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EXTRAÇÃO, IDENTIFICAÇÃO E POTENCIALIDADES FARMACÊUTICAS DE COMPOSTOS OBTIDOS DA FIBRA PRENSADA DA PALMA (*Elaeis guineensis* Jacq)

Santa Maria, RS
2016

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Tese apresentada ao Programa de Pós-Graduação em Ciências Farmacêuticas, Área de Concentração em Controle e Avaliação de Insumos e Produtos Farmacêuticos, da Universidade Federal de Santa Maria (UFSM, RS), como requisito parcial para obtenção do grau de **Doutor em Ciências Farmacêuticas.**

Orientador: Prof. Dr. Marcelo Barcellos da Rosa

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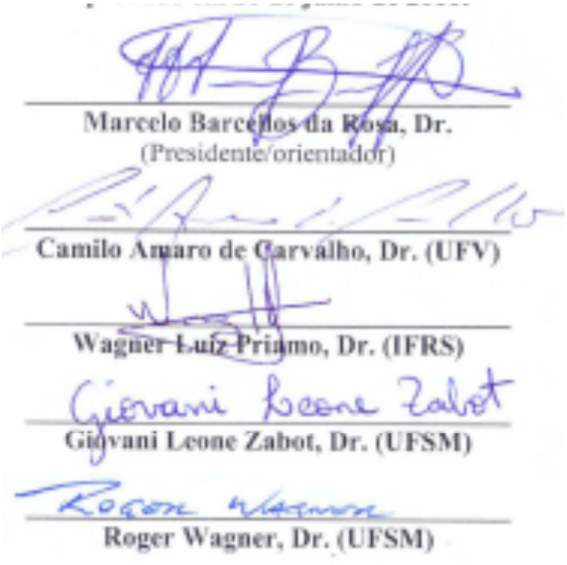

Santa Maria, RS
2016

DEDICATÓRIA

Aos meus pais, Emilia e Aristides, que são meu alicerce. Além de todo amor e ensinamentos me proporcionaram educação, permitindo que eu chegasse até aqui.

Ao meu esposo, Marcio, sem o seu apoio, amor e incentivo não teria realizado este sonho.

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RESUMO

EXTRAÇÃO, IDENTIFICAÇÃO E POTENCIALIDADES FARMACÊUTICAS DE COMPOSTOS OBTIDOS DA FIBRA PRENSADA DA PALMA (*Elaeis guineensis* Jacq.)

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ORIENTADOR: Marcelo Barcellos da Rosa

O aumento na demanda do óleo de palma gera grande quantidade de co-produtos resultantes do processo de extração. Esses co-produtos podem ser alvos de investigação da presença de compostos bioativos, uma vez que não são empregados solventes químicos no processo de extração. Além disso, somente se extrai os componentes oleosos do fruto e da semente, havendo grande quantidade de compostos retidos na fibra e semente prensada da palma e, provavelmente, uma quantidade de óleo residual. Nesse sentido, o objetivo desse trabalho foi avaliar a eficiência de diferentes tipos de extração na caracterização química e atividade biológica de compostos bioativos da fibra e semente prensada de *Elaeis guineensis* Jacq. (palma), bem como otimizar o desenvolvimento de nanoemulsões contendo óleo de palma. Avaliou-se a extração de compostos bioativos da semente prensada da palma utilizando-se CO_2 supercrítico e GLP pressurizado. Este solvente mostrou-se mais efetivo para extração do óleo da semente de palma, uma vez que o tempo gasto no processo foi cerca de 3-4 minutos, enquanto que com CO_2 supercrítico em torno de 75 minutos. Além disso, apresentou maior rendimento de extração e as amostras foram extraídas menor temperatura. A composição química dos extratos apresentou basicamente ácidos graxos (18), em sua maioria ácido láurico, mirístico, oleico, os quais foram encontrados em todas as amostras. Em relação a extração de compostos bioativos da fibra prensada de palma utilizando CO_2 supercrítico e GLP pressurizado, verificou-se que a primeira proporcionou maior rendimento de extração, a 60 °C e 25 MPa, bem como maiores atividades antioxidantes e fator de proteção solar. A composição química para ambos solventes, foi ácido láurico, oleico e palmítico, que juntos representam 80% da constituição química das amostras. Além desses, identificou-se compostos como α-tocoferol, esqualeno, β-sitosterol e carotenoides totais. Na otimização da extração de compostos antioxidantes e fotoprotetores da fibra prensada de palma com etanol utilizando-se sonda de ultrassom, a condição que apresentou maior rendimento (3,24%) foi 120 $W.cm^{-2}$ de intensidade e pulso de 0.4. Obteve-se compostos como ácidos graxos, β-sitosterol, α-tocoferol, esqualeno e fenólicos totais. Além disso, o extrato apresentou atividade antioxidante frente aos radicais sintéticos (DDPH e ABTS) e biológico hidroxil (•OH) e um fator de proteção solar de 15. O óleo de palma foi incorporado em nanoemulsões que demonstraram ser estáveis em diferentes condições de armazenamento (4°C, 25°C e 40°C). A melhor condição foi obtida pela combinação de etapa de pré-formulação utilizando-se ultra turrax e após processador de ultrassom de alta intensidade em uma amplitude de 200 $W.cm^{-2}$ por 15 minutos. A concentração de óleo incorporada foi de 2,8 % bem como de surfactantes (mistura de span 80 e tween 80). Nesse sentido, verificou-se a possibilidade deste óleo ser incorporado em formulações cosméticas, potencializando o fator de proteção solar bem como ser utilizado como antioxidante tanto em produtos cosméticos quanto produtos alimentícios. Em um contexto geral este estudo mostrou uma forma sustentável de aproveitamento da grande quantidade de resíduos gerados pela indústria de processamento de palma, que muitas vezes é desperdiçada.

Palavras-chave:*Elaeis guineensis* Jacq. CO_2 supercrítico. GLP comprimido. Ultrassom. Nanoemulsões. Atividade antioxidante. Atividade fotoprotetora.

ABSTRACT

EXTRACTION, IDENTIFICATION AND POTENTIALS PHARMACEUTICAL COMPOUNDS OBTAINED FROM FIBER PRESSED PALM (*Elaeis guineensis* Jacq)

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ADVISOR: Marcelo Barcellos da Rosa

The increase in palm oil demand in the world market generates a greater amount of co-products resulting from the extraction process. These by-products are being investigated for the presence of bioactive compounds, since they are free of chemical solvents. Furthermore, only the oily components were extracted from the fruit and seed, there is a large quantity of compounds trapped in the palm pressed fiber and palm kernel cake and possibly an amount of residual oil. In this sense, the objective of this study was to evaluate the efficiency of different types of extraction in chemical and biological activity of bioactive compounds of fiber and seed pressed of *Elaeis guineensis* Jacq. (Palm), as well as optimizing the development of nanoemulsions containing palm oil. We evaluated the extraction of bioactive compounds of the pressed palm kernel using supercritical CO_2 and pressurized LPG. The LPG was more effective in palm kernel oil extraction, since the time spent in the process was about 3-4 minutes, while with supercritical CO_2 was about 75 minutes. Extraction yield using LPG was higher than CO_2 at a lower temperature. The chemical composition of the extracts presented primarily fatty acids (18), mostly lauric acid, myristic, oleic, which were found in all samples. Regarding the extraction of bioactive compounds from the palm pressed fiber using supercritical $CO_2$and pressurized LPG, it was found that using CO_2 we obtained the highest extraction yield at 60 °C and 25 MPa, as well as the highest antioxidant activity and sun protection factor. The chemical composition for both solvents was lauric acid, oleic and palmitic acid, which together represent 80% of the chemical composition of the samples. Also it was identified compounds such as α-tocopherol, squalene, β-sitosterol and total carotenoids. Optimization of the extraction of antioxidants and fiber photoprotective palm pressed with ethanol using ultrasound probe, the condition with the highest yield (3.24%) was at ultrasound intensity of 120 $W.cm^{-2}$ and pulse 0.4. Compounds such as fatty acids, β-sitosterol, α-tocopherol, squalene and phenolic were identified. The extract presented antioxidant activity towards synthetic radicals (DDPH and ABTS) and organic hydroxyl (•OH), and a sun protection factor of 15.01. Palm oil was incorporated in nanoemulsions, being stable at different conditions of storage (4°C, 25°C and 40°C). The best operational condition to obtain the nanoemulsion was combining pre-formulation step using the ultra turrax and after high intensity ultrasonic processor at 200 $W.cm^{-2}$ for 15 minutes. The oil and surfactants concentrations were 2.8 wt % and the HLB 6.5 (mixture of Span 80 and Tween 80). The results demonstrated the possibility to incorporate the palm oil in cosmetic formulations to increase the sun protection factor as well as be used both as an antioxidant in cosmetic and/or food products. The use of palm processing residues to obtain bioactive compounds is a sustainable way to obtain value-added products.

Keywords: *Elaeis guineensis* Jacq. Supercritical CO_2. Compressed LPG. Ultrasound. Nanoemulsion. Antioxidant activity. Photoprotective activity.

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LISTA DE ABREVIATURAS E SIGLAS

ABTS- 2,2´-azinobis 3-etilbenzotiazolina-6-ácido sulfônico

CO_2 - Dióxido de carbono

DPPH - 2,2-difenil-1-picril-hidrazila

ESC - Extração supercrítica

FPS- Fator de fotoproteção solar

GC/MS - Cromatografia gasosa acoplada à espectrometria de massas

GC-FID- Cromatografia gasosa equipada com detector de ionização em chama

GLP- Gás liquefeito de petróleo

LPG- *Liquefied petroleum gas*

M_{CER} -*Maximum extraction rate*

HPLC - Cromatografia líquida de alta eficiência

MPa- Mega Pascal

$O_2^{\bullet-}$ - Ânion radical superóxido

$OH^{\bullet}$ - Radical hidroxila

T_{CER-} *Constant extraction period*

UAE- *Ultrassound assisted extraction*

US - Ultrassom

UV-VIS - Ultravioleta visível

$Y_{CER\ -}$ *Mass ratio of solute in the fluid phase at the extractor outlet for the CER period*

SUMÁRIO

1 INTRODUÇÃO

Os benefícios fisiológicos, nutricionais e medicinais à saúde humana atribuídos ao uso de produtos naturais têm motivado o consumo desses em detrimento dos produtos sintéticos. Aliado a isso, soma-se o interesse em diferentes setores, como a indústria farmacêutica, cosmética e de alimentos pela busca de produtos biotecnológicos. Como consequência, gera-se um avanço na fronteira do conhecimento a respeito da utilização desses produtos (CAVALCANTI, 2013).

Elaeis guineensis Jacq. é popularmente conhecida como dendezeiro, palma ou palmeira e o seu fruto como dendê. Dos frutos da palmeira oleaginosa se obtém dois tipos de óleo: o óleo de palma (extraído da polpa) e óleo de palmiste (extraído da amêndoa). Ambos apresentam composições químicas e características físicas diferentes, sendo utilizados na indústria de cosméticos e alimentos (OBAHIAGBON, 2012; MBA et al., 2015). O óleo de palma é obtido por extração mecânica (prensagem) do mesocarpo e epicarpo dos frutos da palma. Após a extração, o subproduto remanescente conhecido como fibra prensada da palma contém cerca de 5-6% de óleo residual e constituintes como carotenoides, tocoferóis, tocotrienóis, fitosteróis e compostos fenólicos (LAU et al., 2006; NEO et al., 2008; TEIXEIRA et al., 2013).

A produção total de óleo de palma no Brasil é de 416.000 ton/ano, o que leva a produção de 318.000 ton/ano de fibra prensada de palma e 15.000 ton/ano de óleo residual. Atualmente, a fibra prensada tem sido usada na nutrição animal e para queima em caldeiras (BRASIL, 2014). Tendo em vista que a extração do óleo é feita sem o uso de solventes químicos, o subproduto está livre de resíduos orgânicos, tornando-se uma opção para obtenção de compostos bioativos com interesse farmacológico. Dessa forma, a utilização da fibra prensada para a extração e identificação de moléculas bioativas com capacidade antioxidante é uma alternativa sustentável para valorização deste subproduto (RAHMAN et al., 2012; TEIXEIRA et al., 2013)

A aplicação da tecnologia supercrítica, utilizando dióxido de carbono (CO_2) como solvente, é uma alternativa eficiente para extração de compostos bioativos. Por se tratar de um processo livre de resíduos tóxicos e conduzidos em baixas temperaturas, não provoca a degradação térmica dos extratos. Além disso, proporciona melhor seletividade e eficiência ao processo e o solvente pode ser facilmente removido no final da extração. Assim, o emprego de fluidos supercríticos tem sido considerado boa opção para a extração e fracionamento de

produtos naturais, particularmente para as indústrias de alimentos e farmacêuticas (PEREIRA et al., 2004).

Outra alternativa é o uso do gás liquefeito de petróleo pressurizado (GLP), que é uma mistura de isômeros de butano e propano. Essa tecnologia proporciona maiores rendimentos de extração em baixas temperaturas, prevenindo a degradação dos compostos bioativos. O gás pode ser facilmente recuperado, possui baixo custo e a seletividade do processo pode ser modificada através de ajustes de pressão (NIMET et al., 2011; JESUS et al., 2013; NOVELLO et al., 2014). Além disso, com base em pesquisa de dados como Scopus, Scielo, PubMed, Google acadêmico foi encontrado apenas um trabalho utilizando este solvente em processos de extração. Soares et al. (2016) avaliaram a extração de óleo de farelo de arroz utilizando GLP pressurizado e obtiveram rendimentos de extração e atividade antioxidante similar ao extrato obtido com CO_2 supercrítico, porém em um tempo bem menor de extração e consequetemente com gasto menor de energia.

A extração assistida por ultrassom (UAE-*ultrassound assisted extraction*) é considerada uma tecnologia limpa, pois sua aplicação incorre em redução de consumo de solventes às demais técnicas, além de proporcionar melhor extração em menor tempo (LUQUE DE CASTRO; PRIEGO-CAPOTE, 2007). A UAE tem sido aplicada na extração de produtos naturais devido ao fenômeno de cavitação, ou seja, acelera a liberação de compostos bioativos da matriz vegetal devido à ruptura da parede celular (VEGGI, 2013; DAL PRÁ et al., 2015).

Embora existam trabalhos abordando a extração de compostos bioativos do fruto de palma por CO_2 supercrítico (LAU et al. 2008; PUAH, 2011), até o presente momento, não foram encontrados trabalhos utilizando GLP nas bases de dados acima citadas. No que se refere ao uso de ultrassom, Juliano et al. (2013) reportaram essa tecnologia para recuperação do óleo da fibra prensada da palma obtendo rendimento superior quando comparado ao processo convencional.

Além disso, poucos reportam à extração de compostos da fibra prensada da palma. Lau et al. (2008) avaliaram a extração seletiva de carotenos e vitamina E por extração supercrítica (ESC), enquanto Lau et al. (2006) avaliaram a qualidade do óleo residual da fibra prensada da palma. Entretanto, nenhum dos trabalhos avaliou, especificamente, a composição da fibra prensada da palma, consequentemente o perfil químico dos extratos e suas respectivas atividades biológicas, abrindo uma lacuna para sua exploração.

O *Stress* oxidativo ocorre de um desequilíbrio entre a produção e consumo de radicais livres e pode levar a danos no DNA de células e tecidos. Os agentes oxidantes podem afetar o

funcionamento normal das células e induzir alterações estruturais e mutações do DNA, que podem levar a doenças degenerativas, câncer, entre outras patologias. Nesse sentido, avaliar atividade antioxidante dos extratos de palma obtidos pelas tecnologias de extração propostas, bem como a atividade fotoprotetora, torna-se uma informação muito valiosa, uma vez que os compostos presentes no óleo são estudados pela ação antioxidante. A ação sobre os radicais livres formados na epiderme pode vir a potencializar a ação fotoprotetora de uma formulação, uma vez que o dano induzido à pele pela radiação UV é em parte mediado pelos intermediários do oxigênio reativo. Além disso, não foram encontrados estudos avaliando o desenvolvimento de nanoemulsões contendo compostos bioativos de óleo de palma da espécie *Elaeis guineensis*.

A presente Tese de Doutorado encontra-se disposta na forma de artigos científicos. Inicialmente é apresentada uma introdução geral, objetivos geral e específicos e revisão da literatura. Na sequência, quatro artigos científicos, referentes aos resultados experimentais. Para finalizar, são apresentadas a discussão integrada e conclusão.

O primeiro artigo científico refere-se a extração de compostos bioativos da fibra prensada de palma, utilizando-se CO_2 supercrítico e GLP pressurizado, bem como caracterização química, atividade antioxidante e determinação do fator de fotoproteção das amostras obtidas. O segundo artigo apresenta resultados da extração de compostos bioativos da semente prensada de palma, utilizando-se CO_2 supercrítico e GLP pressurizado, bem como caracterização química e atividade antioxidante. Após a análise dos dados, optou-se por dar continuidade ao trabalho somente com a fibra prensada de palma, por esta ter apresentado maiores valores de fator de fotoproteção e atividade antioxidante em relação a semente. Nesse sentido, o terceiro artigo aborda a extração de compostos bioativos da fibra prensada da palma usando ultrassom, sendo determinados o perfil químico, fator de fotoproteção e atividade antioxidante dos extratos. Após a comparação do fator de fotoproteção e atividade antioxidante dos compostos polares e apolares, optou-se por otimizar o desenvolvimento de nanoemulsões com atividade fotoprotetora e antioxidante utilizando o óleo obtido de fibra prensada de palma. Assim, estruturou-se o quarto artigo, que aborda o desenvolvimento de nanoemulsões contendo óleo de palma e avaliação da sua estabilidade em diferentes condições de armazenamento.

2 OBJETIVOS

2.1 OBJETIVO GERAL

Avaliar a eficiência de diferentes tipos de extração e atividade biológica de compostos bioativos da fibra e semente prensada de *Elaeis guineensis* Jacq. (palma);

2.2 OBJETIVOS ESPECÍFICOS

1. Avaliar a extração de compostos bioativos da fibra e da semente prensada de palma utilizando CO_2 supercrítico e GLP pressurizado;

2. Avaliar a extração de compostos bioativos da fibra prensada da palma utilizando sonda de ultrassom;

3. Caracterizar os extratos obtidos com relação ao teor de ácidos graxos, esqualeno, β-sitosterol, α-tocoferol;

4. Determinar fenólicos totais nos extratos obtidos por sonda de ultrassom empregando espectrofotometria;

5. Determinar carotenoides totais por espectrofotometria;

6. Investigar a atividade antioxidante dos extratos obtidos, em diferentes concentrações, pelo método *in vitro* baseado na geração do radical superóxido ($O_2^{-\bullet}$) e radical hidroxila ($HO^{\cdot}$), com detecção espectrofotométrica;

7. Investigar a atividade antioxidante dos extratos obtidos, em diferentes concentrações, pelo método clássico baseado na captura do radical 2,2-difenil-1-picril-hidrazila ($DPPH^{\bullet}$) utilizando curva padrão de trolox;

8. Investigar a atividade antioxidante dos extratos obtidos, em diferentes concentrações, pelo método 2,2´-azinobis 3-etilbenzotiazolina-6-ácido sulfônico (ABTS) utilizando curva padrão de trolox;

9. Avaliar o fator de proteção solar (FPS) *in vitro* dos extratos obtidos;

10. Desenvolver nanoemulsões contendo óleo de palma;

11. Caracterizar as nanoemulsões obtidas em termos de pH, tamanho de gotícula, índice de polidispersão e potencial zeta;

12. Avaliar a estabilidade temporal e térmica das nanoemulsões;

13. Avaliar o FPS e atividade antioxidante frente ao radical DPPH das nanoemulsões obtidas;

3 REVISÃO BIBLIOGRÁFICA

3.1 *Elaeis guineensis*

Elaeis guineensis Jacq. é uma espécie pertencente à família Arecaceae. Esta palmeira oleaginosa é nativa do continente africano e atualmente, é cultivada na África, sudeste Asiático (sendo Indonésia e Malásia os maiores produtores mundiais) e América (Central e Sul) (OBAHIAGBON, 2012). Por se desenvolver em regiões de clima tropical úmido, no Brasil, foi naturalizada inicialmente no estado da Bahia, e em seguida, na região Amazônica, onde se encontram as maiores áreas cultivadas (BRAZILIO et al., 2012; RIOS et al., 2012). A figura 1 apresenta uma imagem da palmeira e seu fruto.

