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Fracionamento do Óleo de Café Verde por Destilação Molecular

AUTOR: MELVIN AROLDO DURAN RINCON
ORIENTADOR: Prof. Dr. RUBENS MACIEL FILHO

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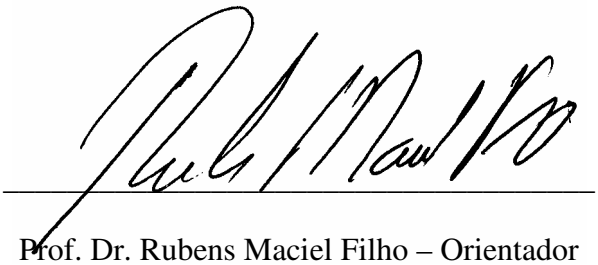

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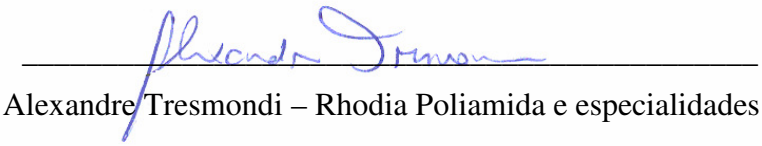

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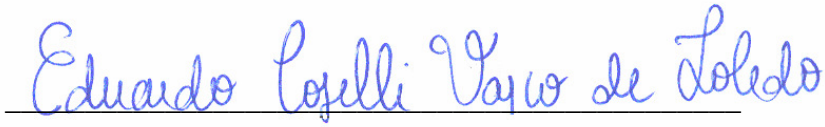

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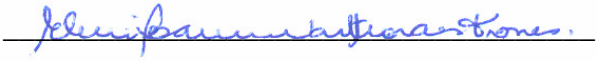

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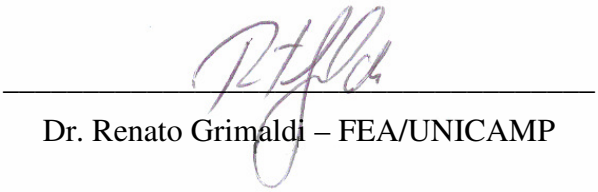

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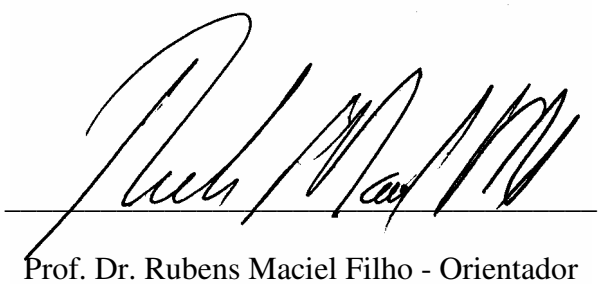

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“ Sublimemos, amor. Assim as flores

No jardim não morreram se o perfume

No cristal da essência se defende.

Passemos nós as provas, os ardores:

Não caldeiam instintos sem o lume

Nem o secreto aroma que rescende”

……. José Saramago

Resumo

Recentemente, a substituição de materiais convencionais utilizados na alimentação, produtos farmacêuticos e cosméticos por produtos naturais vem ganhando mais e mais interesse e importância. As principais indústrias nacionais e internacionais têm procurado fontes naturais alternativas para seus insumos e matérias primas, e uma das grandes dificuldades está na própria obtenção de tais materiais e principalmente, no isolamento e purificação dos agentes bioativos.

O café é uma das bebidas mais conhecidas em todo o mundo, agindo como estimulante nas pessoas devido a sua atividade psicogênica. O café é um dos mais importantes produtos agrícolas representando uma das bases do desenvolvimento econômico e social dos países produtores. O café foi erroneamente considerado no passado como um produto onde basicamente seu principal composto era a cafeína, mas ele tem mais de 1000 compostos tais como vitaminas, aminoácidos, açúcares, lipídios, minerais, diterpenos (cafestol, caveol), ácidos clorogênicos, entre muitos outros ainda a ser estudados. O conteúdo de óleo nos grãos de café é de aproximadamente 18% p/p. Este é composto principalmente de triacilglicerol (TAG) 75%, ésteres diterpênicos de acidos graxos (principalmente cafestol e caveol) 18%, ceras, ácidos graxos livres, esteróis, tocoferóis e diterpenos livres. O óleo contido no grão de café verde tem uma alta composição de acidos linoléico e palmítico, e é classificado como óleo não-secante. Este óleo é um líquido à temperatura ambiente e tem importantes aplicações na indústria de cosméticos, por sua ação como protetor solar, propriedade que é atribuída aos diterpenos. Muitas empresas in Brasil (Café Pelé, Café Iguaçu, Cia Cacique de Café Solúvel, Odebrecht-Comércio e Indústria de Café, e indústrias linax) oferecem o oleo de café torrado e verde no mercado obtido por prensagem mecânica, e este é geralmente acondicionado em recipientes plásticos com capacidade de 5 e 20 kg.

Neste projeto foi fracionado o óleo de café verde da variedade *Coffea arabica*, por meio de destilação molecular para alcançar frações com maior teor de diterpenos. As frações obtidas foram caracterizadas através de procedimentos analíticos para conhecer a influencia dos diterpenos cafestol e caveol sob as propriedades físico-químicas, assim como o comportamento térmico e reológico. Também foi desenvolvido um programa de

simulação baseado no simulador comercial Aspen Plus, para observar a influencia das variáveis mais importantes na destilação molecular. No entanto, não houve informação detalhada, nem estudos sistemáticos, sobre o refino do óleo de café utilizando destilação molecular. A idéia de desenvolver o modelo é compreender o processo e ter uma ferramenta que permita uma extensa avaliação do impacto das diferentes condições operacionais e, em seguida, para implementar a mais adequada condição operacional no destilador molecular.

Palavras chave: Destilação molecular, óleo de café, diterpenos, fracionamento.

Abstract

Recently, the substitution of conventional materials used in the nutrition, pharmaceutical, and cosmetic areas by natural products has gained more and more interest and importance. The main national and international industries have sought alternative natural sources for their inputs and raw materials. One of the great difficulties in obtaining such material is especially the isolation and purification of bioactive agents.

Coffee is one of the most popular beverages worldwide, acting as a stimulant in people due to its activity psychogenic. Coffee represents one of the most important agricultural products for producing countrie's economic and social. Coffee was wrongly considered into the past as having basically or mainly caffeine. Coffee has more than 1.000 compounds such as vitamin, amino acids, sugars, lipids, minerals, cafestol, kahweol, chlorogenic acids, among many others yet to be studied.

The oil content in coffee is about 18% w / w. This consists mainly of triacylglycerol (TAG) 75%, diterpene fatty acid esters (mainly cafestol and kahweol) 18%, waxes, free fatty acids, sterols, tocopherols, and free diterpenes.The oil contained in green coffee bean has a high composition of palmitic and linoleic acids, and is classified as non-drying oil. This oil is a liquid at room temperature and has important applications in the cosmetics industry by acting as a sunscreen, a property that is attributed to the diterpenes. Many companies in Brazil (Café Pelé, Café Iguaçu, Cia Cacique de Café Solúvel, Odebrecht-Comércio e Indústria de Café, e indústrias linax) offer the oil of roasted coffee and green coffee on the market, obtained by mechanical pressing, usually packaged in plastic containers with a capacity of 5 and 20 kg.

In this work, the green coffee oil (*Coffea arabica*), was fractionated by molecular distillation to achieve fractions with higher levels of diterpenes. The obtained fractions were characterized by analytical procedures to know the influence of the diterpenes cafestol and kahweol on the physico-chemical as well as thermal and rheological behavior. We also developed a simulation program based on the commercial simulator Aspen Plus to observe the influence of the most important variables in molecular distillation process. The idea of developing the program is to understand the process and have a tool that allows the

evaluation of the impact of different operating conditions and to implement the results in the experimental tests.

Keywords: Molecular distillation, coffee oil, diterpenes, fractionation.

Sumário

Nomenclatura

Grupos Adimensionais

Re	Número de Reynolds
Kn	Número de Knudsen

Latinas

C_p	Capacidade calorífica
Ea	Energia de ativação
R	Constante universal dos gases
T	Temperatura
t	Tempo
u	Velocidade media

Gregas

ρ	Densidade
γ	Taxa de cisalhamento
η	Viscosidade dinâmica
τ	Tensão de cisalhamento

Acrônimos

TAG	Triacilgliceróis
G	Glicerol
MG	Monoglicerídeos

ROH Álcool

Abreviaturas

DM Destilação molecular

CCD Câmera Digital de Alta Velocidade

CG Cromatografia Gasosa

DSC Calorimetria Diferencial de Varredura

FAME Ésteres Metílico de Ácidos Graxos

FFA Ácidos graxos livres

MEV Microscopia Eletrônica de Varredura

NIR Espectroscopia no infravermelho próximo

PDF Função de distribuição de probabilidade

SEC Cromatografia de Exclusão de Tamanho

SEE Erro Padrão

TG Termogravimetria

Capítulo 1.

Síntese da Tese

1.1. Introdução

Nos últimos anos, um importante ressurgimento do interesse em produtos naturais como uma fonte potencial de novos medicamentos tem sido observada entre o meio acadêmico, bem como entre as empresas farmacêuticas. Aproximadamente 40% das drogas modernas têm sido desenvolvidas a partir de produtos naturais. Em 2000, aproximadamente 60% de todas as drogas em ensaios clínicos para a multiplicidade de cânceres tinham origens naturais [1].

Os óleos vegetais são amplamente utilizados em aplicações industriais, principalmente na indústria cosmética, farmacêutica e alimentar, devido ao seu importante papel nas formulações de produtos. Lipídios podem agir como emolientes, emulsionantes, transportadores, modificadores de viscosidade, agentes espalhadores, ligantes e lubrificantes, em muitos produtos cosméticos [2]. Beveridge et al. [3] relata algumas propriedades terapêuticas dos ácidos graxos linoléico e linolênico para o alívio do eczema crônico e da dermatite. Além disso, constituintes lipídicos não saponificáveis de óleos de sementes contêm de forma natural hidrocarbonetos, álcoois terpenos, esteróis, tocoferóis e outros compostos fenólicos que podem atuar como agentes antioxidantes sob uma variedade de condições [4]. O óleo vegetal bruto obtido por extração das oleaginosas é uma mistura de compostos, sendo difícil aplicar só uma técnica de separação para isolá-los, daí a necessidade de ser inicialmente dividido em varias frações que contêm compostos de características ou tamanhos moleculares semelhantes. Para seu uso na alimentação o óleo bruto tem de ser refinado, a fim de eliminar substâncias indesejáveis sendo raramente usado sem prévio refino, exceto o azeite de oliva virgem. O tradicional refino alcalino é muitas vezes substituído por o refino físico, onde o uso de produtos químicos é reduzido. O método mais usado é o refino com vapor de água. A qualidade do óleo bruto é muito importante, a fim de se obter o óleo refinado de alta qualidade. O óleo vegetal deve ser eficazmente degomado para remover fosfolipídios, assim como os metais pesados e pigmentos. O passo mais importante consiste na aplicação de vapor superaquecido, a baixa

pressão e a temperaturas superiores a 220°C. Ambos, os ácidos graxos livres e voláteis indesejáveis, formados a partir de produtos de oxidação lipídica, são removidos. Uma desvantagem é a perda parcial de tocoferóis [5]. As reações indesejáveis, particularmente a isomerização de ácidos graxos poliinsaturados, devem ser minimizadas. A qualidade de óleo refinado fisicamente a mesma dos óleos refinados com álcalis, mas as perdas de óleo neutro são menores e a contaminação é baixa. Entre outros métodos físicos de refino, a aplicação da destilação molecular é promissora [6]. Motivados pela exigência industrial para conseguir uma funcionalidade reprodutível e previsível, a separação do óleo nas diferentes frações com propriedades funcionais únicas foi amplamente estudada [7]. O fracionamento envolve a alteração do equilíbrio sólido-líquido de gorduras [8], baseado nas diferenças de peso molecular, ponto de fusão, volatilidade e energia de interação intermolecular dos triacilgliceróis constituintes [9]. Diferentes tipos de processos de fracionamento dos óleos têm sido desenvolvidos, incluindo processos de cristalização, fusão, fracionamento com detergentes e solventes, extração com fluidos supercríticos e a destilação molecular.

O processo mais comum é o fracionamento a seco e a frio, em que a separação dos triacilgliceróis (TAG) é realizada com base nos seus pontos de fusão [7, 10-12]. O fracionamento com solventes [10, 13-15] e com detergentes [8, 16] também é realizado com base nos pontos de fusão, mas com a utilização de um solvente (exemplo: acetona) ou uma solução de surfactantes para facilitar a separação das frações. Na extração com fluido supercrítico, a separação é baseada na solubilidade das espécies lipídicas [8, 13, 17-18], enquanto que na destilação molecular a separação ocorre como resultado da volatilidade dos constituintes lipídicos [9, 19-22].

1.1.1. Óleo de café

O nome café é proveniente do nome da província de Keffa, onde pastores da Abissínia (Etiópia) descobriram os grãos de café no século VI. No século XIII os efeitos reparadores do café eram conhecidos e difundidos pelo mundo Islâmico. Duzentos anos mais tarde, o café era vendido na Europa, introduzindo assim a nova bebida na vida e nos costumes ocidentais [23]. O café é um dos produtos básicos mais negociados nos mercados internacionais e é uma cultura agrícola de grande importância econômica. De acordo com a

última estimativa da Organização Internacional do Café (*OIC*), a produção total de café na safra 2009 foi de aproximadamente 120 milhões de sacas de 60 kg, enquanto a produção de café no Brasil contribuiu com 39,5 milhões sacas [24].

A planta de café pertence ao gênero *Coffea* da família *Rubiaceae.* Este gênero inclui cinco grupos distintos, dos quais apenas o grupo *Eucoffea* contém espécies que são importantes como cultivos industriais. Estas são *Coffea arábica Linn* (café arábica) e *Coffea canephora Pierre ex Froehner* (café robusta). O café arábica é cultivado entre 23° de latitude norte e 25° de latitude sul. O café robusta, por outro lado, é cultivada entre 10° de latitude norte e 10° de latitude sul [25]. O café arábica é cultivado em regiões montanhosas (1000-2000m) e representa cerca de 70% da produção mundial de café [24], enquanto o café robusta, representa os 30% restantes, e é cultivado em regiões de planície (0-700m). Entre estas duas espécies, o café arábica fornece uma bebida de qualidade superior (maior acidez e melho sabor), mas é suscetível a pragas (fungos, nematóides e insetos), enquanto o café robusta, produz uma bebida de qualidade inferior (mais amargo), mas é mais resistente a pragas [26]. As espécies de café arábica e robusta divergem não só nas regiões onde são cultivadas, mas também nas suas composições químicas. Por exemplo, existem diferenças [27] nos teores de cafeína, trigonelina e ácidos clorogênicos, assim como na quantidade e composição de lipídios (ver Tablela 1).

Tabela 1. Composição química de grãos de café arábica e robusta.

Composto	*C. arabica*	*C. robusta*
Cafeína	0,8-1,4%	1,7-4,0%
Carboidratos	50-55%	37-47%
Trigonelina	1-1,2%	0,6-0,7%
Lipídeos	12-18%	9-13%
Acidos clorogênicos	5-8%	7-10%
Proteínas	11-13%	11-13%
Minerais	3,0-4,2%	4,0-4,5%

O conteúdo dos lipídeos oscila entre 12 a 18%, com um valor médio de 16% no café arábica e 10% no café robusta [25, 27-28]. A maior parte dos lipídeos está presente na forma líquida no interior das células do grão. Aproximadamente 2% dos lipídios, da chamada cera do café, cobre o grão como um filme fino [29]. Folstar [30] reportou um conteúdo de ceras no óleo de café verde de 0,2-0,3%. Os principais componentes dos lipídios do café são os triacilgliceróis TAG (75%), seguido pelos ésteres diterpênicos num valor de até 18%. Além disso, ésteres de esteróis (1,4-3,4%), esteróis livres (1,5%) e

diterpenos livres (0,1-1,2%), fosfatídios (vestígios), tocoferóis (0,3-0,7%), cafeína (2,7g/kg do óleo de café), igualmente como a 5-hidroxitriptamida estão contidos no óleo de café [31]. O teor de matéria insaponificável (MI) no óleo de café é relativamente alto (9,0 a 14,4 %) comparado com os óleos vegetais que em media apresentam valores abaixo de 1% [30]. Os principais componentes dos ácidos graxos são: linoléico (43,1%), linolênico (1,8%), oléico (9,6%), esteárico (9,6%) e palmítico (31,1%) [30].

A composição dos principais triacilgliceróis (TAG) do óleo de café verde obtido por prensagem mecânico do grão de café arábica é: LLL (7.76%), PLLn (2.35%), OLL(5,84%), PLL (32,74%), PLO + SLL(19,53%), PLP (26,74%), OLO (1,56%), OOO (0,27%), POP (0,65%), OOS (1,77%) e SOS (0,79%) [21]. Os seguintes TAG foram identificados para óleo de café do café robusta: LLL (11,76%), PLnLn (2,94%), OLL (7,77%), PLL (25,90%), PPLn (1,66%), OOL(1,68%), SLL (8,28%), POL (8,76%), PPL (13,74%), ALL (3,51%), OOO (2,33%), PLS (8,73%) e SSLn (2,91%) [32]. Onde, L = ácido linoléico; Ln = ácido linolênico; P = ácido palmítico; O= ácido oléico; S =ácido esteárico; A=ácido araquídico. Exemplo: POP = 1,3 palmitic-2-oleic-glycerol. Na Figura 1, se apresenta o cromatograma da analise de triacilgliceróis por GPC (Cromatografia por permeação em gel) do óleo de café verde (café arábica) obtido neste estudo.

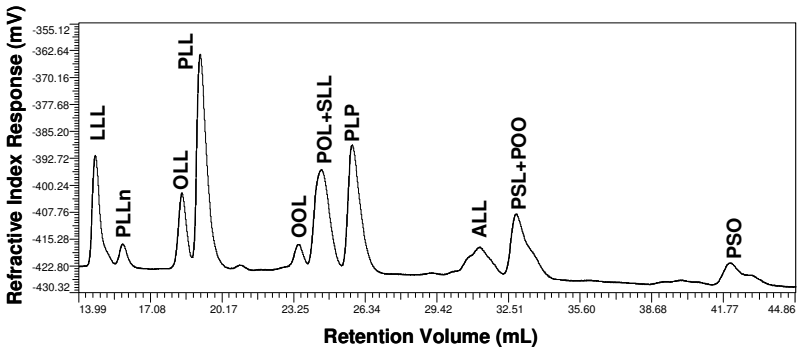

Figura 1. Cromatograma (GPC) dos triacilgliceróis de óleo de café verde (café arábica).

O óleo de café contém diterpenos pentacíclicos baseados na fusão de unidades de isopreno (com 5 átomos de carbono) para formar o esqueleto de caurano (grupo de diterpenos ciclizados em 4 anéis) de 20 átomos carbonos como típicos constituintes lipídicos, que não têm sido detectados em nenhum outro alimento. Os principais representantes são cafestol, caveol e 16-O-Metilcafestol (ver Figura 2).

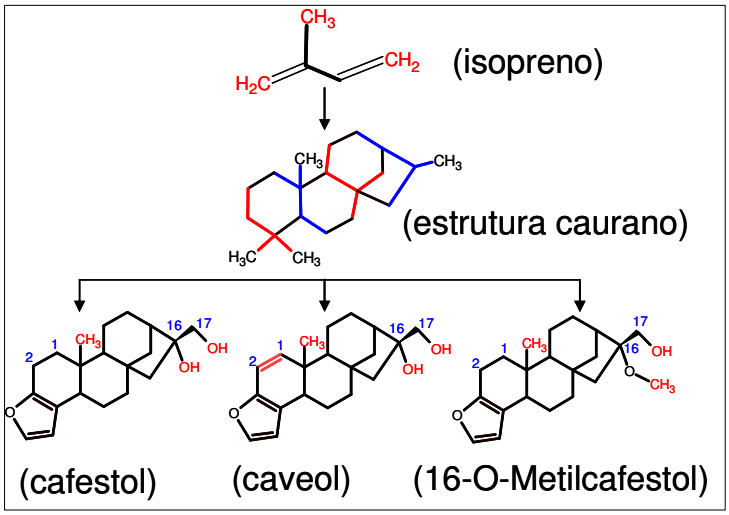

Figura 2. Estruturas dos principais diterpenos do café.

O 16-O-metilcafestol é encontrado apenas em cafés robusta. Cafestol está presente no café arábica, assim como no café robusta. As maiores quantidades de caveol foram detectadas no café arábica, mas só estão presentes em quantidades vestigiais no robusta [33]. A presença de diterpenos no óleo de café foi pesquisada desde 1930. Bengis e Anderson [34] foram os primeiros em detectar o caveol. Slotta e Neisser [35], mais tarde identificaram o cafestol, que originalmente foi designado cafesterol porque foi confundido com um esteróide. Entre 1941 e 1959, o grupo de pesquisa de Wettstein [36-41], Chakravorty [42-44], Djerassi [45-48], e Haworth [49-52] estiveram envolvidos na elucidação da estrutura destes dois diterpenos. Os diterpenos cafestol e caveol são substâncias que apresentam efeitos adversos sobre o metabolismo humano. Estes compostos estimulam a produção de colesterol e, como conseqüência há um aumento de concentração no sangue (hipercolesterolêmia). A estes compostos também têm sido atribuídas propriedades de quimioproteção contra toxinas de ação carcinogênica e propriedades de proteção contra os raios do sol, sendo, portanto utilizado na formulação de filtros solares [53-56]. As concentrações de cafestol determinadas após da saponificação são entre 1,5 e 3,7 g/kg grão seco (g.s) em robusta e entre 2,7 e 6,7 g/kg (g.s) no café arábica [57-59]. As concentrações de 16-O-Metilcafestol no café Robusta estão no intervalo de 0,6 até 1,8 g/kg (g.s) [60], e caveol entre as concentrações de 1,1 e 3,5 g/kg (g.s). no café arábica, e menos de 0,1 g/kg (g.s) no café robusta. O cafestol e o caveol são usados em experiências sensoriais e fisiológicas [31]. O óleo de café contém ambos compostos, e seu

uso foi patenteado como protetor solar [61]. Além disso, o cafestol possui propriedades antiinflamatórias [62].

Em combinação num produto tópico farmacêutico ou cosmético, as composições que contém uma efetiva quantidade de cafestol foram patenteadas para a prevenção e ou tratamento de qualquer doença na qual a barreira lipídica da pele é deficiente ou está danificada (por exemplo, pele seca, casos patológicos, como psoríase e xerose, e de lesões, como queimaduras, feridas e bolhas). As formulações podem também aumentar a adsorção percutânea do medicamento [63]. Uma mistura de cafestol e caveol pode ser usada em aplicações cosméticas [62, 64]. A utilização dos seus ésteres, para tratamento de lesões orais potencialmente malignas, como as leucoplaquias está sob investigação [53-54, 65]. Estudos têm mostrado que uma ingestão de cafestol e caveol provoca um aumento do colesterol total, assim como um aumento da lipoproteína de baixa densidade (LDL) colesterol, triacilgliceróis e a atividade da alanina aminotransferase (ALT) [66]. Quando as soluções e extratos de café foram testados em hamsters, ratos, gerbilos, coelhos, macacos rhesus e cebus, não se observaram efeitos significativos sobre as concentrações de colesterol total e triacilglicerol no sangue [66-67]. Alem, estudos têm demonstrado que o cafestol e o caveol apresentam propriedades anticancerígenas [65].

O óleo de café verde tem um preço relativamente elevado no mercado (US$170/kg) e é atualmente obtido por prensagem mecânica. O fracionamento de óleo de café verde por destilação molecular não só poderá melhorar a qualidade do óleo para uso em aplicações farmacêuticas e de cosméticos, mas também, pode ser uma fonte valiosa de outros produtos, tais como a cafeína, esteróis, tocoferóis e diterpenos. O óleo de café verde bruto obtido pela prensagem a frio tem uma cor marrom escuro esverdeado com um aspecto turvo, a qual é atribuída à presença de pigmentos e outros componentes (por exemplo, clorofila, diterpenos, esteróis, cera, etc.) [21].

1.1.2. Destilação molecular

A Destilação Molecular (DM) é um método de destilação adequado para separação e purificação de materiais termicamente instáveis, assim como para os líquidos com baixa pressão de vapor e alta massa molar [68]. Os ácidos graxos livres do café podem ser separados com sucesso em temperaturas relativamente baixas para produzir praticamente

ácidos graxos de alta pureza. Muitos compostos são sensíveis ao calor [16], como é o caso dos óleos vegetais, dos produtos farmacêuticos e dos cosméticos [69]. Desta forma a destilação convencional não pode ser utilizada com estes materiais. A destilação molecular é um caso particular de evaporação [70], que usa um alto vácuo para a separação de materiais termicamente instáveis. Este é normalmente o método de purificação mais economicamente viável para este tipo de compostos.

Os tipos básicos de destiladores moleculares são as unidades de destilação centrífuga e de filme descendente, que têm uma breve exposição do líquido destilado sobre a superfície do evaporador. O tempo de exposição à alta temperatura na coluna é de poucos segundos a dezenas de segundos, no qual o liquido forma um filme [68]. Estes tipos de unidades de destilação têm sido utilizados com sucesso para concentrar muitos compostos termo-sensíveis, tais como os carotenóides a partir de óleo de palma [71].

A destilação convencional é um dos métodos mais antigos para separar substâncias líquidas ou fundidas. Não obstante, não é recomendada para as substâncias que podem ser degradadas durante as temperaturas de destilação, tais como vitaminas, inseticidas, drogas e aromas (fragrâncias). A destilação molecular é uma técnica de separação utilizada como uma alternativa em diversos processos das indústrias químicas, farmacêuticas, indústria alimentar e de fragrâncias. É um método seguro e adequado para a separação e purificação de materiais termicamente instáveis, e é caracterizada por temperaturas baixas (<250ºC), curtos tempos de permanência do líquido destilado e baixa pressão de destilação. Portanto reduz a decomposição térmica e elimina a oxidação do óleo. Neste método o condensador está separado do evaporador por uma distância menor que o livre percurso médio (λ_L) das moléculas leves, mas maior do que o livre percurso médio das moléculas mais pesadas (λ_P) [71]. O percurso livre médio é definido como a distância média de viagem da uma molécula para a fase de vapor, sem colidir com outra molécula de vapor [72]. Com o aumento da temperatura, o teor de moléculas leves é maior no destilado. Sabe-se que quando aumenta a temperatura de destilação, a pressão de vapor aumenta rapidamente e ao mesmo tempo o caminho livre médio da molécula se torna maior. Imediatamente, as moléculas leves na forma de vapor se condensam sobre a superfície fria do condensador sem colisões intermoleculares e o equilíbrio de fases líquido-vapor, portanto, não pode ser alcançado [73-77]. Em contraste, as moléculas pesadas (como triglicerídeos) não podem alcançar o

condensador e voltam para o evaporador. Uma representação esquemática da destilação molecular é mostrada na Figura 3.

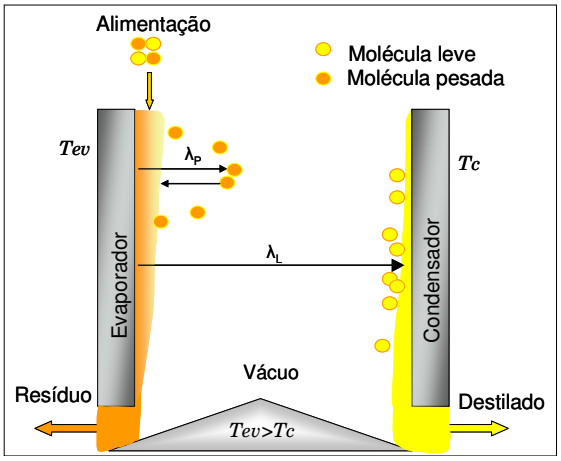

Figura 3. Esquema do processo de destilação molecular.

O curto tempo de permanência do líquido é alcançado devido à distribuição do líquido em uma fina película de espessura de 0,05 a 0,5 (mm) em função da viscosidade e natureza da alimentação [65]. Quando o vácuo é aplicado, há três principais níveis de pressão, que podem ser utilizados para o processo de destilação [72]:

- A destilação a vácuo moderado (estado de equilíbrio), com um limite inferior de pressão de 1 mmHg. Utiliza equipamentos de destilação convencionais. Apresenta equilíbrio entre o líquido e o vapor.
- Destilação de caminho curto (*short path distillation*): precisa de um equipamento especial no qual a separação do evaporador e do condensador permita a livre transferência de moléculas evaporadas, sem obstruções. O nível do vácuo está entre 1 mmHg e 0,01 mmHg (133,32 Pa e 1,3332 Pa) e apresenta equilíbrio entre o líquido e o vapor.
- Destilação molecular: utiliza o mesmo equipamento da "Destilação de caminho curto", mas a distancia de separação entre o evaporador e o condensador é comparável com o livre percurso médio das moléculas evaporadas. A faixa de vácuo esta entre 0,01 mmHg e 0,001 mmHg (1,3332 e 0,13332 Pa). Em comparação com a pressão do processo, se podem eliminar as barreiras de gás na superfície de evaporação com uma pressão perto a 0,1 Pa. Nestas condições não se apresenta equilíbrio nas fases líquido e vapor.

Se o tempo de exposição é curto e a temperatura diminuiu substancialmente, o produto das reações de decomposição é reduzido a um valor insignificante, evitando assim

a decomposição térmica. A curta distância entre as superfícies de evaporação e de condensação de 10 a 50 (mm), em combinação com uma baixa pressão permite que uma alta fração das moléculas evaporadas chegue à superfície esfriada e possam condensar sem dificuldade. Isso irá garantir uma elevada taxa de destilação (de até 40 g/m^2s), que pode ser interessante até mesmo para aplicações industriais [78]. Nestas condições, teoricamente, o retorno das moléculas da fase vapor para a fase líquida não deve ocorrer e a taxa de evaporação é só regulada pela taxa de moléculas que escapam da superfície líquida [79]. Não obstante, em instalações industriais, a distância entre as superfícies de evaporação e de condensação é maior que o percurso livre médio das moléculas. Neste caso, o processo é chamado destilação de caminho curto [80-81]. Na destilação molecular, os conceitos como pratos teóricos, estágios e outras terminologias comuns de destilação não são aplicáveis. Não há, no entanto, nenhuma certeza de que cada molécula que tenha evaporado será capaz de viajar qualquer distância, sem uma colisão [82-83]. Na área das substâncias lipídicas, a DM tem sido utilizado para a purificação de monoglicerídeos [84], fracionamento de ácidos graxos poliinsaturados de óleo de peixe [19], recuperação de carotenóides a partir de óleo de palma [71], recuperação de tocoferóis [85], fracionamento de esqualeno [20], redução do colesterol em manteiga e banha [86], purificação de ésteres do açúcar [20], etc. No entanto, não houve informação detalhada, nem estudos sistemáticos, sobre o refino do óleo de café utilizando destilação molecular.

O desempenho do processo depende do desenho do equipamento, tais como a geometria do espaço de evaporação [72], tipo de condensador [87], presença ou não de arraste no separador [88]. E, também, dos efeitos das variáveis do processo, como a pressão, temperatura do evaporador, vazão de alimentação, temperatura de alimentação, temperatura do condensador, etc. Tradicionalmente, o método de *OFAT* (one-factor-at-a-time) é utilizado para estudar essas variáveis operacionais. Contudo, o planejamento fatorial pode ser uma alternativa, uma vez que, com um reduzido número de experimentos, permite a analisar muitas variáveis simultaneamente e conhecer o impacto de cada uma. Além disso, fornece informações sobre a interação entre as variáveis.

Na destilação molecular, o princípio da separação é o vácuo, permitindo que as moléculas possam migrar do evaporador até o condensador, com a formação de uma fina película líquida que promove as efetivas transferências de energia e massa.

1.1.2.1 Destilador molecular de filme descendente

O destilador de filme descendente é composto por: contém um cilindro duplo encamisado vertical e um condensador interno centrado, e um agitador responsável pela distribuição uniforme do líquido sobre toda a superfície do evaporador. Ele também tem um dispositivo de alimentação com uma bomba e carrosséis de coletores rotatórios, que detêm as amostras de destilado e de resíduo (cada carrossel é constituído por seis coletores que podem ser posicionados e movidos pelo operador, sem interrupção do processo de destilação). Também contem um conjunto de bombas de vácuo com uma armadilha fria de nitrogênio (linha de baixa temperatura) e quatro unidades de aquecimento. Uma bomba de engrenagem que constantemente alimenta a amostra sobre uma placa rotativa de distribuição, um recipiente de armazenamento aquecido, que contém a amostra de trabalho [69].

A força centrífuga distribui o líquido sobre a superfície interior do evaporador, e a gravidade faz que o fluxo descenda; o sistema dispensador tem um espalhador (*wiper*) que constantemente redistribui a amostra como um filme muito fino sobre a superfície interna do evaporador [89]. Os componentes voláteis do material alimentado vaporizam desta película fina e condensam no condensador esfriado. O destilado e o resíduo são coletados nos reservatórios dos respectivos coletores montados nos dois carrosséis. Cada etapa produz um destilado e um resíduo. As temperaturas da destilação molecular (temperaturas do evaporador) variam de 60°C a 340°C. A temperatura final é uma limitação do equipamento. A pressão normalmente se mantém constante (próximo a 10^{-3} mmHg). A vazão de alimentação, a pressão e a temperatura são cuidadosamente monitoradas pelos controladores presentes no equipamento. O esquema de um aparelho de destilação molecular de filme descendente é mostrado na Figura 4.

Então, os líquidos destilados descendem pelo evaporador e formam um filme. Parte deste é vaporizado e as moléculas evaporadas são condensadas no condensador. Se ambos os filmes são muito mais finos do que os raios do evaporador e condensador, eles podem ser considerados como planares [89].

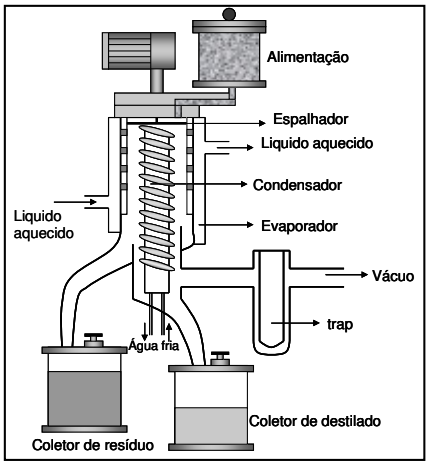

Figura 4. Esquema do destilador molecular de filme descendente.

A temperatura do líquido que entra na periferia do cilindro de evaporação do destilador molecular representa um importante parâmetro operacional [90-91]. Experiências práticas ao longo de muitos anos com a operação de destilador molecular mostraram que a melhor temperatura de entrada do líquido não deve variar muito da temperatura de trabalho no interior do evaporador. Dois produtos são gerados: destilado, rico em moléculas que escapou do evaporador e atingiu o condensador, e o resíduo, rico em moléculas mais pesadas do evaporador. Na Figura 5, observa-se o destilador molecular de filme descendente (*UIC-GmbH KDL 5 unit*) pertencente ao Laboratório de Desenvolvimento de Processos de Separação (*LDPS-FEQ-UNICAMP*).

Figura 5. Destilador molecular de filme descendente do *LDPS*.

1.1.2.2 Destilador molecular centrífugo

O esquema do equipamento típico de destilação molecular do tipo centrífugo é mostrado na Figura 6. O líquido a ser destilado é aquecido até a temperatura de alimentação e conduzido por um tubo até o centro de rotor, onde há uma cavidade para espalhar melhor o líquido. Pela força centrífuga gerada pela rotação do disco, o líquido deslizante é conduzido até as bordas do evaporador formando uma camada liquida muito fina, onde é parcialmente vaporizado, e o vapor é condensado. É possível operar o equipamento usando um refluxo [92].

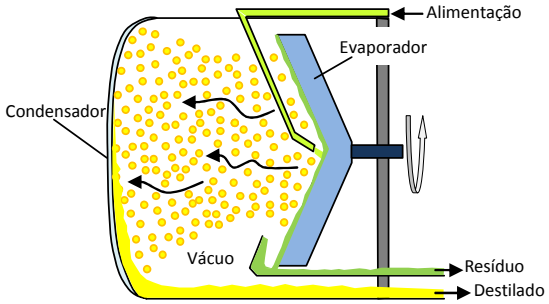

Figura 6. Esquema do destilador molecular centrífugo.

O condensador é apresentado em forma de um disco, com diâmetro tão grande como do evaporador e separado a uma distância de poucos centímetros (livre percurso médio) do evaporador cônico (ver Figura 3). O evaporador é apresentado sob a forma de um disco cônico, onde se fixa na base por meio de rolamentos e de um sistema de juntas, e é movido por um motor elétrico com um movimento de rotação. O evaporador é aquecido pela radiação de um aquecedor elétrico. Uma vez que o destilando é mantido no evaporador pela força centrífuga, a operação é independente da força de gravidade [93].

O destilador molecular centrífugo tem todos os componentes necessários para trabalhar com matéria primas termos-sensíveis da mesma forma que o destilador molecular de filme descendente. A alimentação é fornecida com uma válvula de medição e uma bomba de engrenagens. A vazão de alimentação entra primeiro à unidade de desgaseificação, e logo é aquecida no evaporador, onde tem lugar a destilação molecular (ver Figura 7). O destilado é o resíduo de destilação são continuamente removidos para os respectivos coletores por bombas especiais. O equipamento conta com um sistema de aquecimento com fitas elétricas para evitar a solidificação do material.

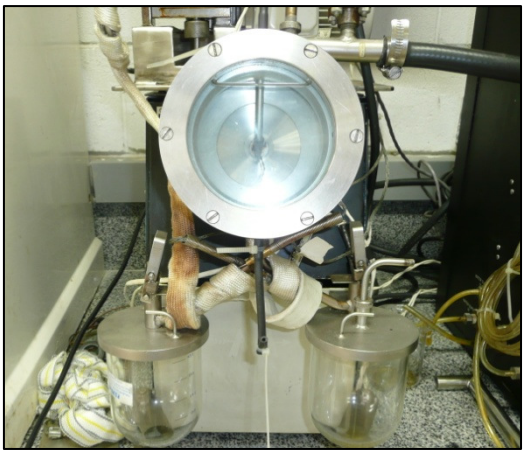

Figura 7. Vista do evaporador cônico no equipamento de destilação.

O maior componente do destilador molecular centrifugo é um evaporador cônico, côncavo e aquecido, que gira dentro da câmara de destilação [94]. Duas bombas de vácuo, uma mecânica de palhetas e uma bomba de difusão de alto vácuo estão acopladas à câmara de destilação. O vapor condensado, o destilado, flui por gravidade por um tubo de saída localizada na parte inferior da câmara de destilação e é bombeado a velocidade constante. Este curto tempo de permanência em altas temperaturas ajuda a evitar a degradação do resíduo e destilado, contrariamente ao que se trabalha a maioria dos destiladores comuns, em que a amostra é mantida à temperatura da destilação por várias horas. A fração destilada é recuperada sobre o condensador, que tem um controle de temperatura ajustado para evitar a solidificação. O destilado sai da câmara e é levado para o coletor. Todas as linhas de transmissão e as bombas estão cobertas com fitas de aquecimento. As fitas de aquecimento e elementos elétricos são controladas digitalmente.

O Laboratório de Desenvolvimento de Processo de Separação (*LDPS-FEQ-UNICAMP*) tem um sistema de destilação molecular centrífugo da *Myers Vacuum Inc* (*Kittanning, PA*), com uma superfície de evaporação total de 0,0046m^2 (ver Figura 8). A temperatura da alimentação, a temperatura do condensador, a pressão e a velocidade de rotação do evaporador podem ser mudadas.

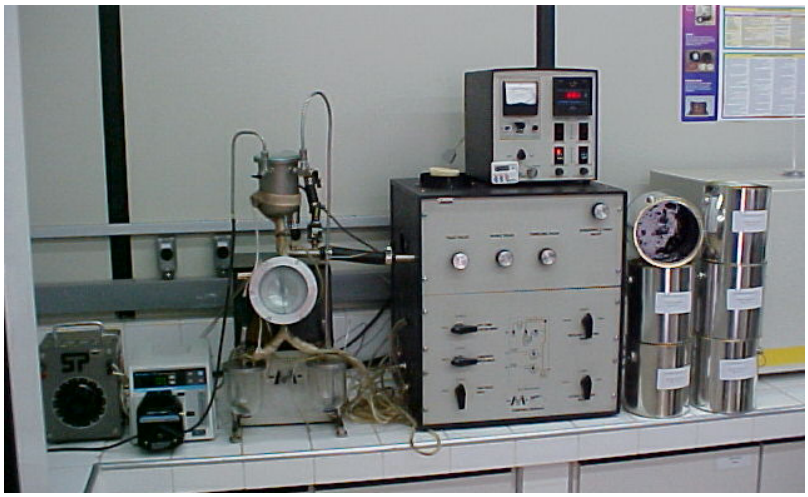

Figura 8. O destilador molecular centrifugo do *LDPS*

1.1.2.3 Modelos matemáticos que descrevem o processo da destilação molecular

Diversos estudos relatam os resultados de modelagem do processo de destilação molecular para diferentes geometrias de evaporadores. A maioria dos modelos reportados foram desenvolvidos para misturas binárias e alguns foram testados com dados experimentais [95-97]. Outros trabalhos concentravam-se em questões específicas de projeto. Por exemplo, Kawala [95], estudou experimentalmente o efeito das propriedades anisotrópicas sobre a taxa de evaporação das misturas binárias. Kawala e Stephan [98] simularam o processo de evaporação molecular de filme descendente em regime adiabático. Batistella e Maciel [69] compararam o desempenho de evaporadores centrífugos e de filme descendente para misturas binárias, utilizando o modelo desenvolvido por Kawala e Stephan [98]. Nguyen e Le Goffic [97] desenvolveram um modelo de separação de misturas binárias sob o pressuposto de que a temperatura é constante na vazão de líquido, desprezando o balanço térmico. Micov *et al.* [68] desenvolveram um modelo para misturas binárias, levando em conta a transferência de massa na fase vapor e a força de gravidade na vazão de filme, a difusão e o balanço energético, mas os efeitos de colisões no espaço da destilação e da pressão diferencial foram desprezados e não houve verificação experimental. Cvengros *et al.* [99] utilizaram o mesmo modelo para estudar o efeito da temperatura sobre a eficiência de evaporação de uma alimentação líquida contendo somente um componente. Cvengros *et al.* [87] estudou também o modo de operação de um

evaporador de cominho curto (*short-path evaporator*) com o condensador dividido, que pode ser utilizado para fracionamento e reciclagem de uma parte do destilado. Recentemente, Lutisan *et al.* [96], investigou o efeito das condições hidrodinâmicas.

