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Dissertação

Estudo da estabilidade oxidativa do biodiesel derivado de óleo de girassol e avaliação de chalconas como antioxidantes

Caroline Tuchtenhagen Rockembach

Pelotas, 2015

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Dissertação apresentada ao Programa de Pós-Graduação em Bioquímica e Bioprospecção da Universidade Federal de Pelotas, como requisito parcial à obtenção do título de Mestre em Ciências (Bioquímica e Bioprospecção).

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Dissertação de Mestrado

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elaborada por
Caroline Tuchtenhagen Rockembacch

Como requisito parcial para a obtenção do grau de
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Para meu esposo com amor e gratidão.

Dedico.

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“Tente ser uma pessoa de sucesso, mas efetivamente tente ser uma pessoa de valor”

Albert Einstein

PARTE I

Resumo

ROCKEMBACH, Caroline. **Estudo da estabilidade oxidativa do biodiesel derivado de óleo de girassol e avaliação de chalconas como antioxidantes.** 2015. 77f. Dissertação - Programa de Pós-Graduação em Bioquímica e Bioprospecção. Universidade Federal de Pelotas, Pelotas.

O biodiesel é constituído por ésteres de ácidos graxos obtidos por transesterificação de triglicerídeos sendo uma alternativa renovável para ser utilizado como biocombustível, mas o biodiesel pode conter ácidos graxos insaturados, que são susceptíveis à oxidação. A reação de transesterificação normalmente é lenta. A aplicação de fontes de energia como o ultrassom tem sido cada vez mais utilizadas em reações orgânicas em substituição as metodologias convencionais. O presente estudo teve como objetivo obter biodiesel metílico de óleo de girassol com o ultrassom como meio de promotor da reação, monitorar a estabilidade do mesmo através de parâmetros físico-químicos (valores de viscosidade, acidez e iodo) e cromatografia gasosa durante 70 dias de armazenamento. Além disso, onze chalconas com diferentes substituintes foram sintetizadas, caracterizadas por infravermelho com transformada de Fourier (FT-IR) e cromatografia gasosa acoplada a espectrometria de massa (GC-MS), e as suas purezas foram determinadas por GC-MS e ponto de fusão. As chalconas estão associadas a diversas atividades biológicas, este estudo investiga o impacto de várias chalconas sintéticas sobre a estabilidade oxidativa do biodiesel. A atividade antioxidante dessas chalconas foi avaliada pelos métodos de 2,2-difenil-1-picril-hidrazila (DPPH) livre radical e Quioluminescência. As chalconas que apresentaram maior atividade antioxidante nestes testes foram avaliadas no biodiesel utilizando rancimat para determinar a estabilidade oxidativa do biodiesel.

Palavras-chave: Biodiesel, Girassol, Ultrassom, Chalcona e Antioxidante.

Abstract

ROCKEMBACH, Caroline. **Study of the oxidative stability of biodiesel from sunflower oil and evaluation chalcones as antioxidants.** 2015. 77p. Dissertação - Programa de Pós-Graduação em Bioquímica e Bioprospecção. Universidade Federal de Pelotas, Pelotas.

Biodiesel is produced by fatty esters obtained by transesterification of triglycerides and it is a renewable alternative to be used as biofuel, but the biodiesel can contain unsaturated fatty acids, which are susceptible to oxidation, being able to change into polymerized compounds. The transesterification reaction is slow and it normally. The application of ultrasound replacing conventional energy sources has been increasingly used in organic reactions. The present study aimed to obtain methyl biodiesel from sunflower oil applying ultrasound as a promoting reaction medium monitor the oxidation of the same through physicochemical parameters (viscosity, acidity and iodine values) and chromatography evaluated during 70 days of storage. Additionally, eleven chalcones having different substituents were synthesized, characterized by Fourier transform infrared (FT-IR) and gas chromatography coupled to mass spectrometry (GC-MS), and their purities were determined by GC-MS and melting point. The chalcone is associated with several biological activities, this study investigates the impact of various synthetic chalcones on the oxidation stability of biodiesel. The evaluation of the antioxidant activity these chalcones was using the methods of 2,2-diphenyl-1-pycril-hydrazyl (DPPH) and chemeluminescence, the chalcones showed the highest antioxidant activity in this tests were evaluated in the biodiesel using rancimat to determine the oxidative stability of biodiesel.

Keywords: Biodiesel, Sunflower, Ultrasound, Chalcone and Antioxidant.

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BHT - Hidroxitolueno butilado

ANP - Agência Nacional de Petróleo, Gás Natural e Biocombustíveis

EN – Norma Européia

BHA - butil hidroxianisol

TBHQ - *terc*-butil-hidroquinona

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

Sumário

PARTE II

1 INTRODUÇÃO

A busca por biocombustíveis provenientes de fontes renováveis vem aumentando gradativamente nas últimas décadas com a finalidade de substituir compostos derivados do petróleo. Neste contexto, a substituição do petrodiesel pelo biodiesel pode ser explicada por dois grandes fatores, a possibilidade de esgotamento das fontes petrolíferas no mundo e pela poluição gerada na queima dos combustíveis fósseis (MARON VICHI, 2009; RAMOS et al., 2009; SUAREZ; MENEGHETTI, 2007). Sendo o biodiesel uma fonte alternativa de energia renovável com potencial aplicação na substituição de combustíveis derivados do petróleo, seu uso é promissor e oferece uma série de vantagens ambientais, econômicas e sociais, além de reduzir a dependência de importação do petróleo (ABBASZAADEH et al., 2012; PINTO et al., 2005; SUAREZ; MENEGHETTI, 2007).