Figura 1- *Elaeis guineensis* (palmeira e fruto) (Fonte: RIOS et al.,2012).

A espécie tem como característica a produção de dois tipos de óleo: óleo de palma ou azeite de dendê extraído do mesocarpo dos frutos (parte externa) e o óleo de palmiste extraído do endocarpo dos frutos (semente). O primeiro é composto por aproximadamente 50% de ácidos graxos saturados e 50% de insaturados, com ampla utilização em diversos alimentos, tais como: margarinas, achocolatados, gorduras para frituras, panificação, biscoitos, também é empregado na indústria saboeira e oleoquímica. O óleo de palmiste é constituído principalmente de ácidos graxos de cadeia curta, sendo empregado na produção de alimentos, cosméticos e indústria oleoquímica. Devido ao baixo grau de insaturação de seus ácidos graxos este óleo apresenta alta estabilidade oxidativa. Ambos são extraídos por métodos

físicos (prensagem mecânica), sem o uso de solventes. O refino desses óleos também é realizado de forma natural (processo físico), assim o produto resultante fica livre de ácidos graxos trans, tornando o óleo de palma uma alternativa saudável às gorduras hidrogenadas (RIOS et al., 2012; TEIXEIRA et al., 2013; MBA et al. 2015).

A cultura de palma se destaca na agricultura mundial por possuir elevada produção por unidade de área, alcançando produtividade média de 4 a 5 toneladas de óleo por hectare/ ano (BRAZILIO et al., 2012; RIOS et al., 2012). A produção total de óleo de palma no Brasil é de 416.000 ton./ano, sendo que as maiores parcelas de cultivo estão situadas na região amazônica, destacando-se o estado do Pará, o maior produtor brasileiro tanto do óleo de palma, quanto de palmiste, responsável por 80% da produção nacional. Esta elevada produtividade gera cerca de 600.000 ton/ano de cachos de frutos vazios, 318.000 ton/ano de fibra prensada de palma, 150.000 ton/ano de caroço dos frutos. O crescimento da demanda do óleo de palma, no mercado mundial, impulsiona o consumo do óleo de palmiste e consequentemente gera maior quantidade de co-produtos resultantes do processo de extração (prensagem) (BRAZILIO et al., 2012). Esses co-produtos podem ser alvo de investigação da presença de compostos bioativos, uma vez que não são empregados solventes químicos no processo de extração. Além disso, somente se extrai os componentes oleosos do fruto, havendo grande quantidade de compostos retidos na fibra prensada da palma e, provavelmente, uma quantidade de óleo residual.

3.2 MÉTODOS DE EXTRAÇÃO E CARACTERIZAÇÃO QUÍMICA

A extração supercrítica (ESC) é um método alternativo de extração, pois utiliza fluidos supercríticos como solventes e seu emprego em processos industriais tem ganhado espaço continuamente, devido aos fatores ambientais e de qualidade envolvidos. É um processo livre de resíduos tóxicos, não provoca a degradação térmica dos extratos e não requer grandes gastos de energia. Além de proporcionar melhor seletividade e eficiência ao processo e o solvente poder ser facilmente removido no final da extração. (BRUNNER,1994. p. 386.KELLNER et al., 2004, p.406; PEREIRA et al., 2004; DAL PRÁ, 2013).

Entre os solventes utilizados para esse processo de extração, o CO_2 é considerado adequado para extração de produtos naturais, pois é atóxico, não inflamável, apresenta baixa reatividade, além de ser de fácil obtenção. O CO_2 possui baixa viscosidade e elevados coeficientes de difusão (PAVIANI, 2004; DAL PRÁ, 2013).

Em relação às metodologias de extração e caracterização de compostos de *Elaeis guineensis*, Teixeira e colaboradores (2013) avaliaram a extração de compostos bioativos do fruto dessa espécie, utilizando processo enzimático aquoso a fim de facilitar a extração dos mesmos. Os extratos obtidos foram caracterizados em termos de carotenoides, tocoferóis e tocotrienóis por HPLC-DAD e detecção por fluorescência. O teor de fenólicos totais nas amostras foi determinado, por método espectrofotométrico e verificou-se um aumento das concentrações do mesmo, tanto nas amostras hidrofílicas como lipofílicas, que pode ser atribuído à presença da enzima tanase.

Em relação à extração utilizando ESC da espécie em questão, foram encontrados poucos trabalhos na literatura. Desses, dois são artigos de revisão em que o primeiro aborda os aspectos tecnológicos aplicados para extração e produção de β-caroteno, e cita a *Elaeis guineensis* como fonte desse composto e a ESC como tecnologia de extração (RIBEIRO; BARRETO; COELHO, 2011). O segundo artigo de revisão encontrado, descreve as tecnologias de extração e recuperação para carotenoides e vitamina E no óleo de palma. Os autores reportam que a ESC utilizando CO_2 como solvente é um processo seletivo e vantajoso em relação aos demais processos de extração, por ser não inflamável, inerte e não tóxico, o uso dessa metodologia evita processos de degradação nos compostos e não deixa nenhum resíduo no produto extraído, o que normalmente ocorre em processos de extração convencionais utilizando solventes orgânicos (OTHMAN et al., 2010).

Lau e colaboradores (2007) avaliaram a qualidade do óleo de palma extraído do mesocarpo do fruto por ESC, como uma forma de processamento alternativa aos métodos convencionais utilizados na indústria. A estabilidade de carotenoides, vitamina E e fitoesteróis (compostos extraídos do óleo de palma) foram avaliados por cromatografia gasosa equipada com detector de ionização em chama (GC-FID), cromatografia líquida acoplada a detector de fluorescência e métodos espectrofotométricos. Foi observado que após utilizar essa técnica de extração, os mesmos permaneciam estáveis.

Wei e colaboradores (2005) também avaliaram a extração de carotenoides do óleo de palma utilizando extração por CO_2 supercrítico e a quantificação dos mesmos foi realizada por métodos espectrofotométricos. Esses mesmos autores, em 2008, avaliaram a extração supercrítica como metodologia na extração de concentrado de carotenoides e tocóis (Vitamina E), a partir do óleo de palma. Os compostos obtidos foram caracterizados por espectrofotometria (carotenoides) e cromatografia líquida de alta eficiência acoplada a detector de fluorescência (tocóis).

Lau et al. (2006) compararam a qualidade do óleo residual, extraído da fibra prensada da palma utilizando ESC-CO_2, ESC-CO_2/etanol e hexano. A avaliação em termos de composição química foi realizada por GC-FID e os compostos identificados foram fitoesteróis (campesterol, estigmasterol e β-sitosterol), vitamina E (α-tocoferol, α-tocotrienol, γ-tocoferol, δ tocotrienol), esqualeno e carotenos, a concentração dos compostos extraídos foi similar para os diferentes métodos de extração empregados.

França e Meireles (2000) abordam a extração de carotenos e lipídeos da fibra prensada da palma. Os compostos extraídos dessas amostras foram determinados através de método espectrofotométrico, ou seja, não utilizou-se metodologias de caracterização mais abrangentes afim de verificar a presença de outros compostos presentes nestes extratos, além disso esses autores não testaram a atividade biológica dos mesmos.

Atawodi et al. (2011) avaliaram a composição química do óleo de palma em termos de compostos fenólicos. A extração foi realizada por agitação em vortex utilizando metanol como solvente, os extratos obtidos foram caracterizados por HPLC-UV e revelou-se a presença de compostos como ácido vanílico, ácido siríngico, ácido ferúlico, ácido p-hidroxibenzoico. Neo et al. (2008) investigaram a composição em termos de compostos fenólicos para o mesocarpo após a extração do óleo da palma, uma vez que essas informações são escassas na literatura. O método de extração escolhido por esses autores foi extração com metanol/acetona e água a temperatura ambiente, seguida de hidrólise ácida. Detectaram-se compostos como catequina, ácido ferúlico, rutina, os quais foram determinados por métodos espectrofotométricos. Monde et al. (2011) investigaram a composição química para frutos maduros de *Elaeis guineensis*, os compostos foram extraídos utilizando etanol/água (70:30) sob aquecimento e caracterizados por HPLC-DAD. Detectaram-se compostos como ácido cumárico, ácido cafeico, rutina, ácido clorogênico e quercetina.

A extração por ultrassom é reconhecida pelo seu potencial de aplicação na indústria fitofarmacêutica para uma grande variedade de extratos de plantas (VILKHU et al., 2008). Em artigo de revisão, reportado por esses mesmos autores, extratos obtidos de“hortelã”, “funcho” e “calêndula” apresentaram rendimento superior de extração quando comparado a métodos convencionais. Hojnik, Skerget e Knez (2007) avaliaram técnicas para extração de compostos da *Urtiga dioica* L. e concluíram que o ultrassom é uma técnica alternativa e promissora por possibilitar maior eficiência na extração. Para especie *Elaeis guineensis* apenas um trabalho utilizando ultrassom foi encontrado na literatura (Juliano et al., 2013).

O ultrassom em soluções aquosas induz a cavitação acústica (formação, crescimento e implosão de bolhas de gás). A energia liberada durante a cavitação fornece perspectivas para

o preparo de amostras. Em sistemas heterogêneos, o tratamento é favorecido devido a fenômenos de emulsão nas interfaces de sistemas líquido-líquido, lixiviação na superfície em sistemas sólido-líquido, erosão, fragmentação e aumento da área superficial de partículas sólidas. Esses ocorrem devido às ondas de choque originadas da implosão das micro-bolhas, além da diminuição do gradiente de concentração pelo aumento do transporte de massas ocasionado pela turbulência e micro-jatos (LUQUE-GARCIA; LUQUE DE CASTRO, 2003; DAL PRÁ, 2013).

Em relação à extração de compostos bioativos de *Elaeis guineensis* obtidos através de extração por GLP, foi encontrado apenas um trabalho utilizando este solvente em processos de extração (Soares et al. (2016). Nesse sentido, há possibilidade concreta de se avaliar a extração de compostos da fibra e semente prensada da palma utilizando CO_2 supercrítico, GLP pressurizado e sonda de ultrassom, além de avaliar a atividade biológica dos extratos da fibra e semente prensada da palma.

3.3 ATIVIDADE BIOLÓGICA EM *Elaeis guineensis*

A Tabela 1 apresenta uma compilação dos trabalhos referentes à atividade biológica dos compostos bioativos de *Elaeis guineenses*. Embora tenha uma quantidade significativa de trabalhos avaliando a atividade biológica, os estudos descritos avaliam a atividade do óleo de palma. Um dos poucos trabalhos reportados na literatura abordando a atividade biológica dos compostos extraídos da fibra prensada da palma é descrito por Nang et al. (2007), onde os autores avaliaram a atividade antioxidante pelos métodos FRAP (*Ferric Reducing Antioxidant Power*) e TEAC (*Trolox Equivalent Antioxidant Capacity*) de compostos polares extraídos da fibra prensada da palma. Os resultados preliminares mostraram que esses compostos possuem elevada capacidade antioxidante.

Tabela 1- Atividade biológica em *Elaeis guineensis.*

Elaeis guineensis	**Atividade biológica**	**Referência**
Vitamina E e β-caroteno	Efeito protetor frente a danos testiculares induzidos em ratos;	Jegede et al. (2015)
Compostos fenólicos do óleo de palma Ácido cafeico, ácido protocatecoico e ácido p-hidroxibenzoico;	Efeito neuroprotetor (melhora das funções cognitivas e motoras);	Leow et al. (2013)
Compostos presentes no óleo de palma α e β-caroteno, licopeno, tocoferóis (α, β, γ e isoformas δ), tocotrienóis (α, β, γ e isoformas δ);	Efeito protetor em modelo de hepatoxicidade oxidativa induzida;	Ajuwon et al. (2013)
Compostos fenólicos do óleo de palma Ácido cafeico, ácido protocatecoico e ácido p-hidroxibenzoico;	Atenua aterosclerose e outras doenças cardiovasculares;	Leow et al. (2013)
Compostos fenólicos extraídos do óleo de palma ácido p-hidroxibenzoico, ácido vanílico, ácido siríngico e ácido ferúlico;	Atividade antioxidante;	Atawodi et al. (2011)
Compostos fenólicos extraídos do fruto de palma Rutina, ácido cafeico e ácido clorogênico;	Atividade antioxidante	Monde et al. (2011)
Vitamina E do óleo de palma	Protetor contra dano oxidativo causado pelo diabetes mellitus	Budin et al. (2006)
Tocotrienóis do óleo de palma	Hipocolesterolemiante	Song e DeBose-Boyd (2006)
Tocotrienóis e tocoferóis do óleo de palma	Atividade antioxidante	Zuzana et al. (2005)
Tocotrienóis do óleo de palma	Antiproliferativo e apoptótico frente à células de câncer de mama	McIntyre et al. (2000)

3.4 NANOEMULSÕES

Muitos compostos naturais, como substâncias extraídas de vegetais, são instáveis. Exemplo disso são compostos que apresentam atividade antioxidante e em razão dessa instabilidade podem sofrer reações que levam à redução, perda de eficácia e até mesmo a degradação. Neste sentido, a nanotecnologia pode ser utilizada para a estabilização desses compostos, aumentando também a estabilidade dos produtos finais. (Daudt et al., 2013). Dispersões coloidais têm sido estudadas devido à sua capacidade de encapsular, proteger e distribuir compostos bioativos, tais como vitaminas, fármacos, antioxidantes, antimicrobianos (Morais et al., 2016).

Nanoemulsões são sistemas coloidais cineticamente estáveis e mais vantajosos que emulsões convencionais devido ao seu menor tamanho de gotícula. São compostas de uma fase aquosa, uma fase oleosa e uma mistura de surfactantes. Quando a fase aquosa é a fase interna e o óleo é fase contínua o sistema é chamado de água em óleo (A/O), ao passo que quando o óleo é disperso na fase aquosa o sistema é óleo em água (O/A). Nanoemulsões do tipo óleo em água têm sido usadas para incorporar ingredientes lipofílicos em base aquosa como alimentos, bebidas e cosméticos (Rebolleda et al., 2015; Silva et al., 2015; do Vale Morais et al., 2016; Rodríguez, 2016).

A emulsificação utilizando ultrassom, método que utiliza alta energia de emulsificação e combina baixa frequência ($\leqslant$100 kHz) com alta potência (> 10 W cm^{-2}), tem sido amplamente utilizanda na indústria, principalmente, alimentícia para geração de micro e nanoestruturas (Sullivan et al., 2015). A energia transferida para o fluido através da propagação das ondas de ultrassom gera diferenças de pressão (zonas de compressão e rarefação) levando a formação e após o colapso de bolhas de cavitação, as quais são responsáveis pela formação das gotículas (Calligaris et al., 2016). Esta técnica é reportada por ser vantajosa no preparo de nanoemulsões mais estáveis, com menor tamanho de gotícula e índice de polidispersão (Gosh et al.,2014). Rebolleda et al. (2015) desenvolveram e caracterizaram nanoemulsões de óleo de farelo de arroz, utilizando ultrassom e planejamento de experimentos para a otimização do processo. Li and Chiang (2012) otimizaram o desenvolvimento de nanoemulsões de limoneno em água avaliando a influência das condições do ultrassom nas características físico-químicas das nanoemulsões obtidas. Alzorqui et al. (2016) desenvolveram nanoemulsões a base de palma-oleína para incorporação de polissacarídeo antioxidante -β-d glucana. Gosh et al., 2014 utilizaram o ultrassom no desenvolvimento de nanoemulsões contendo óleo de sésamo para atividade antibacteriana.

Em relação a nanoestruturas contendo óleo de palma, apenas um artigo foi encontrado e relata o desenvolvimento de nanoestruras carreadoras de lipídeo, além disso Hung et al. (2011) utilizaram a alta pressão de homogeinização como técnica de preparo das formulações.

No entanto, há uma carência de trabalhos com o foco no desenvolvimento de nanoemulsões contendo óleo de palma através do planejamento de experimentos e avaliação das variáveis de processo como concentração de óleo e surfactante, intensidade e tempo de ultrassom. Além de selecioná-las em suas melhores condições através do uso de perfil de desejabilidade torna-se uma oportunidade para obtenção de nanoemulsões estáveis, com pequeno tamanho de gotícula. Uma vez que esse óleo apresenta propriedades antioxidantes e fotoprotetoras, podendo ser incorporado em uma formulação fotoproterora para potencializar o FPS da mesma, bem como ser utilizando em produtos cosméticos bem como alimentícios de base aquosa como antioxidante.

3.5 CONSIDERAÇÕES ACERCA DO ESTADO DA ARTE

Conforme visto na revisão bibliográfica, é elevada a produção total de óleo de palma no Brasil, o que gera grande quantidade de fibra prensada. Dessa forma, a utilização da mesma para a extração, identificação de moléculas bioativas e atividade biológica é uma alternativa sustentável para valorização deste subproduto.

O método de extração escolhido está vinculado com o resultado da atividade biológica, uma vez que os compostos bioativos extraídos estão relacionados ao método selecionado. Há uma carência de trabalhos dessa matriz utilizando sonda de ultrassom. Além disso, até o presente momento, foi encontrado apenas um trabalho utilizando este solvente em processos de extração. Para atividade antioxidante de compostos de *Elaeis guineensis*, existem poucos estudos, no entanto, ainda não se avaliou essa matriz frente sua ação fotoprotetora. Sendo assim, torna-se uma candidata para tal ação e potencializar o FPS de um fotoprotetor sintético em uma nanoemulsão.

4 PUBLICAÇÕES CIENTÍFICAS

4.1 ARTIGO CIENTÍFICO I

Extraction of bioactive compounds from palm (*Elaeis guineensis*) pressed fiber using different compressed fluids

Artigo publicado no periódico The Journal of Supercritical Fluids.

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ABSTRACT

Compressed liquefied petroleum gas (LPG) and CO_2 were used for the extraction of bioactive compounds from palm pressed fiber (*Elaeis guineensis*). The influence of temperature and pressure on extraction yield, chemical composition, antioxidant activities towards superoxide and hydroxyl radicals and the sun protection factor were studied for each solvent. Maximum extraction yield was 4.55 wt% using supercritical CO_2 at 60 °C and 25 MPa, which was about three times higher than the yield using compressed LPG. The highest antioxidant activities and sun protection factor were also obtained at this condition. The chemical profiles were similar for both solvents, where lauric, palmitic and oleic acids corresponded to 80% of the total fatty acids determined in the extract. Antioxidant compounds such as α-tocopherol, squalene, β-carotene and β-sitosterol were also determined. CO_2 was the best solvent for extraction of bioactive compounds from pressed fiber palm, due to the highest extraction yield, antioxidant activities and sun protection factor.