Neste projeto, foi considerada uma coluna de destilação molecular de filme descendente para fracionar o óleo de café verde como caso de estudo. As frações obtidas foram caracterizadas e analisadas experimentalmente e seus resultados apresentados como um aporte ao estudo do óleo de café verde.

1.2. Justificativa

O Brasil é o maior produtor de café do mundo, com 39,4 milhones de sacas de 60 kg em 2009 [24]. Óleos e oleoresinas, obtidas a partir de porções como raízes, sementes, flores e frutos, conformam um importante segmento industrial, que gera produtos de alto valor, úteis para as empresas farmacêutica, cosmética e alimentícia (potencial aromático do café solúvel e odor característico nos bolos, balas e pudins). Além da diversidade de aplicações para o óleo de café é necessário um profundo conhecimento dos seus componentes químicos para compreender seus usos e atividade biológica. No Brasil, os óleos de café verde e torrado são obtidos por processos mecânicos de extrusão de grãos de *Coffea arabica*, mostrando alta qualidade e é amplamente utilizado na indústria alimentar como sabor característico de café. O preço do óleo de café verde no comercio mundial é de US$170/litro, e é muito valorizado na indústria cosmética e farmacêutica pelas propriedades fisiológicas que apresentam os compostos ativos, tais como os diterpenos, esteróis, cafeína e ácidos clorogênicos.

A destilação molecular oferece um método alternativo para fracionar o óleo de café em uma fração pesada (resíduos) e uma fração leve (destilado) com base na volatilidade. Esta produz frações com diferentes composições e propriedades físicas. O resíduo resultante é enriquecido em espécies lipídicas de alta massa molar (TAG), enquanto que no destilado é mais alta a concentração de ácidos graxos livres de baixa massa molar e de ésteres diterpênicos. Estas alterações na composição química afetam as características físicas, como a densidade e comportamentos reológicos, assim como a funcionalidade das frações.

1.3. Objetivos

O principal objetivo desta tese é:

1-) Investigar o fracionamento por destilação molecular de filme descendente do óleo de café verde (*Coffea arabica*), **2-)** Estabelecer as condições operacionais para atingir o produto com características especificadas, **3-)** Caracterizar as frações resultantes.

1.3.1. Objetivos especificos

- Desenvolver e implementar um processo para extrair e isolar compostos de alto valor agregado presentes no óleo de café verde, por destilação molecular.
- Determinar as propriedades físicas e químicas dos produtos do fracionamento de óleo de café verde.
- Estudar a etapa de desacidificação do óleo de café verde por destilação molecular.
- Estudar os efeitos das variáveis de processo no fracionamento de óleo de café verde por destilação molecular.
- Estudar o processo de destilação molecular através da comparação dos dados experimentais e teóricos num simulador comercial.
- Estimar as propriedades termodinâmicas e de transporte dos componentes do óleo de café.

1.4. Metodologia

A metodologia utilizada nesta tese consta de uma parte experimental e outra parte de modelagem computacional. Além disso, esta pode ser dividida em três seções: criação de um banco de dados dos componentes do óleo de café verde e simulação no Aspen Plus® do processo de destilação molecular, aplicação do processo de destilação molecular à desacidificação e ao enriquecimento dos diterpenos do óleo de café verde, e por último o estudo térmico e reológico do óleo de café verde e as frações obtidas na destilação molecular.

Na caracterização do óleo de café verde, análises térmicas e reológicas foram realizadas para investigar o efeito da temperatura sobre as propriedades físico-químicas do óleo. Os estudos reológicos foram realizados para determinar o comportamento mecânico do óleo mediante o monitoramento da variação da tensão de cisalhamento a diferentes taxas

de cisalhamento. Uma correlação empírica foi usada para descrever a variação da viscosidade dinâmica e a densidade com a temperatura e a composição de diterpenos, onde as constantes foram ajustadas com os dados experimentais. As análises térmicas no calorímetro diferencial de varredura (DSC) permitiram avaliar a capacidade calorífica (Cp), temperatura de fusão e degradação e as respectivas entalpias. A composição do óleo foi obtida mediante as análises no cromatógrafo gasoso (*CG*) e por cromatografia de permeação em gel (*CPG*).

Na criação do banco de dados, utilizou-se informação da literatura e dados obtidos de métodos de contribuição de grupos, devido à dificuldade de medir diretamente as propriedades dos compostos, que apresentam degradação térmica abaixo do ponto de ebulição. Na simulação do processo de destilação molecular foi utilizado o programa *Aspen-Plus*® que conta com um modelo matemático de não-equilíbrio (*rate-based*). Encontra-se que os resultados das simulações estão de acordo com os dados experimentais relatados previamente na literatura. Especificamente, o modelo *rate-based* foi capaz de prever o fracionamento do óleo de café verde na faixa de temperatura de 408K a 490K e sob pressões de entre 0,001 e 0,01 mmHg, que são as condições operacionais comumente utilizadas no processo de destilação molecular.

1.5. Organização da Dissertação

As contribuições da tese estão estruturadas em nove capítulos. Em alguns casos, os resultados obtidos em um capítulo são apresentados num outro capítulo e vise versa, em especial entre a parte experimental e modelagem, para sua direta comparação. Finalmente, a síntese de todas as contribuições da tese é resumida no Capítulo 9. Cada capítulo é subdividido em quatro partes: a introdução contendo uma breve descrição do tópico em particular e uma curta revisão da literatura, a metodologia, os resultados e discussões, e finalmente as conclusões do capítulo. Detalha-se a seguir o conteúdo de cada capítulo.

Capítulo 2: Apresenta a criação do banco de dados dos componentes principais do óleo de café e discute tópicos relativos às principais características dos modelos de estimação das propriedades propostos na literatura. As propriedades físicas são importantes para a modelagem e simulação de processos envolvendo esses compostos. Portanto, avaliamos na literatura e estimamos as propriedades criando um conjunto consistente de propriedades físicas de todos os componentes de interesse. Dados de propriedades físicas

dos componentes do óleo de café verde não estão disponíveis na literatura. Devido a que esses compostos têm alta massa molar e apresentam decomposição térmica nas proximidades do ponto de ebulição. Por tanto, determinações experimentais de propriedades críticas das substâncias são difíceis de obter. Devido a esses problemas, numerosos métodos preditivos (métodos de contribuição de grupos) foram utilizados para a sua determinação.

Capítulo 3: Apresenta a modelagem dos principais parâmetros para estimar a melhor separação dos componentes do óleo. Vários experimentos e análises teóricas foram realizados para identificar o impacto dos parâmetros mais importantes (livre percurso médio, taxa de evaporação, volatilidade relativa e número de Knudsen) que determinam o desempenho deste processo e o grau de separação. Neste trabalho, se apresenta um processo baseado na destilação molecular, para o enriquecimento dos diterpenos de café (cafestol e caveol) a partir do óleo de café como matéria-prima. Os primeiros destilados foram enriquecidos em ésteres diterpênicos de ácidos graxos e ácidos graxos livres, enquanto que os resíduos foram enriquecidos com triacilgliceróis de alta massa molar.

Capítulo 4: Este capítulo descreve um modelo matemático de não-equilíbrio e procedimentos de simulação para o fracionamento do óleo de café verde por meio de destilação molecular no simulador comercial Aspen-Plus®. Encontra-se que os resultados das simulações estão de acordo com os dados experimentais relatados previamente na literatura.

Capítulo 5: Apresenta o método de desacidificação do óleo bruto de café verde (CGCO) por destilação molecular (DM). Esta etapa foi delineada desta forma para aproveitar a grande diferença na volatilidade dos compostos indesejáveis e do óleo neutro sob altas temperaturas e alto vácuo, sem causar danos importantes aos compostos termo-sensíveis e sem perda elevada de óleo neutro. A otimização pela Metodologia de Superfície de Resposta (RSM) foi aplicada para projetar os experimentos e avaliar os efeitos interativos de duas variáveis operacionais importantes na destilação molecular: a temperatura do evaporador TEV (120-180ºC) e a vazão volumétrica de alimentação Q (50-10 mL/min).

Capítulo 6: Neste capítulo, se apresenta um estudo do enriquecimento de diterpenos de café de óleo de café verde (cafestol e caveol) utilizando destilação molecular.

Aspectos da análise da composição química são discutidos, assim como propriedades físicas das frações resultantes. Os destilados foram enriquecidos em ésteres diterpênicos de ácidos graxos, enquanto que os resíduos foram enriquecidos com triglicérides de alta massa molar. Os ésteres diterpênicos de ácidos graxos foram concentrados a 42,8% e 38,4% (o óleo de café verde original tinha 24,4%) em fraciones de destilado utilizando o processo de destilação molecular em uma e duas passagens, respectivamente. Comparado ao óleo de café verde, a frações obtidas se comportaram de maneira diferente em suas propriedades químicas e físicas. Essas frações são obtidas em um equipamento de destilação molecular de filme descendente com um fluxo de alimentação de 6 mL/min, em um intervalo de temperaturas do evaporador entre 130ºC e 250ºC.

Capítulo 7: Este capítulo contém procedimentos experimentais para a análise térmico do óleo de café verde e as frações da destilação molecular. Com base nos resultados obtidos neste estudo, o DSC fornece informações úteis sobre as propriedades térmicas de óleo de café verde, que estão associados com teor de ésteres diterpênicos de ácidos graxos. A influência dos ésteres diterpênicos de ácidos graxos, o cafestol e o caveol, foram observadas nas características de fusão e degradação, capacidade térmica e estabilidade térmica nas amostras de estudo. Os parâmetros cinéticos da decomposição térmica foram calculados para todas as amostras. O método de Rogers e Morris foi utilizado para calcular a energia de ativação. Os valores de energia de ativação sugerem a seguinte seqüência de estabilidade térmica: fração 5> fração 6> fração 4> fração 3>óleo original de café verde> fração 2> fração 7> resíduo> fração 1.

Capítulo 8: O capítulo apresenta o estudo reológico do óleo de café verde e as frações da destilação molecular. A influência do teor de diterpenos e da temperatura sobre as propriedades reológicas e densidade do óleo de café verde, frações de destilado e resíduo foi analisada. Essas frações foram obtidas com uma vazão de alimentação de 6 mL/min, em um intervalo de temperaturas do evaporador entre 130ºC e 230ºC. As propriedades reológicas destas amostras foram avaliadas quanto à taxa de cisalhamento de 0,1 a 200 (s^{-1}), e às temperaturas entre 20ºC e 100ºC. Todas as amostras apresentaram comportamento Newtoniano na faixa de temperatura estudada.

Capítulo 9: Neste capítulo é apresentado o desenvolvimento de um novo método para o monitoramento "*on-line*" da reação de transesterificação em microreatores mediante

a utilização de fibra ótica na região do infravermelho próximo. São descritos em detalhe os componentes do dispositivo de análise assim como a avaliação de seu potencial uso.

Capítulo 10: Finalmente, neste capítulo se apresenta as conclusões deste trabalho e sugestões para trabalhos futuros.

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Capítulo 2.

Banco de dados de propriedades físicas dos principais componentes do óleode café verde

2.1. Introdução

As propriedades físicas são importantes para a modelagem e simulação de processos envolvendo esses compostos. Portanto, avaliamos na literatura e estimamos as propriedades criando um conjunto consistente de propriedades físicas de todos os componentes de interesse. A primeira fase deste trabalho está completa. Um conjunto completo de propriedades físicas para os compostos mais importantes atualmente identificados no óleo de café verde foi desenvolvido. Com estes dados como base de partida podemos motivar novas pesquisas em trabalhos futuros para avaliar e estimar novas propriedades, ou determiná-las no laboratório e atualizar o banco de dados.

Dados de propriedades físicas dos componentes do óleo de café verde não estão disponíveis na literatura. Devido a que esses compostos têm alta massa molar e apresentam decomposição térmica nas proximidades do ponto de ebulição. Por tanto, Determinações experimentais de propriedades críticas das substâncias são difíceis de obter. Devido a esses problemas, numerosos métodos preditivos (métodos de contribuição de grupos) foram utilizados para a sua determinação. No método de contribuição de grupos, podemos calcular a propriedade de um composto em função de parâmetros estruturais que conformam a estrutura molecular do composto (óssea, subestruturas moleculares, denominadas grupos, ex. -CH3 , >CH2 , etc.), as propriedades são determinadas pela soma do número de freqüência de cada grupo multiplicado por sua contribuição [1]. Também foi utilizada a base de dados DIPPR (Design Institute for Physical Properties) para estimar algumas propriedades dependentes da temperatura [2]. Os compostos principais do óleo de café verde para este estudo são: os triacilgliceróis, os ésteres diterpênicos de ácidos graxos, os álcoois diterpênicos (cafestol e caveol) e ácidos graxos livres.

2.2. Desenvolvimento

A Tabela 1 se apresenta os compostos incluídos nesta base de dados, a si mesmo a fórmula química e a massa molar. Os isômeros não foram considerados de forma independente, porque as propriedades estimadas não têm grandes diferenças entre eles. Neste trabalho foi utilizado o software ACD/ChemSketch, para a criação das estruturas moleculares de todos os componentes, listados na Tabela 1.

Tabela 1. Principais compostos do óleo de café verde.

Tipo de Composto	***componente***	***Fórmula química***	***Massa molar kg/kmol***
Triacilglicerois	LLL	$C_{57}H_{98}O_6$	879,38
	PLLn	$C_{55}H_{96}O_6$	852,72
	OLL	$C_{57}H_{100}O_6$	881,40
	PLL	$C_{55}H_{98}O_6$	854,74
	OLO	$C_{57}H_{102}O_6$	882,77
	PLO	$C_{55}H_{100}O_6$	856,75
	PLP	$C_{53}H_{98}O_6$	830,73
	POP	$C_{53}H_{100}O_6$	832,7
	SOS	$C_{57}H_{108}O_6$	889,46
Esteres diterpênicos de acidos graxos	Cafestol palmitato	$C_{36}H_{58}O_4$	554,84
	Cafestol linoleato	$C_{38}H_{58}O_4$	578,86
	Caveol palmitato	$C_{36}H_{56}O_4$	552,82
	Caveol linoleato	$C_{38}H_{56}O_4$	576,84
Alcoois Diterpênicos	Cafestol	$C_{20}H_{28}O_3$	316,44
	Caveol	$C_{20}H_{26}O_3$	314,42
Acidos graxos livres	Ácido palmítico	$C_{16}H_{32}O_2$	256,42
	Ácido linoléico	$C_{18}H_{32}O_2$	280,44

As propriedades mais importantes, juntamente com os métodos de estimação são mostradas abaixo:

2.2.1 Propiedades críticas (Tc, Pc, Vc).

As propriedades críticas (temperatura, pressão e volume) dos componentes da Tabela 1, foram estimadas pelo método de contribuição de grupos proposto por Joback e Reid [3], este método foi avaliado por outros autores [4-5], onde o erro de estimativa calculado foi inferior a 5%, mostrando-se bastante satisfatorio para este objetivo. Para este método é

necessário apenas informação da estrutura molecular dos componentes. O método apresenta as seguintes relações (Equações 1-3).

$$T_C = T_b\left[0.584 - 0.965\sum\Delta T - (\sum\Delta T)^2\right]^{-1} \quad (1)$$

$$P_C = (0.113 + 0.0032n_A - \sum\Delta P)^{-2} \quad (2)$$

$$V_C = 17.5 + \sum\Delta V \quad (3)$$

Onde, $\triangle T$, $\triangle P$ e $\triangle V$ são as contribuições do grupo atômico ou molecular (adimensional) do método de Joback, (T_b) é a temperatura normal de ebolição e (n_A) é a massa molar.

2.2.2 Fator acêntrico (ω)

O fator acêntrico (ω) basicamente, dá informação sobre a acentricidade (ou não-esfericidade), e sobre a polaridade das moléculas. Para estimar (ω) foi utilizado o método proposto por Curl e Peizer [6] e representado na Equação 4.

$$\omega = \log(\frac{P^{sat}}{Pc}) - 1.0 \quad (4)$$

Onde, P^{sat} é a pressão de vapor para a temperatura à qual $Tr = 0.7$, (Pc) é a pressão critica, (Tr) é a temperatura reduzida (T/Tc) e (Tc) é a temperatura critica.

2.2.3 Entalpia de formação como gás ideal a 298.15 K ($\triangle H^o_f{}^{(g)}$)

Para estimar a entalpia de formação dos componentes, foi utilizado o método de contribuição de grupos proposto por Joback e Reid [3]. Para aplicar este método também foi preciso conhecer a estrutura molecular dos componentes (Equação 5).

$$\Delta H^\circ_f(298.15K) = 68.29 + \sum N_K \Delta hfk \quad (5)$$

Onde, $N_k \triangle hfk$ são as contribuições dos grupos constituintes na molecula (adimensional).

2.2.4 Energia livre de Gibbs como gás ideal a 298.15 K ($\triangle G^{o}_{f}{}^{(g)}$)

Para calcular a energia livre de Gibbs, foi utilizado o método de contribuição de grupos de Joback e Reid [3]. Com somente a estrutura molecular se pode calcular essa propriedade, segundo a Equação 6.

$$\Delta G^{\circ}_{f}(298.15K) = 53.88 + \sum N_K \Delta Gfk \tag{6}$$

Onde, $N_k \triangle Gfk$ são as contribuições dos grupos constituintes na molecula (adimensional).

2.2.5 Entalpia de vaporização $\triangle H_V$

A entalpia de vaporização à temperatura de ebulição foi estimada pelo método de contribuição de grupos de Kolska, Ruzicka e Gani [7]. O metodo é exposto pela Equação 7.

$$\Delta H_V = 14.876 + \sum N_{hb} C_{hb} + w \sum M_{hb} D_{hb} + z \sum O_{hb} E_{hb} \tag{7}$$

Neste caso, w, z, $N_{hb}C_{hb}$, $M_{hb}D_{hb}$ e $O_{hb}E_{hb}$ Onde, $N_k \triangle Gfk$ são os parâmetros das contribuições dos grupos constituintes na molécula que representam um valor especifico (adimensional).

2.2.6 Temperatura normal de ebulição (T_b)

A temperatura normal de ebulição foi estimada pelo método de contribuição de grupos proposto por Marrero e Gani [8]. Este método tem a similaridade com o método de estimação para a entalpia de vaporização de Kolska, Ruzicka e Gani [7], cada subestrutura tem um peso e uma freqüência, y dependendo destes, pode ser calculado o valor da temperatura de ebulição com erros inferiores a 5% (Equação 8).

$$T_b = 222.543 + Ln\left[\sum N_i Tb1 + \sum M_j Tb2 + \sum O_k Tb3\right] \tag{8}$$

Onde N_iTb1, M_jTb2 e O_kTb3 são os parâmetros das contribuições dos grupos constituintes na molécula que representam um valor especifico (adimensional).

2.2.7 Pressão de vapor (P^{sat})

Os dados de pressão de vapor foram calculados para cada componente pela equação estendida de Antoine [1] na Equação 9, com base em dados obtidos pelo método

de contribuição de grupos proposto por Ceriani e Meirelles [9], que foi especifica para substancias lipídicas.

$$P^{sat} = A + \frac{B}{T} + C(T) + D(LnT) \tag{9}$$

Onde *A*, *B*, *C* e *D* são os parâmetros da equação estendida de Antoine e (*T*) a temperatura absoluta de medida.

2.2.8 Capacidade calorífica do líquido (Cp^L)

Neste caso, os dados de capacidade calorífica do líquido foram estimados utilizando a base de dados DIPPR (Design Institute for Physical Properties). O DIPPR [10] apresenta um modelo polinômico em funsão da temperatura para o calculo da pressão de vapor, da forma da Equação 10.

$$C_P^L = A + BT + CT^2 \text{ para } \mathrm{F} \le \mathrm{T} \le \mathrm{G} \tag{10}$$

Onde *A*, *B* e *C* são os parâmetros da equação, (*T*) é a temperatura absoluta, *F* e *G* são os limites de temperatura inferior e superior, respectivamente, nesta equação.

2.2.9 viscosidade dinâmica do líquido (η^L)

Foi utilizada a base de dados DIPPR (Design Institute for Physical Properties), para estimar a tendência da viscosidade dinâmica dos componentes do óleo de café verde. No DIPPR [2, 10] os dados são correlacionados à equação de Andrade [11], que é apresenta na Equação 11, como um modelo em função da temperatura.

$$Ln(\eta^L) = A + \frac{B}{T} + C(LnT) \text{ para } D \le T \le E \tag{11}$$

Onde *A*, *B*, e *C* são os parâmetros da equação, (*T*) é a temperatura absoluta, *D* e *E* são os limites de temperatura inferior e superior, respectivamente, nesta equação.

2.3. Propriedades físico-químicas

2.3.1 Triacilgliceróis (TAG)

Os triacilgliceróis são moléculas formadas por três cadeias de ácidos graxos (os quais não precisam ser necessariamente iguais) ligadas a uma molécula de glicerol, através de ligação éster. Os ácidos presentes diferem entre si pelo tamanho e pela posição das duplas ligações. A composição dos principais triacilgliceróis (TAG) do óleo de café verde obtido por prensagem mecânico do grão de café arábica é: LLL (7.76%), PLLn (2.35%), OLL(5.84%), PLL (32.74%), PLO + SLL(19.53%), PLP (26.74%), OLO (1.56%), OOO (0.27%), POP (0.65%), OOS (1.77%) e SOS (0.79%) [12]. Aonde, L=acido linoléico; Ln=ácido linolênico; P=ácido palmítico; O=ácido oléico; S=ácido esteárico. Na Figura 1, se apresenta como um exemplo, a estrutura molecular do PLL= 1-hexadecanoyl-2,3-di-(9Z,12Z-octadecadienoyl)-sn-glycerol (ou linoleioilpalmitoil linoleioilglicerol). Os outros triacilgliceróis do óleo de café se conformam da mesma forma. As estruturas dos triacilgliceróis são muito importantes para estimar propriedades baseados nos métodos de contribuição de grupos.

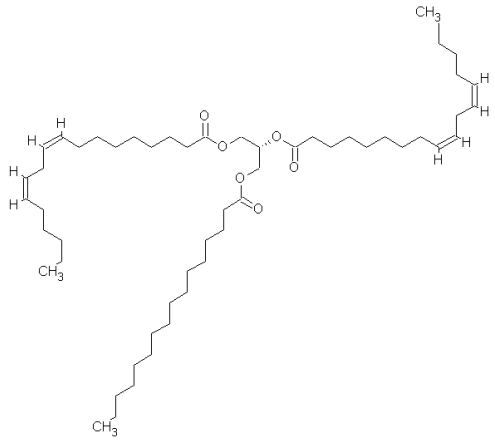

Figura 1. Estrutura molecular do triacilglicerol PLL - linoleioilpalmitoil linoleioilglicerol.

Na Tabela 2, se apresenta os valores das propriedades dos triacilgliceróis que conformam o óleo de café verde calculadas pelos métodos expostos anteriormente. As unidades estão no (SI) sistema internacional para uma melhor compreensão.

Tabela 2. Propriedades dos principais triacilgliceróis no óleo de café verde.

Propriedade	***Eq.***	***Unidade***	***LLL***	***PLLn***	***OLL***	***PLL***	***OLO***	***PLO***	***PLP***	***POP***	***SOS***
Massa molar		kg/kmol	879,38	852,72	881,40	854,74	882,77	856,75	830,73	832,7	889,46
Temperatura crítica (Tc)	***1***	K	1814,81	1691,71	1874,55	1741,82	1939,49	1795,98	1674,56	1723,84	2173,09
Pressão crítica (Pc)	***2***	kPa	247,61	258,89	243,60	254,61	239,70	250,43	261,91	257,56	228,52
Volume crítico (Vc)	***3***	m^3/kmol	3,1795	3,0875	3,1995	3,1075	3,2195	3,1275	3,0355	3,0555	3,2795
Fator acêntrico (ω)	***4***		-3,08525	-2,72453	-3,24343	-2,8743	-3,4042	-3,0259	-2,6701	-2,8117	-3,8953
$\triangle H^{o}_{f}{}^{(g)}$	***5***	J/kmol	-1,4971E+9	-1,5674E+9	-1,6108E+9	-1,6811E+9	-1,7246E+9	-1,7949E+9	-1,8652E+9	-1,9790E+9	-2,0587E+9
$\triangle G^{o}_{f}{}^{(g)}$	***6***	J/kmol	-2,3170E+7	-1,2023E+8	-1,0339E+8	-2,0045E+8	-1,8361E+8	-2,8067E+8	-3,7773E+8	-4,5795E+8	-1,8240E+5
$\triangle H_{V\ (temp.\ Ebulição)}$	***7***	kJ/mol	131,05	130,24	131,1	130,3	131,19	130,48	129,31	129,4	131,3
Temperatura normal de Ebulição (T_b)	***8***	K	843,1	836,6	843,8	837,4	844,6	838,18	831,5	832,3	846,9
Pressão de vapor (kPa) **P^{sat}**	***9***	A	23,275	23,344	23,292	23,302	23,300	23,343	23,278	23,329	23,275
		B	-15762,405	-15665,966	-15771,513	-15678,897	-15780,231	-15694,472	-15551,269	-15573,868	-15801,088
		C	-7,1088E-6	1,6706E-8	-1,90353E-6	-6,03112E-6	1,6752E-8	1,67303E-8	-5,2015E-6	1,6820E-8	1,9669E-8
		D	6,58171E-3	-1,9010E-5	2,5597E-3	6,62427E-3	-1,90616E-5	-1,9040E-5	6,8921E-3	-1,9140E-5	-2,2378E-5
Capacidade calorífica (J/mol.K) **$C_P^{(L)}$**	***10***	A	1593,0401	1538,0895	1582,601	1527,65	1572,162	1517,211	1462,26	1451,822	1544,851
		B	-1,7610414	-1,625154	-1,55797	-1,42208	-1,3549	-1,21902	-1,08313	-0,8800734	-0,898352
		C	5,8311E-3	5,7798E-3	5,62243E-3	5,571E-3	5,4136E-3	5,3623E-3	5,31104E-3	5,1022E-3	5,4574E-3
		F	466,2	462	466	463	467	463	459	460	468
		G	843,1	836	843	837	844	838	831	832	846
Viscosidade (Pa.s) **(η^L)**	***11***	A	-23,214	-22,85	-23,54	-23,191	-23,884	-23,5239	-23,1713	-23,5004	-24,89814
		B	6341,0	6233,0	6431,0	6323,0	6521,0	6413,0	6305,0	6395,0	6791,0
		C	-1,964E-10	-3,610E-10	2,3908E-10	-4,695E-11	-2,223E-10	2,9450E-10	4,3479E-10	9,202E-10	0,0
		D	460	460	460	460	460	460	460	460	460
		E	840	840	840	840	840	840	840	840	840

2.3.2 Cafestol e caveol e seus principais ésteres

Em geral, O teor de matéria insaponificável (MI) em óleos vegetais é ao redor de 1%. No entanto, no óleo de café verde este valor pode variar entre 9% e 14.5%. Cafestol e caveol representam a maior parte da fração lipídica insaponificável no café. Eles estão presentes principalmente sob a forma de ésteres de ácidos graxos (18.5%) e somente uma pequena quantidade (<1%) está presente na forma livre [13]. Os diterpenos cafestol e caveol estão principalmente esterificados com os ácidos graxos palmítico e linoléico [14-15]. Nas Tabelas 3 e 4, se apresentam os valores das propriedades dos diterpenos esterificados e livres, respectivamente. Na Figura 2, se apresentam as estruturas moleculares do cafestol e o caveol, assim como do cafestol palmitato a modo de exemplo.

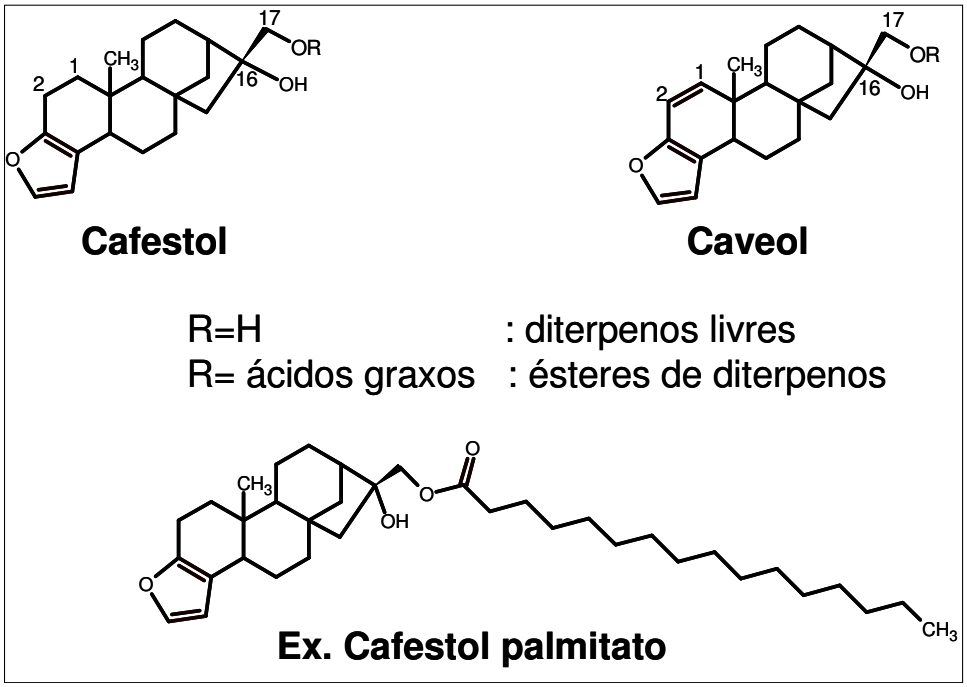

Figura 2. Estrutura molecular do cafestol, caveol e cafestol palmitato.

Tabela 3. Propriedades dos principais ésteres diterpenicos no óleo de café verde.

Propriedade	***Eq.***	***Unidade***	***Cafestol palmitato***	***Cafestol linoleato***	***Caveol palmitato***	***Caveol linoleato***
Massa molar		kg/kmol	554,84	578,86	552,82	576,84
Temperatura crítica (Tc)	***1***	K	939,19	1691,71	1874,55	1741,82
Pressão crítica (Pc)	***2***	kPa	733,6	258,89	243,60	254,61
Volume crítico (Vc)	***3***	m^3/kmol	1,8355	3,0875	3,1995	3,1075
Fator acêntrico (ω)	***4***		-3,08525	-2,72453	-3,24343	-2,8743
$\triangle H^o_{f}{}^{(g)}$	***5***	J/kmol	-1,079E+9	-1,5674E+9	-1,6108E+9	-1,6811E+9
$\triangle G^o_{f}{}^{(g)}$	***6***	J/kmol	-9,24E+6	-1,2023E+8	-1,0339E+8	-2,0045E+8
$\triangle H_{V\ (temp.\ Ebulição)}$	***7***	kJ/mol	101,30	104,0	100,2	103,0
Temperatura normal de Ebulição (T_b)	***8***	K	750	758,3	746,4	755
Pressão de vapor (kPa) **P^{sat}**	***9***	A	20,866	21,129	20,760	21,024
		B	-12184,586	-12520,333	-12048,815	-12386,029
		C	1,96692E-8	1,93912E-8	1,96955E-8	1,93427E-8
		D	-2,2378E-5	-2,206E-5	-2,23977E-5	-2,19938E-5
Viscosidade (Pa.s) **(η^L)**	***11***	A	-22,9614	-23,13	-22,681	-22,624
		B	7474,0	7492,0	7384,0	7402,0
		C	1,07768E-8	0,0	2,9240E-8	6,10075E-8
		D	400	420	410	415
		E	740	750	742	755

Tabela 4. Principais propriedades do cafestol e caveol.

Propriedade	***Eq.***	***Unidade***	***Cafestol***	***Caveol***
Massa molar		kg/kmol	316,44	314,42
Temperatura crítica (Tc)	***1***	K	809,41	804,04
Pressão crítica (Pc)	***2***	kPa	2336,03	2419,49
Volume crítico (Vc)	***3***	m^3/kmol	0,9325	0,9185
Fator acêntrico (ω)	***4***		1,4194	1,4338
$\triangle H^o_{f}{}^{(g)}$	***5***	J/kmol	-5,8769E+8	-4,6267E+8
$\triangle G^o_{f}{}^{(g)}$	***6***	J/kmol	2,959E+7	5,955E+7
$\triangle H_{V\ (temp.\ Ebulição)}$	***7***	kJ/mol	105	106
Temperatura normal de Ebulição (T_b)	***8***	K	650	645
Pressão de vapor (kPa) **P^{sat}**	***9***	A	24,048	24,113
		B	-12629,305	-12749,584
		C	1,6635E-8	1,66490E-8
		D	-1,8922E-5	-1,89417eE5
Viscosidade (Pa.s) **(η^L)**	***11***	A	-21,09	-20,361
		B	7169,0	6829,0
		C	1,5007E-8	2,93847E-8
		D	450	450
		E	650	650

2.3.3 Ácidos graxos livres

No óleo do café verde os principais ácidos graxos livres são: ácido palmítico e acido linoléico [16]. Na Tabela 5, se apresentam os valores das principais propriedades destes ácidos graxos.

Tabela 5. Principais propriedades dos ácidos palmítico e linoléico.

Propriedade	***Eq.***	***Unidade***	***Ac.Palmitico***	***Ac. Linoléico***
Massa molar		kg/kmol	256,42	280,45
Temperatura crítica (Tc)	***1***	K	785,0	775,0
Pressão crítica (Pc)	***2***	kPa	1510,0	1410,0
Volume crítico (Vc)	***3***	m^3/kmol	0,917	0,990
Fator acêntrico (ω)	***4***		0,9827	1,1801
$\triangle H^o_{f\ (g)}$	***5***	J/kmol	-7,23E+8	-5,631E+8
$\triangle G^o_{f\ (g)}$	***6***	J/kmol	-2,6E+8	-1,172E+8
$\triangle H_{V\ (temp.\ Ebulição)}$	***7***	kJ/mol	60,65	68,64
Temperatura normal de Ebulição (T_b)	***8***	K	624,1	628,0
Pressão de vapor (kPa) $\mathbf{P^{sat}}$	***9***	A	16,307221	17,32098
		B	-7294,9317	-8255,9609
		C	2,46690E-8	2,35850E-8
		D	-2,80654E-5	-2,68325E-5
Capacidade calorífica (J/mol.K) $\mathbf{C_P^{(L)}}$	***10***	A	403,636	469,02
		B	-0,0199963	-0,3589482
		C	1,585136E-3	1,845207E-3
		F	340	347
		G	614	625
Viscosidade (Pa.s) $\mathbf{(\eta^L)}$	***11***	A	-12,27094	-12,03770
		B	2529,99	2548,00
		C	-2,85557E-8	0,0
		D	400	410
		E	620	622

2.4. Conclusões

Neste trabalho foi realizada uma estimação das propriedades criticas com o método preditivo de Joback para as temperaturas, pressões e volumes críticos. A energia de formação e a energia livre de Gibbs foram estimadas pelo método de Joback e Reid. Os erros de estimativa para os modelos de Joback, e Joback e Reid foram inferiores a 5%. Também foram obtidos dados do fator acêntrico utilizando o modelo de Curl e Peizer. O método de Marrero e Gani foi utilizado para obter dados estimados da temperatura normal de ebulição, assim como o método Kolska, Ruzicka e Gani foi utilizado na estimativa da entalpia de ebulição. As correlações de pressão de vapor, viscosidade e capacidade calorífica apresentam amplia aplicação nos programas modernos de simulação (ex. *Aspen plus, Hysys* entre outros) y podem ser facilmente adaptados aos algoritmos de calculo em função da temperatura.

Tendo em vista os resultados obtidos, podemos concluir que os modelos utilizados podem ser usados para muitas aplicações industriais, nas quais as propriedades são de difícil determinação experimental pela natureza mesma das substâncias ou não são encontradas com facilidade na literatura.

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Capítulo 3.

Modelagem dos parâmetros da destilação molecular: aplicado ao fracionamento do óleo de café verde (*Coffea arabica*)

3.1. Introdução

Em princípio, os ácidos graxos livres, ésteres diterpênicos de ácidos graxos e triacilgliceróis do óleo de café verde podem ser separados de forma eficaz, por um processo de separação adequada, devido às diferenças entre a massa molar e pressões de vapor destes componentes. Além disso, no caso de separação de componentes por destilação molecular, o ponto de ebulição é substituído pela taxa de evaporação a uma dada temperatura. Vários experimentos e análises teóricas foram realizados para identificar o impacto dos parâmetros mais importantes (livre percurso médio, taxa de evaporação, volatilidade relativa e número de Knudsen) que determinam o desempenho deste processo e o grau de separação. Neste trabalho, se apresenta um processo baseado na destilação molecular, para o enriquecimento dos diterpenos de café (cafestol e caveol) a partir do óleo de café como matéria-prima. Os primeiros destilados foram enriquecidos em ésteres diterpênicos de ácidos graxos e ácidos graxos livres, enquanto que os resíduos foram enriquecidos com triacilgliceróis de alta massa molar.

3.2. Desenvolvimento

O desenvolvimento deste capítulo é apresentado a seguir, no manuscrito intitulado: *Modeling of molecular distillation parameters: case study green coffee oil (Coffea arabica).*

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Modeling of molecular distillation parameters: case study green coffee oil (*Coffea arabica*)

ABSTRACT

In principle, the free fatty acids, diterpene fatty acid esters and triglycerides from green coffee oil can be separated effectively, by a suitable separation process, due to the differences between molar mass and vapor pressures. In addition, in the case of component separation by molecular distillation, boiling point is replaced by evaporation rate at a given temperature. Several experiments and theoretical analyses have been carried out to identify the impact of important parameters (mean free path, evaporation rate, relative volatility and Knudsen number), which determine the performance of these processes and degree of separation. In this work, a process development based on molecular distillation, for the enrichment of coffee diterpenes from green coffee oil is presented. The distillates were enriched in diterpene fatty acid esters and free fatty acids, while the residues were enriched in high molar mass triglycerides.

***Keywords**: diterpenes fatty acid esters, Coffea arabica, molecular distillation, mean free path, cafestol palmitate.*

1. Introduction

Green coffee oil from coffee Arabica (*Coffea arabica*) is a mixture of free fatty acids (FFA), mono-, di-, and triglycerides, diterpene fatty acid esters, phosphatides, pigments, sterols and tocopherols [1, 2]. The unsaponifiable fraction of green coffee oil is rich in diterpene alcohols, mostly cafestol and kahweol, which are mainly esterified with various fatty acids (mainly palmitic and linoleic acids), and only a small amount of the diterpenes is present in the free form [3]. To diterpenes, have been attributed hypercholesterolemic effects, and coronary heart disease risk [4] due to the ingestion of unfiltered coffee drink. On the other hand, various studies have provided further support for the chemoprotective, hepatoprotective, antioxidative, antiinflammatory, and anticancerigenic effects of cafestol and kahweol [5, 6]. Coffee diterpenes are valuable in

the cosmetic and pharmaceutical industries. In this case, molecular distillation technology (MD) has been studied as an alternative technique for recovering and concentrating valuable compounds such as diterpene fatty acid esters from green coffee oil [7].

The molecular distillation process is characterized by direct transfer of molecules from evaporator to condenser without the possibility of come back to evaporator [8-10]. Molecular distillation occurs at low temperatures, high vacuum and short residence times [8], hence reduces the thermal decomposition and eliminates oxidation of the green coffee oil. In a molecular still the condenser is separated from the evaporator a distance less than the mean free path of light molecules (λ_L), but greater than the mean free path of heavy molecules (λ_H). Therefore, with the increase of the evaporator temperature, the light molecules content is increased in the distillate. It is known that when the distilling temperature increases, the vapor pressure rapidly increases; at the same time, the mean free path of the molecule becomes larger [11]. Then, the light molecules (as diterpene fatty acid esters and FFA) in the vapor are condensed on the cooling surface without intermolecular collisions; hence vapor-liquid phase equilibrium cannot be reached. In contrast, the heavy molecules (as triglycerides) cannot reach the condenser and return to the evaporator [11]. Schematic representation of molecular distillation is shown in Fig 1

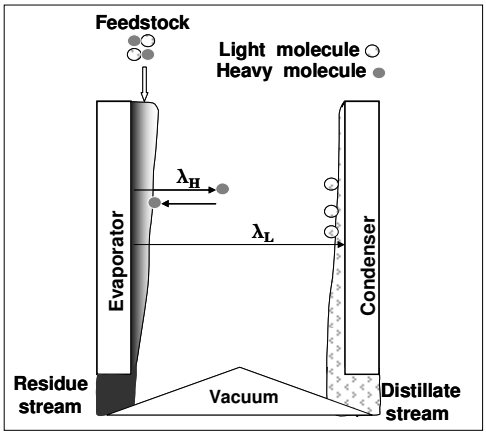

Fig.1. Schematic representation of molecular distillation

The objective of this work is to modeling the molecular distillation parameters in the case of concentration of diterpenes fatty acid esters from green coffee oil. This permits the proposition of suitable operational strategies to separate the desired products and to carry out calculation and evaluation of the evaporation rate and separation efficiency.