Uma das principais desvantagens do biodiesel é a sua instabilidade à oxidação. Por ser proveniente de oleaginosas, o biodiesel apresenta normalmente altas concentrações de ácidos graxos insaturados, o que facilita a oxidação do mesmo dificultando dessa forma o seu armazenamento (KUMAR, R.; KUMAR; CHANDRASHEKAR, 2011; MAYDT et al., 2013).

Cabe salientar que a estabilidade do biodiesel depende da composição dos ácidos graxos do óleo precursor (KARAVALAKIS et al., 2011). A oxidação lipídica é um processo que envolve reações entre radicais livres, o oxigênio e os ácidos graxos insaturados presentes no biodiesel. Durante a decomposição térmica, ocorrem reações de condensação, que geram compostos poliméricos, cujos resultados podem ser o entupimento de filtros de combustíveis e formação de sedimento nos motores (KNOTHE, 2007; RAMALHO; JORGE, 2006).

Os óleos vegetais apresentam normalmente altas concentrações de tocoferóis, os quais são os antioxidantes naturais comuns, contudo, durante o processo de refino, pode ocorrer a diminuição da concentração destes antioxidantes, diminuindo dessa forma, a estabilidade oxidativa destes óleos (MCCORMICK et al., 2007). Nesse contexto, com o intuito de aumentar o tempo de armazenamento do biodiesel, surge à necessidade de adicionar antioxidantes a fim de diminuir o processo oxidativo mantendo, dessa forma, as propriedades físico-químicas destes biocombustível por um tempo maior (BORSATO et al., 2010; KNOTHE, 2007). Os antioxidantes podem ser de origem sintética ou natural. Na Figura 1 são apresentados a estrutura de dois antioxidantes muito estudados, o *α*-tocoferol e hidroxitolueno butilado (DIAS; FIGUEIREDO; D'ELIA, 2012; FERRARI, R. A.; SOUZA, W. L. D., 2009).

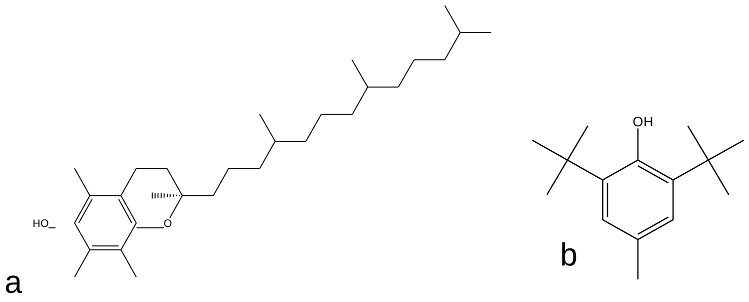


Figura 1 - Moléculas Antioxidantes. a: vitamina E (antioxidante natural), b: BHT-Hidroxitolueno butilado (antioxidante sintético)

A vitamina E (*α*-tocoferol) é conhecida por acentuar a resistência à oxidação de óleos vegetais (TAN; CHE MAN, 2002). O BHT (Hidroxitolueno butilado) é conhecido por retardar efeitos da oxidação (DUNN, 2002). Simkovsky e colaboradores (2003) estudaram o efeito de diversos antioxidantes em biodiesel de óleo de canola a diferentes temperaturas, entretanto os antioxidantes estudados não retardaram os efeitos da oxidação.

2 HIPÓTESE E OBJETIVOS

Hipótese

O biodiesel quando armazenado pode sofrer oxidação, diminuindo assim sua vida útil, entretanto ao adicionar compostos antioxidantes, como chalconas, este processo pode ser retardado.

Objetivo Geral

Avaliar a estabilidade oxidativa do biodiesel de óleo de girassol e retardar o processo de rancificação utilizando chalconas como moléculas antioxidantes.

Objetivos Específicos

- Sintetizar biodiesel de girassol utilizando ultrassom como meio promotor;
- Avaliar o processo oxidativo do biodiesel de girassol;
- Sintetizar e caracterizar diferentes chalconas;
- Avaliar a atividade antioxidante das chalconas por DPPH e Quimioluminescência;
- Avaliar a ação antioxidante das chalconas sintetizadas quando adicionadas no biodiesel.

3 REVISÃO DE LITERATURA

Biodiesel

O inventor do motor com combustão interna foi Rudolph Diesel, no final no século XIX (SOUZA et al., 2009). O petróleo foi empregado neste período por ser encontrado em abundância, tendo assim baixo custo. O uso de óleos vegetais para combustíveis em motores com combustão interna só começou no século XX devido às circunstâncias adversas causadas pelas guerras, como a falta de abastecimento de petróleo (SUAREZ; MENEGHETTI, 2007).

Através do Decreto Presidencial no ano de 2003 iniciaram-se estudos para viabilizar a utilização biodiesel no Brasil. A inserção do biodiesel como fonte de energia foi baseada no desenvolvimento regional, incentivando principalmente a agricultura familiar (DUAILIBE, 2012). O biodiesel é produzido primordialmente a partir de óleos vegetais, como soja, colza, palma, girassol, milho, uva, mamona, óleo residual, além de derivados animais, como óleo de peixe, sebo, banha, graxa entre outros (DE OLIVEIRA et al., 2013; MARQUES et al., 2010; MOTASEMI; ANI, 2012; PEREIRA et al., 2012; ROCKEMBACH et al., 2014).

O girassol é indicado para produção de biodiesel, pois além do óleo extraído possuir excelente qualidade, esta oleaginosa apresenta teores de óleo em torno de 40 a 54 %. Com o alto teor de óleo nos grãos, a obtenção pode ser realizada por simples extração mecânica, desta forma é uma cultura apropriada para agricultora familiar, demonstrando sua importância no que tange ao aspecto social da agricultura (CORRÊA; MAZIERO; ÚNGARO, 2006). O girassol é uma cultura facilmente adaptável e econômica, pois não requer manejo especializado, seu cultivo é recomendado para as regiões Sudeste e Sul do Brasil e pode ser adaptado ao cultivo de safrinha (SLUSZZ; MACHADO, 2006). Além de ser produzido a partir de fontes renováveis, o biodiesel é um recurso biodegradável, não-tóxico e com menores quantidades de elementos particulados depositados nos motores, além disso, é mais lubrificante que o diesel a base de petróleo e menos poluente, pelo fato de a emissão de compostos como SO_2, HC, ser menor quando comparado ao petrodiesel (Tabela 1).