Key-words: Carbon Dioxide; *Elaeis guineensis*; Liquefied petroleum gas; palm pressed fiber.

1. Introduction

Elaeis guineensis Jacq. is popularly known as palm oil, palm, or palm tree and its fruits as palm. From palm fruits we obtain two types of oil: palm oil (extracted from pulp) and palm kernel oil (extracted from kernel) [1]. The chemical composition and physical characteristics of these oils are different. This makes them useful for a wide range of applications, such as in cosmetic and food industries, in the manufacturing of dairy products, margarines, ice creams, etc… [2-3]. Palm oil is obtained by mechanical extraction (pressing) of the epicarp and mesocarp of palm fruits. After the extraction, the by-product remaining, known as palm pressed fiber, contains about 5-6% of residual oil and many high-value constituents such as carotenoids, tocotrienols, tocopherols, phytosterols and phenolic compounds [3-5].

The total production of palm oil in Brazil has been about 416,000 ton/year [6], leading to the production of 318,000 ton/year of palm pressed fiber. Nowadays, the pressed fiber is used as animal feed and as fuel in the boilers. Taking into account that the oil extraction is carried out without the use of chemical solvents, the residue is free from organic residue and is an excellent choice for the extraction of bioactive compounds [3-4]. Therefore, the use of pressed fiber for the extraction and identification of bioactive molecules with antioxidant potential is a sustainable alternative for the valorization of this by-product.

Supercritical technology has been extensively used with success in the extraction of different plants using carbon dioxide (CO_2) as solvent [7-9]. Some investigators reported the use of this methodology for the extraction of bioactive compounds from palm fruits [4;10-14]. However, few of them evaluated the extraction of bioactive compounds from pressed fiber [4, 14].

Although CO_2 is the main solvent used in high-pressure extraction of bioactive compounds from palm and other plant materials, other solvents (e.g. propane, n-butane,

R134a) have been successfully employed [15-17].The extraction with propane has been reported as a promising technology, since it is possible to obtain higher extract yield at a shorter extraction time when compared to supercritical CO_2 [15, 18-19]. However, the cost of this solvent is about twenty times higher than CO_2. A cheaper alternative solvent could be compressed liquefied petroleum gas (LPG) that contains propane and n-butane as the main constituents. LPG is very abundant, cheap and it can be used in much lower pressures compared to carbon dioxide. The usage of LPG has been reported in the high-pressure treatment of some enzymes to increase their catalytic power [20-22].

Recently, Soares et al. [23] noted that the use of compressed LPG as a more promising solvent for extraction of rice bran oil, since the extraction period was considerably reduced in comparison with than supercritical CO_2. However, there are no studies in the literature reporting its use for extraction of bioactive compounds from palm. Based on these aspects, this work is focused on the obtainment bioactive compounds from palm pressed fiber using compressed liquefied petroleum gas and CO_2. The extracts obtained with both solvents were chemically characterized and used for determination of antioxidant activity towards superoxide and hydroxyl radicals as well as the sun protection factor, i.e., effectiveness as a sun screen.

2. Material and methods

2.1. Material

The palm pressed fiber (*Elaeis guineensis*) was take out from the industry of processing of oils and derivatives Agropalma (Tailândia, PA, Brazil). The carbon dioxide (99.9% purity) was purchased from White Martins (Cruz Alta, RS, Brazil), whereas the LPG was purchased from Liquigas (Santa Maria, RS, Brazil). LPG was constituted of a mixture of

propane (50.3 wt%), n-butane (28.4 wt%), isobutane (13.7 wt%), ethane (4.8 wt%) contained other minor constituents (methane, pentane, isopentane).

2.2. Samples

The samples were dried at 50 °C until a constant mass was reached. The final moisture content was 8.5 wt%. Afterwards, the samples were milled and sieved. The particles that passed through a sieve of 16 mesh were collected. The samples were maintained at -12 °C until the moment of the experiments.

2.3. Experimental apparatus and procedure for the extractions

The experiments were performed in a laboratory scale unit consisting of a solvent reservoir, two thermostatic baths, a syringe pump (ISCO 260D), a 100 cm^3 jacketed extraction vessel and an absolute pressure transducer (Smar, LD301) equipped with a portable programmer (Smar, HT 201), a collector device consisting of a vessel with a glass tube and a cold trap.

Ten g of samples were transferred into the extraction vessel. The solvent (CO_2 or LPG) was pumped into the bed, which was supported by two 300 mesh wire disks at both ends, and was kept in contact with the herbaceous matrix for at least 1 h to allow the stabilization of the system. The extract was then collected by opening the micrometering valve. The experiments were carried out isothermally using a constant pressure at a solvent flow rate 4 $g.min^{-1}$ [7].

For experiments carried out with CO_2 as solvent, the temperature ranged from 20–60 °C and over a pressure range of 10.0–25.0 MPa, whereas for LPG temperature was subcritical (20-40 °C) over a pressure range of 0.5-2.5 MPa. All the extractions were performed in triplicate. The overall extraction curves (OEC) were determined by taking into account the

mass of solute extracted in function of process time. The constant-extraction rate (M_{CER}) was determined by determining the angular coefficient (by linear regression) of the constant-extraction rate period of the OEC [24].

2.4. Chemical characterization

2.4.1 Gas chromatography analysis

A 100 mg of extract were solubilized in 1 mL of hexane and centrifuged to separate the insoluble fraction. The solution was divided into two fractions of 0.5 mL, which were used for determinations of fatty acids and α-tocopherol, β-sitosterol and squalene content. For the determination of fatty acids, 250 µL of a solution 4 mg/ml methyl tricosanoato (C23:0 Me) (Sigma-Aldrich, St. Louis, USA) in isooctane as internal standard (Pi) was added to each fraction. Solvents were evaporated at 40 °C under vacuum. Fatty acids methyl esters (FAME) derivatizations were performed according to the method described by Visentainer [20]. FAME's were analyzed by injecting 1 µL into gas chromatograph equipped with flame ionization detector (GC-FID - Varian model Star 3400 CX - CA, USA) and auto sampler (Varian, model 8200 - CA, USA). FAME were separated using a capillary column CP-Wax 52 CB (Middelburg, The Netherlands) (50 m × 0.32 mm × 0.20 µm). The carrier gas used was hydrogen held at a constant pressure of 15 psi. The GC injection port was held at 250 °C in the split mode using a split ratio of 20:1. The column temperature was programmed to 50 °C (1 min); 20 °C min^{-1} up to 200 °C; 10 °C min^{-1} up to 230 °C (8 min). The detector was maintained at a temperature of 240 °C. Identification of FAME were performed by comparison of retention times of compounds with FAME standards Mix-37 (P/N 47885-U; Sigma-Aldrich, St. Louis, USA). The results were expressed in mg of AGs per gram of extract.

Squalene, α-tocopherol and β-sitosterol were determined by injection of 1 µL into the same gas chromatograph system. Compounds were separated by capillary column RTX-5MS (Restek-USA) (30 m × 0.25 mm × 0.25 µm). The carrier gas used was hydrogen at a constant pressure of 15 psi. The injection port temperature was maintained at 320 °C in the splitless mode for 1 minute followed by the opening of the splitter valve set at a ratio of 30:1. The column temperature gradient programming was used: 200 °C (5 min); 15 °C min^{-1} up to 280 °C; 5 °C min^{-1} up to 330°C (10 min). The detector was maintained at 320 °C. The quantification was performed by external standardization method by means of calibration curves for each compound [25].

The compounds were identified by comparison of retention times and mass spectra of standards mix (α- tocopherol, β- sitosterol and squalene) obtained in a gas chromatograph coupled to mass spectrometer (GC/MS) Shimadzu, QP-2010 Plus (Tokyo, Japan) under the same chromatographic conditions described above, using helium as carrier gas, operating in electron ionization mode (EI) at 70 eV with a scan 35 350 m/z.

2.4.2. Total carotene determination

The concentration of β-carotene was measured using a UV-Vis 8453 Hewlett-Packard spectrophotometer (Agilent Technologies, Santa Clara, EUA). About 50 mg of extracts were diluted with 7 ml of hexane. The solution was transferred to a 1-cm quartz cuvette and the absorbance was read at 450 nm. Carotenes in the extracts were calculated in terms of β-carotene, using the following standard curve (R^2=0.9948) over the concentration range of 0.09 to 1.2 µg/mL [26]:

$$TC = 0.8698 \cdot abs \quad (1)$$

where TC is total carotene (µg/mL) and abs is the absorbance of sample.

2.5. Antioxidant activities of extracts

2.5.1. *Superoxide radical scavenging activity – Hypoxanthine/Xanthine Oxidase System (HPX/XOD)*

The activity of extracts toward $O2^{\bullet-}$ radical was evaluated by the hypoxanthine/xanthine oxidase enzymatic system (HPX/XOD) [27]. For this purpose, 100 μL of EDTA (30 mmol L^{-1}), 100 μL of HPX (3 mmol L^{-1}) and 200 μL of nitroblue tetrazolium (NBT) (1.42 mmol.L-1) were mixed with 100 μL of extract. After 3 minutes, 100 μL of enzyme XOD (0.75 U mL^{-1}, diluted in phosphate buffer) was added. The final volume of solution was 3 mL filled with phosphate buffer (0.05 mol L^{-1}, pH 7.4).

The blank sample was prepared in the same manner, but without the presence of NBT. Also, was carried out a control test containing all reagents with the solvent employed in the samples, as well as a blank control. After 40 minutes of reaction time, was carried out the absorbance of samples were read using an UV-Vis 8453 Hewlett-Packard spectrophotometer (Agilent Technologies, Santa Clara, EUA) at 560 nm. The activities towards O2•- (AAO2•-) were calculated according to the following equation:

$$AA_{O_2^{\bullet-}} = \left(1 - \frac{(A - A_B)}{(C - C_B)}\right) \times 100 \qquad (2)$$

where A, AB, C, CB are the absorbance of sample, blank sample, control and blank control, respectively.

2.5.2. Hydroxyl radical scavenging activity

The scavenging activity of extracts extracts toward hydroxyl radical was determined by using the deoxyribose method [27]. $FeCl_3 \bullet 6H_2O$ and ascorbic acid were prepared in degassed deionized water prior to use. The reaction tube contained 100 μL of extract, 100 μL

of 1 mM EDTA, 100 μL of 1 mM $FeCl_3$•$6H_2O$, 100 μL of 36 mM 2-deoxy-d-ribose, 100 μL of 10 mM H_2O_2, and 100 μL of 1 mM l-ascorbic acid in 25 mM phosphate buffer (pH 7.4), and the total volume was made up to 1.0 mL using the same phosphate buffer. After incubation at 37 °C for 1 h, the reaction was stopped by adding 1.0 mL of 10% TCA (w/v) and 1.0 mL of 1.0% TBA (w/v) in buffer phosphate (pH 7.4). The mixture was heated in a boiling water bath for 15 min. Once samples were cooled, the final volume was adjusted to 5.0 mL with deionized water, and the absorbance was read at 532 nm. The radical scavenging capability towards •OH (AA•OH) was calculated using the equation.

$$AA._{OH} = \left(1 - \frac{(S - S_B)}{(C - C_B)}\right) \times 100 \quad (3)$$

where S, S_B, C, C_B are the absorbance of sample, blank sample, control and blank control, respectively.

2.6. Determination of the sun protection factor (SPF)

SPF was determined in vitro by the spectrophotometric method reported by Mansur [28]. The absorption spectra of samples were obtained in the range of 290 to 320 nm every 5 nm, using a 1 cm quartz cell. The observed absorbance values were calculated using the equation:

$$SPF = CF \cdot \sum_{290}^{320} EE(\lambda) \cdot .I(\lambda) \cdot Abs(\lambda) \quad (4)$$

where EE (λ) is the erythemal effect spectrum at wavelength λ, I (λ) is solar intensity spectrum at wavelength λ, Abs (λ) is the absorbance of sample at wavelength λ and CF is the correction factor (= 10).

2.7. Statistical analysis

The statistical analysis was accomplished with the ANOVA test coupled with the Tukey test at a 95% confidence level using the software Statistica® 8.0.

3. Results and discussion

3.1. Influence of solvent on extraction yields and kinetic profiles

Table 1 presents the extraction yields obtained with compressed CO_2 and with LPG as solvents. The yield using CO_2 ranged from 0.08 wt% (run 4) to 4.55 wt% (run 2), that is in good agreement with the works of França and Meireles [29] and Cardenas-Toro et al. [30]. The application of Tukey's test for extraction yield shows that all runs are statistically different ($p<0.05$), indicating that the process variables are influencing on the responses. At isobaric and at isothermal conditions, increments of both temperature and pressure increased the extraction yield. By increasing the pressure of the system, the amount of extracted compounds also increases. This is due to the improvement in the solvating power of the solvent, which provides a greater and better permeability of the solvent into solid matrix. By other hand, the increase of temperature at pressure of 25 MPa (runs 1 and 2) favored the extraction yield, because the influence of the vapor pressure overlapped the influence of the solvent density, increasing the solubility of the extracts in supercritical CO_2.

The extraction yield using compressed LPG ranged from 0.01 (run 7) to 1.52 wt% (run 6). Analyzing the isothermal experiments at 20°C it is seen that there is no statistical difference on extraction yield, independent of system pressure. By other hand, very low extraction yield were obtained at 40°C. The temperature clearly presented a negative effect on the yield. This result is due to the increase on the vapor-pressure of the compounds of the extract and also of the components of LPG. As high as the temperature, higher the quantities of constituents of LPG in the vapor phase, diminishing the density with consequent decreasing on the solvation power.

One important aspect to be considered when studying extraction processes are the overall extraction curves presented in Fig 1. For extractions using CO_2 (Fig. 1a) constant extraction rate was verified in the first 45 minutes. In this period, was recovered 57.6, 82.7, 60.0, 55.2 and 70.43% of total amount of extract available in the raw material for runs, 1-5, respectively. For the constant-rate period, the mass transfer rate was the highest, being the mechanism of mass transfer controlled by convection that took place in the fluid film around the milled particles. The highest extraction rate of the constant-extraction period was obtained in the Run 2, which was 0.0022 g extract /g CO_2. For extractions using LPG (Fig. 1b), only the constant-extraction period was verified, where the highest extraction rate was 0.0083 g extract / g LPG obtained in run 6. The extraction rate using LPG was about 4 times higher than CO_2 and the extractions times faster, although the final yield was lower than using CO_2.

3.2. Chemical characterization of extracts

The extracts obtained in each experimental condition were analyzed by GC-FID. Table 2 presents the chemical composition for extracts using CO_2 and LPG. Nineteen fatty acids were identified in the extract from palm pressed fiber using CO_2. Lauric (C12:0), hexadecanoic (C16:0) and oleic (C18:1n9c) acids were found in high amount in all samples and together represent about 80% of the total fatty acids of the pressed fiber. Besides the fatty acids, others compounds as α-tocoferol, squalene, β-sitosterol and β-carotene also were found in the extract from palm pressed fiber obtained with compressed CO_2. Squalene, α-tocoferol and β-carotene were obtained in major concentrations at run 5, whereas β-sitosterol was detected in low concentration only in the runs carried out at 25 MPa. It is reported in literature that high temperature increases the solubility of the compounds of palm oil in the liquid phase, increasing the pressure increases the solubility as well due to enhancement in the solvating power [31-34]. By example, Brunner and Machado [32] obtained 3.75 wt% of palm

fatty acids distillated in CO_2 vapor phase working at 60°C and solvent density of 825 $kg.m^{-3}$. In this work, the highest concentrations for all compounds were verified in the run 5 (40°C/17.5 MPa) that is in good agreement with literature. At this condition the density of solvent is 823 $kg.m^{-3}$ (estimated from Angus et al [35]) that is higher than 794 $kg.m^{-3}$ verified in run 2 (60°C/25 MPa). In this way, the condition of run 5 was more favorable than that of run 2 to increase the vapor pressure of the components without lost of solvating power. Vapor pressure and density act sinergically, enabling the obtainment of an extract richer in terms of concentration compounds. By the other hand, β-sitosterol was extracted only at runs carried out at the highest pressure (runs 1 and 2), being positively affected by the pressure.

For extractions using LPG as solvent, seventeen identified in extract using LPG. The concentration of the majority compounds (C12:0, C14:0, C16:0, C18:1n9c, C18:2n9c) obtained with compressed LPG was higher than when using CO_2. The same trend was also found for α- tocopherol, squalene and β-carotene. This result might be explained by the highest solubility of palm compounds on LPG in comparison with CO_2. The consequence is verified in the kinetics discussed in the previous section, where extractions using LPG were around 5 minutes and 90 minutes for CO_2. The result obtained here is in good agreement with the work of Jesus et al. [36], which stated that compressed propane is a very good solvent for non-polar compounds and when compared with ethanol, propane showed solubility 2.5 times higher. If compared with CO_2 this difference is more discrepant, since the solubility at 60°C/10 MPa was 1.27 $g_{oil}/g_{solvent}$ (Jesus et al. [36]) whereas for CO_2 was only 0.02 $g_{oil}/g_{solvent}$ at the same condition [37]. For extracts obtained with compressed LPG, the highest concentrations were obtained in run 8 (20 °C and 2.5 MPa) that is the condition that present the highest density among the conditions studied here.

The chemical composition found in this study was in good agreement with other studies available in literature. Jesus et al. [36] reported the presence of lauric acid (dodecanoic

acid), myristic acid (tetradecanoic acid), palmitic acid (hexadecanoic acid), stearic acid (octadecanoic acid) and oleic acid (C18:1n9c) in palm oil obtained by extraction with compressed ethanol and propane. In the same study, the authors reported the presence of 5.5 mg/g of carotene in the extracts using propane. Mba et al. [1] also have been reported palmitic acid, oleic acid and stearic acid as the main compounds and α- tocoferol, squalene and total carotene in lower concentration in the palm oil. França and Meireles [24] detected the presence of fatty acids, although the authors did not specify which over. Teixeira et al. [3] reported palmitic acid (hexadecanoic acid) and oleic acid (C18:1n9c) as the major fatty acids as well as the presence of tocopherol and total carotene. Lau et al. [10] with reported the presence of α-tocoferol, squalene, β-sitosterol and total carotene in fresh palm-pressed mesocarp fiber. Cardenas-Toro et al. [30] reached the maximum value of 0.8 ± 0.2 mg carotene/g extract at 15 MPa and 318K.

3.3. Biological and physical activity of extracts

The results concerning the antioxidant activity towards superoxide and hydroxyl radicals as well sun protection factor for extracts obtained with CO_2 and LPG are presented in Table 1. The results for the antioxidant activities ranged from 44.0-60.0% for OH^- radical and 11.9-35% for $O_2^{\bullet-}$. Extracts from run 2 using CO_2 presented the highest activities with respect to both radical-based assays. This condition was consistent with the highest extraction yield and the highest concentration of β-sitosterol, which is known to be antioxidant. These results are in agreement with other plant extracts obtained by supercritical fluid extraction reported in literature [7], since there are no antioxidant activity against these radicals for palm.