2. Materials and methods

2.1 Material

The crude green coffee oil was obtained from the industry (Linax, Votuporanga-Brazil), where it was obtained by mechanical pressing of arabica coffee beans.

2.2 Molecular distillation equipment

The distillation was performed using a laboratory wiper-film molecular still model KDL 5, GmbH UIC (Alzenau, Germany). The distance between evaporation and condensation surfaces (*h*) is 0.02m. The surface area of the evaporator is 0.048m^2 and the surface area of internal condenser is 0.065m^2. The operational temperature was up to 250°C, and the pressure inside the evaporator achieved up to 0.1 Pa.

2.3 Analysis of diterpene fatty acid esters

For this purpose, a VISCOTEK GPC/SEC TDAmaxTM chromatograph with a refractive index detector was utilized. Samples of green coffee oil and fractions obtained of molecular distillation were dissolved in HPLC grade tetrahydrofuran (THF) and analyzed using THF as the mobile phase at flow rate of 0.8 mL/min. Three GPC/SEC Phenogel analytical columns (Phenomenex, Torrance, CA) with different pore sizes (50-100Å), dimension of (300 mm x 7.8 mm, 5 µm). Sample injection volume was 20 µl, and analyses were carried out at 40°C.

2.4 Determination of mean free path, effective evaporation rate, relative volatility and Knudsen number under molecular distillation

The equations 1 to 5 represent the mean free path, Knudsen number, theoretical evaporation rate, effective evaporation rate, and relative volatility, respectively. Those are given in Table 1.

Table1. Molecular distillation parameters for components of green coffee oil

Parameter	***Equation***	
Mean free path[11]	$\lambda=RT/(2\pi\sigma^2 N_A P^0)^{0.5}$	(1)
Knudsen number[12]	$Kn=\lambda/h$	(2)
Theoretical evaporation rate[12]	$G_T=P^0(M/2\pi RT)^{0.5}$	(3)
Effective evaporation rate[12]	$G=(G_T)(f)$	(4)
Relative volatility[13]	$\alpha=(y_1/x_1)/(y_2/x_2)$	(5)
Where: R(universal gas constant), T(absolute temperature), σ(molecular diameter), N_A(Avogadro constant), P^0(vapor pressure)[7], f(evaporation coefficient), M(molar mass), h(distance between the evaporator and condenser), α(relative volatility), y(mol fraction in the liquid phase), x(mol fraction in the vapor phase).		

The Knudsen number (*Kn*) expresses a ratio of the mean free path (λ) of vapor molecules to the distance between the evaporator and condenser (h), and is useful for determining the range of high-vacuum distillation. At $Kn>10$, evaporation proceeds at the maximal rate (molecular distillation). The intermediate range ($0.05<Kn<10$) is the best to run the process because of the proper distillation rate. At $Kn<0.05$, the distillation is under equilibrium conditions [13, 14].

3. Results and discussions

The composition of original green coffee oil by GPC showed that about 72.2 % are triglycerides, 24.8% are diterpene fatty acid esters, and 3% are (FFA and monoglycerides). Figure 2 shows the mean free path (λ) and Knudsen number at equilibrium conditions for cafestol palmitate (554.84 g/mol), palmitic acid (256.42 g/mol) and PLL (dilinoleoyl palmitoyl glycerol, 854.74 g/mol), as representative components of green coffee oil in this work. The free fatty acids, diterpene fatty acid esters and triglycerides can be separated effectively due to the differences between mean free path and molar mass. The distillation at 0.1 Pa, is in the intermediate range ($0.05<Kn<10$), see Figure 2-b. In these conditions, the process reduces the thermal decomposition of green coffee oil and becomes the proper distillation rate [13]. Figure 3-a shows the effect of temperature on effective evaporation rate (G) for palmitic acid, cafestol palmitate and PLL. The order of volatilities for representative components of green coffee oil based in the effective evaporation rate, was palmitic acid > cafestol palmitate > PLL.

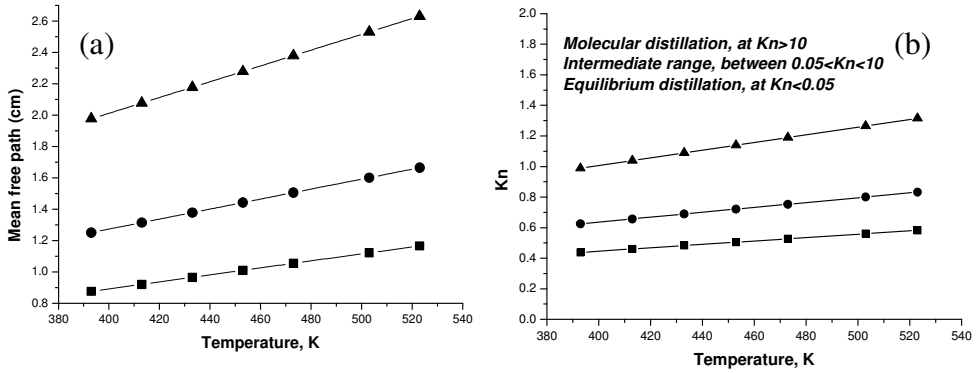

Fig.2. Effect of temperature on (a)mean free path and (b)Knudsen number for (▲)Palmitic acid, (●)Cafestol palmitate, and (■) PLL at 0.1Pa

The experimental relative volatility (α) for cafestol palmitate to PLL obtained in the molecular distillation may be observed in Figure 3-b. The separation of cafestol palmitate from PLL approached maximum value in the distillate fraction at 210ºC and 6 mL/min of feed flow rate, as can be see in Figure 4. It was found that diterpenes fatty esters (ex. cafestol palmitate) were more volatile than triglycerides (ex. PLL) under molecular distillation conditions.

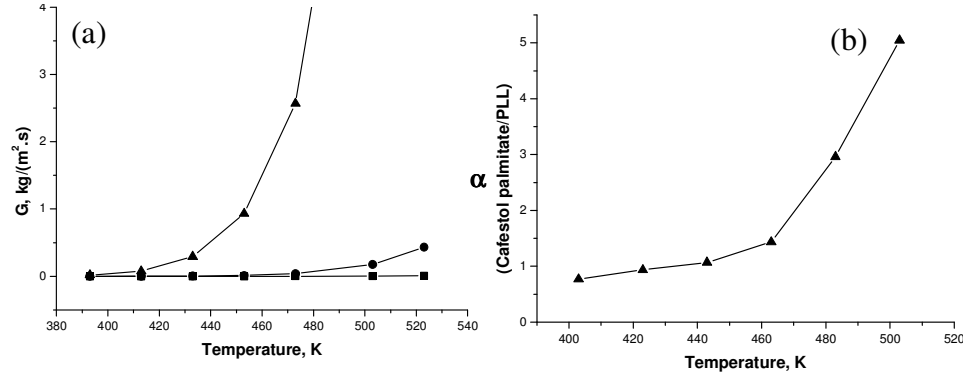

Fig.3. Effect of temperature on (a) effective evaporation rate for (▲)Palmitic acid, (●)Cafestol palmitate, and (■)PLL, and (b) experimental relative volatility (α) for cafestol palmitate to PLL obtained in the molecular distillation at 0.1 Pa

The diterpene fatty acid esters were concentrated in distillate fractions at 210ºC, which were the highest values all the fractions, could achieve about 42.8%. This can be explained by increases of mean free path (λ) of the diterpene esters, causing a high amount of molecule passing to condenser. This is the purpose of the molecular distillation process, in this case to enrich the diterpene esters from green coffee oil.

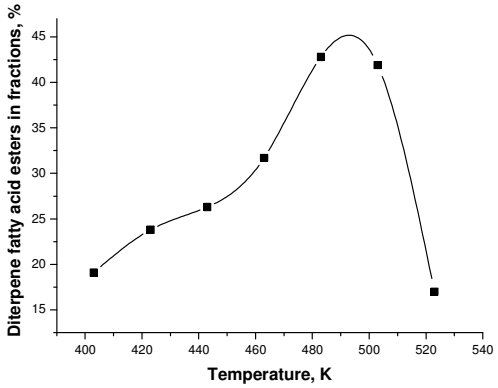

Fig.4. Effect of distillation temperature on the percentage diterpenes fatty acid esters in the distillate fraction, at 0.1 Pa

4. Conclusions

The order of volatilities for representative components of green coffee oil based in the effective evaporation rate was palmitic acid > cafestol palmitate > PLL. The present work has shown that, fractionation of green coffee oil by molecular distillation is an effective tool for yielding several fractions enriched in diterpenes esters which differ markedly in their properties. The best results were obtained for a composition of diterpene fatty acid esters of 42.8% of distillate fractions at 210ºC and 6 mL/min of feed flow rate by molecular distillation. Moreover, the molecular distillation improved the enrichment of the coffee diterpenes as a result of high vacuum and low distillation temperature, since these are components of interest for cosmetic and pharmaceutical industries. The distillation at 0.1 Pa, is in the intermediate range ($0.05<Kn<10$). In these conditions, the process does not lead to the thermal decomposition of green coffee oil. The diterpene fatty acid esters and triglycerides can be separated effectively due to the differences between mean free path, Knudsen number and relative volatility.

5. Acknowledgements

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3.3. Conclusões

Com base na taxa de evaporação efetiva, a ordem das volatilidades dos componentes representativos de óleo de café verde foi o seguinte: ácido palmítico > cafestol palmitato > PLL. O presente trabalho mostrou que o fracionamento de óleo de café verde por destilação molecular é uma ferramenta eficaz para se obter diversas frações enriquecidas em ésteres diterpênicos de ácidos graxos, que diferem significativamente nas suas propriedades físico-químicas. Os melhores resultados foram obtidos para uma composição de ésteres diterpênicos de ácidos graxos de 42,8% na fração de destilado obtida a 210ºC e 6 mL/min de vazão de alimentação. A destilação molecular foi aplicada para o enriquecimento dos diterpenos do café como resultado das condiçõs de alto vácuo e baixa temperatura de destilação. Os diterpenos do café são componentes de interesse para as indústrias cosmética e farmacêutica. A condição de destilação de 0,1 Pa situa o processo na faixa intermediária (0,05 <Kn <10). Nessas condições, o processo não apresenta decomposição térmica do óleo de café verde. Os ésteres diterpênicos de ácido graxos e os triacilgliceróis podem ser separados de forma eficaz devido às diferenças entre o livre percurso médio, número de Knudsen e a volatilidade relativa.

Capítulo 4.

Modelagem e Simulação do Processo de Destilação Molecular de Óleo de Café Verde usando o Modelo de Não-equilíbrio Rate-based

4.1. Introdução

Este trabalho descreve um modelo matemático de não-equilíbrio modelo e procedimentos de simulação para o fracionamento do óleo de café verde por meio de destilação molecular no simulador comercial Aspen-Plus®. Encontra-se que os resultados das simulações estão de acordo com os dados experimentais relatados previamente na literatura. O conteúdo de óleo nos grãos de café é de aproximadamente 18% p/p. Este é composto principalmente de triacilglicerol (TAG) 75%, ésteres diterpênicos de acidos graxos (principalmente cafestol e caveol) 18%, ceras, ácidos graxos livres, esteróis, tocoferóis e diterpenos livres. A grande quantidade de ésteres diterpênicos torna o óleo de café verde impróprio para uso como um óleo vegetal comestível. Na Figura 1, estão apresentados os principais diterpenos do óleo de café.

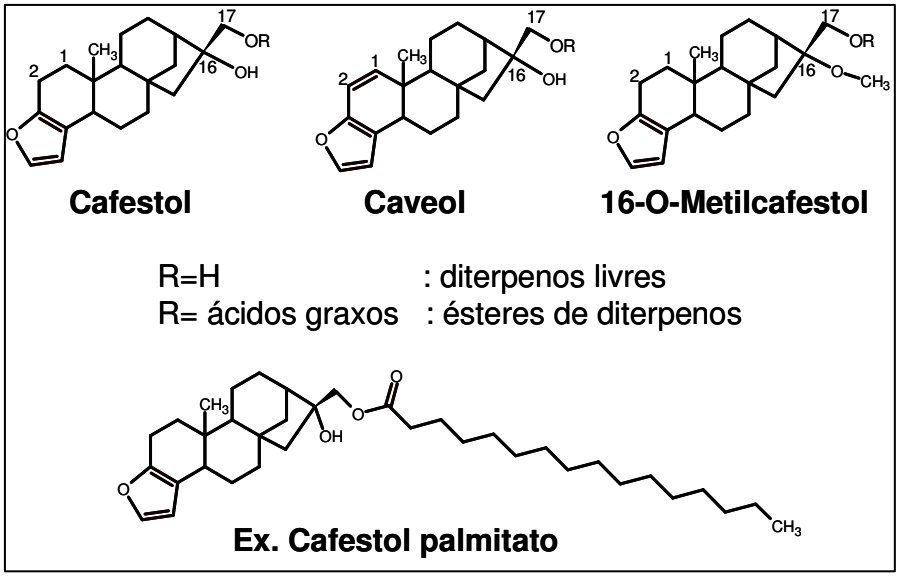

Figura 1. Estrutura dos diterpenos encontrados no óleo de café.

A maioria destes lipídios pode ser encontrada na forma líquida no interior das células dos grãos de café. O fracionamento de óleo de café verde por destilação molecular oferece uma

via para melhorar a qualidade do óleo de café verde, permitindo o seu uso em aplicações alimentícias, farmacêuticas e cosméticas. A destilação molecular também fornece um processo viável para purificar produtos valiosos, tais como os ésteres de diterpenos, que apresentam comprovadas propriedades anticancerígenas.

4.2. Desenvolvimento

O desenvolvimento deste capítulo é apresentado a seguir, no manuscrito intitulado: *Rate-Based Modeling Approach and Simulation for Molecular Distillation of Green Coffee Oil.*

Rate-Based Modeling Approach and Simulation for Molecular Distillation of Green Coffee Oil

ABSTRACT

This work describes a non-equilibrium mathematical model and simulation procedures for the fractionation of green coffee oil via molecular distillation. The simulation results were in quantitative agreement with previously reported experimental. Green coffee oil makes up to 18% (w/w) of coffee beans (Coffea arabica). The main components of the coffee's lipids are triglycerides accounting up to 80% w/w, diterpene fatty acid esters amounting up to 18% w/w. The large amount of diterpene fatty acids renders Green Coffee Oil unsuitable for use as an edible vegetable oil. The majority of these lipids can be found in liquid form inside the cells of the coffee beans. Fractionation of green coffee oil by molecular distillation offers the possibility to improve the quality of green coffee oil allowing its use in nutritional, cosmetic and pharmaceutical applications. Molecular distillation also provides a viable process to purify valuable products such as diterpene esters, which has been reported to exhibit anticarcinogenic properties.

KEYWORDS*: cafestol, kahweol, green coffee oil, molecular distillation, simulation, modeling, diterpene fatty acid esters.*

1. Introduction

Vegetable oils have important market in nutritional, biochemical, cosmetic, pharmaceutical and bioenergy processes. Crude vegetable oils are subject to purification processes before consumer use in order to remove undesirable substances that may influence undesirable taste, appearance, odor, color, etc. Green and roasted coffee oil has a high price in the market and it is commonly obtained by mechanical cold-pressing and/or solvent extraction procedures. The content of triglycerides in the oil originated from green and roasted coffee beans (*Coffea arabica*) are no significantly different [1]. Crude green coffee oil obtained by cold pressing exhibits a dark green color with a cloudy aspect and slight vegetable odor as well as an excessive amount of diterpenes of the kaurane family, mainly cafestol and kahweol. The large amount of diterpenes makes green coffee oil unfit for direct consumer use.

Green coffee oil consists mainly of lipid components such as free fatty acids (1% w/w), free sterols (1.5% w/w), triglycerides (75% w/w), sterol esters (1% w/w), partial glycerides (5% w/w), diterpene fatty acid esters (14% w/w) and polar lipids (<1% w/w) [2]. Diterpenes are receiving significant attention due their demonstrated emollient properties, their ability to increase serum cholesterol levels and block solar radiation as well as potential anticarcinogenic properties [3]. Diterpenes (cafestol and kahweol) are present in the unsaponifiable lipid fraction of coffee oil [4]. Cafestol and kahweol are mainly esterified with various fatty acids, mainly palmitic and linoleic acids, hence only a small amount of the diterpenes is present in the free form [5]. It is well documented that coffee roasting has little influence on the percentage compositions of the diterpene ester fractions [6].

Molecular distillation is well known fractionating process usually used for concentrating vitamins, essential fatty acids, antioxidants and minor components from crude vegetable oils [7-10]. Several studies indicate that fractionation of the triglyceride constituents of vegetable oils via molecular distillation is less effective than chromatography or low-temperature crystallization [9]. However, molecular distillation offers an easier method for the separation of the unsaponifiable fractions and the removal of the free fatty acids without the use of solvents [8, 11, 12]. Molecular distillation occurs at low temperatures, high vacuum, and short residence times, hence reducing thermal

decomposition and eliminating oxidation of the oil. During molecular distillation vapor molecules can reach the condenser without intermolecular collisions hence vapor-liquid phase equilibrium can not be reached [7, 8, 12, 21].

The objective of this work is to model and simulate a fractionation process for the concentration of diterpenes fatty acid esters from green coffee oil via molecular distillation. The modeling steps included the generation of property data for compounds found in the green coffee oil. The needed properties such as normal boiling point and vapour pressure as a function of temperature for the triglycerides and diterpene fatty acids esters were generated using the Marrero and Gani group contribution model [13]. An extrapolative method based on experimental data of vapor pressure for short-chain triglycerides was used to estimate the vapor pressure of coffee oil triglycerides at low temperatures.

2. Characterization of Green coffee oil

We consider that green coffee oil is formed by three key compounds representing its most abundant groups: triglycerides, diterpenes fatty acid esters, and free fatty acids. These are complex compounds and many of their key physical properties are currently not available in the literature. Only the properties of free fatty acids are currently available in the database of Aspen-Plus® process simulation software. Green coffee oil was modeled as a mixture of triglycerides (93.66%), diterpene fatty acid esters (5.84%), and free fatty acids (0.5%), with an average molar mass of 818.57 kg $kmol^{-1}$. The triglycerides profile of green coffee oil has been previously studied [1]. These triglycerides are composed of fatty acids (**L,** linoleic acid, C18:2**; Ln**, linolenic acid, C18:3; **O**, oleic acid, C18:1; **P**, palmitic acid, C16:0; **S**, stearic acid, C18:0; **M**, myristic acid, C14:0). Table 1 shows composition of the triglycerides fraction of the green coffee oil used in this work.

Table.1. Triglycerides composition in green coffee oil

Triglycerides	*Molar mass kg/kmol*	*Composition % (w/w)*
LLL	879.38	7.13
PLLn	852.72	2.45
OLL	881.40	4.49
PLL	854.74	28.59
OLO	882.77	1.57
PLO	856.75	13.28
SLL	882.77	3.91
PLP	830.73	25.64
POP	832.7	5.61
SOS	889.46	0.99

Cafestol is the primary diterpene component in arabica coffe, with kahweol making up to 50% of the cafestol [15]. Diterpenes such as cafestol and kahweol are mainly esterified with palmitic acid (46-50%), linoleic acid (25-29%), stearic acid (8-11%), oleic acid (8-12%), arachidic acid (3-6%), and behenic acid (0.7-1.3%) [6]. Our approach considers that diterpene fatty acid esters are formed by four main components: cafestol palmitate, cafestol linoleate, kahweol palmitate and kahweol linoleate as shown in Table 2

Table 2. Composition of diterpene fatty acid esters in green coffee oil

Diterpenes fatty acid esters	*Molar mass kg/kmol*	*Composition % (w/w)*
Cafestol palmitate	554.84	2.54
Cafestol linoleate	578.86	1.31
Kahweol palmitate	552.82	1.31
Kahweol linoleate	576.84	0.68

Finally, in this work the free fatty acids composition of green coffee oil was set to be palmitic acid 0.35% and linoleic acid 0.15% according to previously reported experimental data [16].

3. Computational model

Advances in the theoretical modeling of this molecular distillation have been reported by several authors [7, 8, 9, 10, 17, 18, 19, 20] with most of the reported models developed only for binary mixtures. Molecular distillation is characterized by direct transfer of molecules from the evaporator to the condenser with no possibility of return of them to evaporator. Under these conditions there is no equilibrium between the vapour and the liquid phases and no true equilibrium pressure of the distilling molecules in the space between evaporator and condenser [7, 8, 9, 10, 12, 21].

The proposed model comprises the following three steps:

- The creation of a property database with the main compounds of green coffee oil.
- Steady state simulation of the molecular distillation process using Aspen-Plus®.
- Model validation with experimental data.

3.1. Creating Database for Simulation

Physical property data for many of the key components of green coffee oil are not available. Only the properties of free fatty acids (palmitic and linoleic acids) are included in the database of Aspen-Plus®. Many of the physical properties of green coffee oil

components can not be determined experimentally due to thermal decomposition of the components at temperatures below their normal boiling point. Aspen-Plus® requires the knowledge of the molecular structure, vapour pressure as a function of temperature, normal boiling point, liquid density, critical temperature, critical pressure, critical volume and acentric factor. Fortunately in cases in which not all of these properties are available Aspen-Plus® can provide accurate estimates using classical group contribution methods.

Prediction of the normal boiling point, which depends only on the molecular structure of pure organic chemicals, was carried out through the Marrero and Gani group contribution method [13]. The estimation of vapour pressure of liquids as a function of temperature was done through the extended Antoine equation with data estimated by the Ceriani and Meirelles group contribution method [14].

3.2. Modeling the molecular distillation process

The non-equilibrium model, (also denoted as rate-based model), was initially presented by Krishnamurthy and Taylor [23] for conventional distillation process and consists of a set of mass and energy balances for vapor and liquid phases, along with rate equations for the evaluation of mass and heat transfer rates. This model use the Maxwell-Stefan equations for description of vapor-liquid mass transfer [23], and it requires information about parameters such as mass and heat transfer coefficients and vapour-liquid interfacial area. The method requires the evaluation of the mass and heat transfer processes for both phases separately. These parameters are usually obtained from semi-empirical correlations.

The following assumptions were made to simplify the rate-based model:

- The molecular distillation process is represented by a distillation column with only one tray and reboiler.
- The process is in steady state.
- Each phase is perfectly mixed in each segment.
- The assumption of phase equilibrium is made only at the vapor-liquid interface. The thermodynamic model UNIQUAC (for liquid phase activity coefficient calculation) is used in this research.
- Chemical reactions are not considered in this process.

- The finite-flux mass transfer coefficients are assumed to be the same as the low-flux mass-transfer coefficients.
- The heat transfer coefficients are assumed to be constant for all segments.
- The reboiler is treated as equilibrium stage.

Figure 1 shows the rate-based concept for a column segment (a stage). In the rate-based model, thermodynamic equilibrium is assumed only at the vapour-liquid interface. The bulk phases of both vapour and liquid are assumed to be perfectly mixed, and the resistance of mass and heat transfer is located in tow films next to the phase boundary. Mass transfer rates are calculated by Maxwell-Stefan equations.

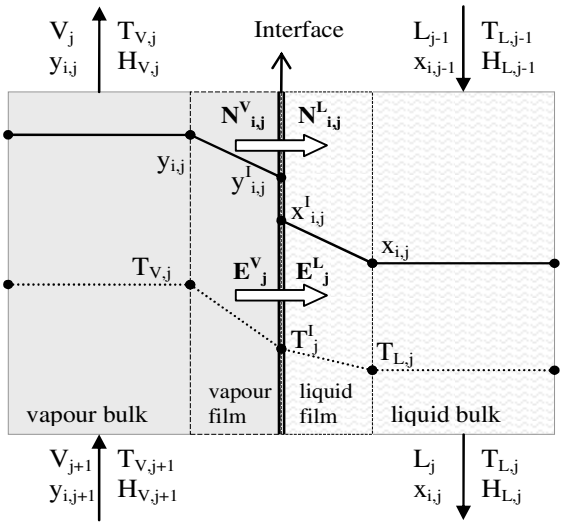

Figure.1. Schematic diagram of rate-based segment

The component molar balance for the vapour and liquid phases are:

$$L_{j-1}x_{i,j-1} - L_j x_{i,j} + N_{i,j}^{L} = 0 \qquad (1)$$

$$V_{j+1}y_{i,j+1} - V_j y_{i,j} - N_{i,j}^{V} = 0 \qquad (2)$$

$$N_{i,j}^{V} = N_{i,j}^{L} \qquad (3)$$

Where $N_{i,j}$ and E_j are the interfacial mass and heat transfer rate of component i on stage j ,where i=1,2,…,c-1. L_j and V_j are the liquid and vapour molar flowrates leaving stage j. $x_{i,j}$ and $y_{i,j}$ are the mole fractions of component i in the liquid and vapour streams leaving stage j. $H_{L,j}$ and $H_{V,j}$ are the liquid and vapour phase enthalpies and $T_{L,j}$ and $T_{V,j}$ are the liquid and vapour phase temperatures. The $N_{i,j}$ are related to the chemical potential gradient in either phase by the Maxwell-Stefan equations [24].

$$\frac{x_{i,j}}{RT_j}\frac{\partial \mu^L_{i,j}}{\partial \eta} = \sum_{k=1}^{c} \frac{x_{i,j}N^L_{k,j} - x_{k,j}N^L_{i,j}}{c^L_{t,j}(k^L_{i,k}A)_j} \qquad (4)$$

$$\frac{y_{i,j}}{RT_j}\frac{\partial \mu^V_{i,j}}{\partial \eta} = \sum_{k=1}^{c} \frac{y_{i,j}N^V_{k,j} - y_{k,j}N^V_{i,j}}{c^V_{t,j}(k^V_{i,k}A)_j} \qquad (5)$$

In these equations, R is the ideal gas constant, μ_i is the chemical potential of species i, η is a dimensionless film coordinate, $c^V_{i,j}$ the total vapour phase concentration and $c^L_{i,j}$ is the total liquid phase concentration. The $k^V_{i,k}$ and $k^L_{i,k}$ represent the mass transfer coefficients of the i-k pair in the liquid and vapour phase. A is the total interfacial area.

The heat balances for both vapour and liquid phases becomes:

$$L_{j-1}H^L_{j-1} - L_jH^L_j + E^L_j = 0 \qquad (6)$$

$$V_{j+1}H^V_{j+1} - V_jH^V_j - E^V_j = 0 \qquad (7)$$

$$E^V_j = E^L_J \qquad (8)$$

The heat transfer rates consist on conductive and convective contributions.

$$E^L_j = -h^L_j A\frac{\partial T^L}{\partial \eta} + \sum_{i=1}^{c} N^L_{i,j}H^L_{i,j} \qquad (9)$$

$$E^V_j = -h^V_j A\frac{\partial T^V}{\partial \eta} + \sum_{i=1}^{c} N^V_{i,j}H^V_{i,j} \qquad (10)$$

Here h^L_k and h^V_k are the transfer coefficients for the liquid and vapour phases. Thermodynamic equilibrium is assumed only at the interface. $K_{i,j}$ is the vapour-liquid equilibrium ratio for component i in the segment j.

$$y^I_{i,j} - K_{i,j}x^I_{i,j} = 0 \qquad (11)$$

The rate-based model has been implemented into the commercial Aspen-Plus® software package in the separation module RadFrac. The process is modeled by simultaneously solving the mass and heat balances, equilibrium equations (interface) and mass transfer rate equations. The heat transfer coefficient in the vapour phase was calculated by the Chilton-Colburn analogy [25] and the mass transfer coefficient was calculated by the correlations of Scheffe and Weiland [26].

4. Results and discussion

4.1. Estimation of vapour pressure of components of green coffee oil

Equation.12 represents the extended Antoine equation used in this study to extrapolate the available estimated data to the temperatures of interest.

$$\ln P^{sat} = A + \frac{B}{T} + CT + D\ln T \qquad (12)$$

Where P^{sat} is vapour pressure in (kPa), temperature T is in (K), and A-D refer to regressed parameters for the extended Antoine equation. The parameters for the extended Antoine vapour pressure equation are summarized in Table 3.

Table 3. Pure component parameters for the extended Antoine equation

Component	*A*	*B*	*C*	*D*
LLL	23.275	-15762.405	-7.1088792E-06	6.5817175E-03
PLLn	23.344	-15665.966	1.6706963E-08	-1.9010551E-05
OLL	23.292	-15771.513	-1.9035309E-06	2.5597721E-03
PLL	23.302	-15678.897	-6.0311229E-06	6.6242770E-03
OLO	23.300	-15780.231	1.6752738E-08	-1.9061677E-05
PLO	23.343	-15694.472	1.6730396E-08	-1.9040678E-05
SLL	23.301	-15780.231	1.6752738E-08	-1.9061677E-05
PLP	23.278	-15551.269	-5.2015317E-06	6.8921692E-03
POP	23.329	-15573.868	1.6820643E-08	-1.9140037E-05
SOS	23.275	-15801.088	1.6670462E-08	-1.8964202E-05
Cafestol palmitate	20.866	-12184.586	1.9669248E-08	-2.2378719E-05
Cafestol linoleate	21.129	-12520.333	1.9391296E-08	-2.2067425E-05
Kahweol palmitate	20.760	-12048.815	1.9695586E-08	-2.2397744E-05
Kahweol linoleate	21.024	-12386.029	1.9342710E-08	-2.1993897E-05

Only limited vapour pressure experimental data could be found for tripalmitin (PPP), tristearin (SSS), and trimyristin (MMM) in the open literature. The predicted vapour pressures were compared to reported experimental data of literature [22] to assess the accuracy of this method. Table 4 illustrates the predicted parameters for the extended Antoine equation of tripalmitin, tristearin and, trimyristin.

Table.4. Pure component parameters for the extended Antoine equation of tripalmitin, tristearin, and trimyristin

Component	*A*	*B*	*C*	*D*
Tristearin	26.637	-18665.869	1.3029228E-08	-1.4812750E-05
Tripalmitin	26.628	-18168.876	1.3265935E-08	-1.5087987E-05
Trimyristin	25.780	-16946.721	1.4332342E-08	-1.6314572E-05

Figure 2 shows the fitting vapour pressure curves obtained for these compounds. It can be noted that the estimated vapour pressures for these triglycerides were in quantitative

agreement with the experimental data with an average relative deviation ($ARD = \frac{\sum \left| (P_i^{sat,\exp} - P_i^{sat,predic}) / P_i^{sat,\exp} \right|}{N} \cdot 100$) of 8.1%.

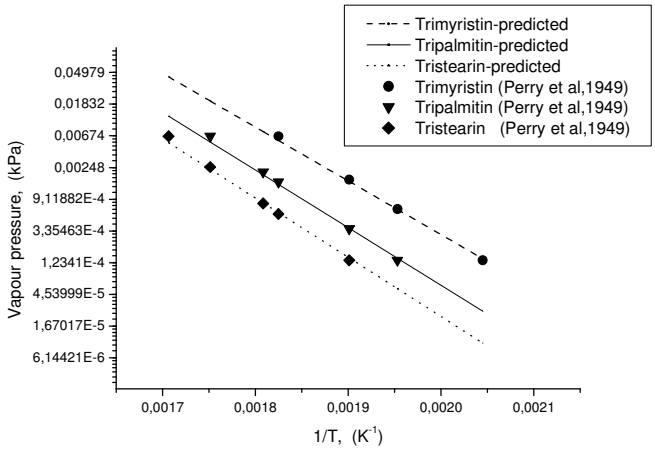

Figure.2. Predicted and experimental vapour pressure of trimyristin, tripalmitin, and tristearin

4.2. Validation of the fractionation process

The equilibrium model and rate-based models were compared with experimental data from literature. Table 5 provides a comparison between the cumulative mass percentage of distillate of coffee oil reported in the literature [27] and that predicted by the equilibrium and rate-based models. The equilibrium model used in this paper consists of the conventional MESH (Mass, Equilibrium, Summation and Heat) equations for evaporation process using the UNIQUAC model for the calculation of the liquid phase activity coefficient.

The cumulative mass percentage of distillate predicted using the rate-based model agrees quantitatively with the experimental data of Khan and Brown [27]. The calculated ARD were 2.65% for the rate-based model, and 33.89% for the equilibrium model.

Table 5. Fractionation process by molecular distillation: comparison of experimental data with equilibrium model and rate-based model.

Fraction	*Temperature (K)*	*Cumulative mass distilled, %*		
		Equilibrium model -	Rate-based model	Experimental data (Khan and Brown, 1953)
1	423	0.429	0.800	0.809
2	448	1.379	2.170	2.226
3	483	15.466	19.548	20.242
Residue	---------	100	100	100

In Figure 3, the predicted and reported data for percent cumulative distilled of coffee oil as a function of distillation temperature at 0.0015 mmHg are highlighted.

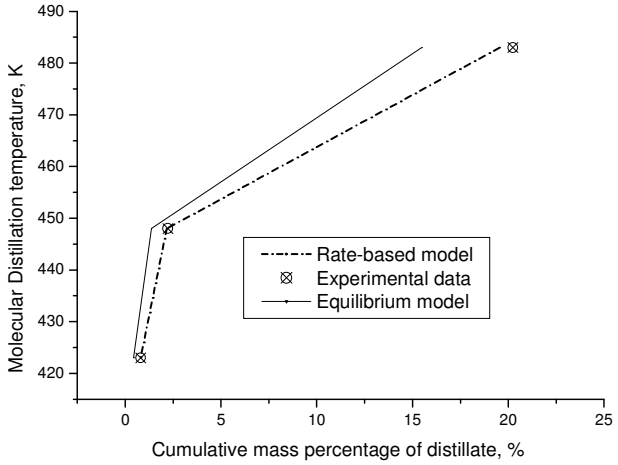

Figure3. Comparison of experimental data with equilibrium model and rate-based model at 0.0015 mmHg

Most of the unsaponifiable matter is recovered in the third distillation fraction at 483K, together with considerable amounts of triglycerides, while free fatty acids were removed in the first and second fractions. It indicates that molecular distillation process would constitute a satisfactory procedure for separation of diterpene fatty acid esters from green coffee oil.

4.3. Evaluation of fractionation Performance

The two models were used for studing the influence of pressure. The distillation temperature and distillation pressure are two major factors affecting the simulation process. The pressure sensitivity of the molecular distillation process can be analyzed through simulation with different pressure profiles. For that purpose, it was performed simulations of the molecular distillation process using a pressure ranging from 0.001 mmHg to 0.01 mmHg at 463 K as illustrated in Figure 4.

The simulation results of the molecular distillation process using a rate-based model depend on the availability and precision of input data. At 463 K, the percentage of distillate yield decreases as the pressure increases, due to increase the collisions between evaporated molecules. When the pressure is increased, the percent yield of distillate is reduced and the molecular distillation (rate-based model) approximates to conventional high-vacuum evaporation with an equilibrium model. The yield of distillate at 463K is appears constant as the distillation pressure increases from 0.001 mmHg to 0.003 mmHg, and then decreases from 0.003 mmHg to 0.01 mmHg, because of greater proportions of the

evaporated molecules are returned to the evaporator by intermolecular collisions, and the process is approached to equilibrium.

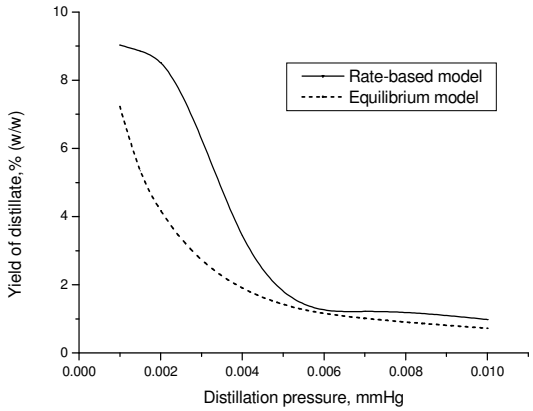

Figure 4. Predicted effect of distillation pressure on the yield of distillate, % (w/w) at 463: equilibrium model vs. rate-based model with Maxwell–Stefan equations

4.4. Elimination curve with rate-based model

The simulation of the fractionation process for green coffee oil was conducted at 0.0015 mmHg. The influence of distillation temperature ranging from 408 K to 490 K on the contents of diterpene fatty acid esters in distillates is shown in Figure 5.

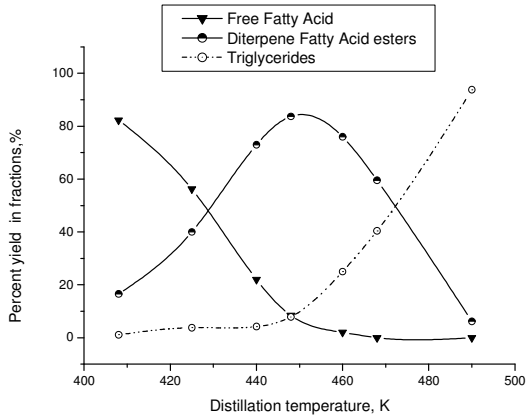

Figure 5. Predicted effect of distillation temperature on diterpene fatty acid esters recovery in fractions (0.0015 mmHg)

The distillation temperature of 453K became a turning point for the changes in diterpene fatty acid esters content in the distillates. At temperatures lower than 453 K, the diterpenes content in distillates decreased slightly while the content of free fatty acids increased. As shown in Figure 5, at 463 K the yield of distillate is 8.85% (w/w) and, the content of diterpene fatty acid esters are about to 60%, what means increasing ten times over that in the raw green coffee oil. When the temperature was above 483 K, the content

of large molecules such as triglycerides increased in the distillates and the percent yield of diterpene fatty acid esters is less than 20% w/w. This behavior indicates that when mass in the distillated fractions is increased, the content of diterpene fatty acid esters diminish due to increase in the content of triglycerides. The components with larger molecular weights such as triglycerides are more difficult to evaporate; these components consisted of a larger proportion in residues than in distillates.

5. Conclusions

A steady-state equilibrium model and a rate-based model were compared with experimental data for fractionation process of green coffee oil. In general, the predictions from the rate-based model were the best ones. The availability of the rate-based model to represent the non-equilibrium process of molecular distillation for green coffee oil was demonstrated through a quantitative agreement between the experimental and simulated data. Specifically, the rate-based model was able to adequately predict the fractionation of green coffee oil in the temperature range of 408 K to 490 K and at pressures between 0.001 and 0.01 mmHg, which are commonly used as operating conditions for molecular distillation process. The purity of diterpenes fatty acid esters of green coffee oil was increased from 5.84% to 60% (w/w) in the fraction to 463 K and 0.0015 mmHg.

The results indicate that molecular distillation process can be effective method for separation of diterpene fatty acid esters of green coffee oil. The characterization of green coffee oil mixture, likewise the estimation of pure component vapour pressure and creation of non-databank compounds into Aspen-Plus® is a reliable alternative for predicting the behavior of green coffee oil. Distillation temperature and distillation pressure have important effect on the purification process by molecular distillation process. When distillation pressure increased, the yield of distillates and the total diterpenes fatty acid esters decreased. In these conditions, the process is approximated to high-vacuum evaporation. The use of thermodynamic models and regression algorithms implemented in commercial Aspen-Plus® software package would expedite design calculations for chemical process development.

Molecular distillation is characterized by a direct transfer of molecules from evaporator to condenser without possibility of return to evaporator. Due to this fact, the system cannot reach equilibrium state and a non-equilibrium model is needed to simulate

the process correctly. For the specific case considered, the rate-based model with the Maxwell–Stefan equations is able to simulate the molecular distillation process of coffee oil, better than the equilibrium model.

6. Acknowledgements

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4.3. Conclusões

Neste estudo foram comparados os modelos de etapa de equilíbrio em estado estacionário e um modelo baseado em Rate-based (não equilíbrio), com dados experimentais para o processo de fracionamento do óleo de café verde. Em geral, as previsões do modelo Rate-Based foram as melhores. A disponibilidade do modelo Rate-Based para representar o processo de não-equilíbrio de destilação molecular no caso de fracionamento do óleo de café verde foi demonstrado satisfatoriamente entre os dados experimentais e simulados. Especificamente, o modelo Rate-Based foi capaz de prever o fracionamento do óleo de café verde na faixa de temperatura de 408 K a 490 K e sob pressões de entre 0,001 e 0,01 mmHg, que são as condições operacionais comumente utilizadas no processo de destilação molecular. O conteúdo de ésteres de diterpenos de ácidos graxos do óleo de café verde foi aumentada de 5,84% a 60% (p/p) na fração de 463 K e 0,0015 mmHg. Os resultados indicam que o processo de destilação molecular pode ser um método eficaz para a separação de ésteres de diterpenos de ácidos graxos do óleo de café verde. A caracterização da mistura de óleo de café verde, igualmente, a estimativa da pressão de vapor do componente puro e a criação de compostos no banco de dados do simulador Aspen-Plus® é uma alternativa confiável para prever o comportamento do óleo de café verde. A temperatura de destilação e a pressão de destilação têm um efeito importante no processo de destilação molecular. Observa-se que quando a pressão aumenta, a produção de destilados e o conteúdo de ésteres de diterpenos diminuem. Nessas condições, o processo se aproxima à evaporação convencional de alto vácuo. O uso de modelos termodinâmicos e os algoritmos de regressão implementados é uma valiosa ferramenta para os cálculos de projeto e para o desenvolvimento de processos químicos baseados no simulador Aspen Plus®.