Tabela 1 - Emissões de compostos do biodiesel comparado ao diesel (%)

	B5	**B20**	**B100**
CO	-7	-15	-48
HC	-5	-20	-67
SO_x	-5	-20	-100
NO_x	<1	2 a 4	10 a 20

B5: 5% de biodiesel no diesel; B20: 20 % de biodiesel no diesel; B100: 100 % de biodisel

Fonte: Adaptado de Duailibe, 2012

Um estudo da Fundação Getulio Vargas relatou que com o uso do biodiesel diminui-se 48 % na emissão de monóxido de carbono, além disso, mesmo quando misturado com diesel, o biodiesel ainda é vantajoso (FERREIRA, 2007). Outrossim, não há necessidade de modificações nos motores com combustão ao misturar até 20 % de biodiesel devido a semelhança com o diesel de petróleo em algumas características como o número cetano, calor de combustão, ponto de fluidez, viscosidade cinemática e lubricidade, apesar das diferentes constituições químicas (FAGUNDES, 2013; KNOTHE; RAMOS, 2006). Com base nos dados deste mesmo estudo, acredita-se que com a mistura de biodiesel ao diesel, problemas com poluentes particulados e suas consequências na área da saúde seriam minimizados.

Recentemente, o Ministério de Minas e Energia anunciou a ampliação para 7 % no total da adição obrigatória de biodiesel ao óleo diesel para o mês de novembro do ano de 2014 (BRASIL, 2014). O biodiesel apresenta ponto de inflamação alto, fazendo com que aumente a segurança do combustível (AMARAL, 2009). De acordo com a Agência Nacional de Petróleo, Gás Natural e Biocombustíveis (ANP), os biocombustíveis mais usados são o etanol e o biodiesel. A síntese do biodiesel se dá por meio de uma reação química chamada transesterificação ou alcoólise (Figura 2), a qual ocorre entre um triacilglicerol com álcool de cadeia curta, sendo os alcoóis mais utilizados o etanol e o metanol, na presença de um catalisador básico ou ácido.

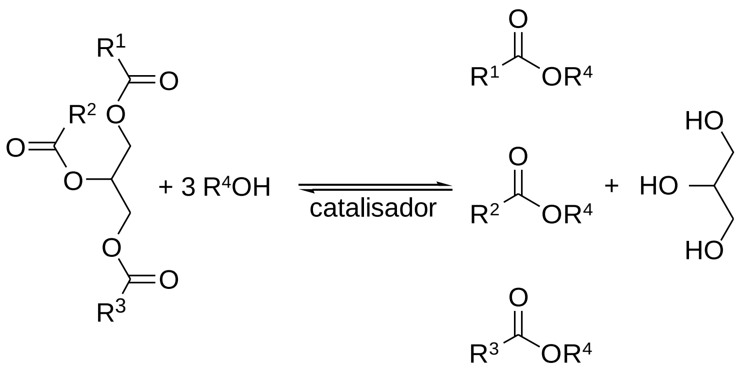


Figura 2 - Reação de transesterificação de um triglicerídeo.

A reação de transesterificação é constituída por uma sequência de três reações consecutivas. Primeiramente, os triglicerídeos são convertidos em diglicerídeos, que posteriormente, são convertidos em monoglicerídeos. Por fim, os monoglicerídeos são convertidos em glicerol. Dessa forma, três monoalquilésteres são obtidos a partir de uma molécula de triglicerídeo, conforme ilustra a Figura 3 (MOTASEMI; ANI, 2012).

$$\text{Triglicerídeos} + \text{ROH} \rightleftharpoons \text{Diglicerídeos} + R^1COOR$$

$$\text{Diglicerídeos} + \text{ROH} \rightleftharpoons \text{Monoglicerídeos} + R^2COOR$$

$$\text{Monoglicerídeos} + \text{ROH} \rightleftharpoons \text{Glicerol} + R^3COOR$$

Figura 3 - Etapas da transesterificação de um triglicerídeo.

Normalmente, catalisadores são utilizados para acelerar a reação e otimizar o rendimento de conversão. Bases, ácidos, e enzima são três tipos de catalisadores utilizados na reação de transesterificação. Hidróxido de potássio (KOH) e hidróxido de sódio (NaOH) são os catalisadores básicos mais utilizados devido a boa relação custo/benefício (FERRARI; OLIVEIRA; SCABIO, 2005). Outros estudos apontam ainda para a possibilidade de empregar outros catalisadores como o ácido sulfúrico (H_2SO_4), zeólitas, enzimas, entre outros (MOTASEMI; ANI, 2012). Como mencionado, os álcoois mais utilizados na transesterificação são o metanol e o etanol, especialmente o metanol devido às vantagens de possuir cadeia mais curta, maior polaridade, facilitando assim a separação do glicerol e biodiesel, outrossim o metanol é adquirido a baixo custo (MOTASEMI; ANI, 2012).

Apesar de o metanol ser oriundo de petróleo, o que representa uma relativa desvantagem, o biodiesel obtido através desse álcool apresenta um maior rendimento quando comparado ao obtido com etanol, um combustível renovável. Além disso, estudos têm demonstrado a possibilidade de produção do metanol a partir de biomassa, o que poderia eliminar a desvantagem desse reagente (DANTAS, M. B., 2006).