Concerning sun protection factor (SPF), the values ranged from 9.93-15.6. The sample that showed the highest value for SPF was run 2 obtained with supercritical CO_2. Similar results were obtained for run 5 (CO_2) and runs 8-10 (LPG). Comparing the chemical

composition of these runs, Run 2 presented the highest concentration of β-sitosterol and similar concentration of β-carotene, although the concentrations of α-tocoferol and squalene were lower than those obtained in the runs 5, 8-10. For runs 5, 8, 9 and 10, β-sitosterol was not extracted, on the other hand the concentration of α-tocopherol, squalene and β-carotene were higher than in run 2. In practice, all these compounds act as SPF, because they are antioxidant compounds, inhibiting the action of the free radicals induced by exposure to sun, which causes the damage to the skin induced by UV radiation, and partly mediated by reactive oxygen intermediates [38]. This result is in good agreement with other plant extracts reported in literature, since there are no SPF for palm. Daher et al. [39] evaluated emulsions containing *Euterpe oleracea* extract and the SPF was 14.97. Badea et al. [38] determined the SPF of pomegranate seed oil and obtained 4.1. Silva et al. [40] found SPF of 3.4 using *P. umbellata* root extract gel.

4. Conclusions

This paper has presented data referring to the extraction of bioactive compounds from palm pressed fiber using supercritical CO_2 and compressed LPG as solvents. Maximum extraction yield was 4.55 wt% using supercritical CO_2 at 60 °C and 25 MPa, which was about three times higher than the yield using compressed LPG. The highest antioxidant activities and sun protection factor were obtained at this condition. Chemical profile was similar for both solvents, where lauric, hexadecanoic and oleic acids correspond to 80% of the total fatty acids of the pressed fiber. Antioxidant compounds such as α-tocoferol, squalene, β-carotene and β-sitosterol were also determined. Supercritical CO_2 was the best solvent for extraction of bioactive compounds from pressed fiber palm, due to the highest yield, antioxidant activities and sun protection factor.

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List of Figure Captions

Fig. 1. Overall kinetic curves for the extraction of palm pressed fiber using supercritical CO_2 (a) and compressed LPG (b) as solvents

Table 1. Extraction yields, antioxidant activities and sun protection factors of extract obtained using CO_2 and LPG as solvents.

Run	Temperature (ºC)	Pressure (MPa)	Yield (wt%)	OH^- (%)	$O_2^{\bullet-}$ (%)	SPF
			CO_2			
1	20	25.0	1.94 ± 0.08^{c}	51.4 ± 0.5	20.8 ± 0.09	9.93 ± 0.8
2	60	25.0	4.55 ± 0.18^{a}	60.0 ± 0.2	35.0 ± 0.3	15.06 ± 0.5
3	20	10.0	$1.32\pm0.05^{d,e,f}$	51.09 ± 0.6	23.0 ± 0.01	9.43 ± 0.3
4	60	10.0	$0.08\pm<0.01^{i}$	Ne	ne	ne
5	40	17.5	3.36 ± 0.16^{b}	46.0 ± 1.1	11.9 ± 0.2	14.45 ± 1.1
			LPG			
6	20	0.5	$1.52\pm0.16^{d,e}$	56.0 ± 0.9	12.5 ± 1.0	14.24 ± 0.6
7	40	0.5	$<0.01\pm<0.01^{g}$	Ne	ne	ne
8	20	2.5	$1.34\pm0.15^{d,e,f}$	57.0 ± 0.08	15.1 ± 0.09	14.04 ± 0.4
9	40	2.5	0.48 ± 0.02^{h}	44.0 ± 0.5	14.6 ± 1.2	12.43 ± 0.3
10	30	1.5	0.86 ± 0.04^{g}	44.0 ± 0.07	14.6 ± 0.8	13.06 ± 0.2

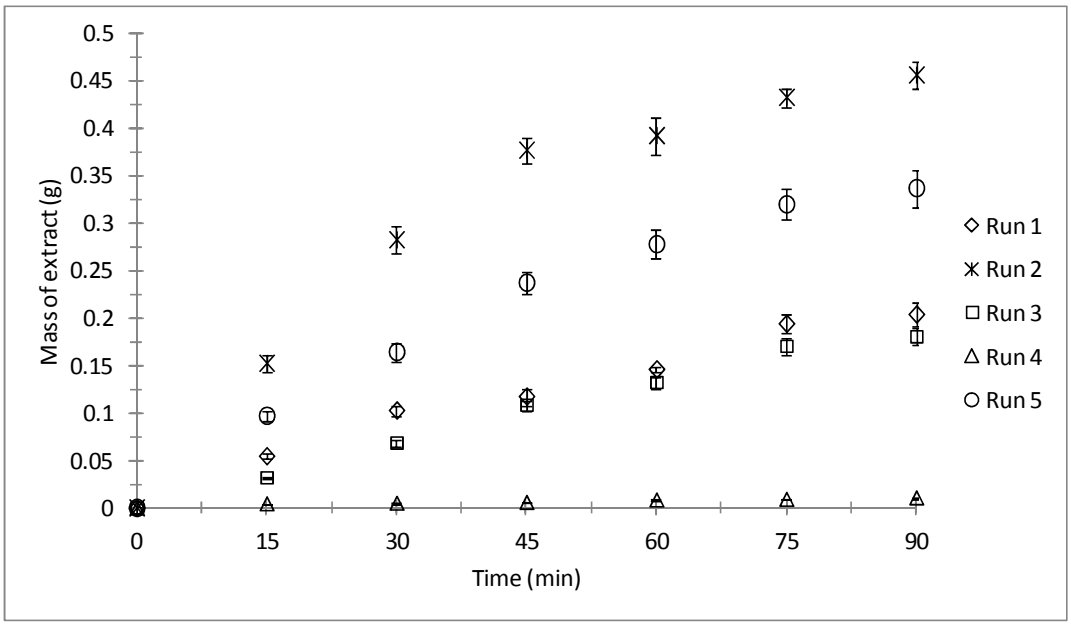
$^{a, b, c, d, e,f,g,h,i}$ different letters in the column represent a significant difference at 95% ($p<0.05$ - Tukey test).

Ne: not evaluated

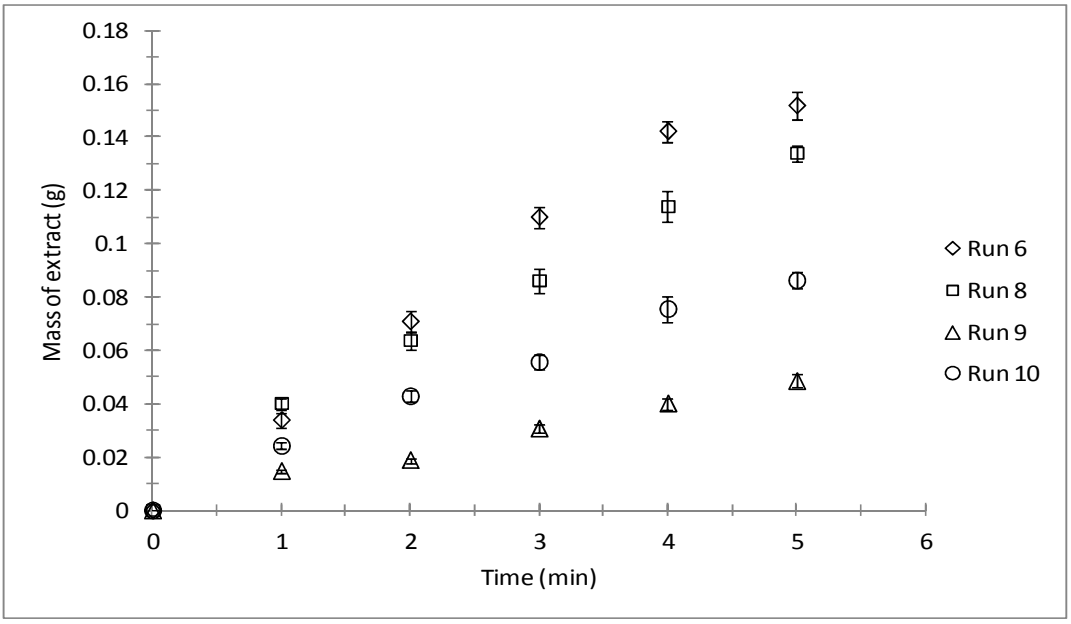
Table 2. Chemical characterization of extract obtained using CO_2 (runs 1-5) and LPG (runs 6-10) as solvents.

Run/compound (mg/g)	Run 1	Run 2	Run 3	Run 5	Run 6	Run 8	Run 9	Run 10
C8:0	3.2 ± 0.13	2.8 ± 0.08	2.2 ± 0.05	5.0 ± 0.70	2.60 ± 0.05	2.60 ± 0.03	3.60 ± 0.02	3.60 ± 0.14
C10:0	3.6 ± 0.11	3.2 ± 0.10	2.4 ± 0.08	5.6 ± 0.90	4.00 ± 0.20	4.00 ± 0.30	5.00 ± 0.39	3.40 ± 0.36
C12:0	69.60 ±1.39	55.0 ± 1.65	39.6 ± 1.58	84.8 ± 1.50	91.0 ± 4.55	87.4 ± 4.35	97.2 ± 4.87	69.4 ± 7.40
C14:0	33.6 ± 1.21	23.6 ± 1.10	18.8 ± 4.29	37 ± 2.20	49.2 ±2.50	40.2 ± 3.80	43.0 ± 5.41	33.6 ± 13.0
C15:0	0.2 ± 0.01	0.2 ± 0.02	0.2 ± 0.02	0.2 ± 0.02	0.40 ± 0.32	0.40 ± 0.39	0.20 ± 0.36	0.40 ± 0.40
C16:0	153.6 ±5.10	155.0 ±5.32	107.4 ±3.37	201.8 ± 12.60	263.0 ± 13.15	205.8 ± 12.80	186.0 ± 19.60	192.6 ± 31.20
C16:1	0.4 ± 0.03	0.4 ± 0.01	0.4 ± 0.02	0.46 ± 0.06	0.60 ± 0.05	0.80 ± 0.07	0.60 ± 0.04	0.80 ± 0.02
C17:0	0.6 ± 0.02	0.6 ± 0.01	0.4 ± 0.01	0.8 ± 0.01	1.0 ± 0.02	0.80 ± 0.05	0.60 ± 0.02	0.60 ± 0.08
C17:1	0.4 ± 0.01	1.4 ± 0.03	0.2 ± 0.01	0.4 ± 0.04	nd	nd	nd	nd
C18:0	22.6 ± 0.80	1.06 ± 0.02	15.2 ± 0.37	29 ± 1.50	40.6 ± 4.1	29.4 ± 1.60	30.2 ± 2.4	28.4 ± 3.80
C18:1n9c	16.4 ± 0.49	21.2 ± 0.06	109.8 ±5.49	169.8 ± 7.60	255.0 ± 12.72	282.6 ± 16.20	250.4 ± 12.27	255.8 ± 15.0
C18:2n6c	25.2 ± 1.73	18.9 ± 0.94	17 ± 0.65	27.2 ± 2.20	40.6 ± 3.70	43.0 ± 2.15	36.8 ± 1.90	38.4 ± 2.40
C18:3n3	1.0 ± 0.09	0.6 ± 0.04	0.6 ± 0.02	1.2 ± 0.12	nd	nd	nd	nd
C20:0	1.8 ± 0.11	1.4 ± 0.08	1.2 ± 0.05	2.2 ± 0.10	11.6 ± 0.58	2.0 ± 0.08	1.6 ± 0.06	1.6 ± 0.09
C20:1	1.0 ± 0.05	0.2 ± <0.01	0.6 ± 0.03	1 ± 0.14	3.4 ± 0.12	2.4 ± 0.03	2.6 ± 0.02	2.4 ± 0.40
C22:0	0.8 ± 0.02	0.4 ± 0.01	0.4 ± 0.02	1.2 ± 0.12	0.2 ± 0.01	0.2 ± <0.01	0.2 ± 0.02	0.2 ± 0.01
C22:1	0.2 ± <0.01	0.2 ± <0.01	0.2 ± 0.01	0.2 ± <0.01	nd	nd	nd	nd
C22:2	2.2 ± 0.15	3.6 ± 0.18	2.2 ± 0.12	4.0 ± 0.40	4.6 ± 0.14	3.8 2 ± 0.31	4.0 ± 0.39	4.2 ± 0.44
C24:0	1.2 ± 0.08	1.0 ± 0.03	0.6 ± 0.02	1.8 ± 0.10	2.8 ± 0.24	2.0 ± 0.41	2.6 ± 0.53	2.0 ± 0.30
α- tocopherol	3.2 ± 0.73	3.2 ± 0.52	4.6 ± 0. 67	7.8 ± 1.40	2.4 ± 0.12	15.2 ± 0.75	7.2 ± 0.28	12.0 ± 1.35
Squalene	3.1 ± 0.06	4.3 ± 0.08	7.5 ± 0.22	9.9 ±1.80	4.23 ± 0.21	15.05 ± 0.05	9.95 ± 0.03	12.04 ± 0.04
β- sitosterol	1.3 ± 0.05	1.4 ± 0.03	-	-	nd	nd	nd	nd
Total carotene (β- carotene)	9.9 ± 0.59	10.8 ± 0.63	7.1 ± 0.43	11.2 ± 1.20	11.4 ± 0.08	10.5 ± 0.82	11.4 ± 0.95	11.4 ± 1.3

Nd: not detected


0.5
0.45
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
Mass of extract (g)
0
15
30
45
60
75
90
Time (min)
Run 1
Run 2
Run 3
Run 4
Run 5

a)


0.18
0.16
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
Mass of extract (g)
0
1
2
3
4
5
6
Time (min)
Run 6
Run 8
Run 9
Run 10

b)

Fig. 1

4.2 ARTIGO CIENTÍFICO II

Influence of different compressed fluids on extraction of residual oil from palm kernel cake

Artigo submetido ao periódico *Journal of Food Engineering*.

Influence of different compressed fluids on extraction of residual oil from palm kernel cake

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A**bstract**

BACKGROUND: The recovery of residual oil from palm kernel cake by supercritical carbon dioxide (CO_2) and compressed liquefied petroleum gas (LPG) was studied. Extractions were carried out in order to investigate the influence of pressure and temperature on the extraction yield, kinetic behavior, chemical composition and antioxidant activity. RESULTS: Maximum extraction yield was about 9.0 wt% regardless the solvent used in the extractions. However, a high variation in the yield depending of extraction condition was observed. Lauric, miristic and oleic acids were the predominant fatty acids. Kinetic parameters such as maximum extraction rate and the mass ratio of solute in the solvent phase at the extractor outlet for extractions with compressed LPG were 27 and 114 times higher than supercritical CO_2, respectively. In extractions carried out with supercritical CO_2, the steady state was reached at about 75 minutes, whereas for LPG at 3-4 minutes. CONCLUSION: Palm kernel cake may be used as a source of oil for industry and compressed LPG showed promising results for industrial oil extraction.

Keywords: palm kernel cake, fatty acids, antioxidant activity, pressurized fluids, high-pressure.

INTRODUCTION

Palm oil agroindustry not only produces the most consumed vegetable oil in the world, but also a significant quantity of residual biomass such as palm kernel cake and pressed palm fiber[1]. Palm kernel cake is used as a medium grade protein feed, containing about 15% crude proteins and up to 12% residual oil, depending on the extraction method. Although the cake provides both protein and energy, it is looked upon more as a source of medium grade protein with high fiber content, more suitable for feeding ruminants[2].

The extraction of residual oil from palm kernel cake before its use as animal feed represents a real opportunity to create value-added products, since palm kernel oil is an important feed stock for the manufacture of oil chemicals including fatty acids, fatty esters and fatty alcohols. In addition, the relatively high content of myristic and lauric fatty acids of the palm kernel oil makes it very suitable for the manufacture of soaps, washing powders and personal care products. Other non-edible applications of the oil include candles manufacture, as well as the pharmaceutical and perfume industries[3].

The utilization of residual palm kernel oil as raw material in the pharmaceutical, perfume and personal care industries requires the use of extraction methods not leave organic residues in the oil[4,5].The supercritical technology using carbon dioxide (CO_2) is a green alternative for the extraction of residual palm kernel oil (Bubalo et al. 2016)[6]. Extraction with supercritical CO_2 constitutes a non-toxic and waste-free process employing an inert gas that does not cause thermal degradation of the bioactive compounds[7,8,9]. Another potential process for oil extraction may be the use of liquefied petroleum gas (LPG), which is a mixture of butane isomers and propane (hydrocarbons) that can increase the extraction yield. Its use at mild temperatures prevents the degradation of the vegetable matrix and their bioactive compounds. Besides the easy recovery of the gas, it is possible to change the properties of solvent of this process through pressure adjustments[10]. Another advantage is the cost, which is

relatively low if compared to other fluid solvents used in the extraction of bioactive compounds as propane and carbon dioxide [11, 12, 13].

The objective of this work was to evaluate two different solvents, supercritical CO_2 and compressed LPG, for extraction of residual oil from palm kernel cake. For both solvents the influence of pressure and temperature was studied on the extraction yield and kinetics, chemical composition and antioxidant activity towards DPPH radical.

MATERIAL AND METHODS

Material

The palm kernel cake (*Elaeis guineensis*) was provided by Agropalma (Tailândia, Pará State, Brazil). The CO_2 (99.9% purity) was purchased from White Martins, whereas the LPG was purchased from Liquigas (Santa Maria, RS, Brazil) and it is constituted of a mixture of propane (50.3 wt%), n-butane (28.4 wt%), isobutane (13.7 wt%), ethane (4.8 wt%) and other minor constituents (methane, pentane, isopentane). The 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical was obtained from Sigma-Aldrich.

Samples

The samples were sieved and the particles that passed through the 16 mesh sieve were collected and used in the extractions. The sample was maintained at -12 ºC until the experimental assays.

Experimental apparatus and procedure for the extractions

The experiments were performed in a laboratory scale unit consisting of a solvent reservoir, two thermostatic baths, a syringe pump (ISCO 260D), a 100 mL jacketed extraction vessel, an absolute pressure transducer (Smar, LD301) equipped with a portable programmer

(Smar, HT 201) with a precision of 0.12 bar, a collector vessel with a glass tube and a cold trap.

Approximately 10 g of the palm kernel cake as charged into the extraction vessel. The solvent (CO_2 or LPG) was pumped into the bed, which was supported by two 300 mesh wire disks at both ends, and it was kept in contact with the herbaceous matrix for at least one hour to allow the stabilization of the system. Afterwards, the extract was collected by opening the micro-metering valve and the solvent mass flow was determined. The experiments were carried out isothermally, at constant pressure and with a rate of solvent mass flow equals to 4 $g.min^{-1}$.[14] For experiments carried out with CO_2 as solvent, the ranges for temperature and pressure were 20–60 °C and 10.0–25.0 MPa, whereas for LPG, they were 20-40 °C and 0.5-2.5 MPa, respectively. All extractions were carried out in triplicate and kinetic curves were determined for all experimental conditions.