A destilação molecular é caracterizada por uma transferência direta de moléculas do evaporador para o condensador sem possibilidade de retorno para o evaporador. Devido a este fato, o sistema não pode atingir o estado de equilíbrio e um modelo de etapas de equilíbrio não serve para simular o processo corretamente. Para o caso específico considerado, o modelo Rate-based, que aplica as equações de Maxwell-Stefan, é capaz de simular o processo de destilação molecular do óleo de café melhor do que o modelo de equilíbrio.

Capítulo 5.

Aplicação da Metodologia de Superfície de Resposta na Otimização do Processo de Desacidificação do Oleo Bruto de Café Verde (*Coffea arabica*) através da Destilação Molecular

5.1. Introdução

A remoção dos ácidos graxos livres ou desacidificação pode ser realizada principalmente por refino químico ou físico. O refino químico, aplicado para a maioria dos óleos vegetais, pode causar grandes perdas de óleo neutro. A desacidificação de óleos vegetais por destilação molecular é um processo alternativo, conduzido à temperatura inferior a 250ºC e alto vácuo. O objetivo deste trabalho é estudar a desacidificação do óleo bruto de café verde (CGCO) por destilação molecular (DM). Esta etapa deve ser delineada de forma e aproveitar a grande diferença na volatilidade dos compostos indesejáveis e do óleo neutro sob altas temperaturas e alto vácuo, sem causar danos importantes aos compostos termo-sensíveis e sem perda elevada de óleo neutro. A otimização pela Metodologia de Superfície de Resposta (RSM) foi aplicada para projetar os experimentos e avaliar os efeitos interativos de duas variáveis operacionais importantes na destilação molecular: a temperatura do evaporador TEV (120-180ºC) e a vazão volumétrica de alimentação Q (50-10 mL/min). Assim, os ensaios foram conduzidos conforme planejamento fatorial 2^2 ensaios mais configuração estrela (4 pontos axiais), com 3 pontos centrais. Um total de 11 experimentos foram realizados para a construção de um modelo não-linear. Os resultados foram apresentados como o conteúdo de ácidos graxos livres (% FFA como ácido oléico) no fluxo de resíduo, e a perda de óleo neutro (NOL%). Uma área ótima foi localizada para temperaturas do evaporador (TEV) entre 142,7ºC e 150ºC com taxa volumétrica de alimentação do fluxo (Q) entre 7 min/mL e 8 mL/min. O conteúdo de ácidos graxos livres nas condições ótimas foram inferior a 0,3% (como ácido oléico), e a perda de óleo neutro (NOL%) foi inferior a 6%.

5.2. Desenvolvimento

O desenvolvimento deste capítulo é apresentado a seguir, no manuscrito intitulado: *Response Surface Methodology Applied to Optimization of Deacidification Process of Crude Green Coffee Oil (Coffea Arabica) by Molecular Distillation.*

Response Surface Methodology Applied to Optimization of Deacidification Process of Crude Green Coffee Oil (*Coffea Arabica*) by Molecular Distillation

ABSTRACT

The aim of this paper is to study the deacidification of crude green coffee oil (CGCO) by molecular distillation (MD).The response surface methodology (RSM) has been applied to design the experiments and to evaluate the interactive effects of two most important operating variables in molecular distillation: the evaporator temperature TEV (120-180ºC) and the volumetric feed flow rate Q (5-10 mL/min). Thus, factorial designs consisting of 2^2 trials plus a star configuration (4 axial points) with 3 central points were carried out. The total 11 experiments were conducted in the present study towards the construction of a non-linear model. Results were presented as free fatty acids content (% FFA as oleic acid) in the residue streams, and neutral oil loss (% NOL). One area of optimum performance was located at evaporator temperatures (TEV) between 142.7ºC and 150ºC with volumetric feed flow rate (Q) between 7 mL/min and 8 mL/min; FFA content under this conditions is less than 0.3% (as oleic acid), and loss of neutral oil (NOL %) is lower than 6%.

***KEYWORDS**: Response Surface Methodology, green coffee oil, molecular distillation, free fatty acids, neutral oil loss, optimization.*

1. Introduction

Crude green coffee oil (CGCO) has a high price in the market and it is mainly obtained by mechanical cold-pressing and/or solvent extraction procedures. Basically, it is characterized as dark green color with a cloudy aspect and slight vegetable odor. CGCO has an important market with a wide variety of economical areas of interest as nutritional,

biochemical, cosmetic, pharmaceutical and bioenergy processes. CGCO is a mixture of free fatty acids (FFA), mono-, di-, and triglycerides, diterpene fatty acid esters, phosphatides, pigments, sterols and tocopherols [1, 2]. Diterpenes have currently received more attention due to their different physiological effects, emollient properties, increase serum cholesterol level, block solar radiation and have anticarcinogenic property [3]. The diterpenes (cafestol and kahweol) are found only in coffee bean, and are present in the unsaponifiable lipid fraction of coffee oil [4,5].The diterpenes cafestol and kahweol are mainly esterified with various fatty acids mainly palmitic and linoleic acids (see Fig.1), and only a small amount of the diterpenes is present in the free form [5].

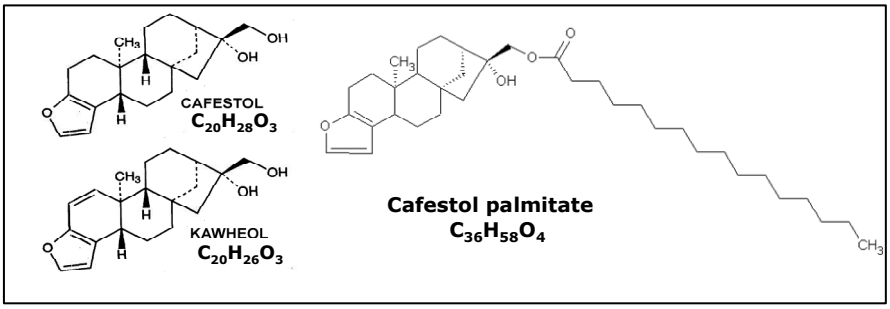

Figure1. Structural formulae of cafestol, kahweol, and cafestol palmitate.

Crude green coffee oil is submitted to purification processes before the use in order to remove undesirable non-triglyceride substances that may influence undesirable taste, appearance, odor, color, or keeping desired quality. The purification process usually refers to the operations of pretreatment, deacidification, bleaching and deodorization [6].

The removal of free fatty acids (deacidification) is important not only for consumer acceptance, but also because it is the most difficult stage of the purification process, mainly because it has the maximum economic impact on oil production price. The FFA represent approximately 2-10 % w/w (expressed as oleic acid) of the crude green coffee oil composition [7]. Chemical, physical and miscella deacidification methods are usually employed industrially for deacidification of vegetable oils [8].

During the chemical deacidification process, considerable losses of neutral oils, vitamins and tocopherols can be achieved due to saponification and emulsification. Besides, the disposal and utilization of high amounts of resulting soapstock may cause serious problems of waste management [8]. Physical deacidification uses steam stripping under vacuum for removes FFA, unsaponifiable substances, and pungent compounds. Additionally, physical

deacidification can lead to good results only when using good quality oils as starting material, since it result in a loss of neutral oil that is lower that the chemical method, but more energy is consumed[9]. The application of miscella deacidification makes possible the production of light-colored oil at a reduced cost, and with a low refining loss. In the process, miscella is formed by 40-60% oil in hexane which is mixed with sodium hydroxide solution for neutralization of free fatty acids. The miscella deacidification is limited by the two-stage solvent removal system and requires all equipment must be explosion-proof, higher investment in solvents, careful operation and greater maintenance [8].

Alternative methods for deacidification of vegetable oils have been proposed [8], such as biological deacidification, chemical reesterification, supercritical fluid extraction (SFE), membrane processing, solvent extraction and molecular distillation, which one will be proposed by CGCO deacidification.

Molecular distillation is well known fractionating process usually used for concentrating vitamins, essential fatty acids, antioxidants and minor components from crude vegetable oils [10-13]. Several studies indicate that fractionation of the triglyceride constituents of vegetable oils via molecular distillation is less effective than chromatography or low-temperature crystallization [12,13]. However, molecular distillation offers an easier method for the separation of the unsaponifiable fractions and the removal of the free fatty acids without the use of solvents, eliminates soapstock and related disposal problems, increases neutral oil yields and lowers capital and operating costs [11,14,15].

Molecular distillation (MD) is a process which takes place at low temperatures, high vacuum, and short residence times, hence reducing thermal decomposition and eliminating oxidation of the oil. Molecular distillation is a non-equilibrium process and relies on the mean free path of different substances. In a molecular still the condenser is separated from the evaporator a distance less than the mean free path of light molecules, but greater than the mean free path of heavy molecules. With the increase of the temperature, the light molecules (FFA) content is increased in the distillates. It is known that when the distilling temperature increases, the vapor pressure rapidly increases; at the same time, the mean free path of the molecule becomes larger. Then, the light molecules in the vapor are condensed on the cooling surface without intermolecular collisions hence vapor-liquid phase equilibrium can not be reached [10,11,15,16]. In contrast, the heavy molecules

(triglycerides) cannot reach the condenser and come back to the evaporator. The mean free path of some lipids of green coffee oil is shown in Fig 2.

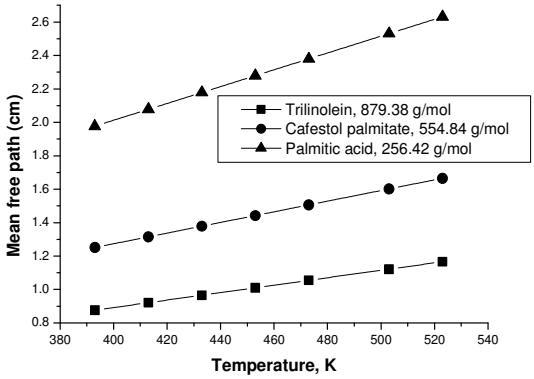

Fig.2. Effect of temperature on mean free path for different lipids at 0.1 Pa.

Removal of FFA from vegetable oils is possible using molecular distillation. In principle, FFA, diterpene fatty acid esters and triglycerides can be separated effectively due to the differences between molar mass, mean free path and vapor pressures (Fig. 3). The FFA which are the lighter substances, are removed of green coffee oil in the distillate stream, and diterpene fatty acid esters and triglycerides are usually concentrated in the residue stream. The FFA are concentrated in the first fractions in the distillations. Laboratory tests with vegetable oil deodorizer distillate (VODD) demonstrated that molecular distillation units can efficiently reduce FFA content of the oils to an acceptable level [17,18]. Distillation tests were carried out in a wiped-film molecular still, with evaporator temperature between 100ºC and 180°C, feed flow rate in the range of 1.5-23 g/min, and 0.1 Pa pressure. The retention time of the oil was less than 10 seconds. The FFA content of the oil was reduced 96.16% and color, and odor compounds were removed [17].

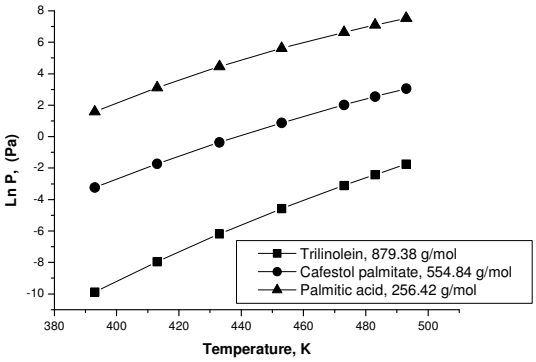

Fig.3. Effect of distilling temperature on vapor pressure for different lipids [15,18].

In the present work, the response surface methodology (RSM) was used to determine the optimum conditions for removal of free fatty acids from green coffee oil (Coffea Arabica) by molecular distillation. With such procedure it is possible to identify the operational

parameter regard to build a statistical model that could describe the effects and relationship of factors such as the evaporator temperature, and volumetric feed flow rate for obtaining the minimum FFA content (as % oleic acid) in the green coffee oil, at the same time, to maintain neutral oil loss (% NOL), as low as possible.

2. Materials and methods

2.1. Material

The crude green coffee oil was obtained from the industry (Linax, Votuporanga-Brazil), where it was obtained by mechanical pressing of arabica coffee beans.

2.2. Raw material analysis

The official methods of the American Oil Chemist Society (AOCS) were used to determine [19]: free fatty acids (AOCS Ca 5a-40), unsaponifiable matter (AOCS Ca 6b-53), saponification value (AOCS Cd 3-25), iodine value (AOCS Cd 1c-85), refractive index (AOCS Cc 7-25), neutral oil and loss (AOCS Ca 9f-57), preparation of methyl esters of fatty acids according to Hartman & Lago [20], and dynamic viscosity and density by Stabinger Viscometer (ASTM D7042–04).

Fatty acid composition and identification were determined with a Gas chromatography coupled to mass spectrometry (Agilent 5975 GC-MSD, Agilent, Santa Clara, United States), connected to a HP-5 capillary column (30 m x 320 µm x 0.25 µm) 5% Phenyl Methyl Siloxan. The column temperature was controlled as follows: initial temperature 70ºC for 5min, then 4°C/min to 230ºC (hold for 3 min), then 4°C/min to 240ºC for a final holding time of 10 min. Helium was used as the carrier gas at a flow rate of 12.26 mL/min. Injection Volume 1 µL. Split Ratio 20:1. The injector and detector temperatures were 250 and 260ºC, respectively. The identification of the fatty acid methyl esters was carried out through the comparison with the retention time of SUPELCO standards (FAME Mix C14-C22) and the quantification by area normalization.

Gel permeation chromatography (GPC) was used for the analysis of triglycerides, diterpene fatty acid esters, monoglycerides, and free fatty acids. For this purpose a VISCOTEK GPC/SEC TDAmax^{TM} chromatograph with a refractive index detector was utilized. Samples of green coffee oil were dissolved in tetrahydrofuran (THF) and analyzed on a GPC system, using THF as the mobile phase at flow rate of 0.8 mL/min. Three

GPC/SEC Phenogel analytical columns (Phenomenex, Torrance, CA) with different pore sizes (50-100Å), dimension of 300 mm x 7.8 mm and packed with spherical styrene divinylbenzene copolymer beads with an average particle size of 5 µm were used. Sample injection volume was 20 µl, and all analyses were carried out at 40°C. All the analyses were performed in triplicate and average values were reported.

2.3. Molecular distillation equipment

The distillation was performed using a laboratory wiper-film molecular still model KDL 5, GmbH UIC (Alzenau, Germany). The equipment components were a evaporator, a condenser, a cooling trap, and the vacuum system (oil diffusion pump and a rotary vane pump). The evaporator is made of glass. The heating of the evaporator was provided by a heating jacket with thermal oil from an oil bath. The distance between evaporation and condensation surfaces is 0.02m. The surface area of the evaporator is 0.048m^2 and the surface area of internal condenser is 0.065m^2. The roller wiper speed inside the evaporator was fixed at 350 rpm. The operational temperature up to 180°C, and the pressure inside the evaporator achieved up to 0.001 mbar. Each step produced one distillate stream and one residue stream. The schematic diagram for this equipment is showed in Fig 4.

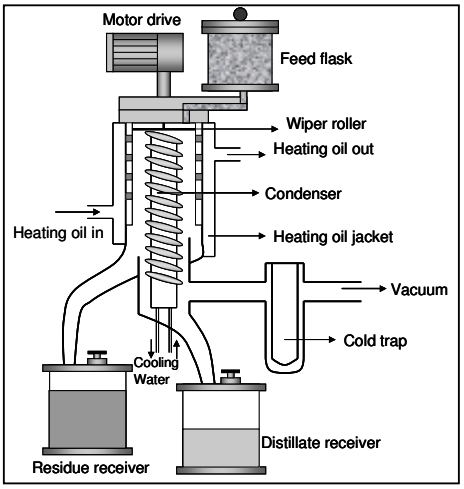

Fig.4. Schematic diagram of wiper-film molecular still.

2.4. Deacidification of green coffee oil (Coffea Arabica) by Molecular Distillation

The green coffee oil obtained by mechanical pressing of arabica coffee beans was fractionally distilled in a wiped-film molecular distillation. Preliminary studies [17,21,22] indicated that the distilling temperature, and feed flow rate were major operative variables

in the molecular distillation, while other factors had little effects on it, and were fixed in typical values for this application: pressure (0.001 mbar), feed temperature (40ºC), roller wiper speed (350 rpm), and condensation temperature (80ºC). The volumetric feed flow rate (Q), and evaporation temperature (TEV) were varied in the range from 5 to 10 mL/min and from 120 to180°C, respectively in order to optimize the operation. The free fatty acids were analyzed in the residue stream as FFA (% as oleic acid), as well as, the loss of neutral oil (NOL%) calculated according to Eq. 1. This loss is determined mainly by the vaporization of monoglycerides (MAG), diterpene fatty acid esters, and triglycerides (TAG) in the distillate stream. The percentage of neutral oil loss is expressed as the relation of neutral oil in the distillate stream to the neutral oil in the crude coffee oil, as follows:

$$NOL\% = \left(\frac{m_D (NO\%)_D}{m_F (NO\%)_F} \right) 100 \qquad (1)$$

Where m_D is the distillate flow rate; m_F is the crude coffee oil feed flow rate, $(NO\%)_D$ and $(NO\%)_F$ are the neutral oil percentages in the feed and distillate streams, respectively.

2.5. Experimental Design

The response surface methodology (RSM) was used to analyze the influence of volumetric feed flow rate, and evaporation temperature on the deacidification of crude green coffee oil and neutral oil loss.

In this work, independent and dependent variables were fitted to a second-degree polynomial equation (Eq. 2), where (Y_1) is the estimated response (FFA, %), and (Y_2) is neutral oil loss (NOL%). The (B_n) values represent corresponding regression coefficients, (X1) and (X2) correspond to the design factors (independent variables), namely, evaporator temperature (TEV °C) and volumetric feed flow rate (Q mL/min), respectively. The data were analyzed by RSM using Statistica software version 7.0 (Statsoft Inc., Tulsa, OK, USA). The significance of the model was tested using coefficients of determination (R^2) and analysis of variances (ANOVA), based on the *F*-test. The effects of the variables were displayed in surface graphs.

$$Y_n = B_0 + B_1 X_1 + B_2 X_2 + B_{11} X_1^2 + B_{22} X_2^2 + B_{12} (X_1 X_2) \qquad (2)$$

A central composite design (CCD) consisting of $\mathbf{2^2}$ trials plus a star configuration (4 axial points) with 3 central points were carried out. The distance of the axial points from the central point is calculated from the equation $\boldsymbol{\alpha} = (\mathbf{2}^{\boldsymbol{k}})^{\mathbf{1/4}}$, where $\boldsymbol{\alpha}$**=1.414** and ($\boldsymbol{k}$) is the number of design factors [22]. The number of trials (N) for the central composite design selected was based on the number of the design factors ($k=2$) as follows (eq.3):

$$N = 2^k + 2k + 3 = 11 \ \text{ trials} \qquad (3)$$

The coded levels and the natural values of these factors set in the statistical experiment are shown in Table 1. The Coded values are dimensionless and shown in bold.

Table1. Coded and natural values of the design factors.

Coded values	TEV (ºC) X_1	Q (mL/min) X_2
+1.414	180	10
+1	171	9.27
0	150	7.5
-1	129	5.73
-1.414	120	5

3. Results and Discussion

3.1 Characterization of crude green coffee oil

In Table 2 some physical and chemical characteristics of crude green coffee oil are presented. The oil shows a low iodine value due to its low content of unsaturated fatty acids (Table 3). The saponification value of the CGCO (162.12 mg KOH/g) was within the saponification value range of all vegetable oils. This value reflected the average length of the fatty acid chain of this oil. The oil contains 14.5% of unsaponifiable matter, which is quite a high value. The main constituents of the unsaponifiable matter are the diterpenoid alcohols, cafestol and kahweol. The high content of unsaponifiable matter is accompanied by high values of density, dynamic viscosity, and refractive index, unusual features in vegetable oils. High oil content of unsaponifiable matter indicated that CGCO is suitable as non-edible vegetable oil, by present important physiological problems [3,23]. High unsaponifiable matters content (14.5%) guarantees the use the oils in cosmetics industry. The CGCO exhibited good physicochemical properties and could be useful as pharmaceutical feedstock and industrial application.

Table 2. Chemical and physical characteristics of CGCO.

Parameter	Value	Method
Density (20ºC, g/mL)	0.9403	ASTM D7042–04
Refractive index (40ºC)	1.4717	AOCS Cc 7-25
Free fatty acids (% as oleic acid)	1.28	AOCS Ca 5a-40
Saponification value (mg KOH/g oil)	162.12	AOCS Cd 3-25
Unsaponifiable matter (%)	14.5	AOCS Ca 6b-53
Iodine value (g I_2/100 g oil)	78.58	AOCS Cd 1c-85
Activation energy for flow (kJ/mol)	30.60	Arrhenius Equation

The fatty acid composition of the CGCO is presented in Table 3. The main fatty acids present in all the samples are linoleic acid, with an average percentage of 39.24%, and palmitic acid, with an average percentage of 38.94%. Minor acids are myristic, pentadecanoic, margaric, linolenic, heneicosanoic and behenic whose contents are lower than 1.0%. These results are in good agreement with those reported in the literature [2,24].

Table 3. Chemical composition (%) of fatty acid from CGCO by GC-MS

Fatty acid	%
Oleic (C18:1)	12.03
Linoleic (C18:2)	39.24
Palmitic (C16:0)	38.94
Stearic (C18:0)	7.53
Linolenic (C18:3)	0.10
Arachidic (C20:0)	1.74
Heneicosanoic (C21:0)	traces
Behenic (C22:0)	0.28
Margaric (C17:0)	traces
Pentadecanoic (C15:0)	traces
Myristic (C14:0)	traces

Fig. 5 shows a typical chromatogram of crude green coffee oil. The retention times (in minutes) for the studied compounds were: 35.3 (myristic), 38.1 (pentadecanoic), 40.8 (palmitic), 43.3 (margaric), 44.9 (linoleic), 45.1 (oleic), 45.7 (stearic), 50.9 (linolenic), 51.7 (arachidic), 55.0 (heneicosanoic) and 59.1 (behenic). The total saturated and unsaturated fatty acid compositions were 48.6 and 51.4%, respectively.

Fig. 6 shows a representative plot of the GPC chromatogram obtained of a sample of CGCO at an eluent (THF) flow rate of 0.8 mL/min. the peak was obtained for each lipid class: triglycerides, diterpene fatty acid esters, and monoglycerides and FFA. CGCO was composed of about 72.2 wt% triglycerides, 24.8% diterpene fatty acid esters, and 3% (FFA and monoglycerides). The retention volumes (in mL) for the studied compounds were: 18.82 (triglycerides), 20.38 (diterpene fatty acid esters), and 20.99 (FFA and monoglycerides). To diterpene fatty acid esters, have been attributed hypercholesterolemic

effects, and coronary heart disease risk [23] due to the ingestion of unfiltered coffee drink. On the other hand, various studies have provided further support for the chemoprotective and anticancerigenic effects of CGCO diterpenes [3,5,25].

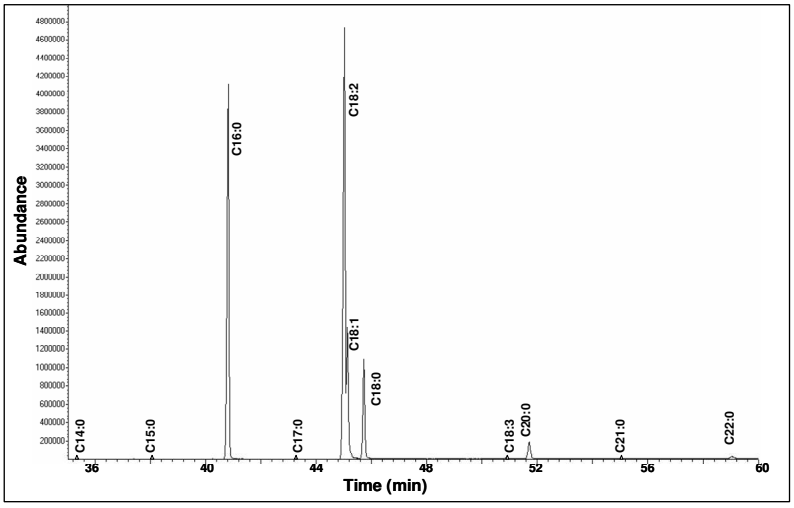

Fig.5. Chromatogram of the methyl esters of fatty acids in CGCO

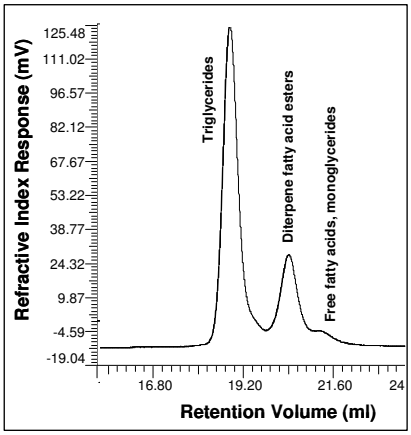

Fig.6. Representative GPC chromatogram of CGCO

Table 4 shows changes in dynamic viscosity of the CGCO as a function of temperature between 293 and 393K.

Table 4. CGCO viscosity at various temperatures

Temperature (K)	Dynamic viscosity (mPa.s)
293	136.29
303	82.251
313	52.915
323	35.941
333	25.533
343	18.854
353	14.384
363	11.304
373	9.0723

The Arrhenius equation (Eq.4) was employed to calculate the average magnitude of activation energy of the CGCO oil from 1/T and the natural logarithm of the dynamic viscosity (Fig.7). Where, (η) is the viscosity, A is the pre-exponential factor, E_a is the activation energy for viscous flow (in kJ/mol), R is the universal gas constant and T is the absolute temperature. R has the value of 8.314 x 10^{-3} (kJ/mol.K).The Arrhenius model is commonly used to model temperature dependence of a property [26]. The E_a indicates the energy barrier that must be overcome before the oil can flow [26].

$$\eta = A.\exp\left(\frac{E_a}{RT}\right) \tag{4}$$

The magnitude of activation energy (E_a) is given in Table 2. The degree of fit, as shown by the R^2 value of 0.9955, indicated that changes in viscosity with temperature could be well described by the Arrhenius equation. When the temperature increases, viscosity decreases exponentially shown that the viscosity magnitude of the unrefined coffee oil was greatly influenced by temperature.

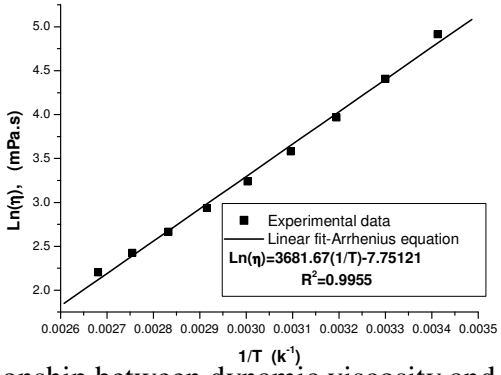

Fig.7. Relationship between dynamic viscosity and temperature for CGCO.

3.2 Optimization

Table 5 shows the studied variables settings and the results based on the experimental design. All 11 of the designed experiments were conducted and the results were analyzed through multiple regression. The coefficients of the full model were evaluated through regression analysis, and tested for significance. A given mathematical model was considered acceptable only when its analysis of variance (ANOVA) reached a high statistical, with F values within a level of confidence of 95% and p values of <0.05. The insignificant coefficients were eliminated, on the basis of the p-values after the testing of the coefficients.

Table 5. Experimental designs.

N	Desing factor X_1		Desing factor X_2		Responses	
	TEV (ºC)	Coded	Q mL/min	Coded	FFA (%) Y_1	NOL (%) Y_2
1	129	-1	5.73	-1	0.363	7.25
2	129	-1	9.27	1	0.460	4.76
3	171	1	5.73	-1	0.240	11.4
4	171	1	9.27	1	0.265	7.9
5	120	-1.414	7.5	0	0.485	4.69
6	180	1.414	7.5	0	0.261	10.3
7	150	0	5	-1.414	0.238	10.6
8	150	0	10	1.414	0.277	6.0
9	150	0	7.5	0	0.247	5.31
10	150	0	7.5	0	0.262	5.22
11	150	0	7.5	0	0.249	5.24

Multiple regression analysis of the experimental data gave the following second-order polynomial equations with natural values (Eqs. 5 and 6):

$$FFA\%(Y_1) = 3.53833581 - 0.04317557(TEV) + 0.00014335(TEV)^2 + 0.0501989(Q) + 0.0022618(Q)^2 - 0.00047861(TEV)(Q) \quad (5)$$

$$NOL\%(Y_2) = 73.21782 - 0.5968(TEV) + 0.00246(TEV)^2 - 7.10751(Q) + 0.48275(Q)^2 - 0.00678(TEV)(Q) \quad (6)$$

The results of the second-order response surface models fitting in the form of ANOVA are given in Table 6. In this case, the values of the correlation coefficients ($R^2_{NOL\%}$=0.9974, and $R^2_{FFA\%}$=0.9831) indicate that the fitted models can be used for prediction with reasonable precision.

The *F*-values for FFA% and NOL% models were 58.3 and 769 at 95% confidence, respectively. In addition, these *F*-values are greater than the critical *F*-value ($F_{0.95,5,5}$=5.05). Furthermore, the results show that the models for FFA% and NOL% are a good predictive in the experimental conditions in this study [27,28].

The *p*-values are used as a tool to check the significance of each of the coefficients, which, in turn, are necessary to understand the pattern of the mutual interactions between the best variables. In addition, the effect of each coefficient is considered to be significant if the *p*-value is lower than 0.05. The less significant effects (*p* >0.05) can be removed so that the regression equation will be the simplest possible. In this

case, the coefficient (B_{22}) in the FFA% model has no effect significant (p =0.175447>0.05) and the model resulting is predicted by Eq. 7 as follows:

$$FFA\%(Y_1) = 3.31200638 - 0.041765298(TEV) + 0.00013865(TEV)^2 + 0.08412651(Q) - 0.00047861(TEV)(Q) \quad (7)$$

Table 6. Analysis of variance (ANOVA) for the quadratic models

Source of variation	Sum of Square	Mean Square	*F*-value	*p*-value
FFA % model	R^2=0.9831			
B1	0.050380	0.050380	759.8	0.001313
B11	0.023277	0.023277	351.0	0.002836
B2	0.003808	0.003808	57.42	0.016971
B22	0.000282	0.000282	4.24	0.175447
B12	0.001266	0.001266	19.0	0.048589
Lack of fit	0.001233	0.000411	6.2004	0.142
Pure error	0.000133	0.000066		
Total	0.081001			
NOL % model	R^2=0.9974			
B1	28.98251	28.98251	12977.24	0.000077
B11	6.85130	6.85130	3067.75	0.000326
B2	19.52231	19.52231	8741.33	0.000114
B22	12.83005	12.83005	5744.80	0.000174
B12	0.25397	0.25397	113.72	0.008679
Lack of fit	0.07908	0.02636	11.80	0.079117
Pure error	0.00447	0.00223		
Total	64.31			

Contour plots and three-dimensional surfaces are graphical representation of the regression equation for the optimization of deacidification conditions of green coffee oil, and convenient to understand the interactions between evaporator temperatures (TEV) and volumetric feed flow rates (Q), and also locate their optimum levels. The response surface plots for FFA% and NOL% are shown in Figs. 8 and 9, respectively.

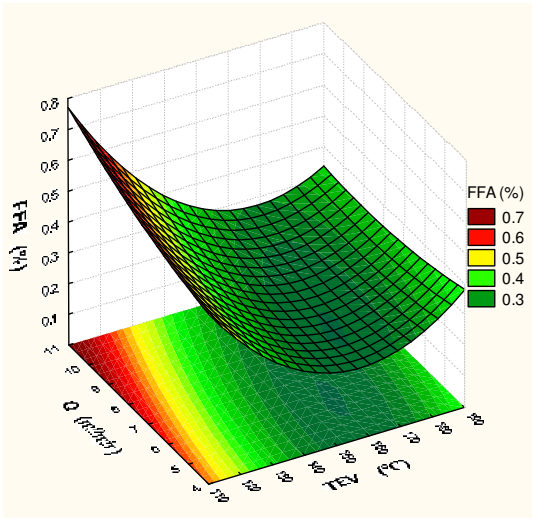

Fig.8. Surface plot of FFA% in the residue stream.

It is deduced from Fig. 8 that FFA% depends strongly on evaporator temperature (TEV) and slightly on volumetric feed flow rates (Q). Accordingly, the minimum value for FFA was 2.25% at 160.39ºC and 5.87 mL/min, which correspond to the evaporator temperature (TEV) and the volumetric feed flow rate (Q), respectively. With the increase of TEV, a decrease in the FFA content in the green coffee oil was observed. The results prove that it was feasible to obtain deacidified green coffee oil with a free fatty acids content lower than 0.3 % (as oleic acid) [29].

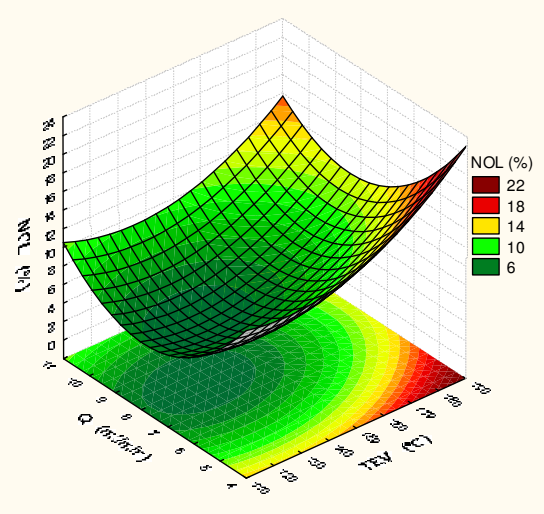

Fig.9. Surface plot of NOL% on deacidification process by MD.

Figure 9 shows that for neutral oil loss in the distillate streams there is a minimum in 4.12% at 132.7ºC and 8.29 mL/min. In this case, the neutral oil loss was strongly influenced by evaporator temperature (TEV) and volumetric feed flow rate (Q). Neutral oil loss in the deacidification process increases apparently with the decrease of volumetric feed flow rate, and increase of the evaporator temperature. Under these conditions, the rates of evaporation of all components increase, due to increase of vapor pressure and high residence times [15]. In these experiments, the neutral oil loss (NOL%) is lower that the results reported in the literature for alkali deacidification of coffee oil [30,9].

Thus, the final optimization of the two parameters was conducted by superimposing the effects in order to obtain the operational conditions for the minimum loss of neutral oil and free fatty acid content lower than 0.3%. One area of optimum performance was located (Fig. 10) at evaporator temperatures (TEV) between 142.7ºC and 150ºC with volumetric feed flow rate (Q) between 7 mL/min and 8 mL/min; FFA content

under this conditions is less than 0.3% (as oleic acid), and loss of neutral oil (NOL %) is lower 6%.

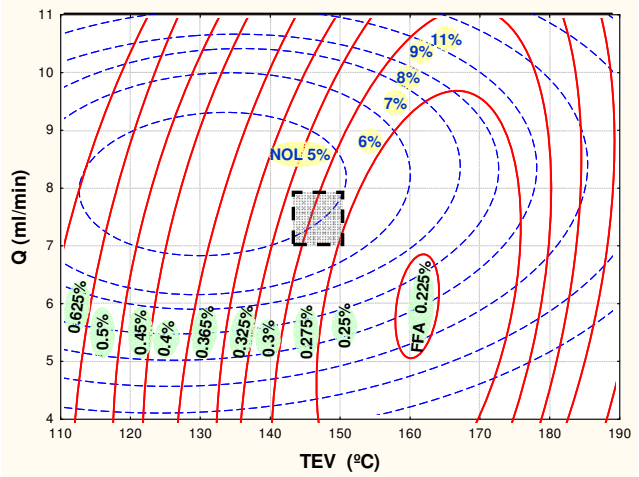

Fig.10. Superimposed contour plot for NOL% and FFA%.

An experiment with an evaporation temperature of 150ºC and 7 mL/min of volumetric feed flow rate was conduced in order to verify the obtained models. The experimental values (FFA content was 2.3% and NOL was about 6.3%) were in good agreement to the predicted values (FFA content was 2.47% and NOL was about 5.77%). The models developed can be used for predicting the FFA content (FFA%), and neutral oil loss (NOL%) in the deacidification process of green coffee oil by molecular distillation.

4. Conclusions

The results show that the deacidification of crude green coffee oil by molecular distillation is possible. RSM was effective for estimating the effect of the evaporator temperature (TEV) and volumetric feed flow rate (Q) of molecular distillation, as well as determining the optimal conditions. It were found the best conditions for guaranteeing a free fatty acids content lower than the required value of 0.3% (as oleic acid) in the green coffee oil, and a loss of neutral oil lower than 6% in the distillate stream. One area of optimum performance was located by superimposing contour plots, inside the variation intervals of the design factors, at evaporator temperatures (TEV) between 142.7ºC and 150ºC with volumetric feed flow rate (Q) between 7 mL/min and 8 mL/min.

5. Acknowledgements

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5.3. Conclusões

Os resultados obtidos mostram que a desacidificação do óleo bruto de café verde por destilação molecular é possível. A otimização pela Metodologia de Superfície de Resposta foi eficaz para estimar os efeitos da temperatura do evaporador (TEV) e vazão volumétrica de alimentação (Q) sob a destilação molecular, assim como para determinar as condições ótimas na desacidificação do óleo de café verde. Também, foram encontradas as melhores condições para garantir um teor de ácidos graxos livres, inferior ao valor exigido de 0,3% (máxima quantidade de ácidos graxos no óleo refinado permitida na legislação), e uma perda de óleo neutro inferior a 6% no fluxo de destilado. Uma área ótima foi localizada pela sobreposição de curvas de nível, dentro dos intervalos das variáveis independentes, a temperatura do evaporador (TEV), entre 142,7ºC e 150ºC, com taxa volumétrica de alimentação do fluxo (Q) entre 7 min/mL e 8 mL / min. Os resultados

obtidos apresentam as melhores condições para a desacidificação através da destilação molecular. A perda de óleo neutro foi muito inferior aos valores encontrados para o refino químico e físico. O planejamento fatorial e a análise de superfície de resposta foram utilizados como potente ferramenta na otimização do processo de desacidificação do óleo bruto de café verde por destilação molecular.

Capítulo 6.

Enriquecimento dos Diterpenos Cafestol e Caveol do Óleo de Café Verde (*Coffea arabica*) por Destilação Molecular

6.1. Introdução

Neste trabalho, se apresenta um estudo do enriquecimento de diterpenos de café de óleo de café verde (cafestol e caveol) utilizando destilação molecular. O cafestol e caveol são dois álcoois diterpênicos baseados na fusão de unidades de isopreno (C5) para formar o esqueleto de caurano de 20 carbonos. A diferença estrutural é pequena (uma dupla ligação entre os carbonos 1 e 2), entre os dois diterpenos. Os diterpenos existem no óleo de café na forma livres e esterificados aos ácidos palmíticos, linoléico e esteárico principalmente. Aspectos da análise da composição química são discutidos, assim como propriedades físicas das frações resultantes. Os destilados foram enriquecidos em ésteres diterpênicos de ácidos graxos, enquanto que os resíduos foram enriquecidos com triglicérides de alta massa molar. Diterpenos de café são importantes para as indústrias cosmética e farmacêutica, devido a suas propriedades emolientes, protetor da radiação solar, e seus efeitos quimioprotetores e anticarcinogênicos. Neste estudo, os ésteres diterpênicos de ácidos graxos foram concentrados a 42,8% e 38,4% (o óleo de café verde original tinha 24,4%) em fraciones de destilado utilizando o processo de destilação molecular em uma e duas passagens, respectivamente. Comparado ao óleo de café verde, a frações obtidas se comportaram de maneira diferente em suas propriedades químicas e físicas. Essas frações são obtidas em um equipamento de destilação molecular de filme descendente com um fluxo de alimentação de 6 mL/min, em um intervalo de temperaturas do evaporador entre 130ºC e 250ºC.

6.2. Desenvolvimento

O desenvolvimento deste capítulo é apresentado a seguir, no manuscrito intitulado: *Enrichment of Coffee Diterpenes from Green Coffee Oil (Coffea arabica) by Molecular Distillation.*

Enrichment of Coffee Diterpenes from Green Coffee Oil (*Coffea arabica*) by Molecular Distillation

ABSTRACT

In this work, enrichment of coffee diterpenes from green coffee oil using molecular distillation is presented. Aspects of chemical composition analysis are discussed as well as physical properties of the resulting fractions. The distillates were enriched in diterpene fatty acid esters and free fatty acids, while the residues were enriched in high molar mass triglycerides. Coffee diterpenes are valuable in the cosmetic and pharmaceutical industries, due to emollient properties, block the solar radiation, and their chemoprotective and anticancerigenic effects. Diterpene fatty acid esters were concentred to 42% and 38.5% (original green coffee oil had 24.4%) in a single-pass, and two-pass molecular distillation, respectively. Compared with green coffee oil, the fractions obtained behaved similarly in their chemical and physical properties. These fractions were obtained in a wiper-film molecular still at feed flows rate of 6 mL/min, and evaporator temperatures between 130ºC and 250 ºC.