Síntese do biodiesel

Diferentes técnicas têm sido utilizadas para obtenção do biodiesel, tais como reatores (JITPUTTI et al., 2006), métodos convencionais que utilizam catalisadores homogêneos e heterogêneos em reatores de vidro e micro-ondas (GAMA; GIL; LACHTER, 2010). No entanto, no aquecimento convencional, uma grande quantidade de energia é necessária para aquecer efetivamente a mistura de reação, o que torna este método menos eficiente. Além disso, é necessário um tempo de reação longo (30 min a 8 h) para obter uma conversão em biodiesel superior ao estipulado pela norma ANP (96,5 %). Outra tecnologia emergente para o processo de transesterificação é a reação assistida por ultrassom. A sonoquímica é conhecida por aumentar as taxas de conversão das reações e seu uso tem se mostrado habitual por se tratar de uma metodologia alternativa e sustentável (HOBUSS et al., 2012; JI et al., 2006; STAVARACHE et al., 2007; STAVARACHE et al., 2005; THANH LE et al., 2010). O princípio de funcionamento do ultrassom é a cavitação, uma fonte de vibração que ocasiona a implosão de cavidades.

A origem da cavitação deve-se ao fato de que, durante a expansão, os gases adsorvidos no líquido ao redor da cavidade ou na interface resultam na expansão da cavidade. Durante a etapa de compressão, estes gases ou vapores não retornam completamente ao líquido, resultando em um aumento efetivo da cavidade. Ciclos periódicos de compressão e expansão causam aumento do tamanho da cavidade que, por fim, ao atingir um tamanho crítico, implode-se, liberando grande quantidade de calor e pressão em curto período de tempo e em pontos localizados do líquido (MARTINES; DAVOLOS; JÚNIOR, 2000; MASON, 1997; ROCKEMBACH et al., 2014). Esta metodologia normalmente reduz o tempo de reação e aumenta a conversão de reagentes em produtos (HOBUSS et al., 2012).

As ondas sonoras para aplicação em sonoquímica têm uma frequência característica num intervalo de 20 kHz a 2 MHz (HOBUSS et al., 2012). A cavitação é um fenômeno físico utilizado na promoção de reações químicas e tem se mostrado eficaz (DANTAS, D. M. M. et al., 2012). Quando aplicado para a obtenção do biodiesel, devido a intensa agitação mecânica do meio reacional, o ultrassom cumpre ainda o papel de facilitar a formação da emulsão e o contato entre os reagentes, uma vez que o álcool e o triglicerídeo são imiscíveis. Esta ferramenta proporciona uma série de vantagens como, por exemplo, a redução do tempo de reação, do consumo de energia e da quantidade de solventes; um maior rendimento das reações e a obtenção de produtos mais limpos e livres de impurezas indesejáveis (DUARTE et al., 2010; ELIZABETH GRANT; GNANESWAR GUDE, 2014; PIZZUTI et al., 2010; PIZZUTI et al., 2009; SILVA et al., 2009; VENZKE et al., 2011).

Parâmetros de qualidade do biodiesel

Com o intuito de averiguar se a síntese do biodiesel é eficaz a resolução nº 45, de 25.8.2014 ANP (Agência Nacional do Petróleo, Gás Natural e Biocombustíveis) estabelece alguns parâmetros de qualidade.

As propriedades físico-químicas do biodiesel estão associadas essencialmente ao número de carbonos constituintes do triglicerídeo, bem como o grau de insaturações dos ácidos graxos (WAN, 1991). Um dos problemas que afeta a qualidade do biodiesel é a oxidação, ocorrendo um aumento na concentração de ácidos graxos saturados, de ácidos graxos livres, entre outros, influenciando desta forma nas propriedades químicas como índice de acidez, índice de iodo e propriedades físicas como a viscosidade, o que pode causar um prejuízo para os motores da matriz automotiva (LÔBO; FERREIRA; CRUZ, 2009).

Conceitualmente, a viscosidade é a resistência à vazão de um líquido, devido ao atrito de suas camadas movendo-se umas sobre as outras (LUTZ, 1976). A viscosidade de um óleo aumenta proporcionalmente com o aumento de carbonos da cadeia de ácidos graxos dos triglicerídeos e diminui com o aumento das insaturações presentes na molécula. Biodiesel com a viscosidade elevada pode provocar sedimentação no interior dos motores. Deste modo, a determinação da viscosidade caracteriza-se como um importante indicador na qualidade do biodiesel (LÔBO et al., 2009). O valor estipulado pela ANP descreve que a viscosidade do biodiesel deve estar entre 3,0-6,0 mm^2s^{-1} e valores de viscosidade acima do especificado, indicam a presença de sabão e oxidação do biodiesel (GONÇALVES et al., 2011; TUBINO; ARICETTI, 2011), os quais dificultam a combustão do biocombustível e aumentam o nível de emissões indesejáveis (COWAN; RACKIS; WOLF, 1973).

O índice de iodo está relacionado ao grau de insaturação das cadeias, deste modo é dependente da fonte de triglicerídeos utilizada na transesterificação (BASHA; RAJA GOPAL, 2012), afetando diretamente a densidade, viscosidade e estabilidade oxidativa do biodiesel (MAKAREVICIENE; SENDZIKIENE; JANULIS, 2005). Entretanto, não há limite estipulado para esta propriedade físico-química na legislação atual da ANP.