Kinetic evaluation

Kinetic study was carried out through analysis of the parameters of the constant-extraction rate period (CER). The process parameters of the CER period that can be fitted from the extraction kinetics are: (1) the extraction rate for the CER period (M_{CER} – g of extract/min); (2) the time span of the CER period (t_{CER} - min); (3) the yield of the CER period (R_{CER} – wt%); and (4) the mass ratio of solute in the solvent phase at the extractor outlet (Y_{CER} – g of extract /g of solvent). [9]

Fatty acids composition

A volume of 10 µL of palm kernel extract were solubilized in 1 mL of hexane were evaporated at 40 °C under vacuum. Fatty acids methyl esters (FAME) were analyzed by injecting 1 µL into gas chromatograph equipped with flame ionization detector (GC-FID - Varian model Star 3400 CX - CA, USA) and autosampler (Varian, model 8200 - CA, USA).

FAME were separated by capillary column CP-Wax 52 CB (Middelburg, The Netherlands) (50 m × 0.32 mm × 0.20 μm.) The carrier gas used was hydrogen at a constant pressure of 15 psi. The injector port was maintained in the split mode with 20:1 ratio and temperature of 250 °C. The following column temperature was programmed at 50 °C (1 min); 20 °C.min^{-1} up to 200 °C; 10 °C.min^{-1} up to 230 °C (8 min). The detector was maintained constantly at 240 °C. Identification of FAME were performed by comparison of retention times of compounds with FAME standards Mix-37 (P/N 47885-U; Sigma-Aldrich, St. Louis, USA). Compositions were expressed as percent of normalized peak areas.[15]

Antioxidant activity of extracts

The antioxidant activity of extracts was evaluated using DPPH radical following the methodology described by Dal Prá et al.[16] The method begins with the addition of 1500 μL of extract to a 1480 μL of a DPPH solution (200 μM) and 20 μL of ethanol. Besides the assay conducted for the DPPH, was performed using 1500 μL of ethanol instead of the extract. The resulting solution was maintained at rest for 30 minutes. The samples absorbance was determined at 522 nm in an UV-Vis 8453 Hewlett-Packard spectrophotometer (Agilent Technologies, Santa Clara, USA). The antiradical activity toward DPPH (AA_{DPPH}) was then calculated according to the following equation:

$$AA_{DPPH} = \left(\frac{A_{DPPH-}(A - A_B)}{A_{DPPH}} \right) \times 100 \quad (1)$$

where A_{DPPH}, A and A_B are the absorbance of the DPPH solution, sample and blank, respectively.

Statistical analysis

All the results were analyzed using the software Statistica® 7.0 (Statsoft Inc., Tulsa, OK, USA), considering a significance level of 95%.

RESULTS AND DISCUSSION

Influence of solvent on extraction yield

Table 1 presents the results in terms of extraction yield obtained with supercritical CO_2 and compressed LPG. The highest yields for CO_2 was 9.2 wt% at run 2 (60 °C/25 MPa) and 9.8 wt% for LPG at run 6 (20 °C/0.5 MPa). The yield obtained at run 6 using LPG was statistically different from other runs, whereas data of runs 2, 9 and 10 were equals ($p<0.05$). Runs 1, 3 and 5 presented extraction yield ranging from 4-6 wt% and runs 4 and 7 presented very low yields (about 0.1 wt%). The yield reported in this study is in good agreement with the study of Rahman et al.[7], who extracted residual oil from the palm kernel cake and achieved an extraction yield of 8.61%, but at higher pressure (41.36 MPa).

Table 1

The process variables (pressure and temperature) presented significant influence on the yield. For CO_2, the increase in pressure maintaining the temperature at 20 °C (runs 1 and 3) did not affect the yield, by other hand, at 60 °C, a significant increase in the yield was verified when the pressure was altered from 10 to 25 MPa. This is verified because at 20 °C there is little variation in the solvent density with the increase of pressure, whereas at 60 °C occurs an accentuated decrease in the solvent density, affecting solvating power. In addition, when combining high pressure with high temperature its verified an increment of the compounds vapor pressure, which are then more easily extracted, justifying the highest yield obtained at 25 MPa and 60 °C. For extractions carried out with LPG, it is seen little variation in the yield with exception for run 7. Combining high temperature (40°C) and low pressure (0.5 MPa) the LPG is predominantly in the vapor phase and the solvating power is

considerably reduced. For other experimental conditions, it is predominantly in the liquid phase, with little influence on pressure and, consequently, in the yield.

The maximum yield obtained in each solvent was similar (approximately 9.50 wt%), but the conditions for LPG were considerably milder than CO_2 making LPG a feasible solvent to be used for extraction of vegetable oils. The benefits to use LPG instead CO_2 for extraction of residual oil from palm kernel cake can be seen on Figure 1, which presents the kinetic profile for the extractions with supercritical CO_2 (Figure 1a) and with compressed LPG (Figure 1b). For extractions carried out with supercritical CO_2, the steady state was reached at about 75 minutes of extraction, whereas for LPG, at 3-4 minutes.

Figure 1

The use of compressed LPG can be an alternative when fast extractions are necessary. The influence of the solvent on extraction parameters is presented in Table 2. Maximum extraction rate (M_{CER}) at constant extraction period (t_{CER}) using supercritical CO_2 was 0.029 g of extract/min, whereas for compressed LPG it was 0.7843 g of extract/min, which is about 27 times greater than for supercritical CO_2. The mass ratio of solute in the solvent phase at the extractor outlet (Y_{CER}) for run 1 using compressed LPG was about 114 times major than for supercritical CO_2. This result implies in the use of 114 times more solvent.

Table 2

Hence, using compressed LPG for extraction leads to lower expenses with solvent in an industrial plant. Besides, the highest yield for supercritical CO_2 was obtained at 25 MPa and for LPG at 0.5 MPa, which is a pressure about 50 times lower. Thus, LPG requires less

energy on recompression compared to carbon dioxide. LPG is plenty available, cheaper and it can be used under much lower pressures compared to carbon dioxide. For instance, at 65 °C and 25 bar, LPG exhibits a density of 9.27 mol/L, while CO_2 will reach a similar density only at 124 bar.[10]

Influence of solvent on extraction antioxidant activity and chemical composition

Table 3 presents the chemical composition and antioxidant activities of the extracts obtained in runs with the highest yield (run 2 and 5 for CO_2 and runs 6, 8, 9 and 10 for LPG). Antioxidant activity ranged from 4.0 to 5.5 % for extractions carried out with LPG and from 13.5 to 14.6 % for extraction with CO_2. Although the antioxidant activity of extracts obtained with CO_2 were higher than those for LPG, the values obtained in this study are considered lower than extracts of other plants. The low antioxidant activity of extracts can be attributed to a chemical composition that is predominately composed of triacylglycerols.

Table 3

Eighteen fatty acids were identified in the extract from palm kernel cake. Lauric (C12:0), myristic (C14:0), hexadecanoic (C16:0), oleic (C18: 1n9c) acids were found in high amount in all samples and together represent about 90% of the total fatty acids of the kernel. The most abundant fatty acid in palm kernel oil samples was lauric acid (C12:0) with 37.15-44.59%. The composition of the samples was similar in terms of compounds and amounts of these compounds.

The fatty acid composition found in this study was in agreement with previous reports. Kok et al.[17] verified that the most abundant fatty acids in palm kernel samples were lauric acid, myristic acid and oleic acid, being lauric acid about 53%. Bora et al.[18] identified

eighteen fatty acids in the palm kernel, where lauric (C12:0) and myristic acid (C14:0) were the most abundant fatty acids determined in the palm kernel oil.

CONCLUSIONS

In this work was investigated the extraction of residual oil from palm kernel cake using supercritical CO_2 and compressed LPG. The last solvent showed to be the best choice for the extraction, since the maximum extraction rate at a constant extraction period and the mass ratio of solute in the solvent phase at the extractor outlet were 27 and 114 times higher than supercritical CO_2. Moreover, for extractions carried out with supercritical CO_2, the steady state was reached at about 75 minutes of extraction, whereas for LPG at 3-4 minutes. In terms of chemical profile were not verified differences between the solvents. This shows that the use of compressed LPG can be a promising alternative to conventional process (pressing or using solvents) for extractions of vegetable oils.

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List of Figure Captions

Figure 1. Overall kinetic curves for the extraction of palm kernel cake using supercritical CO_2 (a) and compressed LPG (b) as solvents.

Table 1. Extraction yields of kernel palm oil obtained using supercritical CO_2 and compressed LPG at different operational conditions

Run	Temperature (°C)	Pressure (MPa)	Yield (wt%)
Supercritical CO_2			
1	20	25.0	4.1±0.1[*d]
2	60	25.0	9.2±0.4[ab]
3	20	10.0	4.1±0.2[d]
4	60	10.0	0.1±0.1[e]
5	40	17.5	6.1±0.3[c]
Compressed LPG			
6	20	0.5	9.8±0.4[a]
7	40	0.5	0.1±0.1[e]
8	20	2.5	8.6±0.3[b]
9	40	2.5	9.1±0.3[ab]
10	30	1.5	9.0±0.4[ab]

[*] different letters at same column represent a significant difference ($p<0.5$).

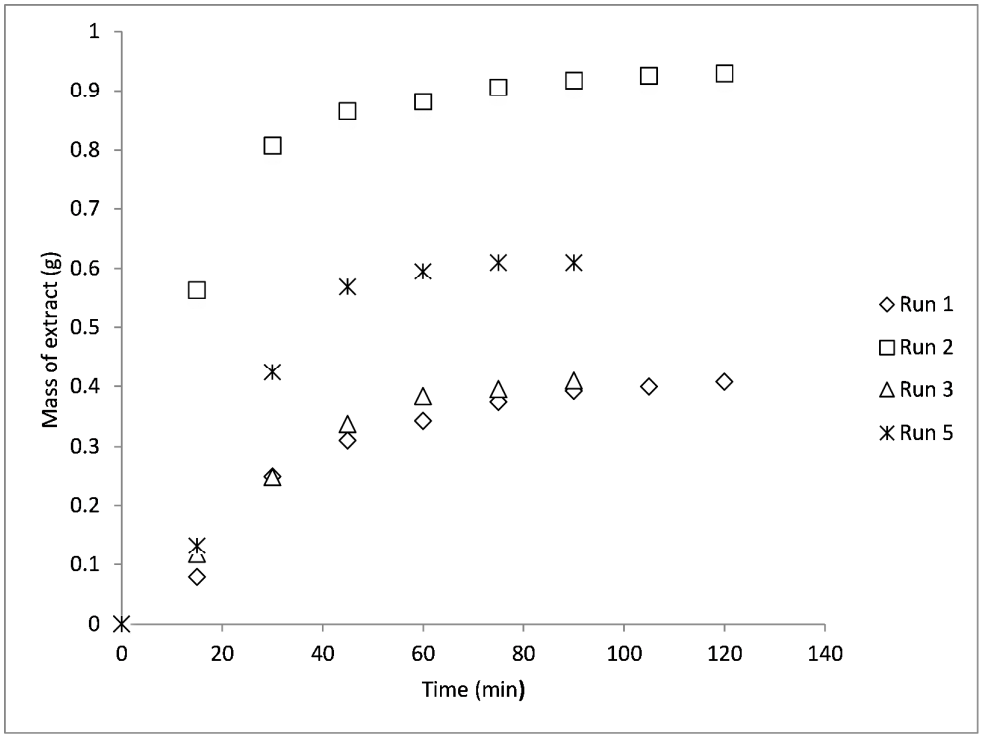
Table 2. Kinetic parameters determined at the constant-extraction rate period for curves determined using supercritical CO_2 and compressed LPG

Run	M_{CER} ($g_{extract}$/min)	t_{CER} (min)	R_{CER} (wt%)	Y_{CER} ($g_{extract}/g_{solvent}$)
		Supercritical CO_2		
1	0.0072	45	76.00	0.0017
2	0.0029	30	86.85	0.0048
3	0.0077	45	82.38	0.0019
4	0.0005	15	80.00	0.0001
5	0.0128	45	93.08	0.0032
		Compressed LPG		
6	0.7843	1	79.90	0.1961
7	Nd	Nd	Nd	Nd
8	0.5518	1	64.31	0.1380
9	0.5096	1	55.79	0.1274
10	0.3477	2	74.83	0.0839

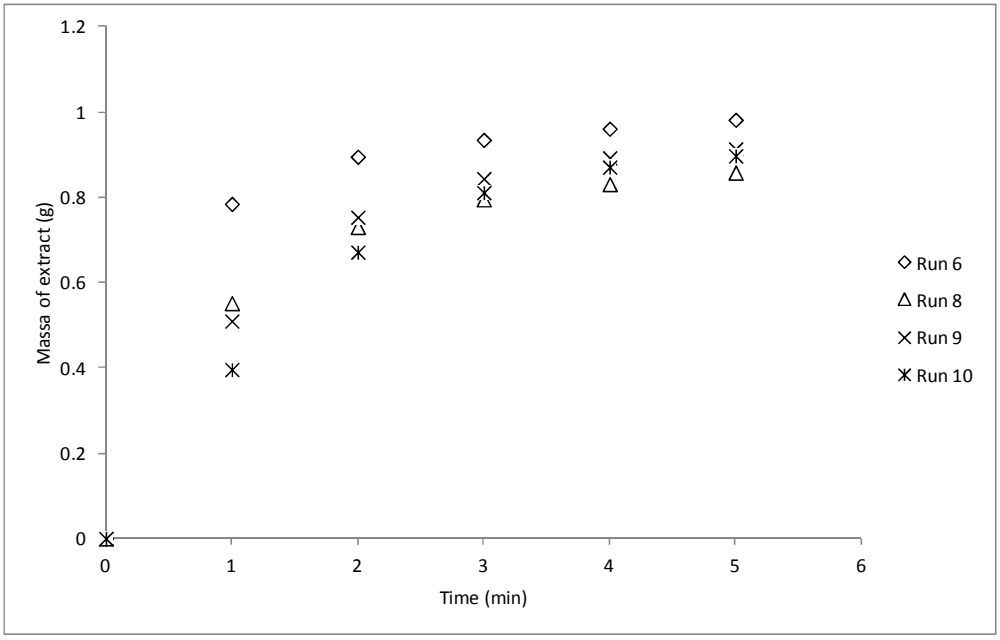
Nd: not determined

Table 3. Chemical characterization (percent of normalized peak area) and antioxidant activities of extract obtained at selected experimental condition

Sample/fatty acid (%)	Run 2 - CO_2	Run 5 - CO_2	Run 6 – LPG	Run 8 - LPG	Run 9 - LPG	Run 10 - LPG
C8:0	1.83	1.49	1.74	1.66	1.64	1.76
C10:0	2.45	1.98	2.35	2.29	2.32	2.38
C11:0	0.03	0.03	0.04	0.03	0.03	0.03
C12:0	44.59	37.15	43.90	44.23	44.80	44.70
C13:0	0.06	0.05	0.06	0.06	0.06	0.06
C14:0	18.55	16.85	18.36	18.57	18.93	18.59
C15:0	0.02	0.06	0.02	0.02	0.02	0.02
C16:0	10.11	12.62	9.98	10.01	10.09	9.83
C17:0	0.02	0.05	0.03	0.03	0.02	0.03
C17:1	0.09	0.06	0.11	0.10	0.08	0.08
C18:0	3.34	4.49	3.39	3.39	3.32	3.30
C18:1n9c	16.01	21.12	16.91	16.58	15.83	16.37
C18:2n6c	2.50	3.54	2.61	2.57	2.47	2.52
C20:1	0.16	0.21	0.16	0.18	0.16	0.15
C20:2	0.11	0.15	0.11	0.11	0.11	0.10
C22:1	0.05	0.05	0.05	0.05	0.04	0.04
C22:2	-	-	0.12	0.03	-	-
C24:0	0.08	0.11	0.06	0.08	0.07	0.05
DPPH (%)	13.52±0.40	14.62±0.03	3.99±0.12	4.71±0.12	5.50±0.16	4.68±0.14


Mass of extract (g)
Time (min)
Run 1
Run 2
Run 3
Run 5

a)


Massa of extract (g)
Time (min)
Run 6
Run 8
Run 9
Run 10

b)Figure. 1.

4.3 ARTIGO CIENTÍFICO III

Ultrasound-assisted extraction of bioactive compounds from palm pressed fiber with high antioxidant and photoprotection activities

Artigo submetido ao periódico *Ultrasonics Sonochemistry* em processo de revisão.

Ultrasound-assisted extraction of bioactive compounds from palm pressed fiber with high antioxidant and photoprotection activities

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ABSTRACT

This work is focused on the optimization of the ultrasound-assisted extraction of antioxidant compounds with photoprotective effect from palm pressed fiber. The influence of ultrasound intensity and pulse cycle was investigated by means of a central composite rotational design. The optimized condition was ultrasound intensity of 120 $W.cm^{-2}$ and pulse factor of 0.4, yielding 3.24 wt%. Compounds such as fatty acids, β-sitosterol, α-tocopherol, squalene, total phenolics and carotene were identified. The extract presented antioxidant activities towards synthetic (DPPH, ABTS) and biological (•OH) radicals besides a sun factor protection of 15. Polar extracts from palm pressed fiber are promising candidates for use in cosmetic and pharmaceutical formulation, since they presented high antioxidant activities towards different radicals combined with the high sun factor protection.

Keywords: natural sunscreen; ultrasound-assisted extraction; synthetic and biological radicals;

1. Introduction

Elaeis guineensis Jacq. (palm tree) is cultivated in 42 countries and presents the highest yielding on edible oil in the world [1]. Crude palm oil is a rich source of fatty acids and other antioxidant constituents as tocopherols, tocotrienols and carotenoids. The process for palm oil extraction based on pressing is well defined [2]. However, residual compounds remain in the palm pressed fiber that possess antioxidant activity and can be recovered by applying different extraction methods.

In recent years, new techniques used for the extraction of bioactive compounds from vegetable raw materials have been studied including ultrasound-assisted extraction (UAE) [3], supercritical fluid extraction (SFE) [4], microwave-assisted extraction (MAE) [5] and accelerated solvent extraction (ASE) [6]. Among them, ultrasound-assisted extraction technology has received considerable attention due to its beneficial properties, involving high extraction efficiency, high reproducibility, low solvent consumption, easy-operating, low cost and low pollution to environment [3, 7]. The heat and mass transfer enhancement induced by ultrasound is mainly attributed to the acoustic effects accompanied by the ultrasonic cavitation phenomenon, such as micro-streaming, local extremely high temperature and pressure, mechanical agitation [8].

Few studies have reported the extraction of bioactive compounds from palm pressed fiber using ultrasound. Juliano et al. [9] obtained higher extraction yield of residual palm oil from palm pressed fiber using ultrasound-assisted extraction. Ofori-Boateng and Lee [10] compared different methods for the extraction of α-tocopherol from the fronds palm oil, verifying that ultrasound was more efficient method. Other studies available in the literature refer to extraction of carotene and α-tocopherol from palm oil [11-13]. However, little attention has been paid on the extraction of the polar components of this by-product.

Other aspect that recently has received great attention is the investigation of potent antioxidant and photoprotective effect of vegetable extracts [14]. These compounds can be used in the development of sunscreen formulations, which remain at the surface of the skin for a longer time and may incorporate antioxidants that can neutralize the reactive species of oxygen (ROS). UV radiations absorbed by the skin surface can produce ROS, causing skin cancer and premature aging [15]. Extracts from different plants have been successfully used for this purpose [14, 16-18]. However, there is no report of extracts from palm pressed fiber.