KEYWORDS*: diterpene fatty acid esters, green coffee oil, molecular distillation, cafestol, triglycerides, kahweol*

1. Introduction

Coffee is one of the most traded commodities in the international markets, is an agricultural crop of significant economic importance. According to the latest estimate of the International Coffee Organization (ICO), total coffee production in the crop year 2009 was approximately 120 million bags of 60 kg, while Brazil's coffee production contributes 39.5 million bags [1]. The two species of coffee with greatest commercial significance are

Coffea arabica L and *Coffea canephora* Pierre, which are known in the trade, respectively, as arabica and robusta. Coffee arabica is grown in highland regions(1000-2000 m) and represent about 70% of coffee world production [1], while the coffee robusta represent remaining 30% which is grown in lowland regions (0-700m). Between these two species, coffee arabica provides a superior quality beverage (acidity, and finer flavour) but is susceptible to pests (fungi, nematodes, and insects), whereas coffee robusta produces a lower quality beverage (bitterness) but is more resistant to pests [2].

The green coffee beans are composed of proteins, carbohydrates, lipids, volatile acids (as acetic and formic acids), nonvolatile acids (as chlorogenic and citric acids), alkaloids (caffeine, trigonelline), minerals, pigments, and volatile aroma compounds [3]. Chemical composition of arabica and robusta coffee beans is shown in Table 1.

Table 1. Chemical composition of arabica and robusta coffee beans [4].

Component	*C. arabica*	*C. canephora*
Caffeine	0.8-1.4%	1.7-4.0%
Carbohydrates	50-55%	37-47%
Trigonelline	1-1.2%	0.6-0.7%
Lipids	12-18%	9-13%
Chlorogenic acids	5-8%	7-10%
Proteins	11-13%	11-13%
Minerals	3.0-4.2%	4.0-4.5%

Lipids in green coffee beans are mainly located in the endosperm while only a small amount of wax is found in the outer layer [5]. Green coffee oil has a high price in the market (about U$170/kg in 2010) with a wide variety of economical areas of interest as nutritional, biochemical, cosmetic, pharmaceutical and bioenergy [6]. Common methods for extracting the oil from green coffee beans include: solvent extraction, mechanical cold-pressing process, and supercritical fluid extraction [7-9]. Green coffee oil is characterized as greenish brown color with a cloudy aspect and an odor characteristic of green beans.

Green coffee oil is a mixture of free fatty acids (FFA), mono-, di-, and triglycerides, diterpene fatty acid esters, phosphatides, pigments, sterols and tocopherols [10-11]. The unsaponifiable fraction of green coffee oil is rich in diterpene alcohols, mostly cafestol, kahweol, and 16-O-methylcafestol, which are mainly esterified with various fatty acids (mainly palmitic and linoleic acids), and only a small amount of the diterpenes is present in the free form [12-13]. To diterpenes, have been attributed hypercholesterolemic effects, and coronary heart disease risk [14-15] due to the ingestion of unfiltered coffee drink. On the other hand, various studies have provided further support for the

chemoprotective, hepatoprotective, antioxidative, antiinflammatory, and anticancerigenic effects of cafestol and kahweol [13, 16-18]. In arabica coffees, both cafestol and kahweol were detected (see Fig.1). The content of cafestol and kahweol were about 6 and 3g/kg dry matter (d.m), respectively. In robusta coffees were detected cafestol (2g/kg d.m) and 16-O-methylcafestol (1-3g/kg d.m), in addition of traces of kahweol [13]. Because of the presence of diterpenes, green coffee oil is appropriate for industrial application and unfit for human consumption [19].

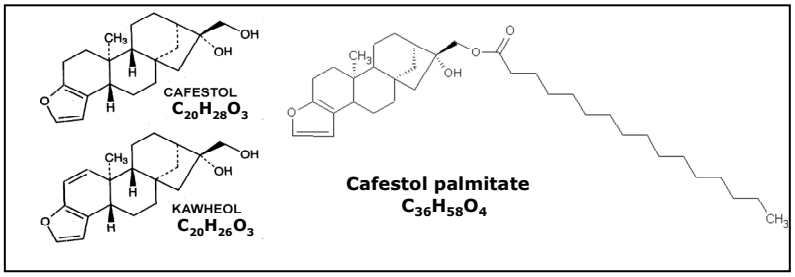

Figure1. Structural formulae of cafestol, kahweol, and cafestol palmitate.

Different methodologies have been investigated to identify and quantify kahweol, cafestol, and their esters. Various studies have used the technique of high performance liquid chromatography (HPLC), with UV detector, for analysis of cafestol and kahweol after saponification [8-9, 20-22]. In general, a reverse-phase column at ambient temperature is employed, and binary mixtures of water and organic solvents (methanol, acetonitrile and isopropanol) are used as the mobile phase. The use of Raman spectroscopy [23-25], gas chromatography (GC) [22, 26-30], thin-layer chromatography (TLC), and spectrophotometric techniques have also been used for diterpene alcohols analysis. Gel permeation chromatography (GPC) proved to be advantageous for the identification and separation of diterpene esters from green coffee oil [20]. Different classes of compounds (triglycerides, diterpene fatty acid esters, diglycerides, and monoglycerides) gave different elution times, each in its own peak, makes quantitation of these compounds simple and accurate [31].

Numerous methods have been proposed and patented for the recovery of diterpene from green coffee oil [32-33]. Conventional diterpenes concentration methods involve a number of complex processes such as liquid–liquid extraction [20], supercritical fluid extraction [8-9], and chromatographic techniques [22, 34-36]. Molecular distillation technology (MD) has been studied as an alternative technique for recovering and concentrating valuable compounds as vitamins, essential fatty acids, antioxidants and minor

components from crude vegetable oils [37-40]. In addition, molecular distillation offers an easier method for the separation of the unsaponifiable fractions and the removal of the free fatty acids without the use of solvents [38, 41-42].

Molecular distillation (MD) occurs at low temperatures, high vacuum, and short residence times, hence reduces thermal decomposition and eliminates oxidation of the oil. Molecular distillation is quite different and relies on the mean free path of different substances. In a molecular still the condenser is separated from the evaporator a distance less than the mean free path of light molecules (λ_L), but greater than the mean free path of heavy molecules (λ_H). With the increase of the temperature, the light molecules content is increased in the distillates. It is known that when the distilling temperature increases, the vapor pressure rapidly increases; at the same time, the mean free path of the molecule becomes larger. Then, the light molecules in the vapor are condensed on the cooling surface without intermolecular collisions hence vapor-liquid phase equilibrium can not be reached [40-41, 43-44]. In contrast, the heavy molecules (as triglycerides) cannot reach the condenser and come back to the evaporator. Schematic representation of molecular distillation is shown in Fig 2.

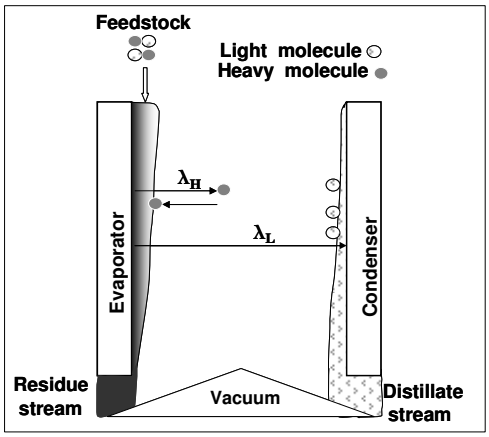

Fig.2. Schematic representation of molecular distillation.

In principle, FFA, diterpene fatty acid esters and triglycerides can be separated effectively due to the differences between molar mass, mean free path and vapor pressures (see Fig 3). The FFA are removed of green coffee oil in the first fractions in the distillations. The diterpene fatty acid esters which are lighter than triglycerides can be concentrated in the distillate stream, and triglycerides are usually concentrated in the residue stream [19].

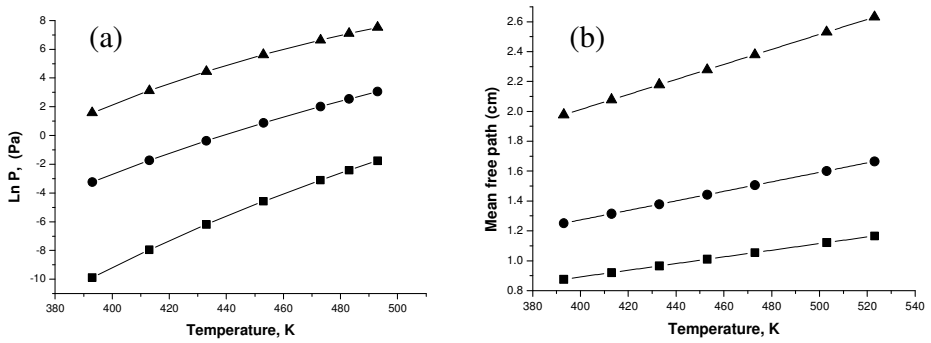

Figure 3. Effect of temperature on (a) vapor pressure and (b) mean free path for different lipids of green coffee oil [44-45]. (▲)Palmitic acid, (●) Cafestol palmitate, and (■)Trilinolein.

The objective of this work was to enrich diterpenes from green coffee oil by means of molecular distillation and to determine the composition and physical properties of fractions obtained at different operating conditions.

2. Materials and methods

2.1. Material

The crude green coffee oil was obtained from the industry (Linax, Votuporanga-Brazil), where it was obtained by mechanical pressing of arabica coffee beans[6].

2.2. Chemical and Physical analysis

The official methods of the American Oil Chemist Society (AOCS) were used to determine [46]: FFA as % oleic acid (AOCS Ca 5a-40), unsaponifiable matter (AOCS Ca 6b-53), saponification value (AOCS Cd 3-25), iodine value (AOCS Cd 1c-85), refractive index (AOCS Cc 7-25), preparation of methyl esters of fatty acids according to Hartman & Lago [47], and dynamic viscosity and density by Stabinger Viscometer (ASTM D7042–04).

2.3. Analysis of Fatty acids (FA)

Fatty acid (FA) composition and identification were determined with a Gas chromatography coupled to mass spectrometry (Agilent 5975 GC-MSD, Agilent, Santa Clara, United States), connected to a HP-5 capillary column (30 m x 0.32 mm x 0.25 µm) 5% Phenyl Methyl Siloxan. The column temperature was controlled as follows: initial

temperature 70ºC for 5min, then 4°C/min to 230ºC (hold for 3 min), then 4°C/min to 240ºC for a final holding time of 10 min. Helium was used as the carrier gas at a flow rate of 12.26 mL/min. Injection Volume 1 µL. Split Ratio 20:1. The injector and detector temperatures were 250 and 260ºC, respectively. The identification of the fatty acid methyl esters was carried out through the comparison with the retention time of SUPELCO standards (FAME Mix C14-C22) and the quantification by area normalization. Fig. 4 shows a typical GC chromatogram of fatty acids composition of original green coffee oil.

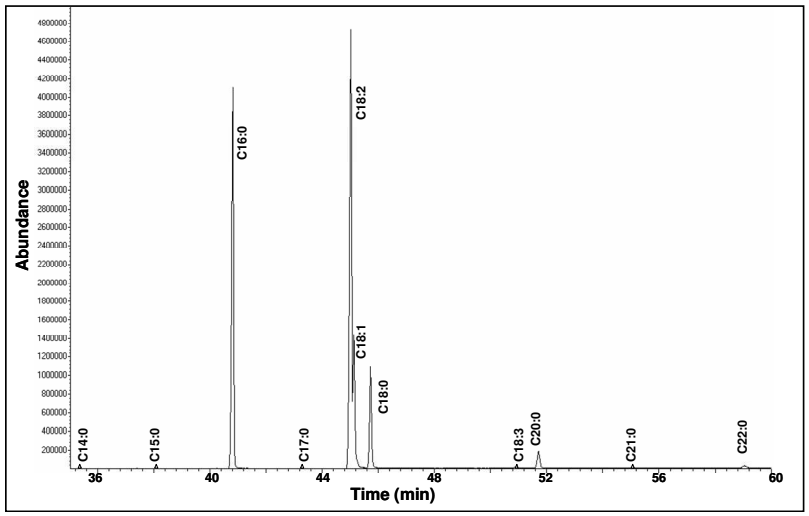

Figure 4. Chromatogram of fatty acids in original green coffee oil.

Green coffee oil has linoleic (C18:2), palmitic (C16:0), myristic (C14:0), pentadecanoic (C15:0), margaric (C17:0), oleic (C18:1), stearic (C18:0), linolenic (C18:3), arachidic (C20:0), heneicosanoic (C21:0) and behenic (C22:0) acids.

2.4. Analysis of coffee oil composition by GPC

Gel permeation chromatography (GPC) was used for the quantitation of triglycerides, diterpene fatty acid esters, monoglycerides (MAG), and free fatty acids (FFA). For this purpose a VISCOTEK GPC/SEC TDAmax^{TM} chromatograph with a refractive index detector was utilized. Samples of green coffee oil and fractions obtained of molecular distillation were dissolved in HPLC grade tetrahydrofuran (THF) and analyzed using THF as the mobile phase at flow rate of 0.8 mL/min. Three GPC/SEC Phenogel analytical columns (Phenomenex, Torrance, CA) with different pore sizes (50-100Å), dimension of 300 mm x 7.8 mm and packed with spherical styrene divinylbenzene copolymer beads with an average particle size of 5 µm were used. Sample injection volume

was 20 µl, and analyses were carried out at 40°C. All the analyses were performed in triplicate and average values were reported. Fig. 5 shows a representative plot of the GPC chromatogram obtained of original green coffee oil.

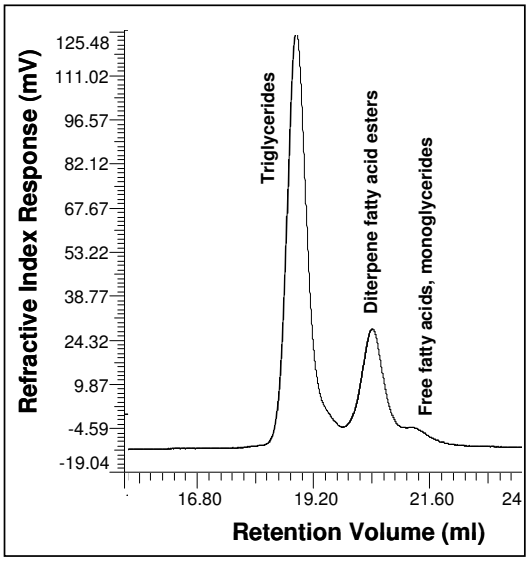

Figure 5. Representative GPC chromatogram of original green coffee oil.

2.5. Analysis of triglycerides

The identification of triglycerides (TAG) were achieved using a *VISCOTEK GPC/SEC TDAmax*^{TM} chromatograph with refractive index detector and a column Hyperclone BDS C18 5 µm 130 Å (250 × 4.6 mm I.D), from Phenomenex Inc (*Torrance, CA-USA*). During analysis, the column was maintained at 40°C. The mobile phase was acetone/acetonitrile in a ratio of 50:50 (v/v) at a flow rate of 0.75 mL/min. For each analysis, the sample was first diluted with acetone and 10 µL of this solution was injected into the chromatograph. Triglycerides were separated according to partition number (*PN=TC-2*DB*), where, *TC* is total acyl carbon number and *DB* is number of double bond. Triglycerides peaks were identified by comparison with retention times of TAG standards (*Sigma Chemical Co. USA*) and with the chromatograms published in the scientific literature [8, 10, 48-49]. Fig. 6 shows a typical chromatogram of triglyceride composition of original green coffee oil.

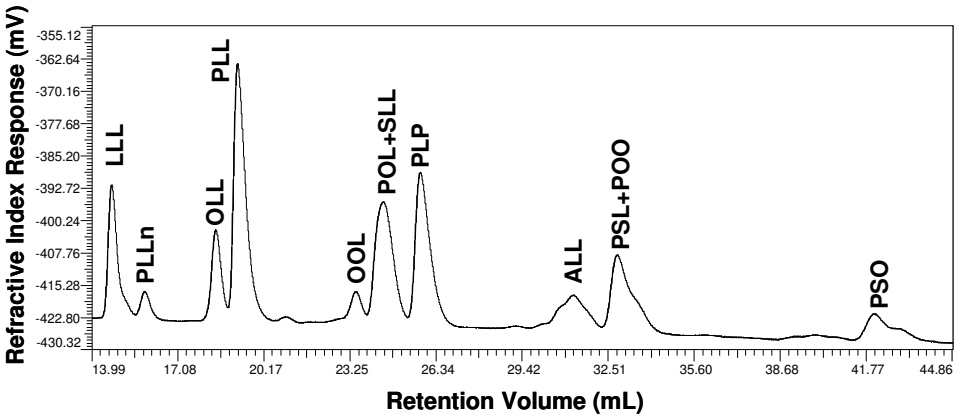

Figure 6. Representative GPC chromatogram of triglycerides of original green coffee oil (A, L, Ln, O, P and S are arachidic, linoleic, linolenic, oleic, palmitic and stearic acids, respectively).

2.6. Analysis of cafestol and kahweol

The identification and quantitation of cafestol and kahweol from coffee oil samples were performed according to the methods described in the literature [9, 36]. Analysis of cafestol and kahweol consisted of direct saponification with subsequent separation of the unsaponifiable fraction. Coffee Oil samples (50 mg) were saponified with 5mL of a solution 0.5M KOH in methanol. The solution was heated for 30 min at 80°C. After saponification, 5 mL water was added and the unsaponifiables were extracted twice with 5 mL of n-hexane. The organic fractions were collected and washed thrice with 5 mL of water, and were transferred to small amber bottles. The solvent was evaporated in a water bath at 70ºC by 30 min, the residue was dissolved in the mobile phase (4 mL), filtered using a nylon membrane filtration (0.45 μm Millipore), and injected into the chromatograph. Samples (20 μL) were analyzed on a *VISCOTEK GPC/SEC TDAmax*^{TM} chromatograph with UV detector and a column Hyperclone BDS C18 5 μm 130 Å (250 × 4.6 mm I.D), from Phenomenex Inc (*Torrance, CA-USA*). During analysis, the column was maintained at 25°C. The mobile phase was acetonitrile/water in a ratio of 55:45 (v/v) at a flow rate of 0.9 mL/min. The identity of the separated diterpenes in the oil fractions was assigned by comparing the retention times and co-chromatography with authentic standards (*Axxora, San Diego, CA*). Cafestol and kahweol were analyzed at 230 nm and 290 nm, respectively. Response factors for each of the standards were obtained by linear regression of known concentrations versus peak areas. Fig. 7 shows a typical chromatogram of cafestol and kahweol of original green coffee oil.

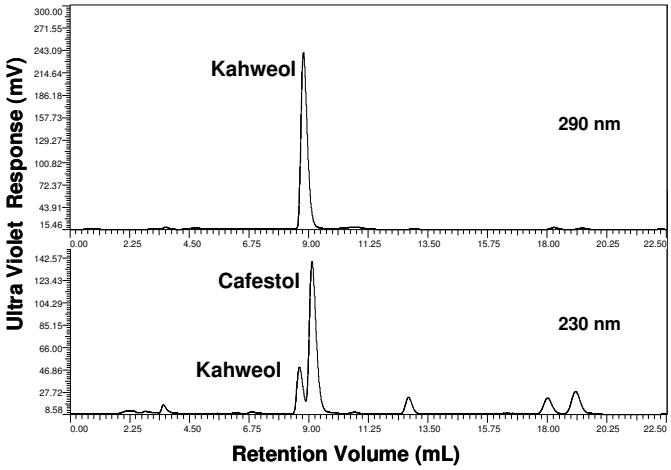

Figure 7. Typical GPC chromatograms with UV detector of cafestol (230 nm) and kahweol (290 nm) from original green coffee oil.

2.7. Molecular distillation equipment

The distillation was performed using a laboratory wiper-film molecular still model *KDL 5, GmbH UIC* (*Alzenau, Germany*). The equipment components were an evaporator, a condenser, a cooling trap, and the vacuum system (oil diffusion pump and a rotary vane pump). The evaporator is made of glass. The heating of the evaporator was provided by a jacket circulated with thermal oil from an oil bath. The distance between evaporation and condensation surfaces is 0.02m. The surface area of the evaporator is 0.048m^2 and the surface area of internal condenser is 0.065m^2. The roller wiper speed inside the evaporator was fixed at 350 rpm. The operational temperature up to 250°C, and the pressure inside the evaporator achieved up to 0.001 mbar. Each step produced one distillate stream and one residue stream. The schematic diagram for this equipment is showed in Fig 8.

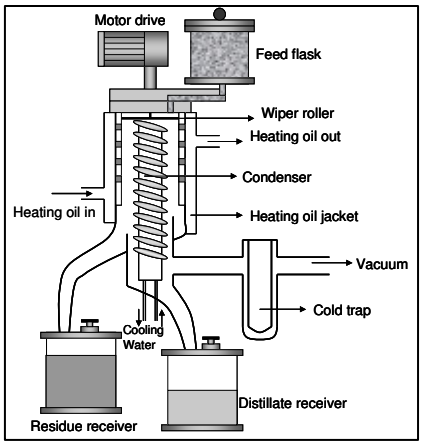

Figure 8. Schematic diagram of wiper-film molecular still.

2.8. Molecular Distillation conditions

The green coffee oil obtained by mechanical pressing of arabica coffee beans was fractionally distilled by single-pass and two-pass molecular distillation. The fractions (residue and distillate) were collected for subsequent analysis. Single-pass distillation (SPMD) is the simplest mode of distillation and produced one fraction of distilled and another one of residue (without recirculating). In the two-pass molecular distillation (TPMD) the residue fraction is redistilled under similar conditions of temperature, pressure and feed flow rate.

Preliminary studies [43, 50-51] indicated that the distilling temperature, and feed flow rate were major operative variables in the molecular distillation. Other factors were fixed in typical values for this application: pressure (0.001 mbar), feed temperature (40ºC), roller wiper speed (350 rpm), and condensation temperature (80ºC). The value of feed flow rate (Q) was fixed in 6 mL/min according to previous experiences in this equipment to form a uniform thin film on the evaporator surface, which promotes efficient mass and energy transfers [51-52]. The evaporation temperature (TEV) in the enrichment process was conducted in the range from 130 to 250°C, with temperature increments of 20ºC.

3. Results and Discussion

3.1. Experimental results

Table 2. Chemical and physical characteristics of distillate fractions obtained by SPMD.

Parameter	Original oil	Distillate obtained at temperature (°C)							Residue
		130	150	170	190	210	230	250	
Percent distilled (%)		8.29	7.63	7.36	7.87	14.89	13.59	11.70	
Cumulative distillate (%)		8.29	15.92	23.28	31.15	46.04	59.63	71.33	100
FFA (% oleic acid)	1.28	9.1	3.6	2.0	0.4	0.19	0.1	0.08	0.07
Saponif. value (mg KOH/g)	162.12	168.4	166.9	164.0	159.9	151.4	157.2	172	179
Unsaponifiable matter (%)	14.5	10.63	13.25	14.64	17.65	23.83	23.33	7.4	ND
Iodine value (g I_2/100 g oil)	78.58	83.2	82.22	83.14	80.65	72.91	76.67	80.2	87.21
Refractive index (40ºC)	1.4717	1.4716	1.4729	1.4738	1.4747	1.4782	1.478	1.4710	1.4665
Density (20ºC, g/mL)	0.9403	0.9361	0.9402	0.942	0.9453	0.9549	0.954	0.9384	0.9261
Viscosity (20ºC, mPa.s)	136.29	120.68	134.67	148.74	157.43	196.44	190.17	132.25	97.761
Cafestol (mg/100 g oil)	369.27	309.76	380.86	433.49	525.42	907.43	683.76	170.2	ND
Kahweol (mg/100g oil)	543.82	392.83	529.44	589.37	754.68	1380.4	950.32	220.6	ND

Seven experiments were conducted at different temperatures for both single-pass and two-pass molecular distillation, respectively. The experiments were carried out until arriving at steady state conditions. Afterwards, samples of the distillate and the residue were characterized and recorded. The results obtained are summarized in Tables 2-5.

Table 3. Composition (%mass) of the distillate fractions obtained by SPMD.

Parameter	Original oil	Distillate obtained at temperature (°C)							Residue
		130	150	170	190	210	230	250	
TAG (%)	72.2	71.5	72.3	70.3	65.7	57.2	58.1	83	100
Diterpene fatty acid esters (%)	24.8	19.1	23.8	26.3	31.7	42.8	41.9	17	0.0
MAG+FFA (%)	3.0	9.4	3.9	3.4	2.6	0.0	0.0	0.0	0.0
TAG composition (%mass)									
LLL	6.37	9.15	8.21	7.11	7.38	6.79	6.95	6.65	7.18
PLLn	1.75	2.1	2.11	2.16	2.18	1.95	2.04	2.12	1.85
OLL	3.9	6.31	5.05	4.43	4.57	3.81	3.68	3.71	3.79
PLL	24.54	22.4	23.58	23.48	24.22	24.49	25.05	25.07	24.92
OLO	1.33	3.58	2.11	1.96	1.77	1.13	1.02	1.1	0.92
PLO+SLL	16.63	16.30	16.42	16.79	16.84	16.26	16.16	16.22	16.41
PLP	20.94	16.93	18.63	19.98	19.85	20.06	20.76	20.67	19.49
ALL	6.16	8.31	7.16	7.62	6.96	6.07	5.73	5.8	6.05
PSL+POO	14.17	10.73	11.89	12.67	12.06	14.71	14.42	13.82	14.46
PSO	4.21	4.21	4.84	3.81	4.16	4.73	4.19	4.84	4.92
Fatty acid (FA) composition (%mass)									
C16:0	34.95	33.3	34.55	33.65	35.72	40.58	38.13	38.2	32.91
C18:0	9.12	8.9	8.4	9.4	9.0	8.22	9.0	9.0	8.75
C18:1	11.33	11.37	12.62	11.87	11.83	10.8	11.0	10.7	11.82
C18:2	41.43	42.6	40.9	42.1	40.47	36.73	38.76	37.8	43.85
C18:3	<0.1	0.19	0.2	<0.1	0.15	<0.1	<0.1	<0.1	<0.1
C20:0	2.44	2.73	2.32	2.13	2.37	2.34	2.51	2.5	2.18
C22:0	0.41	0.61	0.747	0.28	0.32	1.30	0.42	0.6	0.34

Table.4. Chemical and physical characteristics of distillate fractions obtained by TPMD.

Parameter	Original oil	Distillate obtained at temperature (°C)							Residue
		130	150	170	190	210	230	250	
Percent distilled (%)		16.43	14.05	12.07	11.28	12.97	12.58	8.29	
Cumulative distillate (%)		16.43	30.48	42.55	53.83	66.80	79.38	87.67	100
FFA (% oleic acid)	1.28	4.33	2.3	1.5	0.28	0.11	0.08	0.07	0.019
Saponif. value (mg KOH/g)	162.12	164.83	159.92	158.2	154.65	152.41	153.22	170.6	180.7
Unsaponifiable matter (%)	14.5	11.92	13.03	13.81	18.04	21.38	21.16	6.8	ND
Iodine value (g I_2/100 g oil)	78.58	79.57	81.65	81.45	79.59	76.30	77.41	80.10	84.67
Refractive index (40ºC)	1.4717	1.4718	1.4727	1.4729	1.4739	1.4763	1.4762	1.4717	1.4660
Density (20ºC, g/mL)	0.9392	0.9362	0.9404	0.9413	0.9448	0.9512	0.9506	0.9381	0.9231
Viscosity (20ºC, mPa.s)	127.12	132.69	137.62	139.9	151.05	179.60	179.46	131.21	92.85
Cafestol (mg/100 g oil)	369.27	331.7	397.86	455.34	631.86	779.302	657.64	120.1	ND
Kahweol (mg/100 g oil)	543.82	391.62	455.32	489.05	680.36	888.5	694.951	160.4	ND

Table 5. Composition (%mass) of the distillate fractions obtained by TPMD.

Parameter	Original oil	Distillate obtained at temperature (ºC)							Residue
		130	150	170	190	210	230	250	
TAG (%)	72.2	66.9	68.6	69.1	63.6	61.6	62.0	80	100
Diterpene fatty acid esters (%)	24.8	21.5	23.4	24.8	32.4	38.4	38.0	20	0.0
MAG+FFA (%)	3.0	11.6	8	6.1	4	0.0	0.0	0.0	0.0
TAG composition (%mass)									
LLL	6.37	6.56	6.69	6.97	6.77	6.73	6.30	6.56	6.76
PLLn	1.75	1.85	1.85	2.15	1.95	1.94	1.86	1.82	1.94
OLL	3.9	3.79	3.81	4.10	4.0	3.98	3.62	3.72	3.99
PLL	24.54	24.62	24.69	24.69	24.62	24.87	24.38	24.68	24.56
OLO	1.33	1.13	1.23	1.23	1.13	1.22	1.24	1.14	1.23
PLO+SLL	16.63	16.51	16.36	16.5	16.51	16.62	16.22	16.32	16.58
PLP	20.94	20.62	20.58	20.18	20.41	20.69	21.28	20.87	19.96
ALL	6.16	6.05	6.07	5.74	5.95	5.81	5.89	5.84	6.04
PSL+POO	14.17	14.36	14.30	14.14	14.15	13.97	14.67	14.45	14.33
PSO	4.21	4.51	4.42	4.30	4.51	4.18	4.55	4.6	4.61
Fatty acid (FA) composition (%mass)									
C16:0	34.95	37.79	35.54	35.57	36.75	39.52	38.34	38.41	34.28
C18:0	9.12	7.90	8.63	8.62	8.53	8.25	8.46	8.37	8.29
C18:1	11.33	11.81	11.87	12.02	11.84	11.69	11.89	11.72	12.29
C18:2	41.43	40.07	41.04	40.80	39.89	38.12	38.70	38.85	42.70
C18:3	<0.1	<0.1	0.135	0.17	0.11	<0.1	<0.1	<0.1	<0.1
C20:0	2.44	2.0	2.22	2.27	2.30	1.92	2.05	1.93	1.94
C22:0	0.41	0.34	0.3	0.32	0.36	0.21	0.32	0.33	0.27

3.2. Chemical properties of the fractions

The yield of distillate varies with the distillation temperature, and with the mode of distillation (Fig 9, and tables 2 and 4). Cumulative percentage of distillate obtained by TPMD was higher than to SPMD, but the content of diterpenes in distillates did not increase accordingly. Results show increase of distillate yield by SPMD between 190ºC and 230ºC. This is due that at temperatures below 190ºC was more easily remove the FFA and MAG, but a temperatures higher than 190ºC the components with middle molar mass, as diterpene fatty acid esters, were separated and concentrated into distillates, see Fig 10. These findings are in agreement with the results obtained by Khan and Brown [19].

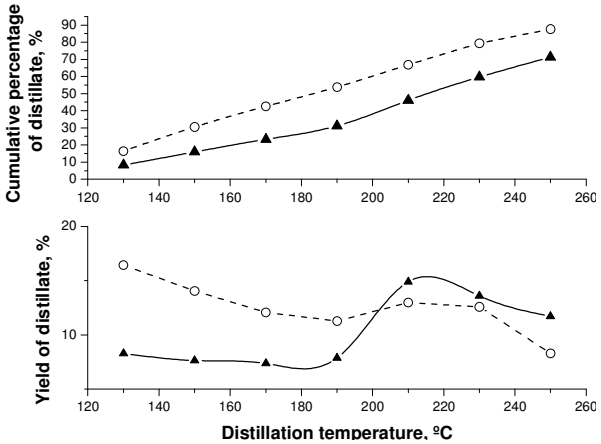

Figure 9. Comparison of distillation curves obtained by (▲) SPMD and (○) TPMD.

In view of the chemical composition (Tables 3 and 5), diterpene fatty acid esters constitute approximately 42.8% and 38.4 % of distillate fractions obtained at 210ºC by SPMD and TPMD, respectively. The distillation temperature of 210ºC became a turning point for the changes in total diterpenes content (cafestol and kahweol) in the distillates. At temperatures lower than 210ºC, the total diterpenes content in distillates decreased slightly while the content of FFA and MAG were increased. As shown in Figures 9-11, at 210ºC the yield of distillates by SPMD and TPMD were 14.89% (with 2287.83 mg of total diterpenes/100 g oil) and 12.97% (with 1667.80 mg of total diterpenes /100g oil), respectively. The kahweol content was higher than that of cafestol for most of the fractions. These results indicate an enrichment of 150.55% (by SPMD) and 82.65% (by TPMD) of total diterpenes, over that in the original green coffee oil (913.09 mg/100g oil).

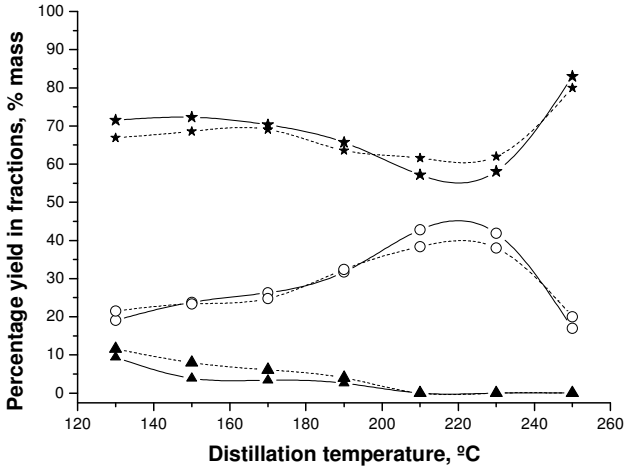

Figure 10. Composition of distillate fractions obtained by (—) SPMD and (---) TPMD. (▲)FFA+MAG, (○) Diterpene fatty acid esters, and (★) TAG.

When the temperature was about 250ºC, the content of large molecules such as triglycerides increased in the distillates and content of total diterpenes were 390.8 and 280.5 mg/100g oil by SPMD and TPMD, respectively. This behavior indicates that when the distillation temperature was increased, the content of diterpene fatty acid esters diminish due to increase in the content of triglycerides. The components with larger molar mass such as triglycerides are more difficult to evaporate; these components consisted of a larger proportion in the residue.

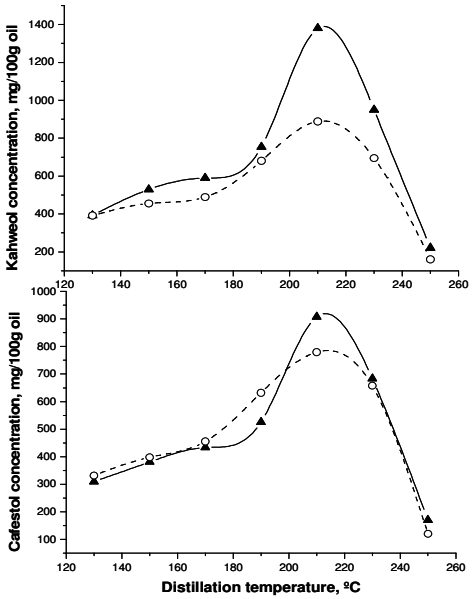

Figure 11. Content of diterpenes (cafestol and kahweol) in distillate fractions obtained by (▲) SPMD and (○) TPMD

Figure 12 shows the percentage of recovery of total diterpenes (cafestol and kahweol), in the distillate fractions. The maximum recovery of diterpenes was reached at the distillation temperature of 210ºC. In these conditions, the percentages of recovery of diterpenes were 37.32% and 22.83% by SPMD and TPMD, respectively, indicating that the molecular distillation process gives high recovery rates. Recovery (%) was calculated by the Eq.1.

$$\text{Diterpene Recovery (\%)} = \frac{\text{(Total diterpene content in each fraction)}}{\text{(Total diterpene content in original coffee oil)}} x(100) \quad (1)$$

The diterpene recovery in distillate fractions at 210ºC, which were the highest values all the fractions, this can be explained by increases of mean free path (MFP) of the diterpene esters, causing a high amount of molecules passing to condenser. This is the purpose of the molecular distillation process, to concentrate the diterpene esters from green coffee oil. So, TAG became the main component in the residue.

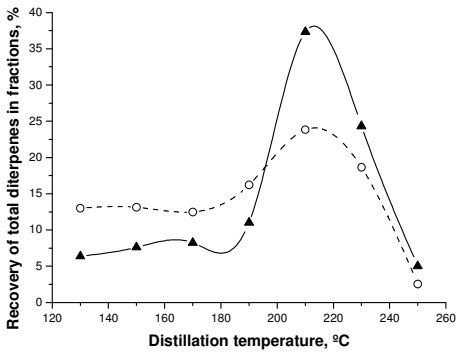

Figure 12. Recovery of total diterpenes in distillate fractions by (▲) SPMD and (○) TPMD.

3.2.1. Fatty acid (FA) composition

Tables 3 and 5 show the fatty acid composition of the fractions obtained by SPMD and TPMD, respectively. Fig. 4 shows a typical chromatogram of fatty acid composition of original green coffee oil (*Coffea arabica*).

The retention times (in minutes) for the fatty acids analysed were: 35.3 (Myristic, C14:0), 38.1 (Pentadecanoic, C15:0), 40.8 (Palmitic, C16:0), 43.3 (Margaric, C17:0), 44.9 (Linoleic, C18:2), 45.1 (Oleic, C18:1), 45.7 (Stearic, C18:0), 50.9 (Linolenic, C18:3), 51.7 (Arachidic, C20:0), 55.0 (Heneicosanoic, C21:0) and 59.1 (Behenic, C22:0). Linoleic, palmitic, oleic, and stearic acids are the major fatty acids found in green coffee oil. Minor fatty acids are myristic, pentadecanoic, margaric, linolenic, heneicosanoic and behenic whose contents are lower than 1.0%. These results are in good agreement with those reported in the literature [11, 53].

No significant differences were observed in the fatty acid composition between fractions obtained in the range from 130 to 250°C by SPMD and TPMD. In general, slight changes in fatty acid composition of the fractions are mainly due to changes that occurred in proportions of palmitic and linoleic acids. Those are the main fatty acids present in the fractions with average percentages of palmitic acid between 36.3% (SPMD) and 37.4% (TPMD), and linoleic acid between 39.9% (SPMD) and 39.6% (TPMD).

3.2.2. Triglycerides (TAG) composition

Tables 3 and 5 show the triglycerides composition of the fractions obtained by SPMD and TPMD, respectively. Generally, green coffee oil is one of the most complex mixtures of natural TAG [49]. Fig. 6 shows a typical chromatogram of triglyceride composition of original green coffee oil (*Coffea arabica*). The GPC conditions used in the present work furnished a good separation allowing the identification of TAG in the fractions of green coffee oil. The identification of the peaks was achieved by comparison with those obtained from the chromatograms of coffee oil reported in the literature. The fatty acid composition of the coffee oil and 1,3-random-2-random distribution hypothesis were used to identify the triglycerides present in the samples [8, 10, 48-49]. It is possible to observe that triglycerides LLL, PLLn, OLL, PLL, OLO, SLL, PLO, PLP, ALL, PSL, POO and PSO were identificated (A, L, Ln, O, P and S are arachidic, linoleic, linolenic, oleic, palmitic and stearic acids, respectively).

The retention volumes (in mL) for triglyceride analysis were: 14.67 (LLL), 15.84 (PLLn), 18.38 (OLL), 19.1 (PLL), 23.37 (OLO), 34.31 (SLL+PLO), 25.58 (PLP), 31.14 (ALL), 32.67 (PSL+POO), and 41.9 (PSO). In this study no significant differences were observed of molecular distillation modes (Tables 3 and 5) on the composition of the TAG identified in the fractions obtained between 130 and 250ºC. Considering the analyzed samples, the major triglycerides present in the fractions obtained by SPMD and TPMD are: PLL, PLP, PLO+SLL, and PSL+POO. The composition of the triglycerides was almost the same in all fractions. It shows that the molecular distillation gives little separation for mixtures of triglycerides of green coffee oil. Several studies indicate that fractionation of the triglyceride constituents of vegetable oils via molecular distillation is less effective than chromatography or low-temperature crystallization [39].

3.2.3. Iodine value (IV)

All fractions, along with the original green coffee oil, were subjected to the method (AOCS Cd 1c-85) of determining iodine values (Tables 2 and 4). The IV is a measure of the unsaturated fatty acids of oils and is expressed as the number of grams of iodine per 100 grams of sample. The higher the amount of unsaturation (number of double bonds), the more iodine is absorbed, therefore, the higher the IV, the greater the degree of unsaturation in the oil, more reactive, less stable, and more susceptible to oxidation and rancidification. The IV is not the best index for oil stability since it does not distinguish the structural differences in fatty compounds such as nature (position in the chain, etc.) and amount of olefinic carbons. However, it still is important in assessing the stability of oil in oleochemical applications. The IV also has developed into a fuel quality parameter, as it has been included in some biodiesel standards, particularly in Europe [54]. With a relatively low IV (less than 125 g I_2/100g), green coffee oil is classed as a nondrying oil, that can be used for soapmaking and in cosmetic products.

Since the original coffee oil had an IV of 78.58(g I_2/100g) and the fractions had IV of 78.22 to 86.6 (g I_2/100g), it is evident that a slight fractionation took place. Thus, it can be concluded that there are only slight variations in composition of unsaturated fatty acids in the fractions obtained by SPMD and TPMD compared to the original green coffee oil. These results are in good agreement with the range published in the literature for coffee oil [55].