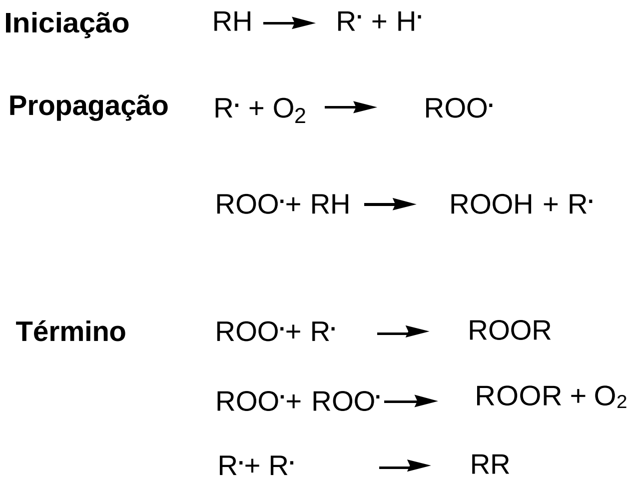
O índice de acidez é a massa de hidróxido de potássio consumida para neutralizar os ácidos graxos livres presentes na amostra. Desta forma é importante ser estudado, pois fornece dados para avaliar o estado de conservação do óleo e do biodiesel e também para averiguar a qualidade do produto, visto que altos índices de acidez podem causar corrosão no tanque de armazenamento e nos motores (KNOTHE, 2005). O índice de acidez pode ser usado para monitorar o grau de degradação do combustível (LÔBO et al., 2009; REDEL-MACÍAS et al., 2012). O limite estabelecido pela ANP é 0,5 mg KOH g^{-1} de amostra (RAMOS et al., 2009). Em particular, o teor de éster é um parâmetro previsto na legislação brasileira a ANP a porcentagem mínima exigida de ésteres no biodiesel é de 96,5 %, a qual é determinada por meio do método cromatográfico EN 14103.

Estabilidade Oxidativa do Biodiesel

Um importante parâmetro analisado para a qualidade do biodiesel é sua estabilidade quanto à oxidação. A oxidação se caracteriza por uma reação de transferência de elétrons ou hidrogênio de um composto para um agente oxidante (MONYEM; H VAN GERPEN, 2001). A estabilidade do biodiesel está relacionada ao tipo e a quantidade de ácidos graxos insaturados presentes na amostra (RAND, 2003). Desta forma, alguns fatores que influenciam a oxidação lipídica são a presença de luz (foto-oxidação), alta temperatura (termo-oxidação), insaturação dos ácidos graxos, enzimas e condições de armazenamento (HERBINET; PITZ; WESTBROOK, 2008). Outra considerável causa da oxidação lipídica é a posição das duplas ligações, sendo assim os ácidos graxos poliinsaturados com dulpas ligações conjugadas são mais propensos à oxidação (PULLEN; SAEED, 2012). A estabilidade oxidativa pode ser explicada como a resistência de uma substância à oxidação (PULLEN; SAEED, 2014). Normalmente expressa por período de indução, que é o período entre o tempo inicial da medição e o momento sucessor à formação de produtos de degradação. De acordo coma ANP, a norma a qual determina a estabilidade oxidativa de biodiesel é EN 14112 (Norma Européia).

A estabilidade oxidativa é uma propriedade a qual indica a qualidade de uma amostra, pois esta está relacionada ao tempo e as condições de armazenamento do biodiesel (PULLEN; SAEED, 2012). Quando o combustível é estocado, o mesmo entra em contato com o ar, que atua como um agente oxidante; além disso, a exposição a altas temperaturas pode potencializar a reação de oxidação, formando radicais livres (XIN; IMAHARA; SAKA, 2009).

A auto-oxidação é o principal meio de oxidação de óleos e gorduras. Para explicar este fenômeno (FARMER et al., 1942), propuseram uma série de reações (Figura 4). A primeira etapa de reações foi denominada iniciação, na qual ocorre a formação dos radicais livres do ácido graxo, na presença de luz e calor, em consequência da remoção de um hidrogênio do ácido graxo. Na propagação, os radicais livres, os quais são propensos ao ataque do oxigênio atmosférico, se convertem em outros radicais, sendo que a estrutura destes depende da origem do ácido graxo. Estes radicais formados comportam-se como propagadores da reação. No término ocorre a combinação de dois radicais livres com o intuito de formar um composto mais estável (PULLEN; SAEED, 2012).



Iniciação $RH \rightarrow R^{\cdot} + H^{\cdot}$

Propagação $R^{\cdot} + O_2 \rightarrow ROO^{\cdot}$

$ROO^{\cdot} + RH \rightarrow ROOH + R^{\cdot}$

Término $ROO^{\cdot} + R^{\cdot} \rightarrow ROOR$

$ROO^{\cdot} + ROO^{\cdot} \rightarrow ROOR + O_2$

$R^{\cdot} + R^{\cdot} \rightarrow RR$

Figura 4 - Mecanismo da oxidação lipídica. RH (ácido graxo insaturado); R. (radical livre); ROO. (radical peróxido) e ROOH (hidroperóxido)

O processo apresentado na Figura 4 também pode ser denominado rancificação, caracterizado por diminuir a qualidade comercial do biodiesel. Nesse contexto, faz-se necessário a pesquisa de novas substâncias antioxidantes para biodiesel, para que estes compostos sofram a oxidação no lugar do componente a ser protegido, aumentando, portanto o tempo de vida útil deste produto (FERRARI, R. A.; SOUZA, W. D., 2009). A oxidação do biodiesel é um dos principais problemas que afeta este biocombustível, por isso tem crescido o interesse por compostos sintéticos que estabilizem esta oxidação. As substâncias antioxidantes são estudadas e abrangidas por diferentes areas da ciência e tecnologia, estas possuem um vasto campo de aplicações (DA SILVA ARAÚJO; DE MOURA; CHAVES, 2010).

Antioxidantes

Antioxidantes são substâncias capazes de impedir ou diminuir um processo de oxidação, sendo este uma reação de transferência de elétrons ou hidrogênio de um composto para um agente oxidante, (ou, qualquer substância) que, quando presente em baixa concentração, comparada à do substrato oxidável, diminui ou inibe significativamente a oxidação daquele substrato, dificultando assim que este processo ocorra em cadeia (DEGÁSPARI; WASZCZYNSKYJ, 2004; MANDAL et al., 2009).