Based on these aspects, the aim of this work is to evaluate the ultrasound-assisted extraction of antioxidant compounds with photoprotective effect from palm pressed fiber. The influence of ultrasound intensity and pulse cycle was investigated by means of a central composite rotational design. The results obtained at optimized condition were validated and chemically characterized.

2. Material and methods

2.1. Materials

The palm pressed fiber (*Elaeis guineensis*) was provided by the industry of processing of oils and derivatives Agropalma (Tailândia, PA, Brazil). The samples of palm pressed fiber were dried at 50ºC until a constant mass was obtained. Afterwards, the samples were milled in a Wiley mill and sieved. The particles that passed through a sieve of 16 mesh were collected. The samples were maintained at -12 ºC for further analysis.

2.2. Experimental apparatus and procedure for the extractions

The experimental apparatus was composed of a jacketed reactor (250 mL capacity) connected to a thermostatic water bath (temperature accuracy of ± 1.0 °C) for temperature control, a high-intensity ultrasound processor of 400 W and frequency of 24 kHz (Hielscher, Model UP 400S). The ultrasound was equipped with a titanium probe of 22 mm (Model H22 Tip 22) presenting a maximum ultrasound intensity of 300 $W.cm^{-2}$. The equipment enables the adjustment of ultrasound intensity from 30 to 300 $W.cm^{-2}$ and pulse cycle between 0 and 1. Pulse cycle is related to the time that ultrasound is on.

For the extractions, the ultrasonic probe was placed at the center of the jacketed reactor containing 10 g of palm pressed fiber and 100 mL of ethanol. Afterwards, the temperature was adjusted to 20 °C ± 2 °C by circulating water through the jacket. All extractions were carried out for 2 h at specified ultrasound power and pulse cycle. The effects of ultrasound intensity (36-204 $W.cm^{-2}$) and pulse cycle (0.12-0.68) in extraction yield of palm pressed fiber were evaluated through a central composite rotational design for two independent variables (CCRD). After the analysis of results of CCRD, three additional experiments were carried out to validate the results.

2.3 Chemical characterization of extracts obtained at optimized conditions

2.3.1. Total phenolics

The assay was performed according to the method described by Cândido et al. [19] with some modifications. The extracts (0.1 mL) were mixed with 2.5 mL of Folin–Ciocalteu reagent (10%) and, after 5 minutes, 2.0 mL of sodium carbonate (7.5%) was added. The solution was kept at room temperature (20 ºC) for 60 min, and then the absorbance was measured at 740 nm using the UV/Vis spectrophotometer (Shimadzu, model UV-2600). The results were expressed in milligrams of GAE (gallic acid equivalents) per 100 g of dried sample.

2.3.2 Gas chromatography analysis

2.3.2.1 Determination of fatty acids

An amount of 40 mL of extract was evaporated at 40 °C under vacuum. Solubilized with 1.5 mL of hexane and withdrew 500 fraction μL for determinations of α- tocopherol, β-sitosterol and squalene, in other fraction added 3 mL of hexane for determinations of fatty acids. For determination of fatty acids, 250 μL of a solution 4 mg/mL methyl tricosanoate (C23:0 Me) (Sigma-Aldrich, St. Louis, USA) in isooctane as internal standard (Pi) was added to fraction. Solvents were evaporated at 40 °C under vacuum. The derivatization was performed according to the method described by Hartman and Lago [20]. Fatty acid methyl esters (FAME) were analyzed by injecting 1 μL into gas chromatograph equipped with flame ionization detector (GC-FID - Varian model Star 3400 CX - CA, USA) and automatic sampler (Varian, model 8200 - CA, USA). FAME were separated by a capillary column CP-Wax 52 CB (Middelburg, The Netherlands) (50 m × 0.32 mm × 0.20 μm) The carrier gas used was hydrogen at a constant pressure of 15 psi. The injector was maintained in the split mode with 20:1 ratio and 250 °C. The following column temperature program was used: 50 °C (1 min); 20 °C/min up to 200 °C; 10 °C/min up to 230 °C (8 min). The detector was maintained at 240 °C. Identification of FAME was performed by comparison of retention times of compounds with FAME standards Mix-37 (P/N 47885-U; Sigma-Aldrich, St. Louis, USA). The results were expressed in mg of FAT ACID per gram of extract.

2.3.2.2 Determination of α- tocopherol, β- sitosterol and squalene

Squalene, α-tocopherol and β- sitosterol were determined by a gas chromatograph equipped with flame ionization detector flame (GC-FID - Varian model Star 3400 CX - CA, USA) and automatic sampler (Varian, model 8200 - CA, USA). Compounds were separated by capillary column RTX-5MS (Restek-USA) (30 m × 0.25 mm × 0.25 μm). The carrier gas used was hydrogen at a constant pressure of 15 psi. The injector was maintained in the

splitless mode for 1 minute followed by the opening of the splitter in the ration 30:1 and 320 °C. The column temperature program was used: 200 °C (5 min); 15 °C/min up to 280 °C; 5 °C/min up to 330 °C (10 min). The detector was maintained of 320 °C and the injection volume was 1 μL. The compounds were identified by comparison of retention times and mass spectra of standards mix (α-tocopherol, β-sitosterol and squalene) obtained in a gas chromatograph coupled to mass spectrometer (GC/MS) Shimadzu, QP-2010 Plus (Tokyo, Japan) under the same chromatographic conditions described above, using helium as carrier gas and GC/MS interface and ionization source temperature were maintained at 280 °C. The detector was operated in the electron impact ionization mode with ionization energy of +70 eV and a scan mass range from 35 to 350 m/z. The quantification was performed by external standard by means of calibration curves for each compound. The results were expressed in mg/g of extract.

2.3.3. Analysis of total carotenoids

The concentration of total carotenoids was measured using a UV/Vis spectrophotometer (Shimadzu, model UV-2600). About 500 μL of extracts were diluted with 3 mL of hexane. The solution was transferred to a 1-cm quartz cuvette and the absorbance was read at 450 nm. Total carotenoid content in the extracts were calculated using external calibration with equivalents of β-carotene (≥ 95%, Sigma Aldrich). The standard curve of β-carotene ranged of 0.09 to 1.2 μg/mL [21].

2.4. Biological activities

2.4.1 DPPH radical

The antioxidant activity of extracts obtained was evaluated using DPPH radical following the methodology described in details by Dal Prá et al. [22]. Initially, 1.5 mL of

extract were added to a 1.48 mL of a DPPH solution (200 μM) and 20 μL of ethanol. A blank assay was performed using 1.5 mL of ethanol and 1.5 mL of extract instead of a DPPH solution, besides a blank assay conducted for the DPPH. The resulting solution was maintained at static for 30 minutes. The absorbance of samples was determined at 522 nm in an UV/Vis spectrophotometer (Shimadzu, model UV-2600). The values were expressed in μmol of trolox equivalents per gram of dried sample (μmol TE g^{-1}).

2.4.2 ABTS method

The ABTS method was carried out according to the methodology described by Candido et al. [19]. The ABTS radical was formed from the reaction of 140 mM potassium persulfate with 7 mM ABTS stock solution, kept in the dark and at room temperature for 16 h. For the analysis, ABTS radical was diluted in ethanol until the solution reached an absorbance of 0.70 ± 0.05 at 734 nm. An aliquot of 30 μL of each extract was then homogenized with 3 mL of the ABTS radical. Absorbance of the samples was read at 734 nm after 6 min of reaction. The results were expressed in μmol of Trolox equivalents per gram of dried sample (μmol TE g^{-1}).

2.4.3. Hydroxyl radical scavenging activity

The scavenging activity of extracts toward hydroxyl radical was determined by using the deoxyribose method [23]. $FeCl_3 \bullet 6H_2O$ and ascorbic acid were prepared in degassed deionized water prior to use. The reaction tube contained 100 μL of extract, 100 μL of 1 mM EDTA, 100 μL of 1 mM $FeCl_3 \bullet 6H_2O$, 100 μL of 36 mM 2-deoxy-d-ribose, 100 μL of 10 mM H_2O_2, and 100 μL of 1 mM l-ascorbic acid in 25 mM phosphate buffer (pH 7.4), and the total volume was made up to 1.0 mL with the same phosphate buffer. After incubation at 37 °C for 1 h, the reaction was stopped by adding 1.0 mL of 10% TCA (w/v) and 1.0 mL of 1.0% TBA

(w/v) in buffer phosphate (pH 7.4). The mixture was heated in a boiling water bath for 15 min. Once samples were cooled, the final volume was adjusted to 5.0 mL with deionized water, and the absorbance was read at 532 nm. The capability to scavenge the •OH ($AA\bullet_{OH}$) was calculated using the equation. (1)

$$AA._{OH} = \left(1 - \frac{(S - S_B)}{(C - C_B)}\right) \times 100 \quad (1)$$

where S, S_B, C, C_B are the absorbance of sample, blank sample, control and blank control, respectively.

2.4.4. Determination of the sun protection factor (SPF)

SPF was determined in vitro by the spectrophotometric method reported by Mansur et al. [24]. The extracts were dissolved in ethanol 99.5% to perform the determination of the in vitro SPF by determining the absorbance in the range of 290-320 nm (5 nm intervals) using a spectrophotometer. The calculation of the FPS was determined the according to the equation: (2)

$$SPF : CF.\sum_{290}^{320} \mathrm{EE}(\lambda) \cdot \mathrm{I}(\lambda) \cdot \mathrm{abs}(\lambda) \quad (2)$$

Where CF, EE, I abs are the correction factor (10), erythematogenic effect and sunlight intensity at wavelength and absorbance, respectively. The EE value (λ) and I (λ) were calculated previously as described by Mansur et al. [24].

2.5. Statistical analysis

The statistical analysis was accomplished with the *ANOVA test* coupled with the *Tukey test at a 90 % confidence level* using the software Statistica® 8.0.

3. Results and discussion

3.1. Optimization of ultrasound-assisted extractions

Table 1 presents the extraction yields obtained in the eleven runs of the CCRD, which ranged from 2.53 wt% (run 3) to 4.30 wt% (run 6). Data of Table 1 were used to compute linear, quadratic and interaction terms of process variables on response, which were expressed in the form of Pareto chart in the Fig.1. Quadratic terms for pulse cycle and ultrasound intensity as well as linear term for ultrasound intensity were statistically significant ($p<0.10$). Ultrasound intensity presented a positive effect on the extraction, indicating that its increase can lead to higher yields. Comparing runs 1-2 and 3-4 (pulse cycle is maintained constant at levels -1 and +1, respectively) it is seen that at the highest ultrasound intensity was obtained the highest yields. However, the positive influence of ultrasound on yield is linear only at 180 $W.cm^{-2}$, since for a wider region the quadratic term for this variable was significant. The negative effect of quadratic term is indicating the presence of a maximum point in the studied region. In this way, the positive effect of ultrasound intensity on the yield is verified only at a determined value within the region studied, which is defined by the quadratic term and will be discussed below. Similar interpretation can be drawn for quadratic term of pulse cycle, where there is a maximum point in the investigated range.

To better understand the influence of ultrasound intensity on the extraction yield, data of Table 1 were used to estimate the terms of a quadratic model for extraction yield, which is presented in Eq. 3 considering the significant terms ($p<0.1$).

$$Y = 4.13 + 0.37 \cdot P - 0.32 \cdot P^2 - 0.60 \cdot C^2 \qquad (3)$$

Where Y is the extraction yield (wt%), P is the coded ultrasound intensity and C is the coded pulse cycle. The model presented above was statistically validated by the analysis of variance (Table 2). The calculated F-test was about 3 times greater than the tabulated one and

the determination coefficient (r^2) was 0.8351. The applicability of this model is explored in Fig. 2, which presents the influence of ultrasound intensity and pulse cycle on the extraction yield. Maximum extraction yield is obtained for ultrasound intensity ranging from 120 to 180 $W.cm^{-2}$ and pulse cycle from 0.35 to 0.45. The results of contour plots are corroborating with the analysis of effects, where the increase of ultrasound intensity increases the yield only until a value of 120 $W.cm^{-2}$. For values higher than 120 $W.cm^{-2}$ yield increases is not significant enough to verify the energy spent. On the other hand, pulse cycle presented a maximum point around the central point of the CCRD, explaining the reason why the linear term was not significant in the evaluated range.

From Fig. 2, the range for both variables that lead to maximum extraction yield was predicted. However, the optimization of process only is achieved (and proved) after validation of predicted results. In this way, three additional experiments were carried out at different conditions. Pulse cycle was maintained at 0.4 whereas ultrasound intensity was increased from 120 to 180 $W.cm^{-2}$. The results obtained are presented in Table 3, where a good agreement can be seen among the data of CCRD and the validation experiments. For this reason, the optimized conditions for ultrasound-assisted extraction of bioactive compounds from palm pressed fiber is an ultrasound intensity of 120 $W.cm^{-2}$ and a pulse factor of 0.4.

3.2 Chemical characterization and biological activities

Table 4 presents data referring to chemical composition of the extracts obtained at runs 12-14 (validation experiments). Seventeen fatty acids were determined in the extract. Lauric (C12:0) with 3.51-4.84 mg/g, palmitic (C16:0) with 7.79- 8.83 mg/g and oleic (C18:1n9c) acids with 8.03-8.52 mg/g were found in high amounts in all samples and together represent about 80% of the total fatty acids of the pressed fiber. The concentrations obtained

here for these fatty acids are about 10-20 times lower than those obtained by in the previous work of Dal Prá et al. [25] using non-polar solvents as CO_2 and liquefied petroleum gas (LPG). This result might be explained by the highest solubility of palm compounds on LPG and CO_2 in comparison with ethanol. However, the results obtained in this study are in good agreement with other works using polar solvents for extraction of palm extracts. Jesus et al. [26] found palmitic and oleic acids as the majority compounds in palm oil obtained by extraction with compressed ethanol. Similar result was obtained Teixeira et al. [1] using aqueous oil palm extraction.

Squalene, α-tocopherol and β-sitosterol were found in the extracts from palm pressed fiber. Squalene was detected ranging from 1.42 to 1.73 mg/g, α-tocopherol with 2.92-9.46 mg/g and β-sitosterol with 2.77-8.09 mg/g. Total carotene was determined in the range of 2.53-4.07 mg/g. Squalene and α-tocopherol, which are non-polar compounds were obtained in high concentration using supercritical CO_2 or compressed LPG, whereas β-sitosterol presented the highest concentration in the extraction using ethanol, since it is a more polar compound. In the work of Dal Prá et al. [25] squalene and α-tocopherol ranged from 3.0-15.0 mg/g in the extractions with supercritical CO_2 and compressed LPG. By other hand. β-sitosterol was only detected in two extractions with supercritical CO_2, because it is more soluble in ethanol than LPG and CO_2.

Teixeira et al. [1] also reported the presence of tocopherol and total carotene within a similar range. Lau et al. [13] reported the presence of α- tocopherol, squalene, β-sitosterol and total carotene in palm-pressed mesocarp fiber. Mba et al. [2] reported the presence of the α-tocopherol, squalene and total carotene, but in lower concentrations than the concentrations obtained in the present work (squalene ranging from 0.2-0.5 mg/g and total carotene about 0.6 mg/g). Teixeira et al. [1] obtained total carotenes in the range of 0.46-1.27 mg/g. Total phenolic content ranged from 0.85-1.07 mg GAE/g of sample, which is in good agreement

with that reported by Neo et al. [27] (0.31-7.53 mg/g) and Teixeira et al. [1] (0.015-0.026 mg/g).

The results concerning the antioxidant activities towards DPPH, ABTS and •OH radicals of extracts of palm pressed fiber are presented in Table 5. The antioxidant activities are 247-346 μM of trolox equivalents for ABTS and 50-63 μM trolox equivalents for DPPH and from 65-73% for •OH. The sample that has the highest values for both radicals was the run 14 that presented the highest extraction yield and the highest concentrations of β-sitosterol, squalene, carotene and total phenolic content. The antioxidant activities of extracts obtained in this study are in good agreement with other studies reported in literature. Teixeira et al. [1] verified values in the range of 266-991 μM trolox equivalent/g oil.

Table 5 also presents the results for sun protection factor (SPF) for extracts of palm pressed fiber obtained in the validation experiments. The values for SPF ranged from 14.03-15.01, with the highest SPF at run 14. The reasons for the highest SPF obtained in the run 14 may be attributed to the presence of β-sitosterol, α-tocopherol, squalene, total phenolics and carotene, which are compounds that also present antioxidant activities. The antioxidant activities towards different radicals combined with the sun factor protection make the extracts of palm pressed fiber a promising candidate for use in cosmetic and pharmaceutical formulation for sun protection. One possibility is the development of sun protection gel enriched with palm extracts, a natural product with high antioxidant and photoprotective activities.

The SPF obtained in this study is promising since the protection obtained is higher than reported by other studied also using plant extracts. Wagemaker et al. [28] characterized the lipid fraction and determined the sun protection factor of 10 species of *Coffea*, obtained a sun protection factor from 0.0 to 4.1. Maske et al. [29] investigated the chemical stability and the *in vitro* sun protection factors of crude extract of *Rosa kordesii* petal and a gel

formulation, obtaining a SPF of 20.15 and 3.25, respectively. Dévéhat et al. [30] obtained a SPF ~5 with *Lasallia pustulata* extract. Badea et al. [31] designed new nanostructured lipid carriers containing various vegetable oils (Pomegranate seed oil, wheat germ oil, blackcurrant seed oil, sesame seed oil, carrot root oil, raspberry seed oil and rice bran oil and their combinations in order to obtain efficient formulations with UV protection performance and antioxidant activity. The best UV protection was assured by the pomegranate seed oil based cream resulting in a SPF of 4.1. Combining pomegranate seed oil and wheat germ oil have shown a SPF of 5.1.

4. Conclusions

In this work the ultrasound-assisted extraction of bioactive compounds from palm pressed fiber was optimized. The optimized conditions were ultrasound intensity of 120 $W.cm^{-2}$ and pulse factor of 0.4, yielding 3.24 wt%. In the extract various bioactive compounds were identified and quantified such as β-sitosterol, α-tocopherol, squalene, total phenolics and carotene, which presented antioxidant activities towards synthetic (DPPH, ABTS) and biological (•OH) radicals. The extracts also presented a sun factor protection of 15.01. Due to its antioxidant activities towards different radicals combined with the sun factor protection obtained, the extracts from palm pressed fiber are promising active substances for use in cosmetic and pharmaceutical formulation, being incorporated in a gel formulation for increasing photoprotective activity of a commercial formulation.

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Fig. 1. Pareto chart expressing the effect of process variables on the extraction yield.

Fig. 2. Contour plots expressing the influence of process variables on the extraction yield

Table 1. Matrix of the experimental results obtained in the CCRD for ultrasound assisted extraction the palm pressed fiber

Run	Ultrasound Intensity ($W.cm^{-2}$)	Pulse Cycle (-)	Yield (wt%)
1	60 (-1)	0.2 (-1)	2.68
2	180 (1)	0.2 (-1)	2.94
3	60 (-1)	0.6 (1)	2.53
4	180 (1)	0.6 (1)	3.62
5	36 (-1.41)	0.4 (0)	3.18
6	204 (1.41)	0.4 (0)	4.30
7	120 (0)	0.12 (-1.41)	3.04
8	120 (0)	0.68 (1.41)	3.02
9	120 (0)	0.4 (0)	4.0
10	120 (0)	0.4 (0)	3.78
11	120 (0)	0.4 (0)	4.22

Table 2. ANOVA for validation of model presented in Eq. 2.