3.2.4. Saponification value (SV)

The SV determined for the original green coffee oil presented a value of 162.12 mg KOH/g slightly smaller than the range encountered in the literature [55]. SV of original green coffee oil and the fractions obtained by SPMD and TPMD are presented in Tables 2 and 4, respectively. As seen from figure 13-A, the saponification value decreases with increasing of distillation temperature. The minimum SV for SPMD (151.4 mgKOH/g) and TPMD (152.4 mgKOH/g) were attained at 210ºC. This behavior is influenced by the decrease of the composition of TAG in the distillate fractions. Figure 13-B shows a moderate relationship between SV and percentage of diterpene fatty acid esters. When the percentage of diterpene fatty acid esters increases, SV decreases lineally shown that the SV of the distillate fractions is influenced by percentage of diterpene fatty acid esters, according to Equation 2. In this case, the value of the correlation coefficient (R^2=0.7837) indicates that the fitted model can be used moderately for prediction with reasonable precision.

$$SV = 180.853 - 0.69415(Diterpene\ fatty\ acid\ esters, \%) \quad (2)$$

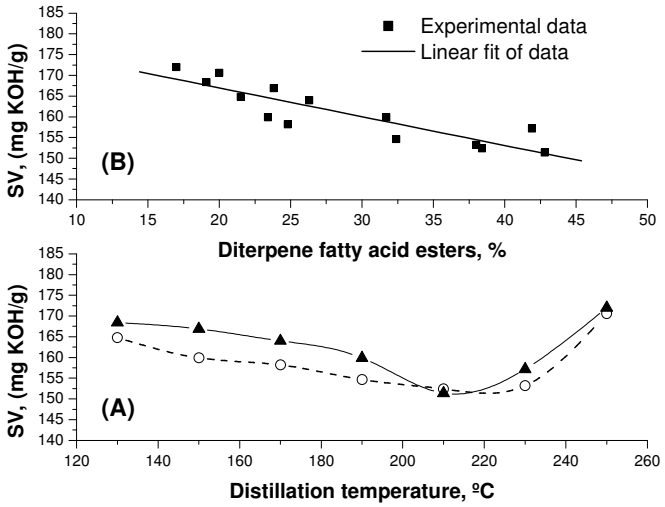

Figure 13. (A) Variation of saponification value SV in the distillate fractions obtained by SPMD (▲), and TPMD (○), and (B) Linear relationship for SV and diterpene fatty acid esters (%).

3.2.5. Unsaponifiable matter

The unsaponifiable fraction of vegetable oils comprises those constituents which, after saponification (i.e. base-catalysed hydrolysis) of the oil exhibit low solubility in water and high solubility in organic solvents. Literature data of molecular distillation of various vegetable oils show that the unsaponifiable matter is concentrated in the first fractions [56]. In the case of green coffee oil (*Coffea arabica*), the unsaponifiable fraction (rich in cafestol and kahweol) is

esterified with various fatty acids, and these esters have almost the same distillation characteristics as the triglycerides [45]. This behavior is parallel to trend observed in the content of diterpene fatty acid ester in the fractions obtained by SPMD and TPMD. In general, the unsaponifiable matter is concentrated in fractions about of 210ºC, as shown in Figure 14.

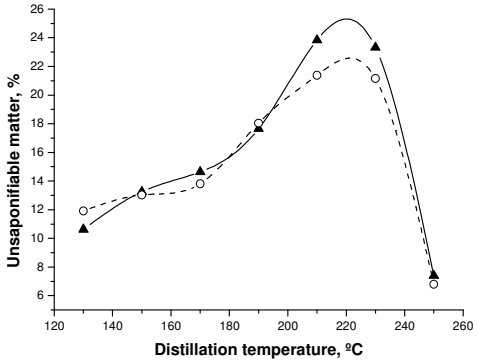

Figure 14. Variation of content of unsaponifiable matter in the distillate fractions obtained by SPMD (▲) and TPMD (○) with the distillation temperature.

3.2.6. Free Fatty Acids (FFA)

Most of free fatty acids were separated in the first fractions at temperatures lower than 190ºC (Tables 2 and 4). As shown in Figure 15, the content of FFA in the residue streams obtained by molecular distillation show low values (<0.3%) after the first distillation. According to the Codex Alimentarius [57] the indicated value of FFA in refined oils is 0.3 mass % as the maximum recommended by the human consumption.

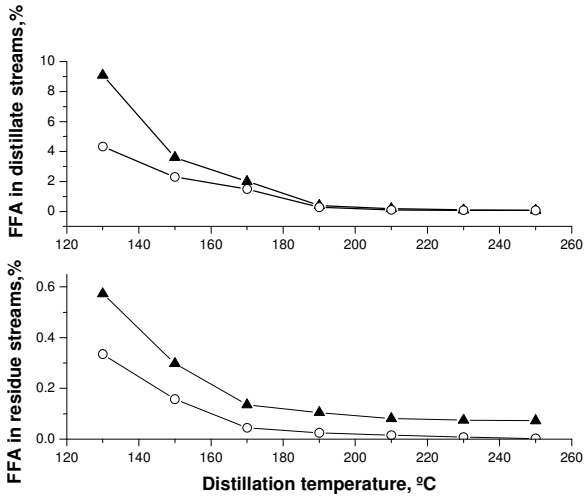

Figure 15. Variation of content of FFA in the distillate and residue streams with the distillation temperature obtained by SPMD (▲) and TPMD (○)

Free fatty acids content in the distillate and the residue decreased as distillation temperature increased. This can be explained considering that these compounds were evaporated before diterpene fatty acid esters [56]. The best removal of free fatty acids

(more than 58 %) occurred at condition of 130ºC by SPMD. These results suggest that the molecular distillation can be an alternative method for deacidification process of vegetable oils.

3.3. Physical properties of the fractions

3.3.1. The density, viscosity, and refractive index

In general, the density, viscosity, and refractive index values of the distillate fractions show substantial differences with the original oil. The density, viscosity, and refractive index values increase with increasing of distillation temperature, until they reach a maximum value. The maximum values obtained by SPMD and TPMD were attained at 210ºC. This behavior is influenced by the increase of the content of diterpene fatty acid esters. Figures 16-18 show a linear relationship between refractive index, viscosity and density with the percentage of diterpene fatty acid esters. When the percentage of diterpene fatty acid esters increases, these properties increase lineally shown that are influenced by content of diterpene fatty acid esters, according to Equations 3, 4 and 5. The value of the correlation coefficient (R^2) were (0.9839), (0.9864), and (0.9846) obtained for refractive index, viscosity and density, respectively. This relatively good fit to the models suggest that the regression equations may be used to predict these properties within these size intervals.

$$\text{Refractive index} = 1.46634 + 2.68203E-4(\textit{Diterpene fatty acid esters},\%) \quad (3)$$

$$\text{Viscosity(mPas)} = 67.11951 + 2.93507(\textit{Diterpene fatty acid esters},\%) \quad (4)$$

$$\text{Density(g/mL)} = 0.92191 + 7.57633E-4(\textit{Diterpene fatty acid esters},\%) \quad (5)$$

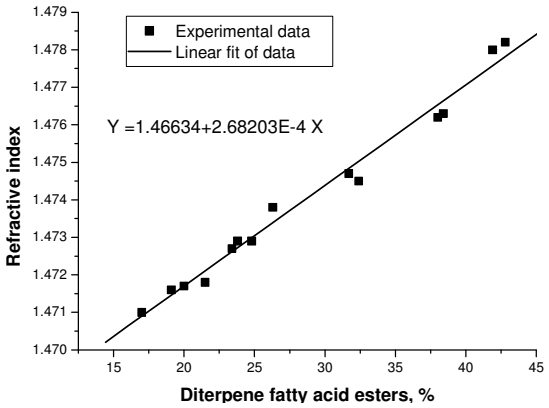

Figure 16. Relationship between the refractive index (40ºC) and percentage of diterpene fatty acid esters.

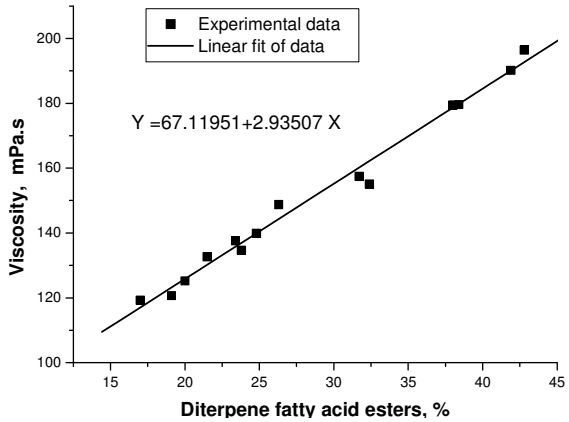

Figure 17. Relationship between the viscosity (20ºC) and percentage of diterpene fatty acid esters.

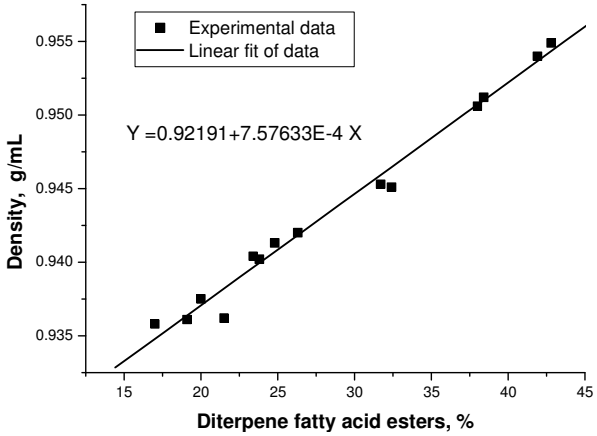

Figure 18. Relationship between the density (20ºC) and percentage of diterpene fatty acid esters.

4. Conclusions

The present work has shown that, fractionation of green coffee oil by molecular distillation is an effective tool for yielding several fractions which differ markedly in their properties. Aspects of chemical composition analysis are discussed as well as physical properties. Moreover, the molecular distillation improved the enrichment of the coffee diterpenes as a result of high vacuum and low distillation temperature, since these are components of interest for cosmetic and pharmaceutical industries. The best results were obtained for a purity of diterpene fatty acid esters of 42.8% and 38.4 % of distillate fractions at 210ºC and 6 mL/min of feed flow rate by SPMD and TPMD, respectively. The mode SPMD showed a higher Enrichment of diterpenes (cafestol and kahweol) than TPMD. But, the TPMD is more effective to remove free fatty acids. At temperatures lower than 210ºC, the total diterpenes content in distillates decreased slightly while the content of

FFA and MAG were increased. At 210ºC the yield of distillates by SPMD and TPMD were 14.89% (with 2287.83 mg of total diterpenes/100 g oil) and 12.97% (with 1667.80 mg of total diterpenes /100g oil), respectively. These results indicate an enrichment of 150.55% (by SPMD) and 82.65% (by TPMD) of total diterpenes, over that in the original green coffee oil (913.09 mg/100g oil). The kahweol content was higher than that of cafestol for most of the fractions. The influence of composition of the diterpene fatty acid esters on the physical and chemical properties of the distillate fractions, were observed.

5. Acknowledgements

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6.3. Conclusões

O presente trabalho mostrou que a destilação molecular é uma ferramenta eficaz para o fracionamento de óleo de café verde, e no enriquecimento dos diterpenos de café obtendo frações com propriedades marcadamente diferentes em comparação ao o óleo original. Aspectos da análise da composição química são discutidos, bem como propriedades físicas. Além disso, a destilação molecular alcançou o objetivo de enriquecimento dos diterpenos do café sob condições brandas de baixa temperatura de destilação e alto vácuo. Este procedimento tem aplicações importantes para obter produtos de alto valor agregado e de interesse para as indústrias cosmética e farmacêutica. Os melhores resultados de enriquecimento de ésteres diterpênicos em frações foram obtidos a 210ºC e 6 mL/min de vazão de alimentação com uma pureza de 42,8% e 38,4% por uma e duas passagens no destilador molecular, respectivamente. O método de destilação molecular com uma única passagem apresentou maior enriquecimento de diterpenos de café (cafestol e caveol) que o método de recirculação com duas passagens. Mas, com o método de duas passagens de destilação, observou-se mais eficácia para a eliminação dos ácidos graxos livres. Ás temperaturas de destilação inferiores a 210ºC, o teor de diterpenos totais diminuíram ligeiramente nos destilados, enquanto que o teor de ácidos graxos livres e monoglicerídeos aumentaram. A 210ºC a produção de destilados por uma e duas passagens pelo destilador foram de 14,89% (com 2287,83 mg diterpenos total/100 g de óleo) e 12,97% (com 1667,80 mg de diterpenos total/100g óleo), respectivamente. Estes resultados indicam um enriquecimento de 150,55% (numa passagem) e 82,65% (em duas passagens) de diterpenos total, mais que no óleo de café verde original (913,09 mg/100g de óleo). O teor de caveol foi maior do que o cafestol na maioria das frações. A influência da composição de ésteres diterpênicos de ácido graxos sobre as propriedades físicas e químicas das frações de destilado, também foi estudada.

Capítulo 7.

Estudo por Calorimetria Diferencial de Varredura (DSC) das propriedades térmicas do óleo de café verde (*Coffea arabica*) e suas frações obtidas por destilação molecular

7.1. Introdução

Estudos relacionados às propriedades térmicas de óleo de café verde são limitados se comparados com outros óleos vegetais. O óleo bruto de café verde foi fracionado por destilação molecular num evaporador de filme descendente. Essas frações foram obtidas com uma vazão de alimentação de 6 mL/min, em um intervalo de temperaturas do evaporador entre 130ºC e 230ºC. O estudo térmico das frações obtidas foi avaliado utilizando o processo não-isotérmico de calorimetria diferencial de varredura (DSC-823e pela Mettler Toledo), a uma taxa constante de aquecimento (10ºC/min), com uma vazão constante de nitrogênio (50mL/min). O perfil térmico do óleo de café verde e as frações apresentaram dois picos endotérmicos (atribuído ao ponto de fusão e da degradação térmica). Os parâmetros cinéticos da degradação térmica do óleo e as frações foram calculados utilizando o método de Roger e Morris, e uma relação da energia de ativação com a composição de ésteres diterpênicos foi observada. Alem disso, as capacidades caloríficas foram medidas entre 35 e 240°C. A influência dos ésteres diterpênicos de ácidos graxos nas propriedades térmicas de óleo de café verde foi analisada. Os resultados obtidos sugerem que o conteúdo de ésteres diterpênicos de ácidos graxos desempenha um papel importante na estabilidade térmica de frações. Comparado com o óleo de café verde, a frações obtidas se comportaram de maneira semelhante em suas propriedades térmicas. Os diterpenos de café são importantes para as indústrias cosmética e farmacêutica, devido a suas propriedades emolientes, protetor da radiação solar, e seus efeitos quiomoprotetores e anticarcinogênicos.

7.2. Desenvolvimento

O desenvolvimento deste capítulo é apresentado a seguir, no manuscrito intitulado: *Differential Scanning Calorimetry (DSC) Studies on the Thermal Properties of green coffee oil (Coffea arabica) and fractions obtained by Molecular Distillation.*

Differential Scanning Calorimetry (DSC) Studies on the Thermal Properties of Green Coffee Oil (*Coffea arabica*) and Fractions Obtained by Molecular Distillation

ABSTRACT

Studies related to the thermal properties of the green coffee oil are limited if compared with other vegetable oils. Crude green coffee oil was fractioned by molecular distillation (MD) in a wiped-film evaporator. These fractions were obtained at feed flows rate of 6 mL/min, and evaporator temperatures between 130ºC and 250 ºC. The thermal stability of the fractions obtained has been evaluated using non-isothermal differential scanning calorimetry (DSC-823e from Mettler-Toledo) at a constant heating rate (10ºC/min) under nitrogen atmosphere. The thermal profile of green coffee oil and fractions showed two endothermic peaks (assigned to the melting and thermal degradation). The activation energy of thermal degradation of the oil and the fractions were calculated using the Rogers and Morris method, and an increase was observed with increase of content of diterpene fatty acid esters. The heat capacities have been measured between 35 and 240°C. The influence of diterpene fatty acid esters on thermal properties of green coffee oil was analyzed. Results obtained suggest that content diterpene fatty acid esters play an important role in thermal stability of fractions. Compared with green coffee oil, the fractions obtained behaved similarly in their thermal properties. Coffee diterpenes are valuable in the cosmetic and pharmaceutical industries, due to emollient properties, block the solar radiation, and their chemoprotective and anticancerigenic effects

KEYWORDS*: diterpene fatty acid esters, green coffee oil, molecular distillation, cafestol, kahwol, Differential Scanning Calorimetry (DSC).*

1. Introduction

Coffee is one of the most traded commodities in the international markets, is an agricultural crop of significant economic importance. According to the latest estimate of the International Coffee Organization (ICO), total coffee production in the crop year 2009 was approximately 120 million bags of 60 kg, while Brazil's coffee production contributes 39.5 million bags [1]. *Coffea arabica* L (arabica) is grown in highland regions(1000-2000 m) and represent about 70% of coffee world production [1], while the *Coffea canephora* Pierre (robusta) represent remaining 30% which is grown in lowland regions (0-700m). Between these two species, coffee arabica provides a superior quality beverage (acidity, and finer flavour) but is susceptible to pests (fungi, nematodes, and insects), whereas coffee robusta produces a lower quality beverage (bitterness) but is more resistant to pests [2].

Green coffee oil has a high price in the market (about U$170/kg in 2010) with a wide variety of economical areas of interest as nutritional, biochemical, cosmetic, pharmaceutical and bioenergy [3]. Common methods for extracting the oil from green coffee beans include: solvent extraction, mechanical cold-pressing process, and supercritical fluid extraction [4-6]. Green coffee oil is characterized as greenish brown color with a cloudy aspect and an odor characteristic of green beans. Green coffee oil is a mixture of free fatty acids (FFA), mono-, di-, and triglycerides, diterpene fatty acid esters, phosphatides, pigments, sterols and tocopherols [7-8]. The unsaponifiable fraction of green coffee oil (*Coffea Arabica)* is rich in diterpene alcohols (see Figure 1), mostly cafestol and kahweol, which are mainly esterified with palmitic and linoleic acids, and only a small amount of the diterpenes is present in the free form [9-10]. To diterpenes, have been attributed hypercholesterolemic effects, and coronary heart disease risk [11-12] due to the ingestion of unfiltered coffee drink. On the other hand, various studies have provided further support for the chemoprotective, hepatoprotective, antioxidative, antiinflammatory, and anticancerigenic effects of cafestol and kahweol [10, 13-15]. Because of the presence of diterpenes, green coffee oil is appropriate for industrial application and unfit for human consumption [16]. Therefore, an understanding of the thermal behavior of green coffee oil and their fractions is important for many practical applications in the oils and fats industry. Kinetic data are essential for predicting thermal stability of oil under various heat processing, storage, and distribution conditions.

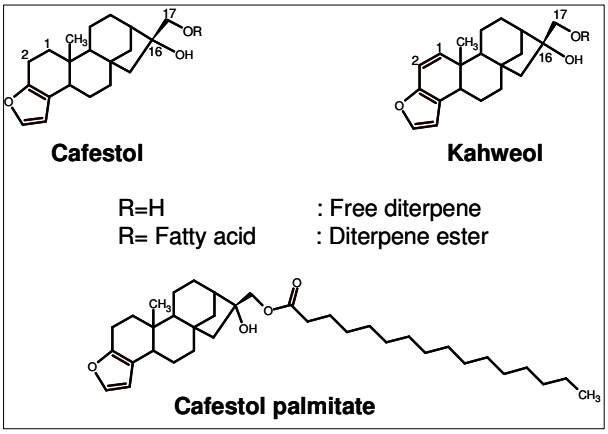

Fig.1. Structural formulae of cafestol, kahweol, and cafestol palmitate

Actually, thermal analysis techniques as differential scanning calorimetry (DSC) and thermogravimetry (TGA) have been used for characterization of edible oils and fats [17]. These methods are advantageous in relation to the conventional ones because present a higher precision and sensitivity for measuring several properties such as heat capacities [18], thermo-oxidative behavior and thermal stability [19-21], thermal degradation kinetics [22], action of antioxidants in oil thermal stability [22-23], temperature and enthalpy of crystallization and melting [23-28], and high-pressure oxidation induction time measurements [29]. Application of statistical methods to DSC data can be used to measure content of fat solid [30], to determine the country of origin [31], to detect adulterants [32], to estimate the amount of saturation present in transesterified blends of esters [33], to quantify the iodine value [34], and to determine total polar compounds in original and heated oils [35].

The objectives of this study were to evaluate by DSC, heat capacities, melting and degradation points, enthalpy of melting and degradation, activation energy of thermal degradation, and pre-exponential factor, and to determine the influence of temperature and content of diterpene fatty acid esters in the thermal behavior of green coffee oil and the fractions obtained by molecular distillation (MD). For the evaluation of activation energy, the non-isothermal DSC method was used and the treatment of experimental data was carried out by Rogers and Morris method [36].

2. Materials and methods

2.1. Material

The crude green coffee oil was obtained from the industry (Linax, Votuporanga-Brazil), where it was obtained by mechanical pressing of arabica coffee beans [3].

2.2. Thermal analysis by DSC

A differential scanning calorimeter (DSC823e, Mettler Toledo, Switzerland) with the STARe thermal analysis software v9.0x was used. The DSC instrument was calibrated with indium (melting point 156.6°C, $\triangle H_f$ = 28.45 J/g). The measurements were carried out in 40 μL hermetically sealed aluminum pans with a pinhole on the lid. Samples of 10-20 mg were weighed into aluminum pans to the nearest 0.1 mg. An empty, hermetically sealed aluminum pan was used as reference (blank). Samples were subjected to the following temperature program: from -30 to 550 ºC at 10°C/min with nitrogen (99.999% purity) as purge gas (50mL/min). The thermal melting and degradation characteristics of each sample were indicated by various temperatures. The onset temperature (T_{on}), the offset temperature (T_{off}), and the peak temperatures (T_{max}), temperatures of maximum heat flow between T_{on} and T_{off} were determined. The higher is the onset temperature of decomposition of sample, the higher is its thermal stability. The enthalpy values ($\triangle H$) were obtained by integration of the area under the transition thermogram peak.

2.3. Heat capacity by DSC

Measurements of heat capacities were carried out in the temperature range of 35–240ºC. A thin disc of sapphire (Al_2O_3) was used as the heat capacity standard. Sapphire is the traditional material used to calibrate DSC instruments for heat capacity measurements [37]. Measurements were performed for blank, sapphire and samples under the same operating condition including a heating rate of 10 ºC /min under a constant nitrogen flow (50 mL/min). The heat capacities were estimated automatically using the STARe software by Sapphire method (see Eq. 1). All DSC values reported are the average of three.

$$(c_p)_{sam} = \frac{(HF_{sam})(m_{sapp})}{(m_{sam})(HF_{sapp})}(c_p)_{sapp} \quad (1)$$

where $(c_p)_{sam}$ and $(c_p)_{sapp}$ are the heat capacities of sapphire and sample, respectively; m_{sam} and m_{sapp} refer to the mass of sample and of the sapphire, respectively, and $(HF)_{sam}$ and $(HF)_{sapp}$ are the heat flow of sample and of the sapphire, respectively. The DSC signal of the sample is compared with the DSC signal of the sapphire of known heat capacity. Both curves are blank curve corrected.

2.4. Kinetic study

The calculation of kinetic data is based on the formal kinetic equation (Eq. 2):

$$\frac{d\alpha}{dt} = k\alpha^n \quad (2)$$

where α is the amount of sample undergoing reaction, n is the order of reaction and k is the rate constant. The temperature dependence of (k) is expressed by the Arrhenius equation (Eq. 3):

$$k = A\exp(-E_a / RT) \quad (3)$$

where A is the Arrhenius constant, (E_a) is the activation energy, T is the absolute temperature, and R is the universal gas constant. The kinetic model developed by Rogers and Morris gives a means of estimating activation energies from a single DSC curve [36]. This method uses the data from DSC curve in the form of distances (D) between the sample curve and the baseline at points below the degradation temperature. This distance is proportional to the rate constant (k). In this case, the distances are only a ratio; therefore, the proportionality constants are canceled. Neither sample mass nor heat of reaction needs to be known to calculate the activation energy. The activation energy for the degradation reaction of first-order in the reactant can be estimated from Equation 4:

$$-E_a = R\frac{(\ln D_1 - \ln D_2)}{(\frac{1}{T_1} - \frac{1}{T_2})} \quad (4)$$

where D_1 and D_2 are two distances from the baseline at the associated temperatures T_1 and T_2. Rogers and Smith [38] provide a means of estimating the Arrhenius constant or pre-exponential factor (A) for the Arrhenius expression using DSC data (Eq. 5):

$$A = \frac{\beta E_a}{RT_{\max}^2} \exp\left(\frac{E_a}{RT_{\max}}\right) \quad (5)$$

where β is the heating rate, A is the Arrhenius constant, E_a is the activation energy, T_{max} is the absolute temperature of maximum peak, and R is the universal gas constant.

2.5. Analysis of diterpene fatty acid esters composition by GPC

Gel permeation chromatography (GPC) was used for the quantitation of triglycerides, diterpene fatty acid esters, monoglycerides (MAG), and free fatty acids (FFA). For this purpose a *VISCOTEK GPC/SEC TDAmax*^{TM} chromatograph with a refractive index detector was utilized. Samples of green coffee oil and fractions obtained of molecular distillation were dissolved in HPLC grade tetrahydrofuran (THF) and analyzed using THF as the mobile phase at flow rate of 0.8 mL/min. Three GPC/SEC Phenogel analytical columns (*Phenomenex, Torrance, CA*) with different pore sizes (50-100Å), dimension of 300 mm x 7.8 mm and packed with spherical styrene divinylbenzene copolymer beads with an average particle size of 5 µm were used. Sample injection volume was 20 µl, and analyses were carried out at 40°C. All the analyses were performed in triplicate and average values were reported.

2.6. Analysis of Fatty acids (FA)

Fatty acid (FA) composition and identification were determined with a gas chromatography coupled to mass spectrometry (*Agilent 5975 GC-MSD, Agilent, Santa Clara, United States*), connected to a HP-5 capillary column (30 m x 0.32 mm x 0.25 µm) 5% Phenyl Methyl Siloxan. The column temperature was controlled as follows: initial temperature 70ºC for 5min, then 4°C/min to 230ºC (hold for 3 min), then 4°C/min to 240ºC for a final holding time of 10 min. Helium was used as the carrier gas at a flow rate of 12.26 mL/min. Injection Volume 1 µL. Split Ratio 20:1. The injector and detector temperatures were 250 and 260ºC, respectively. The identification of the fatty acid methyl

esters was carried out through the comparison with the retention time of *SUPELCO standards* (FAME Mix C14-C22) and the quantification by area normalization. Green coffee oil has linoleic (C18:2), palmitic (C16:0), myristic (C14:0), pentadecanoic (C15:0), margaric (C17:0), oleic (C18:1), stearic (C18:0), linolenic (C18:3), arachidic (C20:0), heneicosanoic (C21:0) and behenic (C22:0) acids.

2.7. Analysis of triglycerides

The identification of triglycerides (TAG) were achieved using a *VISCOTEK GPC/SEC TDAmax*^{TM} chromatograph with refractive index detector and a column Hyperclone BDS C18 5 μm 130 Å (250 × 4.6 mm I.D), from Phenomenex Inc (*Torrance, CA-USA*). During analysis, the column was maintained at 40°C. The mobile phase was acetone/acetonitrile in a ratio of 50:50 (v/v) at a flow rate of 0.75 mL/min. For each analysis, the sample was first diluted with acetone and 10 μL of this solution was injected into the chromatograph. Triglycerides were separated according to their degree of unsaturation and molar mass. Triglycerides peaks were identified by comparison with retention times of TAG standards (*Sigma Chemical Co. USA*) and with the chromatograms published in the scientific literature [5, 7, 39-40].

2.8. Analysis of cafestol and kahweol

Samples (20 μL) were analyzed on a VISCOTEK GPC/SEC TDAmax^{TM} chromatograph with UV detector and a column Hyperclone BDS C18 5 μm 130 Å (250 × 4.6 mm I.D), from Phenomenex Inc (Torrance, CA-USA). During analysis, the column was maintained at 25°C. The mobile phase was acetonitrile/water in a ratio of 55:45 (v/v) at a flow rate of 0.9 mL/min. The identity of the separated diterpenes in the oil fractions was assigned by comparing the retention times and co-chromatography with authentic standards (*Axxora, San Diego, CA*). Cafestol and kahweol were analyzed at 230 nm and 290 nm, respectively. Response factors for each of the standards were obtained by linear regression of known concentrations versus peak areas. Figure 2 shows a typical chromatogram of cafestol and kahweol of original green coffee oil.

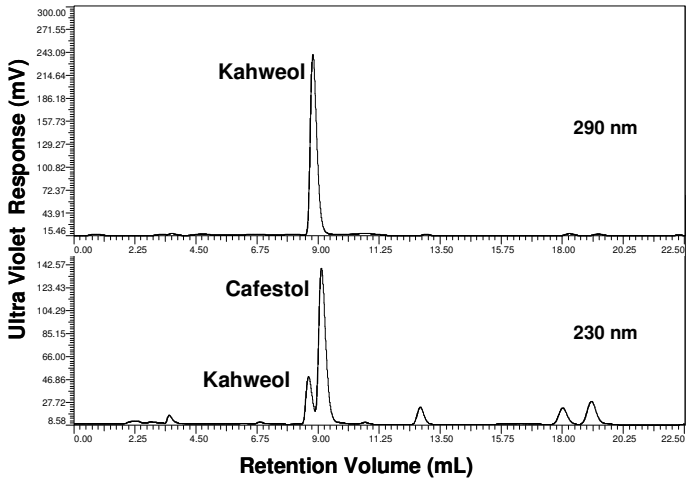

Fig.2. GPC chromatograms with UV detector of cafestol (230 nm) and kahweol (290 nm) from original green coffee oil

2.9. Molecular distillation (MD) equipment

The distillation was performed using a laboratory wiper-film molecular still model KDL 5, GmbH UIC (Alzenau, Germany). The evaporation temperature (TEV) in the enrichment process was conducted in the range from 130 to 230°C, with temperature increments of 20ºC. The operational pressure inside the evaporator achieved up to 0.001 mbar. Each step produced one distillate stream and one residue stream. The value of feed flow rate (Q) was fixed in 6 mL/min according to previous experiences in this equipment to form a uniform thin film on the evaporator surface, which promotes efficient mass and energy transfers.

3. Results and Discussion

Seven experiments were conducted at different temperatures by molecular distillation (MD). The experiments were carried out until arriving at steady state conditions. Afterwards, samples of the distillates were characterized and recorded. The results obtained are summarized in Table 1.

The results show that the diterpene fatty acid esters content was different in each distillate fraction. In view of the chemical composition (Table 1), diterpene fatty acid esters constitute approximately 42.8% of distillate fractions obtained at 210ºC. The distillation temperature of 210ºC became a turning point for the changes in total diterpenes content (cafestol and kahweol) in the distillates. At temperatures lower than 210ºC, the total diterpenes content in distillates decreased slightly while the content of FFA and MAG were

increased. The kahweol content was higher than that of cafestol for most of the fractions. These results indicate an enrichment of 150.55% of total diterpenes, over that in the original green coffee oil (913.09 mg/100g oil). According to these results, the molecular distillation process can be effective method for separation of diterpene fatty acid esters of green coffee oil.

Table 1. Chemical and physical characteristics of distillate fractions obtained by MD

Parameter	Original oil	Distillate fractions obtained at temperature (°C)							Residue
		130°C	150°C	170°C	190°C	210°C	230°C	250°C	
# Fraction		**1**	**2**	**3**	**4**	**5**	**6**	**7**	
TAG (%)	72.2	71.5	72.3	70.3	65.7	57.2	58.1	83	100
Diterpene fatty acid esters (%)	24.8	19.1	23.8	26.3	31.7	42.8	41.9	17	ND*
MAG+FFA (%)	3.0	9.4	3.9	3.4	2.6	0.0	0.0	0.0	ND
Cafestol (mg/100 g oil)	369.27	309.76	380.86	433.49	525.42	907.43	683.76	170.2	ND
Kahweol (mg/100g oil)	543.82	392.83	529.44	589.37	754.68	1380.4	950.32	220.6	ND
TAG composition (%mass)									
LLL	6.37	9.15	8.21	7.11	7.38	6.79	6.95	6.65	7.18
PLLn	1.75	2.1	2.11	2.16	2.18	1.95	2.04	2.12	1.85
OLL	3.9	6.31	5.05	4.43	4.57	3.81	3.68	3.71	3.79
PLL	24.54	22.4	23.58	23.48	24.22	24.49	25.05	25.07	24.92
OLO	1.33	3.58	2.11	1.96	1.77	1.13	1.02	1.1	0.92
PLO+SLL	16.63	16.30	16.42	16.79	16.84	16.26	16.16	16.22	16.41
PLP	20.94	16.93	18.63	19.98	19.85	20.06	20.76	20.67	19.49
ALL	6.16	8.31	7.16	7.62	6.96	6.07	5.73	5.8	6.05
PSL+POO	14.17	10.73	11.89	12.67	12.06	14.71	14.42	13.82	14.46
PSO	4.21	4.21	4.84	3.81	4.16	4.73	4.19	4.84	4.92
Fatty acid (FA) composition (%mass)									
C16:0	34.95	33.3	34.55	33.65	35.72	40.58	38.13	38.2	32.91
C18:0	9.12	8.9	8.4	9.4	9.0	8.22	9.0	9.0	8.75
C18:1	11.33	11.37	12.62	11.87	11.83	10.8	11.0	10.7	11.82
C18:2	41.43	42.6	40.9	42.1	40.47	36.73	38.76	37.8	43.85
C18:3	<0.1	0.19	0.2	<0.1	0.15	<0.1	<0.1	<0.1	<0.1
C20:0	2.44	2.73	2.32	2.13	2.37	2.34	2.51	2.5	2.18
C22:0	0.41	0.61	0.747	0.28	0.32	1.30	0.42	0.6	0.34

* (ND) Not detected

No significant differences were observed in the fatty acid composition between fractions obtained in the range from 130 to 250°C by MD. In general, slight changes in fatty acid composition of the fractions are mainly due to changes that occurred in proportions of palmitic and linoleic acids.

The green coffee oil is one of the most complex mixtures of natural TAG [40-41]. The GPC conditions used in the present work furnished a good separation allowing the identification of TAG in the fractions of green coffee oil. The identification of the peaks was achieved by comparison with those obtained from the chromatograms of coffee oil reported in the literature. The fatty acid composition of the coffee oil and 1,3-random-2-random distribution hypothesis were used to identify the triglycerides present in the samples [5, 7, 39-40]. It is possible to observe that triglycerides LLL, PLLn, OLL, PLL,

OLO, SLL, PLO, PLP, ALL, PSL, POO and PSO were identificated (A, L, Ln, O, P and S are arachidic, linoleic, linolenic, oleic, palmitic and stearic acids, respectively). In this study no significant differences were observed on the composition of the TAG identified in the fractions obtained between 130 and 250ºC. Considering the analyzed samples, the major triglycerides present in the fractions obtained by SPMD and TPMD are: PLL, PLP, PLO+SLL, and PSL+POO. The composition of the triglycerides was almost the same in all fractions. It shows that the molecular distillation gives little separation for mixtures of triglycerides of green coffee oil. Several studies indicate that fractionation of the triglyceride constituents of vegetable oils via molecular distillation is less effective than chromatography or low-temperature crystallization [42-45].

3.1 Thermal behavior

The DSC heating thermograms under nitrogen atmosphere (Fig. 3) for green coffee oil, fractions 1, 5, 7, and residue show two endothermic peaks (melting and thermal degradation) at heating rate of 10ºC/min. Those two events characterize the thermal behavior of the green coffee oil and the fractions obtained by molecular distillation process. These characteristics illustrate the complex nature of the composition of the samples. Table 1 shows the composition of the samples of green coffee oil, distillate fractions, and residue used in the present study. In order to compare different thermal characteristics, DSC data were normalized by dividing the heat flow (mW) by the sample mass (mg).

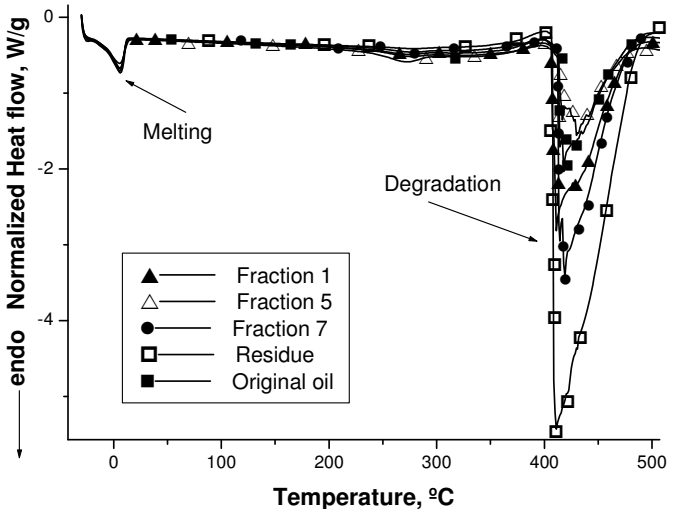

Fig.3. DSC heating thermogram of the green coffee oil under nitrogen atmosphere at 10ºC/min

The DSC heating curve of the green coffee oil shows an endothermic event of melting, at temperatures between -9.47ºC and 11.51ºC. Those results are in agreement with previous literature data on roasted coffee oil [46]. At temperatures between 412ºC and 427ºC presented the thermal degradation. The enthalpy of melting ($\triangle H_m$) and heat of degradation ($\triangle H_d$) were determined by the software of the DSC instrument from the melting and degradation endotherm areas, respectively. The thermal behavior of samples obtained by MD process presented the same trend to the green coffee oil (original oil), but with differences in melting and degradation temperatures, and their respective enthalpies. This may be due to variation in composition of samples during fractionation process by MD.

3.2 Melting characteristics

Table 2 shows the onset temperature (T_{on}), the peak temperature (T_{max}) and the melting enthalpy ($\triangle H_m$) of green coffee oil, distillate fractions, and residue obtained by MD. Figure 4 shows the melting curves obtained during heating at 10 ºC/min from -30 to 30 ºC. It can be noted that a single endothermic event was recorded during heating. It can be observed that the melting enthalpies of fractions 5 and 6 were considerably higher than those of the fractions 1, 2, 3, 4 and 7. It is also worth noting that the higher the melting enthalpy, the higher the peak temperature (T_{max}) obtained. Examination of the composition of the samples in Table 1 reveals that those with the highest diterpene fatty acid esters content also have the highest melting enthalpy, which might account for the higher peak temperature.

Table 2. Onset temperature (T_{on}), peak temperature (T_{max}), and enthalpy ($\triangle H_m$) associated with the melting of green coffee oil, distillates, and residue obtained by MD. DSC data were obtained under nitrogen purge at heating rate of 10ºC/min.

Sample	Melting characteristics		
	T_{on} (ºC)	T_{max} (ºC)	$\triangle H_m$ (kJ/kg)
Original oil	-9.47	5.71	34.16
Distillate fraction 1	-12.16	5.74	24.37
Distillate Fraction 2	-11.91	5.71	27.8
Distillate Fraction 3	-10.38	5.78	28.7
Distillate Fraction 4	-8.49	6.02	29.42
Distillate Fraction 5	-7.34	6.09	32.51
Distillate Fraction 6	-8.53	6.07	32.49
Distillate Fraction 7	-12.4	5.66	24.71
Residue	-11.85	4.51	30.86

A linear mathematical relationship (Eq. 6) was developed that relates the melting characteristics (y) to the mass percentage of diterpene fatty acid esters, content of cafestol or kahweol (x-obtained from Table 1 for the fractions) using the regression procedure in Origin V. 6.0 (Microcal Software, USA). There were good correlations (R^2) between the melting characteristics (T_{on}, T_{pk} *and* $\triangle H_m$), and the amount of diterpene fatty acid esters, cafestol, and kahweol in the distillate fractions (Table 3). The results from the correlation analysis therefore, indicated that those have a high influence on the melting characteristics of the fractions of green coffee oil.

$$y = m(x) + b \tag{6}$$

where (m) and (b) are coefficients. Their estimated values are given in Table 3. The results indicate that the values of peak temperature (T_{max}), and enthalpy ($\triangle H_m$) were increased with the increase in amount of diterpene fatty acid esters, cafestol and kahweol, while the values of onset temperature (T_{on}) were decreased.

Table 3. Values of coefficients (Eq. 6) that can be used to predict the melting characteristics based on content of diterpene fatty acid ester (%), cafestol and kahweol

Parameter	**Characteristic**	***y=m(x)+b***		
		m	***b***	***R^2***
Diterpene fatty acid esters (%)	onset temperature (T_{on})	0.192756	-15.7375	0.8576
	peak temperature (T_{max})	0.017262	5.369967	0.8732
	melting enthalpy ($\triangle H_m$)	0.312445	19.59169	0.9390
Cafestol (mg/100g oil)	onset temperature (T_{on})	0.007805	-13.9761	0.8660
	peak temperature (T_{max})	0.000686	5.532788	0.8311
	melting enthalpy ($\triangle H_m$)	0.012408	22.525129	0.8626
Kahweol (mg/100g oil)	onset temperature (T_{on})	0.004957	-13.58502	0.8600
	peak temperature (T_{max})	0.000431	5.570027	0.8082
	melting enthalpy ($\triangle H_m$)	0.007799	23.203402	0.8389

In the case of the fractions obtained, the increased in the content (%) of diterpene fatty acid esters increased the melting point. For example, comparing the fraction 1 with the fraction 5, there was a increase in the melting point of 5.74 to 6.09ºC for an increased of diterpene fatty acid esters of 19.1 to 42.8%, respectively. The lowest melting temperature (4.51ºC) was observed for the residue.