Segunda a literatura durante a I Guerra Mundial foram testadas a atividade antioxidante de mais de 500 compostos (RAMALHO; JORGE, 2006). A reação de oxidação acontece em praticamente todas as operações de manufatura, desta forma esta pesquisa elementar com estudos clássicos, agregada com a importância de estudar a oxidação, desencadeou novas pesquisas por moléculas antioxidantes a fim de controlar a oxidação (BAILEY; HUI, 1996). As espécies reativas (ER) mais estudadas são as de oxigênio (ERO), de nitrogênio (ERN) e os radicais derivados de tióis (RS•) (GUTTERIDGE; HALLIWELL, 1999). Em condições aeróbias, o O_2 sofre uma redução tetravalente formando H_2O (Figura 5). Durante este processo, intermediários reativos são formados, como os radicais superóxido (O_2^-), peróxido de hidrogênio (H_2O_2), radical hidroxila (OH•) (ULRICH-MERZENICH et al., 2009).

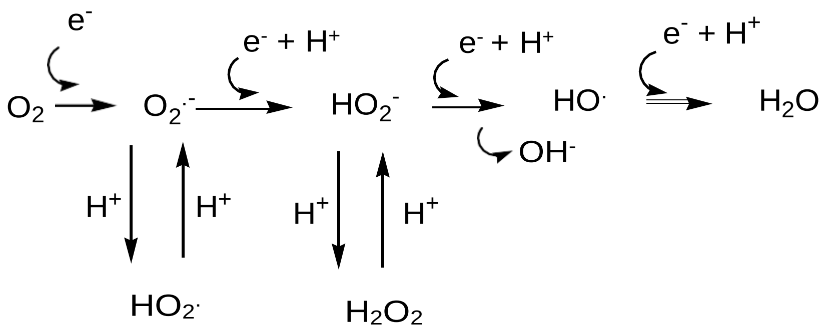


Figura 5 - Formação de espécies reativas de oxigênio.

Compostos com ação antioxidante são divididos em duas categorias básicas, sendo estas sintéticas e naturais, podendo ser derivados de distintas matrizes, entretanto, estes antioxidantes possuem funções similares: desativar radicais livres auxiliando compostos propensos à oxidação (YEO et al., 2010).

Para aumentar o tempo de armazenamento do biodiesel, os tocoferóis, butil hidroxianisol (BHA), *terc*-butil-hidroquinona (TBHQ) e butil hidroxitolueno (BHT) são antioxidantes conhecidos por aumentar a resistência à oxidação e por retardarem os efeitos do processo oxidativo no biodiesel, como viscosidade, índice de acidez, índice de iodo entre outros (DOMINGOS et al., 2007). Demonstrando desta forma a necessidade de estudar o efeito dos antioxidantes no biodiesel.

3.2.1 Chalcona

As chalconas podem ser encontradas em produtos naturais ou obtidas por meio sintético, são consideradas pigmentos naturais comuns e importantes intermediários na biossíntese de flavonóides (BANO et al, 2013). São classificadas como cetonas *α,β*-insaturadas com um anel aromático ligado à carbonila e outro ligado à insaturação (ZHANG et al, 2013) geralmente obtidas através de uma reação de condensação aldólica. As chalconas são conhecidas por possuírem diferentes atividades farmacológicas, desta forma apresentam interessantes aplicações em diferentes áreas. Dentre as atividades farmacológicas, cabe salientar que as chalconas e seus derivados possuem atividade antioxidante, anti-inflamatória, antimicrobiana, antifúngica, antibacteriana, citotóxica, antimalariana dentre outros (KIM et al., 2007; KUMAR, V. et al., 2011; MARTINS et al., 2004; MAYDT et al., 2013; SALEHI et al., 2003).

Avanços na área da química sintética, juntamente com modelos biológicos *in vitro* têm auxiliado efetivamente na descoberta de novos fármacos, uma vez que com a síntese é possível obter uma diversidade de moléculas com estruturas conhecidas e alto grau de pureza. (RISHTON, 2008). Dentre as distintas classes de compostos estudadas estão as chalconas, atualmente o número de pesquisas em torno dessas moléculas cresceu muito (EL-SAYED; GABER, 2015; KUMAR, C. C. et al., 2015; LI et al., 1995). Em especial a síntese clássica de chalconas ocorre através de uma condensação de Claisen-Schmidt (GUIDA et al., 1997), utilizando acetofenona e aldeídos aromáticos, catalisada por KOH, conforme Figura 6.

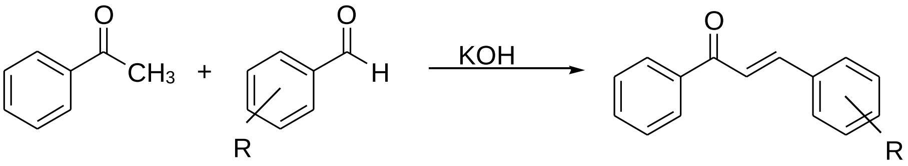


Figura 6 - Síntese de chalconas.

Tendo em vista a versatitilade sintética e possibilidade de variação química das chalconas, se amplia a possibilidade na investigação dessas moléculas na aplicação no setor produtivo, como na área de antioxidante. Pesquisas recentes revelaram a atividade de vários exemplos destes compostos com atividade antioxidante (EL-SAYED; GABER, 2015 ; KIM, 1007; MAYDT, 2013). Haja visto a necessidade de estudos referente a estabilidade oxidativa do biodiesel e a necessidade de moléculas antioxidantes capazes de estabilizar este biocombustível.