Source of Variation	Sum of squares	Degrees of freedom	Mean Square	F test
Regression	3.11	3	1.04	9.96
Residual	0.73	7	0.10	
Total	3.84	10		

$R^2 = 0.8351$; $F\text{-tab}_{0.90;3;7} = 3.07$

Table 3. Additional experiments for validation of the results of CCRD

Run	Ultrasound Intensity ($W.cm^{-2}$)	Pulse Cycle (-)	Yield (wt%)
12	120	0.4	3.24±0.11
13	150	0.4	3.83±0.13
14	180	0.4	3.62±0.17

Table 4. Chemical characterization of extract obtained at ultrasound-assisted extraction

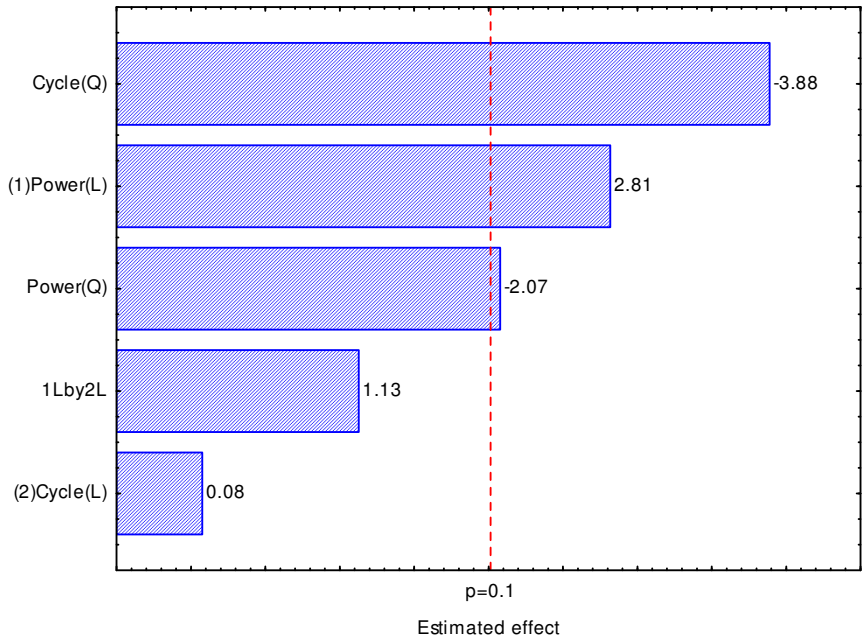
Run/Compound (mg/g)	Run 12	Run 13	Run 14
C8:0	0.23 ± 0.03	0.21 ± 0.03	0.24 ± 0.01
C10:0	0.25 ± 0.04	0.28 ± 0.05	0.26 ± 0.02
C12:0	3.51 ± 1.20	4.84 ± 0.68	3.51 ± 0.36
C14:0	1.41 ± 0.58	2.07 ± 0.26	1.28 ± 0.09
C15:0	0.02 ± < 0.01	0.02 ± < 0.01	0.02 ± < 0.01
C16:0	8.30 ± 0.5	7.79 ± 1.01	8.83 ± 0.56
C16:1	0.03 ± < 0.01	0.03 ± < 0.01	0.03 ± < 0.01
C17:0	0.03 ± < 0.01	0.03 ± < 0.01	0.04 ± 0.02
C18:0	1.14 ± 0.12	1.18 ± 0.18	1.18 ± 0.08
C18:1n9c	8.24 ± 0.61	8.03 ± 1.25	8.52 ± 0.72
C18:2n6c	1.41 ± 0.09	1.38 ± 0.21	1.46 ± 0.12
C20:0	0.09 ± < 0.01	0.07 ± < 0.01	0.10 ± 0.01
C20:1	0.09 ± < 0.01	0.09 ± < 0.01	0.10 ± 0.01
C20:2	0.04 ± < 0.01	0.04 ± < 0.01	0.04 ± < 0.01
C22:1	0.05 ± < 0.01	0.05 ± < 0.01	0.07 ± < 0.01
C22:2	0.24 ± 0.02	0.22 ± 0.03	0.26 ± 0.03
C24:0	0.08 ± < 0.01	0.08 ± 0.02	0.09 ± 0.01
α- tocopherol	2.92 ± 0.07	9.46 ± 0.65	3.84 ± 0.05
Squalene	1.42 ± 0.13	1.73 ± 0.02	1.78 ± 0.05
β- sitosterol	2.77 ± 0.06	4.73 ± 0.04	8.09 ± 0.52
Total carotene (β-carotene)	3.43 ± 0.05	2.53 ± 0.01	4.07 ± 0.1
Phenolic total* (mg GA Equivalent/g)	1.03 ± 0.01	0.85 ± 0.02	1.07 ± < 0.01

* The results for total phenolic content are expressed as mg of GAE /g instead mg/g

Table 5. Antioxidant activities and sun protection factor of extract obtained at ultrasound-assisted extraction

Run	OH^- (%)	ABTS (µM TE)*	DPPH (µM TE)*	SPF
Run 12	69	268	63	14.03
Run 13	65	247	50	13.79
Run 14	73	346	66	15.01

*Micromolar of Trolox Equivalents



L: linear and Q: quadratic

Fig. 1.

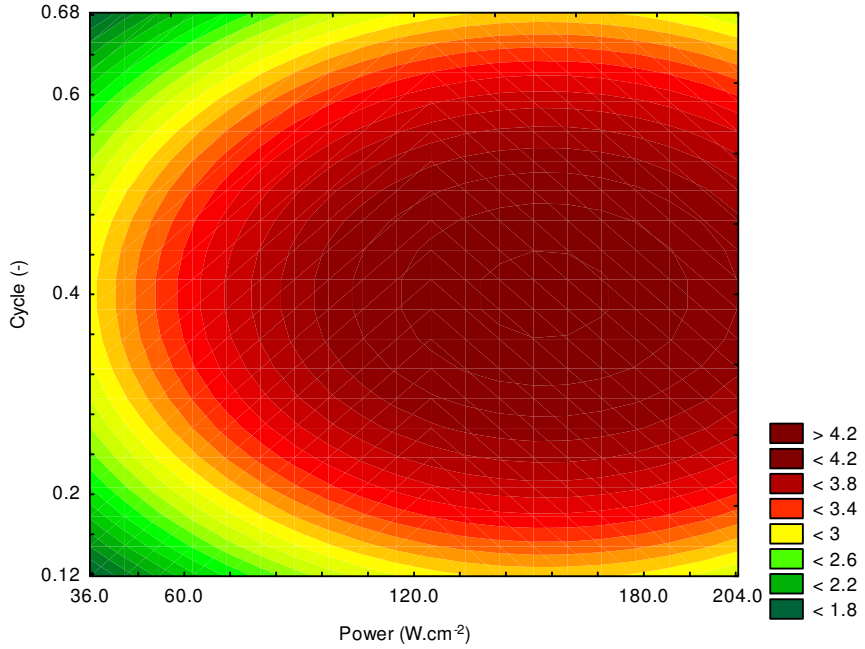

0.68
0.6
0.4
0.2
0.12
Cycle (-)
36.0
60.0
120.0
180.0
204.0
Power (W.cm-2)
> 4.2
< 4.2
< 3.8
< 3.4
< 3
< 2.6
< 2.2
< 1.8

Fig. 2.

4.4 ARTIGO CIENTÍFICO IV

Formulation and characterization of ultrasound-assisted nanoemulsions containing palm oil in water

Artigo a ser submetido ao periódico *Industrial Crops and Products*.

Formulation and characterization of ultrasound-assisted nanoemulsions containing palm oil in water

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ABSTRACT

This study aimed to optimize the ultrasound-assisted palm oil-in-water nanoemulsions. The influence of hydrophilic–lipophilic balance (HLB), oil and surfactant concentration, ultrasound intensity and processing time was investigated by means of two experimental designs. Profiles of the desirability were applied to detect the optimal conditions for preparing the nanoemulsion. Stable nanoemulsions with low droplet size and PDI was obtained at HLB of 6.5, oil and surfactant concentrations of 2.8 wt%, ultrasound intensity of 200 $W.cm^{-2}$ and processing time of 15 minutes. The nanoemulsion presented stability for 30 days at three different temperatures (4°C, 25°C and 40°C), which make it suitable for use in food and comestic applications. Ultrasound showed to be a promising technology to produce nanoemulsions containing palm oil.

Key-words: palm oil; nanoemulsion; ultrasound; formulation.

1. Introduction

In the recent years, there is a growing interest for natural products in pharmaceutical and food industries due to the increase consumer demand for substitution of synthetic compounds by natural substances. Many vegetable oils are good source of functional ingredients and exhibit a great interest as raw materials in the development of natural and eco-friendly products (Badea, et al., 2015; Rebolleda et al., 2015).

Palm (*Elaeis guineensis* Jacq) is cultivated in 42 countries and presents the highest yielding on edible oil in the world (palm oil). The palm oil is one rich source of the fatty acids and other constituents as tocopherols, tocotrienols and carotenoids (Teixeira et al., 2013; Mba, Dumont, Ngadi, 2015). These compounds present high antioxidant and photoprotective activities (Dal Prá et al., 2016). However, the low stability and solubility of these compounds difficulties their utilization in the industry.

One alternative is the development and stabilization of an emulsion to incorporate these compounds in a formulation (Silva et al., 2015). The incorporation of lipophilic active ingredients in aqueous media is by oil-in-water emulsions (Rebolleda et al., 2015; Silva et al., 2015; Rodríguez, 2016). Besides, nanoemulsions are preferable because are colloidal systems more stable than microemulsions.

Among the methods to obtain nanoemulsions, high-intensity ultrasound presents effective and promising results in the preparation of food/pharmaceutical nanoemulsion (Kaur et al., 2016). The ultrasound-assisted nanoemulsions present lower index of polydispersity and are more stable in comparison with those from mechanical devices (Izquierdo et al., 2005 and Li and Chiang, 2012). Factors as the type of oil and process parameters may influence the physicochemical properties and stability (Rebolleda et al., 2015). Although some authors optimized nanoemulsions prepared by ultrasound (Li and Chiang 2012; Rebolleda et al.,

2015; Alzorqui et al. 2016), there is none study reporting its use for nanoemulsion of bioactive compounds from palm pressed fiber.

Based on these aspects, the main purpose of this study was to optimize the process variables (oil and surfactants concentrations, hydrophilic–lipophilic balance, ultrasound intensity and time of ultrasound application) to obtain stable palm oil in-water nanoemulsion by ultrasound.

2. Materials and methods

2.1. Materials

The palm oil (*Elaeis guineensis*) was provided by the industry of processing of oils and derivatives Agropalma (Tailândia, PA, Brazil). Tween 80 (polyoxyethylene sorbitan monooleate) and Span 80 (sorbitan monooleate) were purchase from Sigma–Aldrich.

2.2. Experimental apparatus and procedure

The high intensity ultrasonic processor of 400 W and frequency of 24 kHz (Hielscher, Model UP 400S) with a titanium probe of 7 mm diameter and ultra-turrax Metabo (GE 700 Nurtigen-Alemanha) were the apparatuses used to prepare the nanoemulsions. Palm oil and span 80 (oil phase) and water and tween 80 (aqueous phase) were homogenized separately in ultra-turrax for 1 minute at 7000 rpm. In the following, mixed oil phase in aqueous one in ultra-turrax for 1 minute at 7000 rpm. Afterwards, the pre-emulsion was subjected the high intensity ultrasonic processor at different intensities and processing time. The total mass of nanoemusion (palm oil + water + surfactants) was 30 g.

The effect of process variables on characteristic of nanoemulsion (droplet size, polidispersity index and zeta potential) was determined by means of two experimental designs. In the first one, a central composite rotational design (CCRD) was used to evaluate

the effects of oil concentration (1–10% w/w), emulsifier concentration (1–10% w/w) and hydrophilic–lipophilic balance (HLB) (4.3-15). Ultrasound intensity and processing time were 200 $W.cm^{-2}$ and 10 minutes, respectively. From the results of the CCRD, a central composite design (CCD) was used to study the effects of time (5-15 minutes) and ultrasound intensity (200-400 $W.cm^{-2}$).

At optimized condition, the stability of palm oil in water nanoemulsions was determined by measuring the change of droplet size, polydispersity index, zeta potential and pH at 0, 7, 15, 30, 60, 90 days. Three different storage conditions were evaluated: 4 ºC, 25 ºC and 40 ºC.

2.3 Nanoemulsion characterization

The droplet size and polydispesity index (PDI) of nanoemulsions were analyzed by dynamic light scattering (DLS) using Zetasizer Nano ZS (Malvern Instruments Ltd. UK). Formulations were first diluted 1:500 with ultra-pure water to obtain an adequate scattering intensity. Zeta potential (ZP) was measured with the same apparatus by using electrophoretic light scattering technique. The formulations were diluted 1:500 in 10 mM NaCl solution. The pH of the nanoemulsions was measured by means of a potentiometer Digimed®, previously calibrated with pH 4.0 buffer and 7.0 and the measurements were performed directly on the formulations.

2.4. Statistical analysis

All statistical analysis were performed using Statistica 8.0 software (Statsoft Inc., Tulsa, OK, USA), considering a 95% significance level. Statistical differences between treatments were determined by one-way analysis of variance and means separated using the least significant difference test ($p < 0.05$).

3. Results and discussion

Table 1 presents the results referring to the effect of oil and surfactants concentrations and HLB on droplet size (DS), polidispersity index (PDI) and zeta potential (ZP) of the nanoemulsions. For DS, the values ranged from 98.35 nm (run 5) to 346.80 nm (run 4), 0.186 nm (run 11) to 0.631 nm (run 14) for PDI, and - 4.96 (run 14) to -28.5 (run 13) for ZP.

One way to calculate the effects of process variables is the estimation of parameters of a quadratic a model expressing the response in function of linear, quadratic and interaction terms. Table 2 presents the three models with significant terms ($p<0.1$) as well as the ANOVA for each one of them. Analyzing the terms of the three models simultaneously it is seen that the increase of HLB may reduce the droplet size. However, PDI and zeta potential were negatively affected, since higher PDI and less stable emulsions may be obtained.

The validated models were used to make predictions and to understand better the influence of process variables on responses. These predictions were presented in Fig. 1 in the form of desirability profiles. Low DS and PDI and high ZP (in module) were obtained for HLB of 6.5 and for oil and surfactant concentrations of 2.8 wt%. At this condition, the concentrations of oil and surfactant favored the formation of low droplets with adequate rate of diffusion and the adsorption of surfactant onto the newly formed droplets. Excessive amount of surfactant and oil might lead to a lower diffusion rate of surfactants and result in the coalescence of emulsion droplets (Li and Chiang, 2012).

After the selection of oil and surfactant concentrations and HLB, a central composite design (CCD) was used to study the effects of ultrasound time (5-15 minutes) and ultrasound intensity (200-400 $W.cm^{-2}$) on the characteristics of nanoemulsions. Table 3 presents the DS, PDI and ZP obtained in the eight runs of the CCD. DS ranged from 167.43 nm (run 1) to 212.47 nm (run 8), PDI from 0.200 nm (run 6) to 0.358 nm (run 8) and ZP from -14.87 (run

5) to -22.0 (run 2). The effects of process variables were presented in Table 4. Although the ultrasound intensity and treatment time were not significant in the studied range, they affect the rate of emulsifier adsorption onto the droplet surface as well as the droplet size distribution of newly formed droplets (Jena and Das 2006; Li et al. 2012). The formation of smaller droplet size requires high shear rates (Alzorqui et al., 2016), which may be obtained with low ultrasound intensity for longer periods. In this work, stable emulsion with low droplet size and PDI was obtained at ultrasound intensity of 200 $W.cm^{-2}$ and processing time of 15 minutes (run 2).

After two experimental designs, the best condition for the ultrasound-assisted palm oil in water nanoemulsion was HLB of 6.5, oil and surfactant concentrations of 2.8 wt%, ultrasound intensity of 200 $W.cm^{-2}$ and processing time of 15 minutes. At this condition, the physical stability of nanoemulsion was examined under different storage conditions (4, 25 and 40 ºC) by monitoring their droplet size, polydispersity index, zeta potential and pH for 0, 7, 15, 30 days. The stability results are showed in Fig. 2. Independent of storage temperature and time, no visible creaming or flocculation was observed. At 4 ºC, the nanoemulsion exhibited good stability, without significant ($p<0.05$) changes in the droplet size and PDI. However, zeta potential presented a significant decrease in its value. At room temperature (25 ºC), the nanoemulsion presented good stability for 30 days of storage and presented behavior similar to that maintained at 4 °C. The droplet diameter increased in this period, and zeta potential decreased. At 40 ºC was verified the greatest variation in the properties of nanoemulsion. These results are in accordance with the literature (Alzorqui et al. 201; Reboledda et al. 2015)

4. Conclusion

Two experimental designs were performed to evaluate the influence of process varibales on the ultrasound-assisted palm oil water nanoemulsions. Stable nanoemulsions with low droplet size and PDI was obtained at HLB of 6.5, oil and surfactant concentrations of 2.8 wt%, ultrasound intensity of 200 $W.cm^{-2}$ and processing time of 15 minutes. The nanoemulsion presented stability for 30 days at three different temperatures (4, 25 and 40 °C), which makes it suitable for use in food and comestic applications. Ultrasound showed to be a promising technology to produce nanoemulsions containing palm oil.

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Fig. 1. Profiles the desirability expressing the influence of process variables HLB, oil and surfactant concentration on the droplet size, polydispersity index and zeta potential.

Fig. 2. Graph columns expressing the physical stability of nanoemulsion examined under different storage conditions (4 ºC, 25 ºC and 40 ºC) by monitoring their droplet size (a), polydispersity index (b), zeta potential (c) and for 0, 7, 15, 30 days.