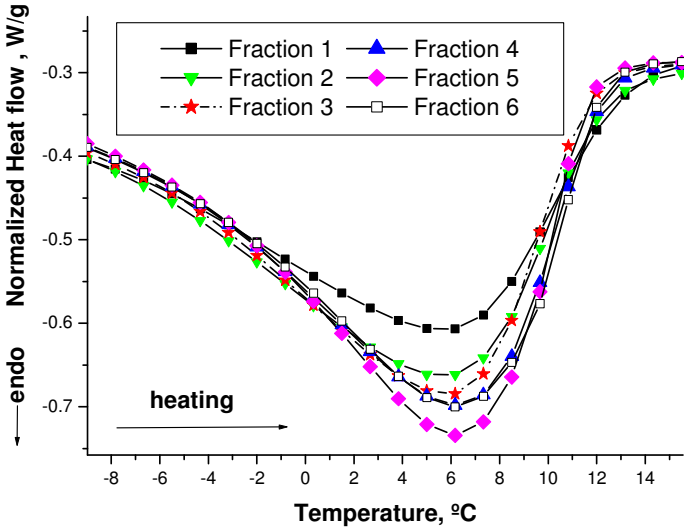

Fig.4. DSC melting curves of the fractions 1 - 6, under nitrogen atmosphere at 10ºC/min.

3.3 Heat capacity (C_p)

Measured values of the heat capacity (C_p) for the fractions 1, 3, 5, residue and original oil, at temperatures between 36ºC and 240°C (309–513 K) are shown in Figure 5. The selection of this temperature was necessary because the DSC curves obtained showed some irregularities at lower temperatures than 30°C and higher than 240ºC presumably because of the occurrence of melting and thermal degradation processes, which can be attributed to the composition of the green coffee oil (see Fig. 3).

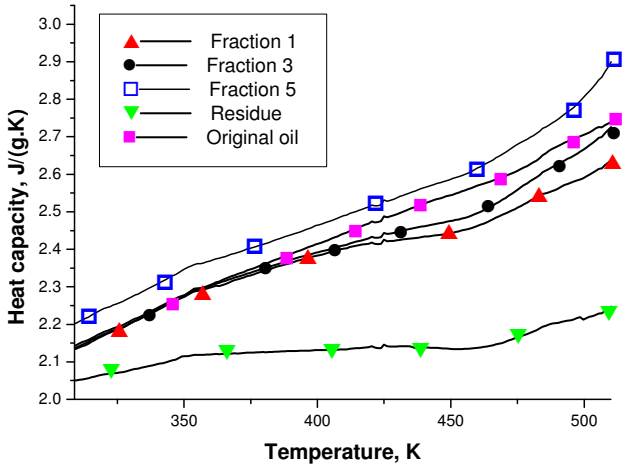

Fig.5. Comparison of heat capacities of the fractions 1, 3, 5, residue and original green coffee oil

The heat capacity of samples depends of the temperature and the content of diterpene fatty acid esters (see Fig.5). For example, the fraction 5 (42.8% of diterpene esters) had higher values, and the residue had lower values than the other samples on temperature range. Additionally, the increase in the temperature increased the heat capacity values on all samples. The heat capacity values for the samples were fitted by the least square method and the polynomial equation (Eq.7) of heat capacity as a function of the absolute temperature.

$$C_p = a + b(T) + c(T^2) + d(T^3) + e(T^4) \quad (7)$$

where (*a*), (*b*), (*c*), (*d*) and (*e*) are coefficients, and (*T*) is the absolute temperature. The estimated values are given in Table 4.

Table 4. Values of coefficients (Eq. 7) that can be used to predict the heat capacities of the samples based on absolute temperature between 309 and 513K.

Sample	$Cp=a+b(T)+c(T^2)+d(T^3)+e(T^4)$					
	a	***b***	***c***	***d***	***e***	R^2
Original oil	1.1542381	-0.004754	6.03643E-05	-1.5007887E-07	1.2150414E-10	0.9994
Distillate fraction 1	6.8695537	-0.070333	0.000338698	-6.6542324E-07	4.7028811E-10	0.9980
Distillate Fraction 2	15.010611	-0.159245	0.00070166815	-1.3192632E-06	9.0868269E-10	0.9935
Distillate Fraction 3	6.6668072	-0.068499	0.00033436978	-6.6618424E-07	4.7890238E-10	0.9987
Distillate Fraction 4	10.06533	-0.103445	0.00046857923	-8.9338301E-07	6.2200876E-10	0.9987
Distillate Fraction 5	11.476815	-0.115024	0.00050416594	-9.41306E-07	6.4754019E-10	0.9993
Distillate Fraction 6	11.78779	-0.117041	0.00050379268	-9.2195532E-07	6.2130793E-10	0.9990
Distillate Fraction 7	2.8456179	-0.027274	0.00017042202	-3.8250988E-07	2.9528239E-10	0.9965
Residue	1.8337019	-0.013976	0.00011167884	-2.7839412E-07	2.2803673E-10	0.9850

The results indicate that the values of heat capacity were increased with the increase of temperature and with the content of diterpene fatty acid esters, cafestol and kahweol. In this case, the values of the correlation coefficients (R^2>0.985) indicate that the fitted equations can be used for prediction the heat capacities as a function of the absolute temperature with good precision.

3.4 Thermal degradation

Figure 6 shows the DSC thermograms for the original green coffee oil, fractions 1, 5, 7, and residue obtained by MD. The thermograms were obtained at 10ºC/min from -30 to 550ºC, under nitrogen atmosphere. It can be noted that the thermal degradation occurred in a single endothermic event between 400 and 500ºC. Table 5 shows the details for the onset temperature (T_{on}), the peak temperature (T_{max}) and the degradation enthalpy ($\triangle H_d$) of study samples. It can be observed that the degradation enthalpies of fractions 5 and 6 were lower

than those of the fractions 1, 2, 3, 4 and 7. It is also worth noting that the lower the degradation enthalpy, the higher the peak temperature (T_{max}) of the samples. Table 1 reveals that those samples with the highest diterpene fatty acid esters content have the lowest degradation enthalpy, which might account for the higher peak temperature and onset temperature.

Table 5. Onset temperature (T_{on}), peak temperature (T_{max}), and enthalpy ($\triangle H_d$) associated with the thermal degradation of green coffee oil, distillates, and residue obtained by MD. DSC data were obtained under nitrogen purge at heating rate of 10ºC/min.

Sample	Thermal degradation characteristics		
	T_{on} (ºC)	T_{max} (ºC)	$\triangle H_d$ (kJ/kg)
Original oil	412.39	415.66	369.88
Distillate fraction 1	406.86	411	585.46
Distillate Fraction 2	406.21	408.66	413.91
Distillate Fraction 3	406.75	408.66	387.7
Distillate Fraction 4	409.58	413.33	391.56
Distillate Fraction 5	412.28	429.66	265.59
Distillate Fraction 6	411.65	430.83	281.1
Distillate Fraction 7	410.53	415.41	686.35
Residue	407.5	411	1393.69

Onset temperatures (T_{on}) can be used to indicate the resistance of the samples to thermal degradation [47]. The onset temperature for the fraction 5 and 6 were higher than those samples 1, 2, 3, 4, 5, and residue. It can be seen for the samples that, the higher is the onset temperature, the lower is the enthalpy of degradation. This shows that the diterpene esters of cafestol and kahweol can act as protective agents from thermal degradation on the green coffee oil.

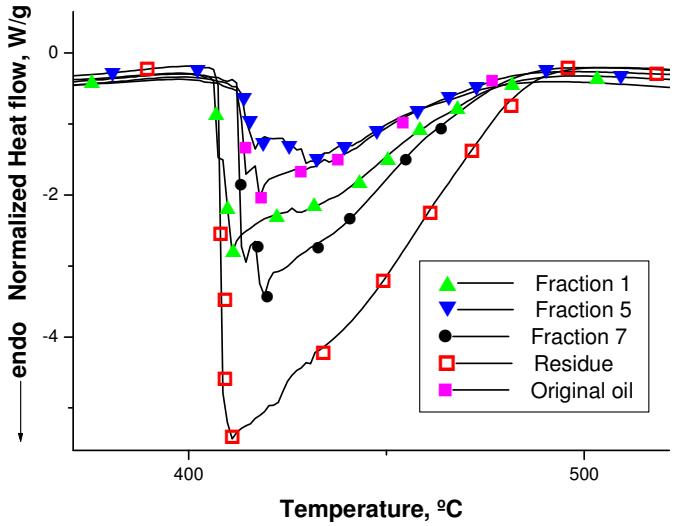

Fig.6. DSC degradation curves of the fractions 1, 5, 7, residue and original green coffee oil, under nitrogen atmosphere at 10ºC/min.

Table 6 shows the linear correlation analysis data (Eq. 6) that relates the degradation characteristics (y) to the mass percentage of diterpene fatty acid esters, content of cafestol or kahweol (x-obtained from Table 1 for the samples).The results from the correlation analysis indicated that the content of diterpene fatty acid esters, cafestol, and kahweol have a high influence on the degradation characteristics. The results indicate that the values of peak temperature (T_{max}), and onset temperature (T_{on}) were increased with the increase in amount of diterpene fatty acid esters, cafestol and kahweol, while the values of enthalpy ($\triangle Hd$) were decreased.

Table 6. Values of coefficients (Eq. 6) that can be used to predict the degradation characteristics based on content of diterpene fatty acid ester (%), cafestol and kahweol

Parameter	**Characteristic**	***y=m(x)+b***		
		m	***b***	***R^2***
Diterpene fatty acid esters (%)	onset temperature (T_{on})	0.261741	400.7918	0.9156
	peak temperature (T_{max})	0.987697	386.4705	0.8562
	degradation enthalpy ($\triangle H_d$)	-10.8474	723.0998	0.8440
Cafestol (mg/100g oil)	onset temperature (T_{on})	0.011215	402.8306	0.8708
	peak temperature (T_{max})	0.041904	394.3897	0.7983
	degradation enthalpy ($\triangle H_d$)	-0.44774	629.3912	0.7449
Kahweol (mg/100g oil)	onset temperature (T_{on})	0.006823	403.6605	0.8374
	peak temperature (T_{max})	0.025162	397.7442	0.7479
	degradation enthalpy ($\triangle H_d$)	-0.27384	597.3668	0.7239

3.5 Kinetic parameters

Table 7 compares the activation energy and frequency factors for all the samples. Activation energy is the minimum amount of energy that is required to activate atoms or molecules to a condition in which they can undergo chemical transformation or physical transport. The activation energy of original green coffee oil was about 128.15 kJ/mol, while the activation energy for the fraction 5 was increased by 54.64 kJ/mol. Since high decomposition activation energy is associated with high thermal stability. Based on the activation energy, the thermal stability of fraction 5 was better than those other samples in this study. Increasing the content of diterpene fatty acid esters increases the activation energy in the samples. The onset temperature (T_{on}) discussed earlier shows a similar pattern.

Table 7. Kinetic parameters of the samples from DSC data in nitrogen atmosphere. Activation energy (E_a) from Rogers and Morris method [36], and frequency factor (A) from Rogers and Smiths method [38]

Sample	Kinetic parameters	
	E_a (kJ/mol)	A (min^{-1})
Original oil	128.15	1.5704E+09
Distillate fraction 1	102.19	1.6742E+07
Distillate Fraction 2	127.10	1.8075E+08
Distillate Fraction 3	129.68	2.9066E+09
Distillate Fraction 4	131.52	3.4324E+09
Distillate Fraction 5	182.79	1.7238E+13
Distillate Fraction 6	177.11	5.9918E+12
Distillate Fraction 7	122.45	5.3519E+08
Residue	80.38	2.8407E+05

In order to compare the kinetic parameters (Table 7), a plot was shows in Figure 7. In the samples, both parameters raise with the increase of the diterpene fatty acid esters content on the samples. This correlated increment implies that any increase in the activation energy is accompanied by an increase in the frequency factor to keep the rate constant nearly unchanged. This tendency is known as the kinetic compensation effect [48]. A good linearity of estimated results was observed. Both the natural logarithm of the frequency factor ($\ln A$) and the activation energy (E_a) indicating a good precision in the analyses (Fig. 7).

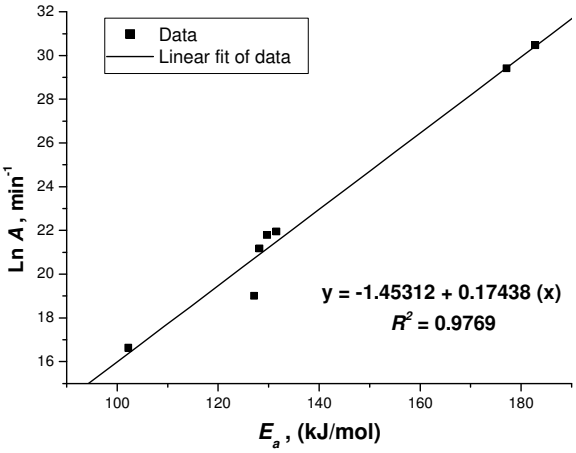

Fig.7. Linear relationship between natural logarithm of the frequency factor and the activation energy (E_a)

As it can be noticed in Figure 8, increasing the content of diterpene fatty acid esters increases the activation energy in the samples. The degree of fit, as shown by the R^2 value of 0.94, indicated that the activation energy in relation to content of diterpene fatty acid esters (%) could be well described by linear relationship for the study samples.

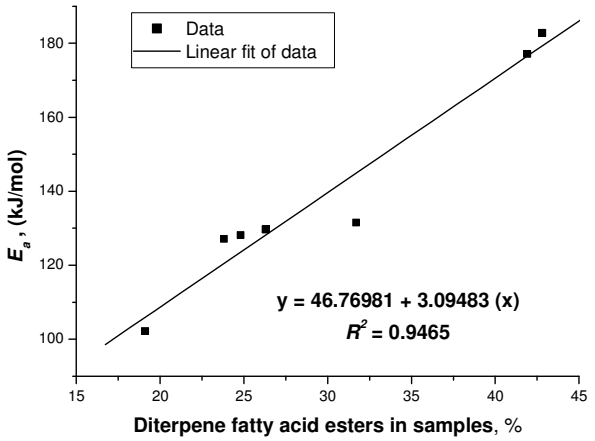

Fig.8. Linear relationship between activation energy and the content of diterpene fatty acid esters (%)

Figure 9 shows a plot of the activation energy as a function of their respective enthalpy of degradation. It can be seen that, the higher is the enthalpy degradation, the lower is the activation energy. This correlation suggested that both of the parameters may relate to the composition of samples. When the content of diterpene fatty acid esters is high, higher activation energy is needed to degradation of samples. As it can be noticed, the correlation coefficient (R^2) was 0.8324.

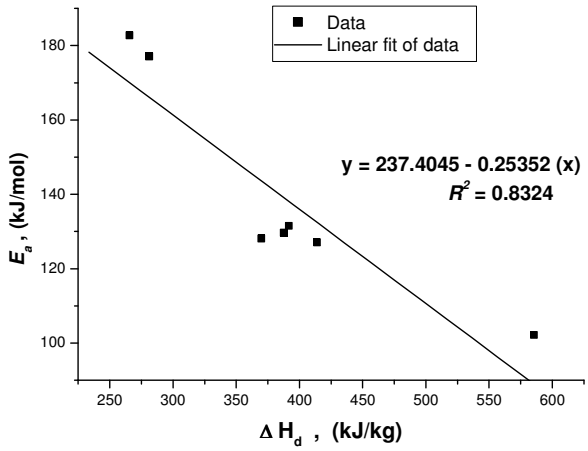

Fig.9. Linear relationship between activation energy and the enthalpy degradation

On the base of the respective decomposition activation energy, obtained by Rogers and Morris method [36] from DSC thermograms in nitrogen, the fraction 5 presents the highest thermal stability, followed in decreasing stability order by fraction 6, fraction 4, fraction 3, original green coffee oil, fraction 2, fraction 7, fraction 1, and residue.

The thermal stability of the vegetable oils [49] dependent of the proportion of saturated and unsaturated fatty acids in the triglycerides, and other substances present in the oil such as terpenes, carotenoids, sterols, phospholipids, phenolic substances, tocopherols, etc. The results show that the proportion of saturated and unsaturated fatty acids was almost the same in all fractions. In this case, the diterpene fatty acid esters and consequently the diterpene alcohols (cafestol and kahweol) present in the green coffee oil were responsible for thermal stability of the study samples.

4. Conclusions

The present work has shown that, fractionation of green coffee oil by molecular distillation is an effective tool for yielding several fractions which differ markedly in their properties. Aspects of chemical composition analysis are discussed as well as thermal properties. Moreover, the molecular distillation improved the enrichment of the coffee diterpenes as a result of high vacuum and low distillation temperature, since these are components of interest for cosmetic and pharmaceutical industries. The best results were obtained for a purity of diterpene fatty acid esters of 42.8% of distillate fractions at 210ºC and 6 mL/min of feed flow rate. At 210ºC the yield of cafestol and kahweol were 907.43 mg /100 g oil and 1380.4 mg /100g oil, respectively. These results indicate an enrichment of 150.55% of total diterpenes, over that in the original green coffee oil of 913.09 mg/100g oil. The kahweol content was higher than that of cafestol for most of the fractions.

Based on the results obtained in this study, DSC provides useful information regarding the thermal properties of green coffee oil that are associated with content of diterpene fatty acid esters. The influence of the diterpene fatty acid esters, cafestol and kahweol, were observed in the melting and degradation characteristics, heat capacity, and thermal stability on study samples. The kinetic parameters were calculated for the thermal decomposition of the samples. Rogers and Morris method was used to calculate activation energy. The activation energy values suggested the following sequence of thermal stability: fraction 5 > fraction 6 >fraction 4 >fraction 3 > original green coffee oil > fraction 2 > fraction 7 > fraction 1 > residue. This result concludes that the thermal stability is principally due to the presence of diterpene components (as cafestol and kahweol) that compose green coffee oil and are obtained during the extraction process.

5. Acknowledgements

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7.3. Conclusões

O presente trabalho mostrou que a destilação molecular é uma ferramenta eficaz para o fracionamento de óleo de café verde, e no enriquecimento dos diterpenos de café obtendo frações com propriedades marcadamente diferentes em comparação com o óleo original. Além disso, a destilação molecular alcançou o objetivo de enriquecimento dos diterpenos do café sob condições brandas de baixa temperatura de destilação e alto vácuo. Este procedimento tem aplicações importantes para obter produtos de alto valor agregado e de interesse para as indústrias cosmética e farmacêutica. Os melhores resultados de enriquecimento de ésteres diterpênicos em frações foram obtidos a 210ºC e 6 mL/min de vazão de alimentação com uma pureza de 42,8%. Aspectos da análise da composição química e propriedades térmicas são discutidos. Estes resultados indicam um enriquecimento de 150,55% de diterpenos total, mais que no óleo de café verde original que foi de 913,09 mg/100g de óleo. O conteúdo de caveol foi maior do que o cafestol para a maioria das frações. Com base nos resultados obtidos neste estudo, o DSC fornece informações úteis sobre as propriedades térmicas de óleo de café verde, que estão associados com teor de ésteres diterpênicos de ácidos graxos. A influência dos ésteres diterpênicos de ácidos graxos, o cafestol e o caveol, foram observadas nas características de fusão e degradação, capacidade térmica e estabilidade térmica nas amostras de estudo. Os parâmetros cinéticos da decomposição térmica foram calculados para todas as amostras. O método de Rogers e Morris foi utilizado para calcular a energia de ativação. Os valores de energia de ativação sugerem a seguinte seqüência de estabilidade térmica: fração 5> fração 6> fração 4> fração 3>óleo original de café verde> fração 2> fração 7> resíduo> fração 1. Este resultado conclui que a estabilidade térmica é devida principalmente pela presença dos diterpenos (como o cafestol e o caveol) que compõem o óleo de café verde e são obtidos durante o processo de extração.

Capítulo 8.

Estudo das propriedades reológicas do óleo de café verde (*Coffea arabica*) e suas frações obtidas por destilação molecular

8.1. Introdução

Uma das maiores dificuldades na utilização de óleos vegetais brutos em processos industriais é a sua viscosidade relativamente alta. Estudos relacionados às propriedades reológicas do óleo de café verde são limitados se comparadas com outros óleos vegetais. Neste estudo, o óleo de café verde bruto foi fracionado por destilação molecular (MD), num evaporador de filme descendente. A influência do teor de diterpenos e da temperatura sobre as propriedades reológicas e densidade do óleo de café verde, frações de destilado e resíduo foi analisada. Essas frações foram obtidas com uma vazão de alimentação de 6 mL/min, em um intervalo de temperaturas do evaporador entre 130ºC e 230ºC. As propriedades reológicas destas amostras foram avaliadas quanto à taxa de cisalhamento de 0,1 a 200 (s^{-1}), e às temperaturas entre 20ºC e 100ºC. Todas as amostras apresentaram comportamento Newtoniano na faixa de temperatura estudada, e uma relação entre o teor de diterpenos e viscosidade dinâmica foi observada. Os resultados indicam que a presença de diterpenos de café tem uma influência direta sobre o comportamento viscoso das amostras de estudo. A viscosidade e a densidade das amostras aumentaram consideravelmente com o aumento do teor de diterpenos. A viscosidade diminuiu com o aumento da temperatura e a equação de Arrhenius descreveu adequadamente o efeito da temperatura sobre a viscosidade, também a energia de ativação de fluxo (E_a) foi determinada. Comparado com o óleo de café verde, a frações obtidas se comportaram de forma semelhante em suas propriedades reológicas. Uma dependência exponencial entre a viscosidade e a temperatura e os ésteres diterpênicos de ácidos graxos foi determinada. Fazendo uso de regressão múltipla foi possível criar modelos matemáticos para descrever a viscosidade e densidade de amostras, em função da temperatura e os ésteres diterpênicos de ácidos graxos. Os diterpenos de café são importantes para as indústrias cosmética e farmacêutica, devido a suas propriedades

emolientes na pele, também como bloqueador da radiação solar, além de seus efeitos quiomoprotetores e anticarcinogênicos.

8.2. Desenvolvimento

O desenvolvimento deste capítulo é apresentado a seguir, no manuscrito intitulado: *Influence of Composition of Diterpenes on the Rheological Properties and Density of Green Coffee Oil (Coffea Arabica L) and Fractions Obtained by Molecular Distillation.*

Influence of composition of Diterpenes on the Rheological Properties and Density of Green Coffee Oil (*Coffea arabica*) and Fractions Obtained by Molecular Distillation

ABSTRACT

One of the major difficulties in using crude vegetable oils in industrial processes is their relatively high viscosities. Studies related to the rheological properties of the green coffee oil are limited if compared with other vegetable oils. In this study, Crude green coffee oil was fractioned by molecular distillation (MD) in a wiped-film evaporator. The influence of diterpene content and temperature on rheological properties and density of the green coffee oil, distillate fractions and residue was analyzed. These fractions were obtained at feed flows rate of 6 mL/min, and evaporator temperatures between 130ºC and 250ºC. Rheological properties of these samples were evaluated for shear rates of 0.1 to 200 s^{-1} at temperatures between 20ºC and 100ºC. All samples presented Newtonian behavior at the temperature range studied, and a relationship between diterpene content and dynamic viscosity was observed. The results indicate that the presence of coffee diterpenes has a direct influence on the viscous behaviours of the study samples. The viscosity and density of samples increased considerably with increase in diterpene content. The viscosity decreased with an increase in temperature, and the Arrhenius equation described adequately the effect of temperature on the viscosity, also the activation energy of flow (E_a) was determined. Compared with green coffee oil, the fractions obtained behaved similarly in

their rheological properties. An exponential dependence between viscosity and temperature and diterpene fatty acid esters content was determined. Making use of multiple regression it was possible to create mathematical models to describe viscosity and density of samples as a function of temperature and diterpene fatty acid esters content. Coffee diterpenes are valuable in the cosmetic and pharmaceutical industries, due to emollient properties, block the solar radiation, and their chemoprotective and anticancerigenic effects.

***KEYWORDS**: diterpene fatty acid esters, green coffee oil, molecular distillation, cafestol, kahweol, viscosity, density, rheological properties.*

1. Introduction

Coffee is one of the most traded commodities in the international markets, is an agricultural crop of significant economic importance. According to the latest estimate of the International Coffee Organization (ICO), total coffee production in the crop year 2009 was approximately 120 million bags of 60 kg, while Brazil's coffee production contributes 39.5 million bags [1]. The genus Coffea (*Rubiaceae*) hás about 100 species. Among these, two commercially important species are *Coffea arabica* L and *Coffea canephora* Pierre. *Coffea arabica* (arabica) is grown in highland regions(1000-2000 m) and represent about 70% of coffee world production [1], while the *Coffea canephora* (robusta) represent remaining 30% which is grown in lowland regions (0-700m).

The green coffee beans are composed of proteins, carbohydrates, lipids, volatile acids (as acetic and formic acids), nonvolatile acids (as chlorogenic and citric acids), alkaloids (caffeine, trigonelline), minerals, pigments, and volatile aroma compounds [2]. Caffeine and chlorogenic acids are the major bioactive metabolites in beans of coffee, which also contains diterpene compounds (mainly cafestol, kahweol, and 16-O-methylcafestol) known to be responsible for various physiological effects (see Figure 1). The naturally occurring diterpenes cafestol, kahweol and 16-O-methylcafestol are unique to coffee, and are present in the unsaponifiable fraction of coffee oil mostly esterified to 14 different fatty acids (mainly esterified with palmitic and linoleic acids) and only a small portion is present in the free form [3-4].

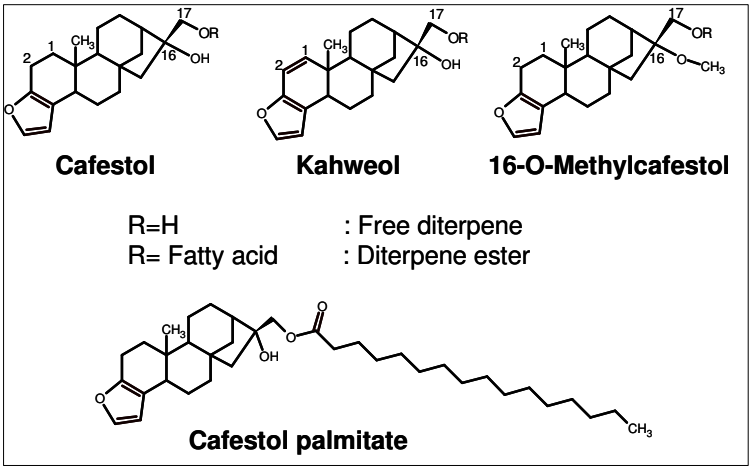

Fig.1. Structural formulas of cafestol, kahweol, 16-O-Methylcafestol and cafestol palmitate

Variation in diterpenes content in coffee beans depends on the geographical origin and of the *Coffea* species [5]. It is known that cafestol and kahweol are present in arabica coffee, whereas robusta coffee contains additionally 16-O-methyl cafestol. The content of cafestol and kahweol were about 6 and 3g/kg dry matter (d.m), respectively. In robusta coffees were detected cafestol (2g/kg d.m) and 16-O-methylcafestol (1-3g/kg d.m), in addition of traces of kahweol [4].

Green coffee oil has a high price in the market (about U$170/kg in 2010) with a wide variety of economical areas of interest as nutritional, biochemical, cosmetic, pharmaceutical and bioenergy [6]. Common methods for extracting the oil from green coffee beans include: solvent extraction, mechanical cold-pressing process, and supercritical fluid extraction [7-9]. Green coffee oil is characterized as greenish brown color with a cloudy aspect and an odor characteristic of green beans. Green coffee oil is a mixture of free fatty acids (FFA), mono-, di-, and triglycerides, diterpene fatty acid esters, phosphatides, pigments, sterols and tocopherols [10-11].

To diterpenes, have been attributed hypercholesterolemic effects, and coronary heart disease risk [12-13] due to the ingestion of unfiltered coffee drink. On the other hand, various studies have provided further support for the chemoprotective, hepatoprotective, antioxidative, antiinflammatory, and anticancerigenic effects of cafestol and kahweol [4, 14-16]. Because of the presence of diterpenes, green coffee oil is appropriate for industrial application in skin care as a sun filter and also in the treatment of dry skin, psoriasis, burns, wounds and blisters. [17-21]. Therefore, an understanding of the rheological behavior of

green coffee oil and their fractions is important, especially for processing and handling applications involving pumping and mixing.

The importance of vegetable oils in industries such as foods, energy, cosmetics, and pharmaceuticals has been well documented [22-23]. Rheological behavior and physical properties of vegetable oils (i.e., viscosity, density, melting, crystallization, degradation, and specific heat capacity) play a major role in describing heat transfer, design, evaluation, and modeling of continuous process such as distillation, heat exchangers, piping, and reactors [22, 24]. These experimental data are useful for estimating properties and predicting behavior for various products, as well as knowledge of the effect of processing, formulation changes and aging phenomena. Therefore, it is necessary to have theoretical knowledge as related to rheological aspects [25]. Rheological behavior and physical properties of vegetable oils have been evaluated in preceding studies [24, 26-32]. One of the most important characteristics of the the rheological properties is the material properties dependence on temperature.

Normally, the power law equation (Eq.1) is used to analyze the flow behavior index (n) of samples at different temperatures [33]. Viscosity is the measurement of the internal flow resistance of a liquid, constitutes an intrinsic property of vegetable oils. The dynamic viscosity of any fluid is equal to the ratio of the shear stress (τ) to the applied shear rate (γ). For Newtonian fluids this ratio is a constant, and the viscosity does not depend on the shear rate.

$$\tau = (\eta)(\gamma)^n \tag{1}$$

where (τ) is the shear stress (Pa), (γ) is the shear rate (s^{-1}), (η) is the dynamic viscosity (Pa.s) and (n) is the flow behaviour index (dimensionless). A flow behavior index (n) less than unity indicates pseudoplastic behavior, an index greater than unity corresponds to dilatant behavior. Newtonian fluids have a flow behavior index of unity, which indicates that for these fluids the viscosity remains constant for different shear rates [34]. Therefore, (η) and (n) are determined experimentally from isothermal viscosity and shear rate data. Studies related to the rheological properties of the green coffee oil are limited if compared with other vegetable oils.

The physical properties of vegetable oils depend primarily on composition and temperature. The high content of diterpene esters (>18%) in the green coffee oil may alter

these properties [35]. Taking into account this consideration, the overall objective of this research was to study the influence of temperature and content of diterpene esters on density and rheological behavior of green coffee oil, and distillate fractions obtained by molecular distillation. In the present work was developed a generalized equation for calculating the viscosities and density of green coffee oil based on the temperature and content of diterpene esters.

2. Materials and methods

2.1. Material

The crude green coffee oil was obtained from the industry (Linax, Votuporanga-Brazil), where it was obtained by mechanical pressing of arabica coffee beans [6].

2.2. Rheological measurements

The rheological measurements were performed in a controlled-stress rheometer (*HAAKE RheoStress 6000, Germany*) with parallel plate geometry (60 mm diameter). The measurements were conducted using a gap distance of 1mm. The shear rate range used was 0.1–200 s^{-1}.The sample compartment was controlled by a *Haake Universal Temperature Controler system-UTC (Haake, Germany*) with an accuracy of ± 0.1°C. The measurement was started 3 min after the temperature reached its value over the range of (20 to 100) °C with 10 °C intervals. Each measurement was repeated twice. Data were analyzed using the Rheowin Data Manager software, version 4.00.

2.3. Density measurements

Density measurements were carried out using an *Anton-Paar (Graz, Austria) SVM3000 Stabinger-type* dual viscometer/density meter. The adjustment of the density meter was checked using degassed bidistilled water; the measured value at 25°C was compared with the corresponding value and the difference was 0.00005 g/mL. The specified accuracy of the instrument is ±5 x 10^{-6} g/mL and ± 0.01ºC. The specified reproducibility of the instrument is ±1 x 10^{-6} g/mL and ± 0.001ºC. Each measurement was repeated twice.

2.5. Analysis of diterpene fatty acid esters composition by GPC

Gel permeation chromatography (GPC) was used for the quantitation of triglycerides, diterpene fatty acid esters, monoglycerides (MAG), and free fatty acids (FFA). For this purpose a *VISCOTEK GPC/SEC TDAmax*^{TM} chromatograph with a refractive index detector was utilized. Samples of green coffee oil and fractions obtained of molecular distillation were dissolved in HPLC grade tetrahydrofuran (THF) and analyzed using THF as the mobile phase at flow rate of 0.8 mL/min. Three GPC/SEC Phenogel analytical columns (*Phenomenex, Torrance, CA*) with different pore sizes (50-100Å), dimension of 300 mm x 7.8 mm and packed with spherical styrene divinylbenzene copolymer beads with an average particle size of 5 µm were used. Sample injection volume was 20 µl, and analyses were carried out at 40°C. All the analyses were performed in triplicate and average values were reported.

2.6. Analysis of Fatty acids (FA)

Fatty acid (FA) composition and identification were determined with a Gas chromatography coupled to mass spectrometry (*Agilent 5975 GC-MSD, Agilent, Santa Clara, United States*), connected to a HP-5 capillary column (30 m x 0.32 mm x 0.25 µm) 5% Phenyl Methyl Siloxan. The column temperature was controlled as follows: initial temperature 70ºC for 5min, then 4°C/min to 230ºC (hold for 3 min), then 4°C/min to 240ºC for a final holding time of 10 min. Helium was used as the carrier gas at a flow rate of 12.26 mL/min. Injection Volume 1 µL. Split Ratio 20:1. The injector and detector temperatures were 250 and 260ºC, respectively. The identification of the fatty acid methyl esters was carried out through the comparison with the retention time of *SUPELCO standards* (FAME Mix C14-C22) and the quantification by area normalization. Green coffee oil has linoleic (C18:2), palmitic (C16:0), myristic (C14:0), pentadecanoic (C15:0), margaric (C17:0), oleic (C18:1), stearic (C18:0), linolenic (C18:3), arachidic (C20:0), heneicosanoic (C21:0) and behenic (C22:0) acids.

2.7. Analysis of triglycerides

The identification of triglycerides (TAG) were achieved using a *VISCOTEK GPC/SEC TDAmax*^{TM} chromatograph with refractive index detector and a column

Hyperclone BDS C18 5 μm 130 Å (250 × 4.6 mm I.D), from *Phenomenex Inc* (*Torrance, CA-USA*). During analysis, the column was maintained at 40°C. The mobile phase was acetone/acetonitrile in a ratio of 50:50 (v/v) at a flow rate of 0.75 mL/min. For each analysis, the sample was first diluted with acetone and 10 μL of this solution was injected into the chromatograph. Triglycerides were separated according to their degree of unsaturation and molar mass. Triglycerides peaks were identified by comparison with retention times of TAG standards (*Sigma Chemical Co. USA*) and with the chromatograms published in the scientific literature [8, 10, 36-37].

2.8. Analysis of cafestol and kahweol

Samples (20 μL) were analyzed on a *VISCOTEK GPC/SEC TDAmax*^{TM} chromatograph with UV detector and a column Hyperclone BDS C18 5 μm 130 Å (250 × 4.6 mm I.D), from *Phenomenex Inc* (*Torrance, CA-USA*). During analysis, the column was maintained at 25°C. The mobile phase was acetonitrile/water in a ratio of 55:45 (v/v) at a flow rate of 0.9 mL/min. The identity of the separated diterpenes in the oil fractions was assigned by comparing the retention times and co-chromatography with authentic standards (*Axxora, San Diego, CA*). Cafestol and kahweol were analyzed at 230 nm and 290 nm, respectively. Response factors for each of the standards were obtained by linear regression of known concentrations versus peak areas. Fig. 2 shows a typical chromatogram of cafestol and kahweol of original green coffee oil.

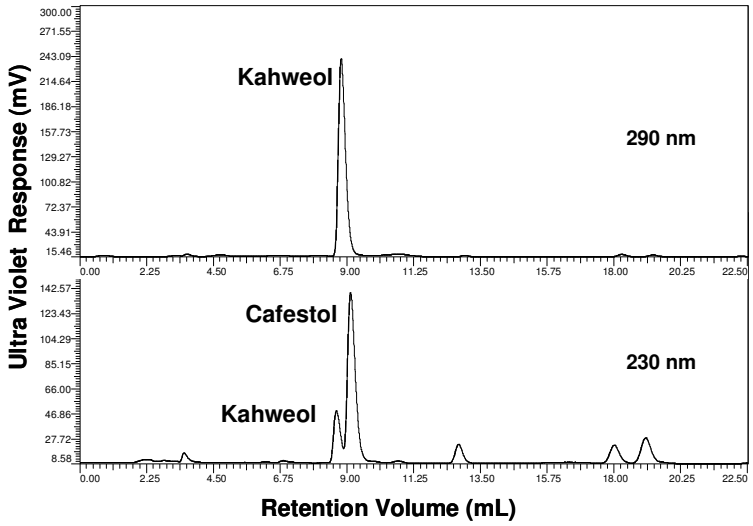

Fig.2. GPC chromatograms with UV detector of cafestol (230 nm) and kahweol (290 nm) from original green coffee oil

2.9. Molecular distillation (MD) equipment

The distillation was performed using a laboratory wiper-film molecular still model KDL 5, GmbH UIC (Alzenau, Germany). The evaporation temperature (TEV) in the enrichment process was conducted in the range from 130 to 230°C, with temperature increments of 20ºC. The operational pressure inside the evaporator achieved up to 0.001 mbar. Each step produced one distillate stream and one residue stream. The value of feed flow rate (Q) was fixed in 6 mL/min according to previous experiences in this equipment to form a uniform thin film on the evaporator surface, which promotes efficient mass and energy transfers.

3. Results and Discussion

Seven experiments were conducted at different temperatures by molecular distillation (MD). The experiments were carried out until arriving at steady state conditions. Afterwards, samples of the distillates were characterized and recorded. The results obtained are summarized in Table 1. The results show that the diterpene fatty acid esters content was different in each distillate fraction. In view of the chemical composition (Table 1), diterpene fatty acid esters constitute approximately 42.8% of distillate fractions obtained at 210ºC. The distillation temperature of 210ºC became a turning point for the changes in total diterpenes content (cafestol and kahweol) in the distillates. At temperatures lower than 210ºC, the total diterpenes content in distillates decreased slightly while the content of FFA and MAG were increased. The kahweol content was higher than that of cafestol for most of the fractions. These results indicate an enrichment of 150.55% of total diterpenes, over that in the original green coffee oil (913.09 mg/100g oil). According to these results, the molecular distillation process can be effective method for separation of diterpene fatty acid esters of green coffee oil.

No significant differences were observed in the fatty acid composition between fractions obtained in the range from 130 to 250°C by MD. In general, slight changes in fatty acid composition of the fractions are mainly due to changes that occurred in proportions of palmitic and linoleic acids.

Table 1. Chemical and physical characteristics of distillate fractions obtained by MD

Parameter	Original oil	Distillate fractions obtained at temperature (°C)							Residue
		130ºC	150ºC	170ºC	190ºC	210ºC	230ºC	250ºC	
# Fraction		**1**	**2**	**3**	**4**	**5**	**6**	**7**	
TAG (%)	72.2	71.5	72.3	70.3	65.7	57.2	58.1	83	100
Diterpene fatty acid esters (%)	24.8	19.1	23.8	26.3	31.7	42.8	41.9	17	ND*
MAG+FFA (%)	3.0	9.4	3.9	3.4	2.6	0.0	0.0	0.0	ND
Cafestol (mg/100 g oil)	369.27	309.76	380.86	433.49	525.42	907.43	683.76	170.2	ND
Kahweol (mg/100g oil)	543.82	392.83	529.44	589.37	754.68	1380.4	950.32	220.6	ND
TAG composition (%mass)									
LLL	6.37	9.15	8.21	7.11	7.38	6.79	6.95	6.65	7.18
PLLn	1.75	2.1	2.11	2.16	2.18	1.95	2.04	2.12	1.85
OLL	3.9	6.31	5.05	4.43	4.57	3.81	3.68	3.71	3.79
PLL	24.54	22.4	23.58	23.48	24.22	24.49	25.05	25.07	24.92
OLO	1.33	3.58	2.11	1.96	1.77	1.13	1.02	1.1	0.92
PLO+SLL	16.63	16.30	16.42	16.79	16.84	16.26	16.16	16.22	16.41
PLP	20.94	16.93	18.63	19.98	19.85	20.06	20.76	20.67	19.49
ALL	6.16	8.31	7.16	7.62	6.96	6.07	5.73	5.8	6.05
PSL+POO	14.17	10.73	11.89	12.67	12.06	14.71	14.42	13.82	14.46
PSO	4.21	4.21	4.84	3.81	4.16	4.73	4.19	4.84	4.92
Fatty acid (FA) composition (%mass)									
C16:0	34.95	33.3	34.55	33.65	35.72	40.58	38.13	38.2	32.91
C18:0	9.12	8.9	8.4	9.4	9.0	8.22	9.0	9.0	8.75
C18:1	11.33	11.37	12.62	11.87	11.83	10.8	11.0	10.7	11.82
C18:2	41.43	42.6	40.9	42.1	40.47	36.73	38.76	37.8	43.85
C18:3	<0.1	0.19	0.2	<0.1	0.15	<0.1	<0.1	<0.1	<0.1
C20:0	2.44	2.73	2.32	2.13	2.37	2.34	2.51	2.5	2.18
C22:0	0.41	0.61	0.747	0.28	0.32	1.30	0.42	0.6	0.34

* (ND) not detected

The green coffee oil is one of the most complex mixtures of natural TAG [37-38]. The GPC conditions used in the present work furnished a good separation allowing the identification of TAG in the fractions of green coffee oil. The identification of the peaks was achieved by comparison with those obtained from the chromatograms of coffee oil reported in the literature. The fatty acid composition of the coffee oil and 1,3-random-2-random distribution hypothesis were used to identify the triglycerides present in the samples [8, 10, 36-37]. It is possible to observe that triglycerides LLL, PLLn, OLL, PLL, OLO, SLL, PLO, PLP, ALL, PSL, POO and PSO were identificated (A, L, Ln, O, P and S are arachidic, linoleic, linolenic, oleic, palmitic and stearic acids, respectively). In this study no significant differences were observed on the composition of the TAG identified in the fractions obtained between 130 and 250ºC. Considering the analyzed samples, the major triglycerides present in the fractions obtained by SPMD and TPMD are: PLL, PLP, PLO+SLL, and PSL+POO. The composition of the triglycerides was almost the same in all fractions. It shows that the molecular distillation gives little separation for mixtures of triglycerides of the green coffee oil. Several studies indicate that fractionation of the triglyceride constituents of vegetable oils via molecular distillation is less effective than chromatography or low-temperature crystallization [39-42].