PARTE III

Capítulo 1

4 ARTIGO CIENTÍFICO 1

Os resultados dessa dissertação serão apresentados na forma de artigo científico.

BIODIESEL FROM SUNFLOWER OIL: IMPROVED SYNTHESYS IN ULTRASOUND AND MONITORING OF THE OXIDATIVE STABILITY

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Abstract:

The present study aimed to optimize the ultrasound-assisted biodiesel synthesis of refined sunflower oil and monitor the oxidation of the same through physicochemical parameters. For the synthesis, the influence of amplitude, reaction time and amount of catalyst were evaluated, also the physicochemical parameters evaluated during 70 days of storage were viscosity, acidity and iodine values. In addition, fatty acid profiles were also obtained from the biodiesel before and after the storage period. It can be seen that the use of ultrasound proved to be efficient in obtaining biodiesel in a an amplitude of 30 % and with only 10 min reaction time with 0.15 g of catalyst (99 % yield). Through evaluation of the physicochemical parameters, it can be seen that when stored at 50 °C, the product can be used for a period of 49 days. After this period, the viscosity value exceeds the permitted limits. However, the acid value remained within the limit during 63 days. With the fatty acid profile, the results obtained in the analysis of the viscosity can be confirmed, since with chromatography, a degradation of unsaturated fatty acids was observed, thereby increasing the percentage of saturated fatty acids and hence the viscosity.

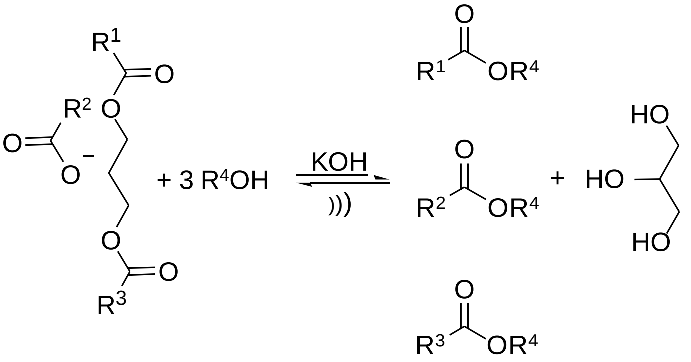
Keywords: Biodiesel, sunflower, oxidation and ultrasound

1. Introduction:

The search for renewable fuels is intensely growing because these present several advantages. They are environmentally friendly, biodegradable, non-toxic and have less emission of polluting gases when compared with petroleum diesel [1, 2]. Biodiesel is usually produced by the transesterification of vegetable oil or materials that contain a high concentration of triacylglycerol with alcohol in the presence of a catalyst [3]. Soybean, grape seed, rapeseed, palm, sunflower, castor oil, residual oil, algae, fish oil, tallow, lard and grease are some of the materials used for this purpose [4-6]. Despite the physicochemical characteristics of biodiesel being similar with diesel [7], biodiesel produced from vegetable oils is more susceptible to oxidation when exposed to oxygen in the air and high temperature, mainly due to the presence of varying numbers of double bonds in the free fatty acid chains [8]. The autoxidation reaction produces hydroperoxides that can polymerize with other radicals to produce high molecular weight insoluble sediments [9]. In some cases, the oxidized fatty acid chains can break producing shorter chain acids and aldehydes [10]. However, the oxidation can be prevented by the use of synthetic antioxidants, such as tocopherols that increase the oxidative stability of biodiesel, maintaining the physicochemical properties during a long period of time [11, 12].

Conventional methods using homogeneous or heterogeneous catalysts have been used to obtain biodiesel, but these methods need prolonged reaction times, overspending of energy and show low conversion rates [4, 13, 14]. Sonochemistry, is a new technique in the production of high quality biodiesel through more efficient reactions, generating higher conversion rates, short reaction times and cleaner products [15-17].

In ultrasound-assisted reactions, the frequency range used to promote the process of cavitation is 20 kHz to 2 MHz, with low frequency and high power [18]. The cavitation phenomenon is based on the formation, growth and implosion of gas bubbles [19]. In general, the waves consist of continuous compression-expansion cycles; the compression exerts positive pressure on the molecules, while the expansion occurs during the removal of the molecules due to negative pressure [20]. The periodicity of these cycles causes an increase in bubbles, until these reach a critical size and implode, thus releasing a lot of energy at certain points in the reaction medium [21-24]. In this biodiesel synthesis setting, the ultrasound is the promoter of the reaction, facilitating contact between the reactants, due to intense mechanical agitation. This makes emulsification and the contact between the reactants easier, since alcohol and triglycerides are immiscible [20]. The scheme represents the ultrasound-assisted transesterification of triglycerides in biodiesel.



Scheme 1: Biodiesel synthesis reaction.

Since the use of biodiesel obtained from different sources has increased and with the knowledge of the problems that it may have regarding oxidation, the main idea of the present work was to optimize the ultrasound-assisted biodiesel synthesis of refined sunflower oil and assess its stability during 70 days of storage at 50 °C. During this period, the gas chromatographic (GC), viscosity, iodine index and acid value were the analysis used to monitoring the process.

2. Methods and materials:

Biodiesel synthesis

The reactions of commercial sunflower oil (Suavit, Brazil) was conducted using an ultrasound probe (Sonics, model VC 500, serial 480357, 500 Watts, frequency 20 kHz, 220 V). Studies on the effect of reaction time (10, 15, 20 and 25 minutes), amplitude (20, 25, 30 and 35%) and the KOH (Synth) catalyst concentration (0.15; 0.20; 0.25 and 0.30 g) during sonication were performed. The procedure consisted of adding different concentrations of KOH and 13 mL of methanol (Sigma-Aldrich) in 25 mL of commercially available sunflower oil. Subsequently, the mixture was transferred to a separatory funnel and the glycerin separated. The biodiesel was washed with 15 mL of aqueous HCl (5 % v/v - Sigma-Aldrich) and with 15 mL of saturated NaCl (Vetec). Finally the residual methanol was evaporated in vacuum.

