the chemical composition of *Duguetia furfuracea* extract obtained by the SFE and Soxhlet with ethanol and hexane by GC-MS. In the composition of the extract obtained by SFE prevail the sesquiterpenes (+)- spathulenol and β -caryophyllene oxide with 39.53 and 11.68 % of relative area, respectively. For the ethanol extract, the main component was the 3-Deoxyestradiol with 28.41 % of relative area.

In the three extracts, it is important to emphasize the presence of spathulenol, a component that has been shown multiple biological activities, including, antimicrobial, insecticidal and cytotoxic [32]. This component also predominates in the chemical composition of the essential oil of *Duguetia furfuracea* leaves, according to Valter et al. [33].

Table 4.4. Chemical composition of supercritical (SFE) and Soxhlet extracts of leaves of *Duguetia furfuracea*.

Compound	Relative Area %		
	SFE*	Ethanol	Hexane
Alloaromadendrene oxide-1	2.33		
β -Caryophyllene oxide	11.68	5.18	4.95
(+)-Spathulenol	39.53	20.35	13.80
Ethyl palmitate		3.61	
Spathulenol	1.64		
(-)-Caryophyllene oxide	3.08		
Methyl elaidate	2.19	10.47	2.63
Aromadendrene oxide-2	3.63		
Alloaromadendrene oxide-2	2.34		
(-)-Spathulenol	2.53		
Isoaromadendrene epoxide	3.22		
2-Metilenocolestan-3-ol	3.15	5.33	
α -Tocoferol		2.87	
Palmitic acid	6.32		
3-Deoxyestradiol		28.41	7.12
2-Metilhexadecan-1-ol	3.55		
Not identified	14.81	23.79	71.54

* Average value of three extractions at the center point (19 MPa, 323 K and 4.5 mL min⁻¹).

4.3.4 Total phenolic content (TPC)

The values of total phenolic content of SFE and Soxhlet extracts are shown in the Table 4.2. In the supercritical extraction model, the results presented no significant difference, showing no dependence of the total phenolic content with pressure, temperature and volumetric flow. The values of TPC ranged from 28.08 to 62.91 mg GAE g⁻¹ of extract and showed no statistical difference at 5% significance level. The TPC values for ethanol and hexane extracts differed significantly at 5 % of the extracts obtained by SFE, and presented values of 253.14 and 209.56 mg GAE g⁻¹ of extract, respectively. The TPC of the ethanol extract agrees with the results of other species of the Annonaceae family, such as the methanol extract of the twigs of the species *Duguetia riparia* [34] and the ethanol extract of *Annona cherimoya* Mill. [35].

4.3.5 Antioxidant activity

Table 4.2 shows the values of the antioxidant activity of *Duguetia furfuracea* extracts performed by the ABTS method. The highest values obtained were 605.99 and 530.92 $\mu\text{M Trolox g}^{-1}$ extract for ethanol and hexane extracts, respectively. For the supercritical extraction the antioxidant activity values were not significantly different, showing no dependence of the antioxidant activity with pressure, temperature and volumetric flow. The ABTS values for the extractions ranged from 67.47 to 169.54 $\mu\text{mol Trolox g}^{-1}$ of extract. As expected, the extracts with higher total phenolic content showed the highest antioxidant activity. Pereira et al. [36] also obtained higher values of ABTS for ethanolic extract of *Myrtus communis L.* in comparison with the supercritical extract.

4.4 Conclusion

The best extraction yield result with supercritical CO_2 of *Duguetia furfuracea* was 1.21 %, with the highest pressure (23 MPa), highest temperature (333 K), and highest volume flow rate (6 ml min^{-1}). The values of yield were adjusted to a polynomial model with all the positive effects of variables, so that the pressure was the variable that had the greatest effect. The regression coefficient of 98.6 % indicates that the polynomial model can be used under the evaluated conditions. The total phenolic content and antioxidant activity showed no significant difference between the experiments with SFE, however showed higher values for Soxhlet extraction, and ethanol was the solvent that showed the highest results for TPC and ABTS. The chemical profile of the extracts obtained showed high levels of spathulenol, especially for SFE. The mathematical simple model proposed by Pardo-Castaño et al. correlated satisfactorily the experimental data with the calculated values.

4.5 References

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5. CONCLUSÃO GERAL

A extração dos componentes químicos de maiores atividades biológicas de frutos de sucupira empregado scCO₂ foram superiores a hidrodestilação comprovando a maior efetividade na extração de compostos bioativos. O conhecimento do diagrama de fases possibilitou melhor definição das condições de extração supercrítica, sendo a condição de 22 MPa e 313 K resultando em maiores rendimentos mássicos, concentração de diterpenos vouacapanos e alta atividade antioxidante. Para a extração supercrítica de folhas de *Duguetia furfuracea* o rendimento da extração obteve o melhor resultado nas condições com a maior pressão (23MPa), maior temperatura (333 K) e maior vazão volumétrica (6 mL min⁻¹). Os valores dos rendimentos foram ajustados ao modelo polinomial com todos os efeitos das variáveis positivos, sendo a pressão a variável que apresentou maior efeito. O teor de fenóis totais e das atividades antioxidantes não apresentaram diferença significativas entre os experimentos com FSC, entretanto apresentaram maiores valores para a extração por Soxhlet, sendo o etanol o solvente que apresentou os maiores resultados. As composições químicas dos extratos de sucupira e de *D. furfuracea* obtidos utilizando diferentes métodos apresentaram diferenças. A equação de estado de Peng-Robinson com a regra de mistura quadrática de Wong-Sandler, o modelo de Sovová e o modelo de Pardo-Castaño et al. representaram adequadamente os valores experimentais do extrato de sucupira e de *D. furfuracea*.