Table 1. Matrix of the experimental results obtained in the CCRD for nanoemulsion

Run	Oil (wt%)	Surfactant (wt%)	HLB (-)	Droplet size (nm)	Polydispersity index	Zeta Potential (mV)	pH
1	(-1) 2.8	(-1) 2.8	(-1) 6.5	163.37 ±1.32	0.195 ± 0.016	-16.33 ± 0.58	5.24
2	(1) 8.2	(-1) 2.8	(-1) 6.5	182.70 ±1.21	0.198 ± 0.009	-13.27 ± 1.18	5.21
3	(-1) 2.8	(1) 8.2	(-1) 6.5	190.37 ±0.35	0.254 ± 0.011	-14.77 ± 0.71	5.24
4	(1) 8.2	(1) 8.2	(-1) 6.5	346.80 ±0.28	0.399 ± 0.018	-14.23 ± 0.21	5.32
5	(-1) 2.8	(-1) 2.8	(1) 12.8	98.35 ± 1.38	0.401 ± 0.003	-8.93 ± 0.75	5.70
6	(1) 8.2	(-1) 2.8	(1) 12.8	136.53 ±1.55	0.224 ± 0.013	-9.67 ± 0.26	5.74
7	(-1) 2.8	(1) 8.2	(1) 12.8	140.57 ±1.12	0.526 ± 0.004	-8.92 ± 0.61	5.72
8	(1) 8.2	(1) 8.2	(1) 12.8	128.83 ±3.92	0.321 ± 0.045	-7.18 ± 0.48	5.86
9	(-1.68) 1	(0) 5.5	(0) 9.7	105.20 ±4.95	0.440 ± 0.098	-8.88 ± 0.78	5.38
10	(1.68) 10	(0) 5.5	(0) 9.7	155.01 ±1.40	0.277 ± 0.007	-10.28 ± 0.38	5.53
11	(0) 5.5	(-1.68) 1	(0) 9.7	158.60 ±1.75	0.186 ± 0.017	-9.49 ± 0.68	5.53
12	(0) 5.5	(1.68) 10	(0) 9.7	128.67 ±1.25	0.397 ± 0.015	-8.65 ± 0.61	5.47
13	(0) 5.5	(0) 5.5	(-1.68) 4.3	214.10 ±3.98	0.486 ± 0.011	-28.5 ± 0.98	4.82
14	(0) 5.5	(0) 5.5	(1.68) 15	137.30 ±6.08	0.631 ± 0.135	-4.96 ± 0.45	6.14
15	(0) 5.5	(0) 5.5	(0) 9.7	120.50 ±2.23	0.351 ± 0.014	-10.87 ± 0.65	5.48
16	(0) 5.5	(0) 5.5	(0) 9.7	119.57 ±1.60	0.291 ± 0.036	-8.58 ± 0.68	4.8
17	(0) 5.5	(0) 5.5	(0) 9.7	111.70 ±0.91	0.279 ± 0.015	-11.03 ± 0.40	5.43

Table 2. Models represented in equations and ANOVA for validation.

Equation	Source of variation	Sum of squares	Degrees of freedon	Mean squares	F test	F test tablet	R^2
$DS = 115.93 - 37.39 \cdot P + 24.31 \cdot P^2$	**Regression**	23625.32	2	11802.66	5.86	2.73	0.7677
	Residual	28172.46	14	2012.32			
	Total	51797.78	16				
$PDI = 0.31 + 0.049 \cdot P + 0.068 \cdot P^2 + 0.061 \cdot L - 0.037.O - 0.066 \cdot P \cdot O$	**Regression**	0.2107	5	0.0421	10.02	2.45	0.8587
	Residual	0.0463	11	0.0042			
	Total	0.2570	16				
$ZP = 10.18 - 4.71 \cdot P + 2.25 \cdot P^2$	**Regression**	379.49	2	189.74	39.61	2.73	0.8667
	Residual	67.07	14	4.79			
	Total	446.56	16				

P is coded HLB, L: is surfactant, O: is oil

Table 3. Matrix of the experimental results obtained in the CCD for nanoemulsion

Run	Time (min)	Ultrasound Intensity ($W.cm^{-2}$)	Droplet size (nm)	Polydispersity index	Zeta Potential (mV)	pH
1	5	200	167.43	0.228	-15.70	5.72
2	15	200	176.40	0.257	-22.00	5.65
3	5	400	212.17	0.371	-18.37	5.79
4	15	400	181.90	0.243	-15.57	5.66
5	10	300	208.93	0.326	-14.87	4.51
6	10	300	169.33	0.200	-16.07	5.45
7	10	300	168.60	0.203	-16.5	5.32
8	10	400	212.47	0.358	-17.4	4.98

Table 4: Effect of variables time and ultrasound intensity in droplet size, polydispersity index and zeta potential

	Droplet size			Polydispersity index			Zeta potential		
Source of variation	**Effect**	**Std. Error**	**p-value**	**Effect**	**Std. Error**	**p-value**	**Effect**	**Std. Error**	**p-value**
Time	-10.65	18.91	0.61	-0.049	0.063	0.49	1.75	1.73	0.38
Ultrasound Intensity	25.12	18.91	0.27	0.064	0.063	0.38	-1.88	1.73	0.35
Time X Ultrasound intensity	-19.62	18.91	0.37	-0.08	0.063	0.30	-4.55	1.73	0.08

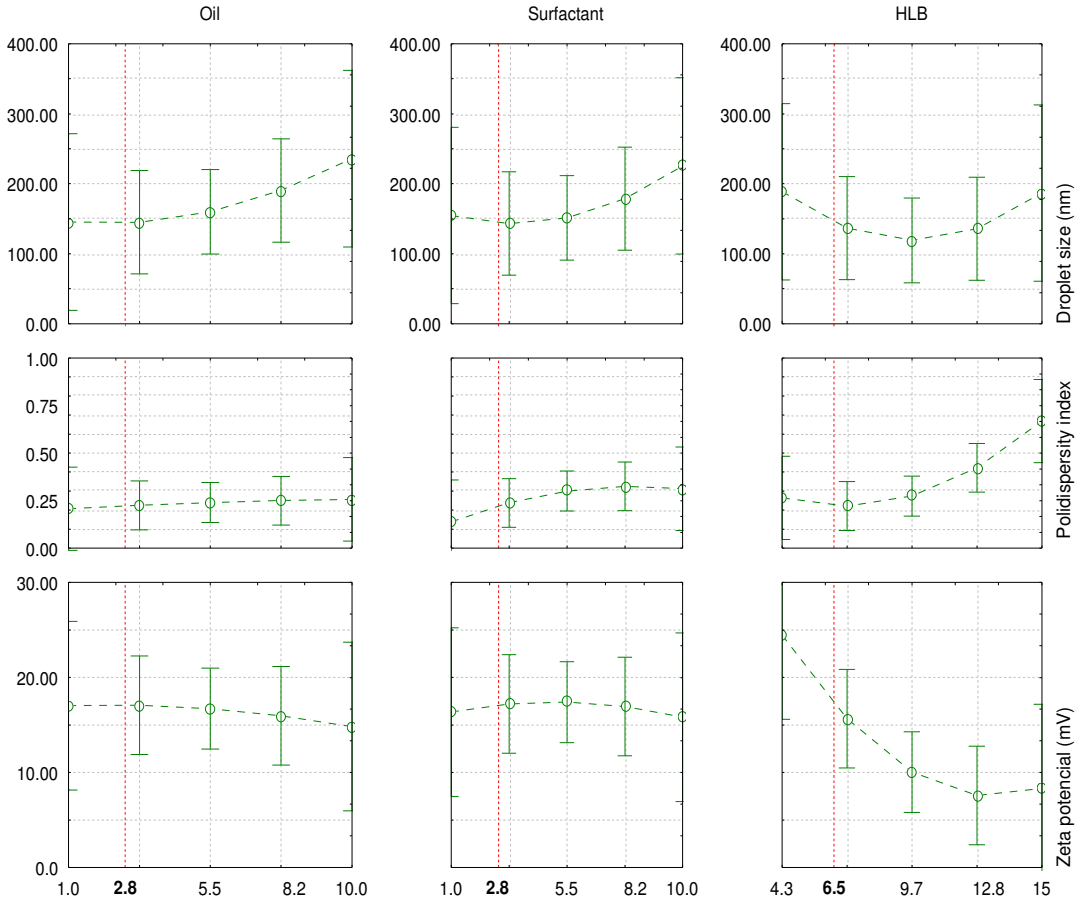
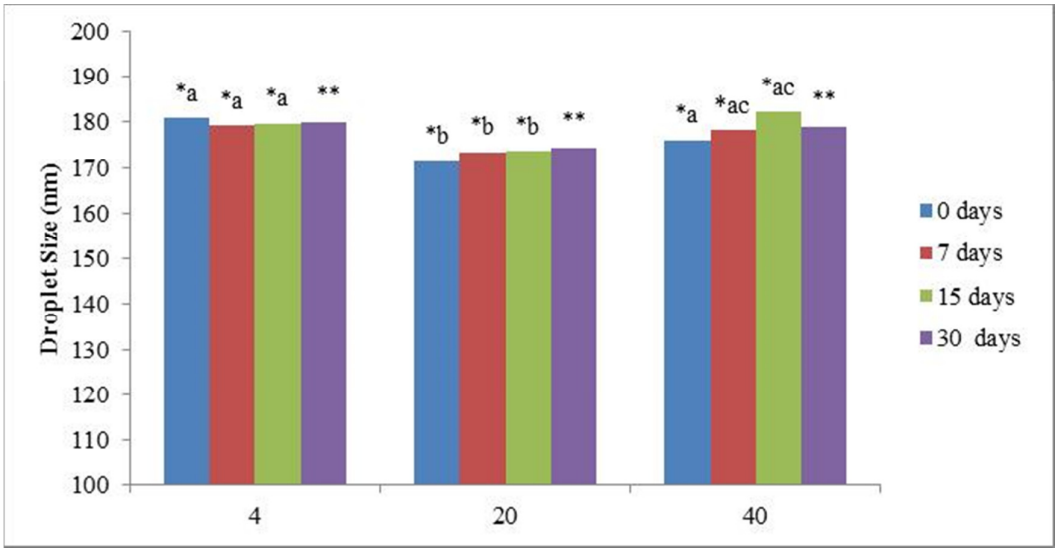
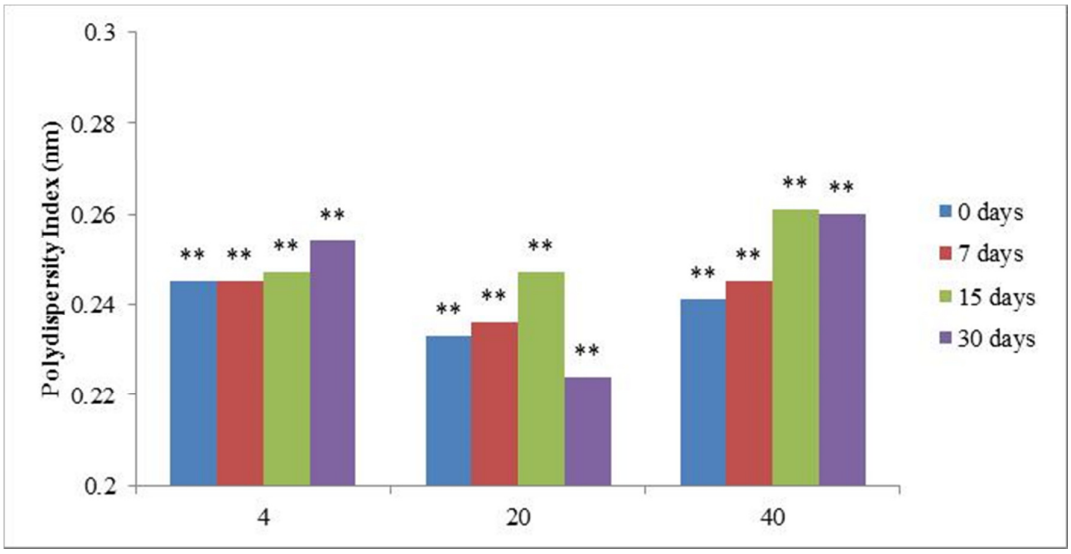

Oil
Surfactant
HLB
400.00
300.00
200.00
100.00
0.00
Droplet size (nm)
1.00
0.75
0.50
0.25
0.00
Polidispersity index
30.00
20.00
10.00
0.0
Zeta potencial (mV)
1.0
2.8
5.5
8.2
10.0
4.3
6.5
9.7
12.8
15

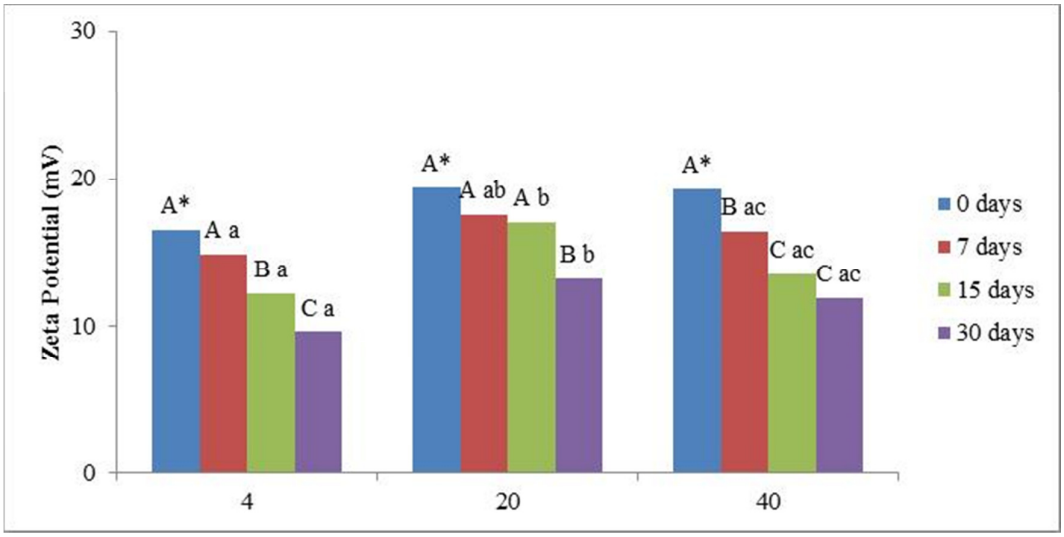
Fig. 1


Droplet Size (nm)
200
190
180
170
160
150
140
130
120
110
100
*a *a *a **
*b *b *b **
*a *ac *ac **
4
20
40
0 days
7 days
15 days
30 days

a)


Polydispersity Index (nm)
0.3
0.28
0.26
0.24
0.22
0.2
** ** ** **
** ** ** **
** ** ** **
4
20
40
0 days
7 days
15 days
30 days

b)


30
20
10
0
Zeta Potential (mV)
A*
A a
B a
C a
A*
A ab
A b
B b
A*
B ac
C ac
C ac
0 days
7 days
15 days
30 days
4
20
40

c)

Fig. 2

5 DISCUSSÃO INTEGRADA

A atividade biológica de extratos vegetais está relacionada com o método de extração e solvente utilizado, uma vez que ambos afetam a seletividade dos compostos extraídos. Em relação às amostras obtidas a partir da torta de processamento da semente de palma, por ambos métodos de extração (GLP pressurizado e CO_2 supercrítico), observou-se menor atividade antioxidante em relação àquelas que foram extraídas a partir da fibra prensada de palma. Além disso, o extrato obtido a partir desta matriz por CO_2 supercrítico, foi a melhor opção para extração de compostos antioxidantes e fotoprotetores como α-tocoferol, esqualeno, β-sitosterol e carotenoides totais, compostos que não foram obtidos da semente, a qual apresentou em sua composição basicamente ácidos graxos. Diante disso, optou-se por dar continuidade ao trabalho apenas com a fibra prensada da palma, otimizando-se a extração de compostos químicos polares através da sonda de ultrassom.

Na otimização do processo de extração de fibra prensada de palma utilizando sonda de ultrassom, compostos químicos como ácidos graxos, β-sitosterol, α-tocoferol, fenólicos totais e carotenoides totais foram identificados. O extrato apresentou atividade antioxidante frente aos radicais sintéticos (DDPH e ABTS), bem como ao radical biológico (•OH) e apresentou fator de poteção solar de 15.01, mostrando-se um promissor candidato para o desenvolvimento de um produto cosmético. Porém, apesar de ter apresentado excelentes resultados optou-se por otimizar o desenvolvimento de nanoemulsões contendo óleo de palma, uma vez que o extrato obtido a partir da fibra prensada apresentou um FPS de 15.06, valor similiar ao obtido pela extração com sonda de ultrassom e utilizando etanol como solvente. No entanto, devido ao baixo rendimento dos métodos de extração utilizados, optou-se pelo desenvolvimento de nanoemuslões contendo óleo de palma, que apresenta composição similar a óleo residual extraído da fibra prensada.

O óleo de palma foi incorporado em nanoemulsões que demonstraram ser altamente estáveis em diferentes condições de armazenamento (4 °C, 25 C and 40°C). A melhor condição foi obtida pela combinação de etapa de pré-formulação utilizando-se ultra turrax e após processador de ultrassom de alta intensidade em uma amplitude de 200 $W.cm^{-2}$ por 15 minutos. A concentração de óleo incorporada foi de 2,8 % bem como de surfactantes (mistura de span 80 e tween 80). Nesse sentido, verifica-se a real possibilidade deste óleo ser empregado tanto no desenvolvimento de formulações cosméticas, potencializando o fator de proteção de solar bem como ser utilizando como antioxidante em produtos alimentícios.

Certamente é uma forma sustentável de aproveitamento da grande quantidade de resíduos gerados pela indústria de processamento de palma, que muitas vezes é desperdiçada.

6 CONCLUSÕES

- ✓ Avaliou-se a extração de compostos bioativos da semente prensada da palma utilizando-se CO_2 supercrítico e GLP pressurizado. Este solvente mostrou-se mais efetivo para extração do óleo da semente de palma, uma vez que o tempo gasto no processo foi cerca de 3-4 minutos, enquanto que com CO_2 supercrítico em torno de 75 minutos. Além disso, apresentou maior rendimento de extração e as amostras foram extraídas em menor temperatura, o que acaba protegendo-as de processos de degradação;

- ✓ A composição química dos extratos acima obtidos foi similiar em relação a compostos e quantidade. Apresentou basicamente ácidos graxos (18), em sua maioria ácido láurico, mirístico, oleico, os quais foram encontrados em todas as amostras;

- ✓ Após avaliar a extração de compostos bioativos da fibra prensada de palma utilizando CO_2 supercrítico e GLP pressurizado, verificou-se que aquela proporcionou maior rendimento de extração, três vezes maior que com GLP, a 60 °C e 25 MPa, bem como maiores atividades antioxidantes e fator de proteção solar. O CO_2 mostrou-se melhor opção para extração de compostos bioativos dessa matriz vegetal;

- ✓ A composição química para ambos solventes, foi ácido láurico, oleico e palmítico, que juntos representam 80% da constituição química das amostras. Além desses, identificou-se compostos como α-tocoferol, esqualeno, β-sitosterol e carotenoides totais;

- ✓ Otimizou-se a extração de compostos antioxidantes e fotoprotetores da fibra prensada de palma com solvente etanol utilizando-se sonda de ultrassom. A condição que apresentou maior rendimento (3,24%) foi 120 $W.cm^{-2}$ de intensidade e pulso de 0.4;

- ✓ Obteve-se compostos como ácidos graxos, β-sitosterol, α-tocoferol, esqualeno e fenólicos totais. Além disso, o extrato apresentou atividade antioxidante frente aos radicais sintéticos (DDPH e ABTS) e biológico hidroxil (OH^-) e fator de proteção solar de 15.01. Tornando-se um promissor candidato para o desenvolvimento de um produto cosmético;

- ✓ O óleo de palma foi incorporado em nanoemulsões que demonstraram ser altamente estáveis em diferentes condições de armazenamento (4 °C, 25°C and 40°C). A melhor condição foi obtida pela combinação de etapa de pré-formulação utilizando-se ultra turrax e após processador de ultrassom de alta intensidade em uma amplitude de 200 $W.cm^{-2}$ por 15 minutos.

- ✓ A concentração de óleo incorporada foi de 2,8 % bem como de surfactantes (mistura de span 80 e tween 80). Nesse sentido, verificou-se a real possibilidade deste óleo ser incorporado em formulações cosméticas, potencializando o fator de proteção de solar bem como ser utilizando como antioxidante em tanto em produtos cosméticos quanto produtos alimentícios. Certamente é uma forma sustentável de aproveitamento da grande quantidade de resíduos gerados pela indústria de processamento de palma, que muitas vezes é desperdiçada.

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