3.1. Rheological behavior

Rheological behavior of the green coffee oil oils may be observed in Fig. 3 that presents a linear relationship between shear stress and shear rate over the temperature range of 20ºC to 100ºC. The flow behavior index (*n*) calculated by the Rheowin Data Manager software (Haake, Germany) was from 1, which indicated Newtonian behavior. All of the samples (fractions and residue obtained by molecular distillation) showed the similar flow pattern at 40ºC, which were characterised by straight lines as shown in Fig. 4. Consequently, the linear relationship indicated a Newtonian behavior for the samples, therefore the viscosities at each temperature can be obtained from the slope of the fit of experimental shear stress vs shear rates data to Newton's law of viscosity equation (Eq. 1, for n =1). However, the values of viscosity (that is, the slope of each straight line) varied distinctly depending on the type of sample (diferent composition) and of temperature (see Figs. 3 and 4). This rheological behavior is similar to the results obtained to some vegetable oils, because of their long chain molecules [43-44].

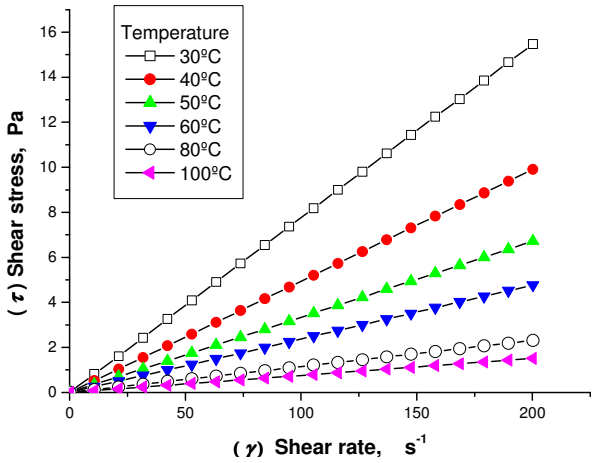

Fig.3. Shear stress vs. Shear rate for green coffee oil at various temperatures.

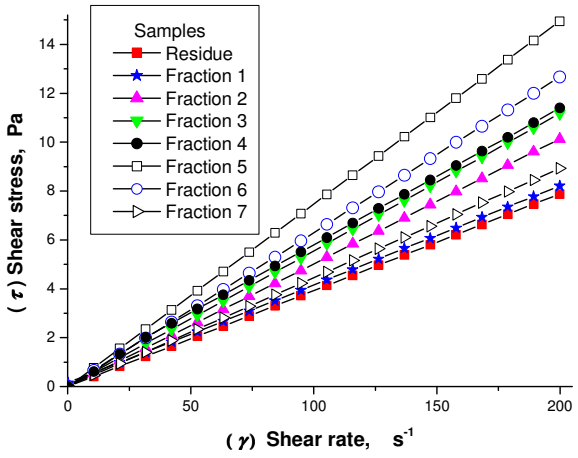

Fig.4. Shear stress vs. Shear rate for study samples at 40ºC

Figure 5 shows the dynamic viscosity of the samples, respectively, as function of shear rate at 40°C. The dynamic viscosity of samples remained constant with increasing shear rate, confirming Newtonian behavior.

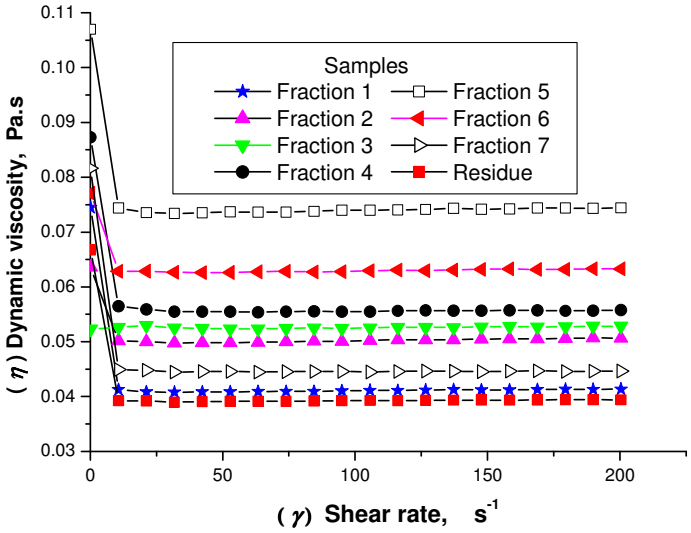

Fig.5. Relation between dynamic viscosity and shear rate for the samples at 40ºC

3.2. Influence of temperature and composition on dynamic viscosity

The experimental values of dynamic viscosity and density for the study samples, at different temperatures are given in Table 2.

Table 2. Dynamic viscosity and density data as a function of temperature for the study samples.

Parameter	Original oil	Distillate fractions obtained by Molecular Distillation							Residue
		Fraction 1	Fraction 2	Fraction 3	Fraction 4	Fraction 5	Fraction 6	Fraction 7	
Dynamic viscosity (mPa.s)									
Temperature		**1**	**2**	**3**	**4**	**5**	**6**	**7**	
20ºC (293K)	136.29	120.19	134.67	148.74	157.43	196.44	190.17	132.25	97.761
30ºC (303K)	82.251	73.981	81.347	88.519	93.192	112.6	109.78	80.487	62.017
40ºC (313K)	52.915	48.393	52.356	56.274	59.013	69.52	68.044	52.247	41.578
50ºC (323K)	35.941	33.315	35.538	37.837	39.457	45.566	44.749	35.654	29.249
60ºC (333K)	25.533	23.957	25.263	26.679	27.682	31.434	30.965	25.48	21.419
70ºC (343K)	18.854	17.869	18.662	19.532	20.219	22.616	22.371	18.796	16.192
80ºC (353K)	14.384	13.761	14.239	14.803	15.259	16.89	16.756	14.46	12.611
90ºC (363K)	11.304	10.867	11.169	11.546	11.885	13.017	12.945	11.368	10.07
100ºC (373K)	9.0723	8.7789	8.9878	9.2307	9.4946	10.297	10.253	9.1636	8.2117
Density (g/mL)									
20ºC (293K)	0.9403	0.9359	0.9402	0.942	0.9453	0.9549	0.954	0.9384	0.9261
30ºC (303K)	0.9336	0.9292	0.9335	0.9353	0.9386	0.9482	0.9472	0.9317	0.9193
40ºC (313K)	0.9268	0.9224	0.9268	0.9285	0.9318	0.9414	0.9404	0.9249	0.9126
50ºC (323K)	0.92	0.9157	0.92	0.9218	0.9251	0.9346	0.9337	0.9182	0.9058
60ºC (333K)	0.9133	0.9089	0.9133	0.915	0.9183	0.9278	0.9269	0.9114	0.899
70ºC (343K)	0.9065	0.9021	0.9065	0.9083	0.9115	0.9211	0.9201	0.9047	0.8922
80ºC (353K)	0.8997	0.8954	0.8998	0.9015	0.9048	0.9143	0.9133	0.8978	0.8855
90ºC (363K)	0.893	0.8887	0.8931	0.8948	0.8981	0.9075	0.9066	0.891	0.8787
100ºC (373K)	0.8864	0.882	0.8865	0.8882	0.8915	0.9009	0.9	0.8843	0.8721

As can be seen in Figure 6 and Table 2, all of the samples exhibited the same viscosity pattern over temperature, which was a non-linear decrease in viscosity with

increasing temperature. The increase in temperature tends to increase molecular interchange and reduce attractive forces between molecules, and therefore viscosity of the samples decreases [24, 44-47].

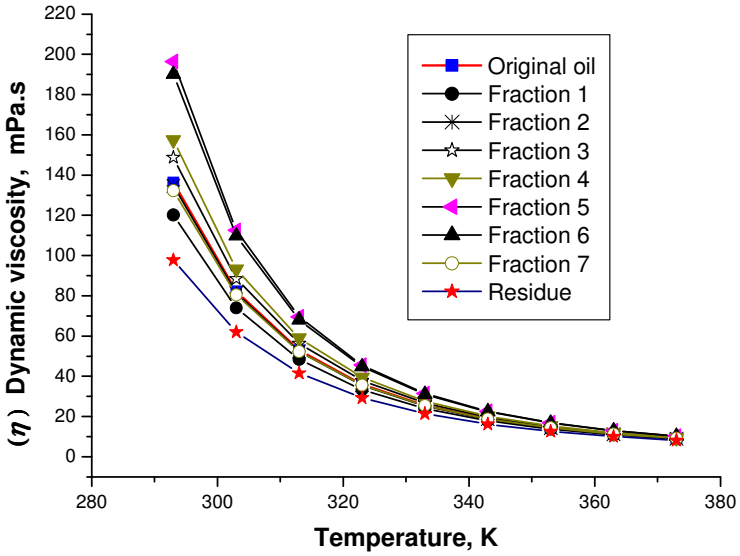

Fig.6. Effect of temperature on dynamic viscosity of the study samples

From Figure 6, we can see that the reduction of the viscosity is greater at the initial stage of the temperature increment, and subsequent increases in the temperature during the latter part had less influence on reducing the viscosity, as was observed for all samples. The effect of temperature on the oil viscosity was evaluated by means of the Arrhenius-type relationship (because of Newtonian behavior) [44, 46, 48], which describes the exponential decrease of the viscosity over temperature (Eq. 2).

$$\eta = (A)\exp^{(\frac{Ea}{RT})} \qquad (2)$$

where, (η) is the dynamic viscosity, A is the pre-exponential factor, E_a is the activation energy for viscous flow (in kJ/mol), R is the universal gas constant and T is the absolute temperature. R has the value of 8.314 x 10^{-3} (kJ/mol.K). The Arrhenius model is commonly used to model temperature dependence of a property [48]. The viscosity activation energy (E_a) is a characteristic of fluids. The E_a indicates the energy barrier that must be overcome before the oil can flow and reflects the viscosity-temperature stability (sensitivity of the oil to the influence of temperature) [48]. The Arrhenius equation can be linearized into the following form (Eq. 3).

$$Ln(\eta) = Ln(A) + (\frac{E_a}{RT}) \quad (3)$$

Linear regresion analysis was applied to determine the parameter of the relation Ln (η) versus (1/T), where (E_a /R) is the slope from which (E_a) was evaluated. The values of the pre-exponential factor (A) and the activation energy (E_a) estimated for study samples are given in Table 3. Figure 7 shows the linear relationship between Ln (η) versus (1/T), for original green coffee oil, Residue and Fractions 1 and 5.

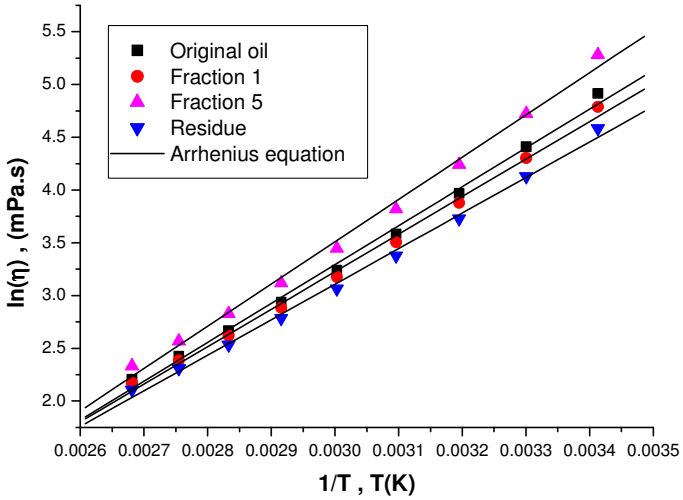

Fig.7. Linear relationship between Ln (η) versus (1/T) of original green coffee oil, Residue and Fractions 1 and 5

Table 3. Values of pre-exponential factor (A) and the activation energy (E_a) obtained from Arrhenius equation (Eqn. 3) for the study samples

Sample	***E*$_a$ (kJ/mol)**	***A* (mPa.s)**	***R*^{2}**
Original oil	30.61	4.3E-4	0.9945
Distillate fraction 1	29.55	5.92E-4	0.9949
Distillate Fraction 2	30.58	4.32E-4	0.9946
Distillate Fraction 3	31.39	3.41E-4	0.9945
Distillate Fraction 4	31.73	3.13E-4	0.9944
Distillate Fraction 5	33.29	2.04E-4	0.9940
Distillate Fraction 6	32.97	2.26E-4	0.9940
Distillate Fraction 7	30.16	5.05E-4	0.9947
Residue	27.99	9.24E-4	0.9953

The E_a is ranked in a descending order as follows: Fraction 5 > Fraction 6 > Fraction 4 > Fraction 3 > Original green coffee oil > Fraction 2 > Fraction 7 > Fraction 1 > Residue (Table 2). This trend indicates that the viscosity of Residue and Fraction 5 is respectively the least and the most sensitive to the influence of temperature. It is observed that the value of activation energy increases with the diterpene fatty acid esters content in the samples, as shown in the Figure 8. The degree of fit, as shown by the R^2 value of

0.9547, indicated that the activation energy in relation to content of diterpene fatty acid esters (%) could be well described by linear relationship.

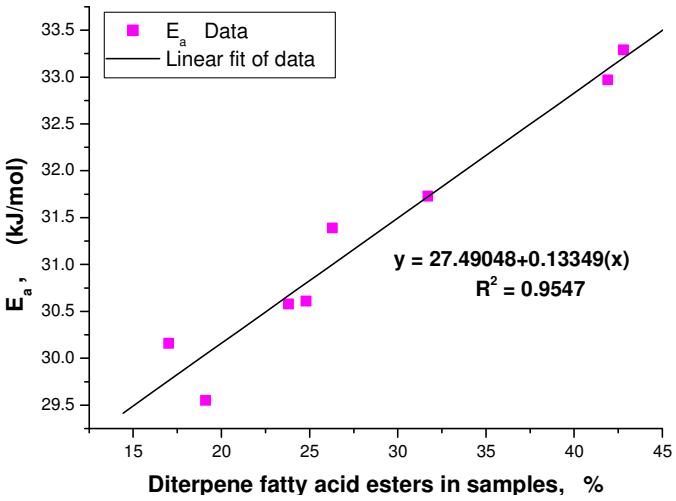

Fig.8. Linear relationship between activation energy and the content of diterpene fatty acid esters (%). Straight line is linear fit to the observed data.

There are a number of dependencies that include, apart from temperature, the influence of diterpenes content on the dynamic viscosity of samples. The results were fitted to a mathematical model [49-50] of the form of the Equation 4:

$$Ln(\eta) = a + b(T) + (\frac{c}{T}) + d(x) \tag{4}$$

where (a), (b), (c), and (d) are coefficients, (η) is the dynamic viscosity in (mPa.s), (T) is the absolute temperature and (x) is the mass percentage of diterpene fatty acid esters, content of cafestol or kahweol (x-obtained from Table 1 for the samples). In this work was possible to verify dependence of temperature and composition by making use of the Statistica 7.1 software. The standard error of estimate (SEE) was calculated from Equation 5 and used to compare the goodness of fit of the mathematical model to experimental data [51]. The lower SEE value for an equation gives a better fit to experimental data compared to an equation with higher SEE value.

$$SEE = \sqrt{\frac{\sum_{i=1}^{n}(Y_i - Y_i')}{n-p}} \tag{5}$$

where (Y_i) is the value of $Ln(\eta)$ at a particular temperature, (Y'_i) is the value predicted from Equation 4, (n) is the number of data points, and (p) is the number of parameters in each equation. The values of the estimated constants for Equations 4 and 5 are given in Table 4.

Table.4. Results of multiple regression of dependent variable Ln(η) with respect to independent variables temperature and diterpenes content (x).

Parameter (x)	***Ln(η)= a + b (T)+ (c/T)+ d (x)***					
	a	***b***	***c***	***d***	***R^2***	Std Error of Estimate ***SEE***
Diterpene fatty acid esters (%)	-31.05	0.034	7521.037	0.011	0.9976	0.04540
Cafestol (mg/100g oil)	14.60512	-0.03415	6E-8	0.00042	0.9781	0.13539
Kahweol (mg/100g oil)	14.62472	-0.03415	5E-8	0.00027	0.9780	0.13598

The results from the correlation analysis indicated that the content of diterpene fatty acid esters, cafestol, and kahweol have influence on the dynamic viscosity. It is observed that there is excellent agreement between values obtained by experiment and those predicted by the Equation 4 (Fig. 9). A high value of correlation coefficients R^2>0.978 confirms the right choice of the adopted mathematical model. The model for diterpene fatty acid esters gives a better fit to experimental data (SEE = 0.04540) compared to cafestol and kahweol models which show a higher SEE value.

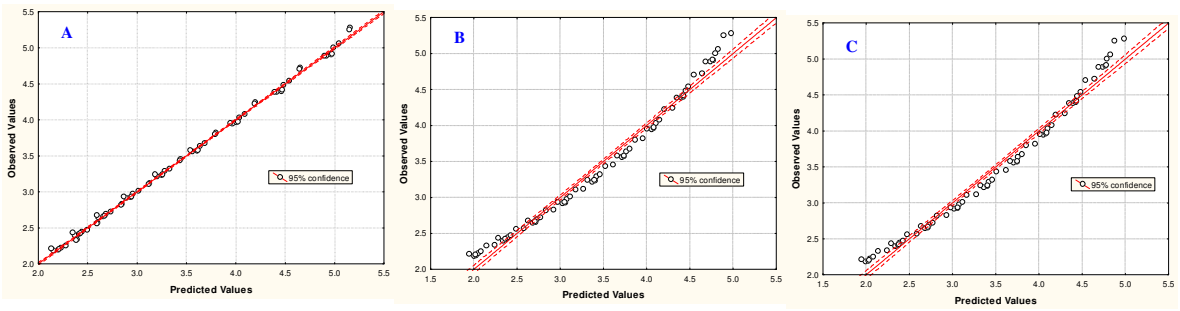

Fig.9. Observed values of Ln (η) are compared against predicted values by Equation 4, for (A) Diterpene fatty acid esters %, (B) Cafestol and (C) Kahweol

As it can be seen in Fig 10, the viscosity was increased with an increase in diterpenes content and with a decrease in temperature. The temperature range of the conducted measurements was between 293 K to 373K and the diterpenes content varied as shown in the Table 1 for cafestol, kahweol and diterpene esters. The analysis of Figure 10 evidently shows that the influence of temperature on viscosity is even more significant than diterpenes content. The viscosity of all investigated samples reaches the value of about 9 mPa s at 293 K. At higher temperature values the curve gets even steeper demonstrating a high influence of temperature and a slight influence of diterpenes content.

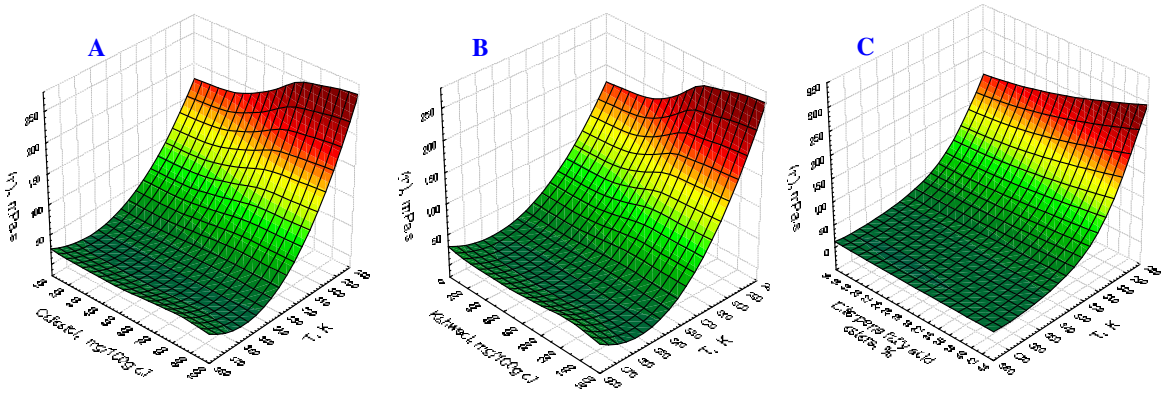

Fig.10. Graphical representation of viscosity measurements within temperature range of 293 to 373 K and diterpenes content, (A) Cafestol, (B) Kahweol and (C) Diterpene fatty acid esters %.

3.3. Influence of temperature and composition on density

The experimental data of density of the original green coffee oil, residue and distillate fractions obtained by MD, at different temperatures are listed in Table 2. As can be seen in Figure 11, the samples showed temperature dependent behavior and the density decreases linearly with the increase in temperature.

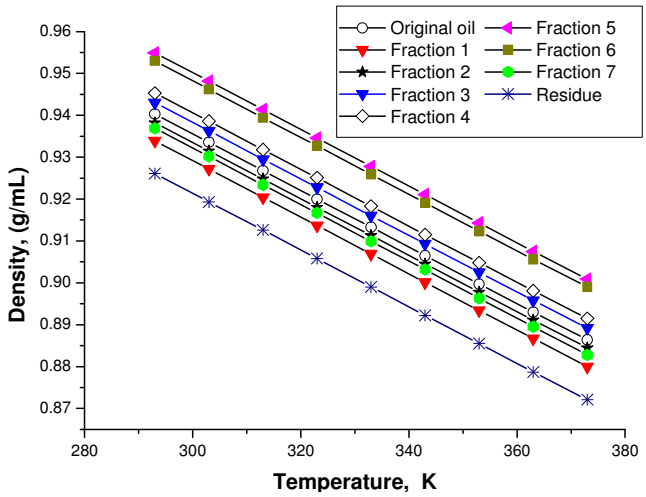

Fig.11. Effect of temperature on density of the study samples

The accuracy of the density data was further evaluated by correlating them with temperature by means of a linear equation (Eq. 6), and good correlation coefficients were obtained ($R^2 > 0.999$).

$$\rho = a + b(T) \quad (6)$$

where (a) and (b) are coefficients, (ρ) is the density in (g/mL), and (T) is the absolute temperature. The estimated values are given in Table 5. It is observed that at a given temperature, density increases with increasing diterpenes content (Tables 1 and 2). Therefore, density must be closely correlated with the composition and temperature of the samples.

Table 5. Values of coefficients (a), and (b) (Eq. 5) for empirical correlation of density in function of temperature

Sample	*a*	*b*	R^2
Original oil	1.1381194	-0.00067516667	0.99998
Distillate fraction 1	1.1315307	-0.0006745	0.99999
Distillate Fraction 2	1.1372425	-0.0006725	0.99999
Distillate Fraction 3	1.1393754	-0.00067366667	0.99999
Distillate Fraction 4	1.1427198	-0.00067383333	0.99998
Distillate Fraction 5	1.1530746	-0.00067633333	0.99999
Distillate Fraction 6	1.1520191	-0.000676	0.99998
Distillate Fraction 7	1.1368188	-0.000677	0.99999
Residue	1.1241413	-0.000676	0.99999

The influence of temperature and diterpenes content on density was modeled using Equation 7.

$$\rho = a + b(T) + c(x) \quad (7)$$

where (a), (b), and (c) are coefficients, (ρ) is the density in (g/mL), (T) is the absolute temperature and (x) is the mass percentage of diterpene fatty acid esters, content of cafestol or kahweol (x-obtained from Table 1 for the samples). The estimated values are given in Table 6.

Table 6. Results of multiple regression of dependent variable (ρ) with respect to independent variables temperature and diterpenes content (x).

Parameter (x)	***(ρ)= a + b (T)+ c (x)***				
	a	***b***	***c***	R^2	Std Error of Estimate ***SEE***
Diterpene fatty acid esters (%)	1.121307	-6.749E-4	7.1444E-4	0.9958	0.00122
Cafestol (mg/100g oil)	1.128133	-6.749E-4	2.853E-5	0.9853	0.00230
Kahweol (mg/100g oil)	1.129468	-6.749E-4	1.812E-5	0.9831	0.00247

An examination of the results of Table 6 shows that the mathematical model (Eq.6) could to predict density of samples with different diterpenes content within temperature range with a high degree of accuracy ($R^2 > 0.983$). The results indicated that the content of diterpene fatty acid esters, cafestol, and kahweol have influence on the

density. The good correlation between the calculated and experimentally determined densities is shown in Figure 12. The model for diterpene fatty acid esters gives a better fit to experimental data (SEE = 0.00122) compared to cafestol and kahweol models which show a higher SEE value.

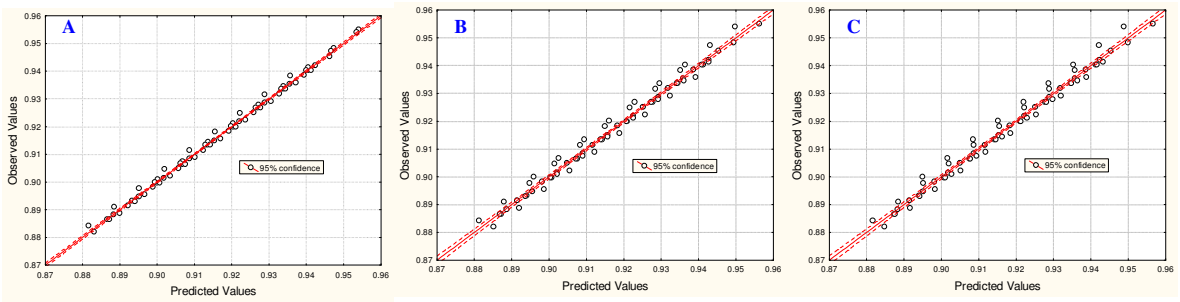

Fig.12. Observed values of density (ρ) are compared against predicted values by Equation 7, for (A) Diterpene fatty acid esters %, (B) Cafestol and (C) Kahweol

As it can be seen in Fig 13, the density was increased with an increase in diterpenes content and with a decrease in temperature. The temperature range of the conducted measurements was between 293 K to 373K and the diterpenes content varied as shown in the Table 1 for cafestol, kahweol and diterpene esters. The analysis of Figure 13 evidently shows that the influence of temperature on density is even more significant than diterpenes content. The density of all investigated samples reaches the value of about 0.88 g/mL at 293 K. At higher temperature values the curve gets even steeper demonstrating a high influence of temperature and a slight influence of diterpenes content.

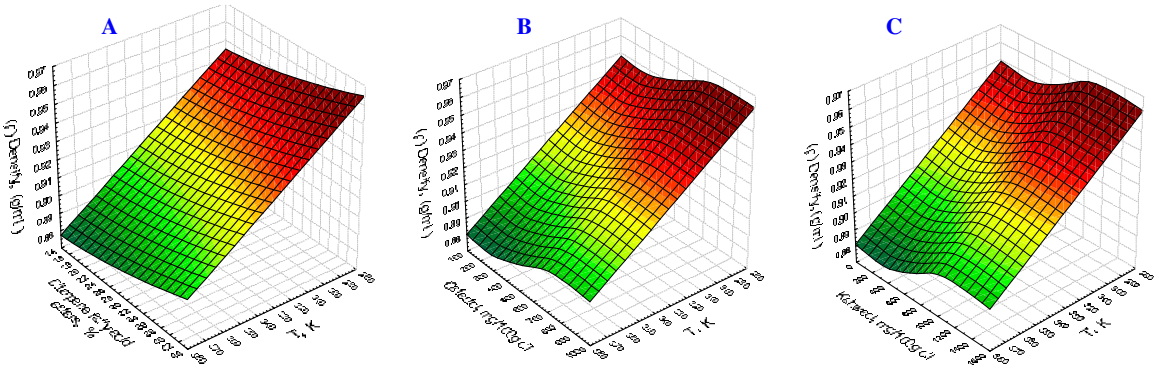

Fig.13. Graphical representation of density measurements within temperature range of 293 to 373 K and diterpenes content, (A) Diterpene fatty acid esters %, (B) Cafestol and (C) Kahweol

3.4. Prediction of diterpenes content

To predict the diterpene content of the study samples was used Equation 8, which relates diterpenes content (y) to viscosity, density, and temperature data of Tables 1 and 2.

$$\text{Diterpene content (y)} = a + b(Ln(\eta)) + c(\rho) + d(T) \tag{8}$$

where (a), (b), (c) and (d) are coefficients, (ρ) is the density in (g/mL), (η) is the dynamic viscosity in (mPa.s), (T) is the absolute temperature and (y) is the mass percentage of diterpene fatty acid esters, content of cafestol or kahweol. The estimated values are given in Table 7.

Table.7. Results of multiple regression of diterpene content (y) with respect to viscosity, density and temperature of samples

Parameter (y)	***Diterpene content (y) =a + b Ln(η)+c (ρ)+d (T)***					
	a	***b***	***c***	***d***	R^2	Std Error of Estimate ***SEE***
Diterpene fatty acid esters (%)	-1519.01	-0.86	1366.58	0.89	0.9671	1.69
Cafestol (mg/100g oil)	-34905.2	-28.6	31359.9	20.2	0.8825	76.35
Kahweol (mg/100g oil)	-53937.9	-43.0	48391.8	31.2	0.8653	127.43

The correlation between the calculated and experimentally determined densities is shown in Figure 14. As well as in the previous cases, the model for diterpene fatty acid esters presented a better fit to experimental data (SEE = 1.69) as compared to cafestol and kahweol models which show a higher SEE value.

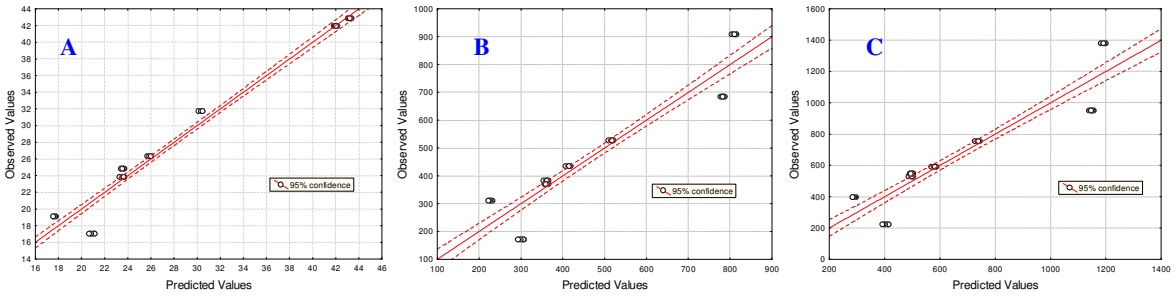

Fig.14. Observed values of diterpenes content (y) are compared against predicted values by Equation 8, for (A) Diterpene fatty acid esters, (B) Cafestol and (C) Kahweol

The main advantage of the Equation 8 is the possibility of determining diterpenes content in a relatively wide range by simple measurements of viscosity, density and temperature. In this way we can obtain an approximated value of diterpenes on green coffee oil without the need to chemical composition analysis.

4. Conclusions

In this study, samples of fractionation of green coffee oil, as well as the original green coffee oil were subjected to rheological evaluations. The fractionation of green coffee oil by molecular distillation has proved to be an effective tool for yielding several fractions which differ markedly in their physicochemical properties. Moreover, the molecular distillation improved the enrichment of the coffee diterpenes as a result of high vacuum and low distillation temperature, since these are components of interest for cosmetic and pharmaceutical industries. The best results were obtained for a purity of diterpene fatty acid esters of 42.8% of distillate fractions at 210ºC and 6 mL/min of feed flow rate. At 210ºC the yield of cafestol and kahweol were 907.43 mg /100 g oil and 1380.4 mg /100g oil, respectively. These results indicate an enrichment of 150.55% of total diterpenes (cafestol and kahweol), over that in the original green coffee oil of 913.09 mg/100g oil.

The results demonstrated that all samples presented a linear relationship between between shear stress and shear rate (flow behavior index n=1) over the temperature range of 20ºC to 100ºC, which indicated Newtonian behavior. Also, their flow behaviours over temperature could be well characterised by the Arrhenius model. The rheological properties and density of the investigated samples showed dependency on the temperature and diterpenes content (diterpene fatty acid esters, cafestol and kahweol). Both dynamic viscosity and density of the samples decreased with increasing temperature, in agreement with behavior data for other vegetable oils. Making use of multiple regression was possible to create mathematical models (linear and exponential correlations for density and dynamic viscosity, respectively) to describe dynamic viscosity and density in the function of temperature and diterpenes content. The good correlation ($R^2 > 0.98$) between the calculated and experimentally determined data suggests the possibility of determining dynamic viscosity and density of samples by measurements of temperature and diterpenes content. Additionally, we developed a model to estimate the composition of the diterpenes in the oil of green coffee, as a function of viscosity, density and temperature.This information can be used for industrial applications, especially in the selection and design of equipment and processes that use the green coffee oil in their formulations.

5. Acknowledgements

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8.3. Conclusões

Neste estudo, amostras do fracionamento do óleo de café verde, assim como o óleo de café verde original foram submetidas a avaliações reológicas. O fracionamento do óleo de café verde por destilação molecular tem se mostrado uma ferramenta eficaz para rendendo várias frações que diferem marcadamente em suas propriedades físico-químicas. Além disso, a destilação molecular melhorou o enriquecimento dos diterpenos do café como um resultado do alto vácuo e as baixas temperaturas de destilação, uma vez que estas são componentes de interesse para as indústrias cosmética e farmacêutica. Os melhores resultados foram obtidos com um grau de pureza de ésteres diterpenicos de ácidos graxos de 42,8% nas frações de destilado a 210 ° C e 6 mL/min de vazão de alimentação. A 210ºC, o rendimento de cafestol e caveol foram 907,43mg/100g de óleo e 1380,4mg/100g de óleo, respectivamente. Estes resultados indicam um enriquecimento de 150,55% de diterpenos totais (cafestol e caveol), mais que no óleo de café verde original de 913,09 mg/100g de óleo. Os resultados demonstraram que todas as amostras apresentaram uma relação linear entre a tensão de cisalhamento e taxa de cisalhamento (índice de comportamento do fluxo $n = 1$) na faixa de temperatura de 20ºC a 100ºC, o que indica um comportamento newtoniano. Além disso, seus comportamentos de fluxo com a temperatura podem ser bem caracterizados pelo modelo de *Arrhenius*. As propriedades reológicas e a densidade das amostras investigadas apresentaram dependência da temperatura e teor de diterpenos (ésteres diterpênicos de ácidos graxos, o cafestol e o caveol). Tanto a viscosidade dinâmica e a densidade das amostras diminuíram com o aumento da temperatura, o que esta de acordo com os dados de comportamento de outros óleos vegetais. Fazendo uso de regressão múltipla foi possível criar modelos matemáticos (correlação linear e exponencial para a densidade e viscosidade dinâmica, respectivamente) para descrever a viscosidade dinâmica e densidade em função da temperatura e o teor de diterpenos. A boa correlação ($R^2 > 0,98$) entre os dados calculados e determinados experimentalmente sugerem a possibilidade de obter os valores da viscosidade dinâmica e da densidade de amostras, por meio de medições de temperatura e teor de diterpenos. Alem, foi possível criar modelos matemáticos para estimar o teor de diterpenos no óleo de café em função de dados de temperatura, viscosidade dinâmica e densidade. Esta informação pode ser utilizada para aplicações

industriais, em especial na seleção e o projeto de equipamentos e processos que utilizam o óleo de café verde em suas formulações.

Capítulo 9.

Conclusões e Sugestões de Trabalhos Futuros

9.1. Conclusões

Nesta tese foi apresentada uma breve revisão bibliográfica dos principais conceitos envolvidos no fracionamento do óleo vegetal por destilação molecular, além da caracterização das frações. Pode ser visto que o processo de destilação molecular traz fundamentais vantagens na separação e enriquecimento de produtos altamente valorizados na indústria. Nesta seção se apresenta uma síntese com as principais conclusões que se depreendem do estudo.

- A criação de um banco de dados para os componentes do óleo de café verde é uma ferramenta confiável para prever o comportamento do óleo de café verde utilizando um software de processos químicos como o *Aspen-Plus*®.

- A disponibilidade do modelo baseado em taxas (rate-based) para representar o processo de não-equilíbrio de destilação molecular foi demonstrada através de um ajuste quantitativo entre os dados experimentais e simulados.

- A pressão, a temperatura e a vazão de alimentação são os principais fatores operacionais que afetam o processo de destilação molecular e a qualidade dos produtos.

- Os resultados mostram que a desacidificação do óleo de café verde pode ser realizado por o processo de destilação molecular, com baixa perda de óleo neutro.

- A metodologia de superfície de resposta (RSM) foi efetiva para determinar as condições ótimas na desacidificação do óleo de café verde.

- Pode-se observar que a influência da temperatura do evaporador é muito mais expressiva que a vazão de alimentação.

- A destilação molecular é um método eficaz para o enriquecimento dos diterpenos (cafestol e caveol) utilizando condições brandas de pressão e temperatura, uma vez que estas são moléculas de interesse para as indústrias cosmética e farmacêutica.

- Os melhores resultados foram obtidos a 210ºC e 6 mL/min de vazão de alimentação, com um teor de ésteres diterpênicos de ácidos graxos de 42,8% e 38,4% das frações de destilado para uma e duas passadas pelo destilador molecular, respectivamente.

- Os resultados indicam um enriquecimento dos diterpenos totais em 150,56% (2287,83 mg/100g de óleo, uma passada) e 83,75% (1667,8 mg/100g de óleo, duas passadas), em relação com o teor no óleo original de café verde que foi de (913,09 mg/100g de óleo).

- A influência da composição dos ésteres diterpênicos de ácidos graxos, sob a densidade, viscosidade, índice de saponificação e índice de refração das frações destiladas, foi observada.

- A influência dos ésteres diterpênicos de ácidos graxos, o cafestol e o caveol, foram observadas nas características de fusão e degradação, capacidade térmica e estabilidade térmica das amostras de estudo.

- Os parâmetros cinéticos da decomposição térmica foram calculados para as amostras. O método de Rogers e Morris foi utilizado para calcular a energia de ativação. Este resultado conclui que a estabilidade térmica é principalmente devido à presença dos diterpenos (como o cafestol e o caveol) que compõem o óleo de café verde e são obtidos durante o processo de extração.

- Os resultados demonstraram que todas as amostras de óleo de café verde apresentaram um comportamento newtoniano.

- As propriedades reológicas e a densidade das amostras investigadas apresentaram dependência da temperatura e teor de diterpenos (ésteres diterpênicos de ácidos graxos, o cafestol e o caveol). Tanto a viscosidade dinâmica e a densidade das amostras diminuíram com o aumento da temperatura, o que esta de acordo com os dados de comportamento de outros óleos vegetais.

- Fazendo uso de regressão múltipla foi possível criar modelos matemáticos para descrever a viscosidade dinâmica e densidade em função da temperatura e o teor de diterpenos. Alem, foi possível criar modelos matemáticos para estimar o teor de diterpenos no óleo de café em função de dados de temperatura, viscosidade dinâmica e densidade.

- O presente trabalho é um aporte ao estudo do óleo de café e o primeiro que mostra a viabilidade da aplicação da destilação molecular no melhoramento de propriedades a nível laboratorial e promissório para uso industrial.

- As propriedades do óleo de café verde permitem sua utilização na indústria cosmética e farmacêutica, e pode ser classificado como um óleo não-secante.

- Esta informação pode ser utilizada para aplicações industriais, em especial na seleção de equipamentos e processos que utilizam o óleo de café verde em suas formulações.

9.2. Sugestão para Trabalhos Futuros

Com o desenvolvimento desta tese, grandes avanços no entendimento da aplicação de intensificação de processos mediante a utilização da destilação molecular foram realizados. No entanto, estudos ainda precisam ser realizados com o intuito de viabilizar o processo e projetar novos processos adicionais para a obtenção de cafestol e caveol e seus ésteres no futuro. A seguir são apresentadas algumas sugestões para trabalhos futuros:

- Fazer experimentação com o destilador molecular de tipo centrifugo, para comparar os valores aqui obtidos das frações e corroborar os resultados.

- Tomar como matéria prima o óleo de café torrado e analisar a viabilidade para uso na indústria alimentícia.

- Implementar métodos mistos para isolar os ésteres diterpênicos (>90% pureza), para uma aplicação farmacêutica.

- Adiantar trabalhos com a faculdade de medicina (FCM) para testar os produtos obtidos nos tratamentos anticancerígenos e desenvolver novas drogas nesta área.