Analytical methods

The biodiesel samples were analyzed by viscosity, acid number, iodine value and saponification number, according to Instituto Adolfo Lutz standards [26] and the chromatographic profile was also evaluated. The fatty esters content in biodiesel was estimated by gas chromatography (EN 14103) [27].

Viscosity

To determine the viscosity, a Saybolt viscometer (Q288SR model) was used. The methodology is in accordance with the standards employed to determine fuel viscosity (ASTM-D-88-MB326) [26].

Acid value

4 g of sample were dissolved in 25 mL of ethyl ether-ethanol (2:1, Synth) biodiesel was added, followed by the addition of two drops of phenolphthalein indicator (1%) and titrated with a 0.01 mol L^{-1} aqueous NaOH (Sigma-Aldrich), until a pink color appeared, to determine the amount of free acids present in a gram of oil sample [26].

Iodine index

To determine the iodine index, 0.25 g of biodiesel was added to 10 mL of cyclohexane and 25 mL of Wijis solution (Synth). For homogenization of the solution, the system was shaken. The mixture was allowed to stand in the dark and after 30 minutes, a solution of 10 mL of 15 % KI (Sigma-Aldrich) and 100 mL of water were added, the obtained mixture was titrated with 0.1 mol L^{-1} $Na_2S_2O_3$ (Vetec) until the appearance of a yellowish coloration. Finally, 2 mL of 1 % starch indicator solution was added and the solution was titrated again until the disappearance of the dark blue coloring. A blank was performed by repeating the above procedure without adding the sample [26].

Fatty acid profile

To perform the analysis of the total converted esters a Gas Chromatograph was used following the EN 14103:2003 standard [27]. A gas chromatograph (GC-17A - Shimadzu/Class GC 10) equipped with split/splitless injector, flame ionization detector (FID) and RTX-Wax (30 m x 0.32 mm x 0.25 µm) capillary column was used. The following conditions were used: Hydrogen carrier gas, flow rate of 1.2 mL min^{-1}, Split 1:50, sample volume 1µL, programmed oven temperature: initial temperature 100 °C kept at this temperature for 0.5 min with a heating ramp at 7 °C min^{-1} until 175 °C, then heating at 5° C min^{-1} until 190 °C keeping this temperature for 1 minute and then going to 1.2 °C min^{-1} until 230 °C keeping this temperature for 12 minutes, with a total time of 60 min, with the injector and detector temperatures of 250 °C.

3. Results and discussion

Biodiesel synthesis

The ester conversion was estimated accordingly to the European Standard – EN14103. As it can be seen from the ester content shown in Table 1, were obtained in most assays where the amplitude and the reaction time were varied.

Since fast analysis methods with good rates of conversion are desired, using 30 % amplitude within 10 minutes of reaction time was established as the best working condition.

It is known that the amount of catalyst can increase the conversion rates in chemical reactions [28, 29, 30]. Thus, the mass variation of the KOH catalyst (0.15; 0.20; 0.25 and 0.30 g) was evaluated and the synthesis was carried out by varying the amplitude and the reaction time. As it can be seen in

Table 1, the conversion rate values were very close even with the change in the catalyst mass, and thus, the lowest catalyst concentration was adopted.

Table 1: Values of Conversion to methyl esters under different conditions

Average ester content (%) using 0.15 g KOH				
Amplitude (%)	**10 minutes**	**15 minutes**	**20 minutes**	**25 minutes**
20	93±1.6	97±0.6	98±0.9	94±1.6
25	96±1.6	96±1.5	98±1.6	99±1.0
30	99±0.1	98±1.1	96±1.8	99±0.2
35	99±0.7	98±0.9	99±0.3	96±1.9
Average ester content (%) using 0.2 g KOH				
Amplitude (%)	**10 minutes**	**15 minutes**	**20 minutes**	**25 minutes**
20	95±0.8	96±0.7	97±1.1	96±0.9
25	97±1.1	97±1.3	98±0.7	98±1.1
30	98±0.9	97±1.1	97±1.0	98±0.7
35	98±1.2	98±0.7	98±0.9	97±1.2
Average ester content (%) using 0.25 g KOH				
Amplitude (%)	**10 minutes**	**15 minutes**	**20 minutes**	**25 minutes**
20	95±0.9	96±0.9	98±0.8	96±0.7
25	97±1.3	96±1.1	97±0.9	97±1.0
30	99±0.5	97±1.4	97±1.2	98±1.1
35	98±0.9	98±0.8	98±0.5	98±0.4
Average ester content (%) using 0.3 g KOH				
Amplitude (%)	**10 minutes**	**15 minutes**	**20 minutes**	**25 minutes**
20	96±1.1	98±0.9	97±0.5	95±1.2
25	96±1.3	99±1.0	97±1.3	97±1.0
30	98±0.8	99±1.3	98±0.9	97±1.2
35	98±0.9	99±0.5	98±0.7	98±0.8

Thus, it is established that using ultrasound with an amplitude of 30 %, 10 minutes of reaction and 0.15 g of KOH, it is possible to obtain biodiesel of quality accepted by current legislation that calls for minimum conversion rates of 96.5 % [31] from sunflower oil.

Studies of biodiesel stability

Biodiesel is an unstable product [32], so it is important to study the oxidative stability. Thus, in this study, parameters such as viscosity, acid and iodine values were evaluated. Additionally, the characterization of the fatty acid profile by GC-FID was performed.

Oxidation leads cleavage of the double increasing the viscosity of biodiesel [33] that makes it an important indicator of product quality [34]. In Figure 1, the results of the regular analyses (every 7 days) of biodiesel viscosity are shown.

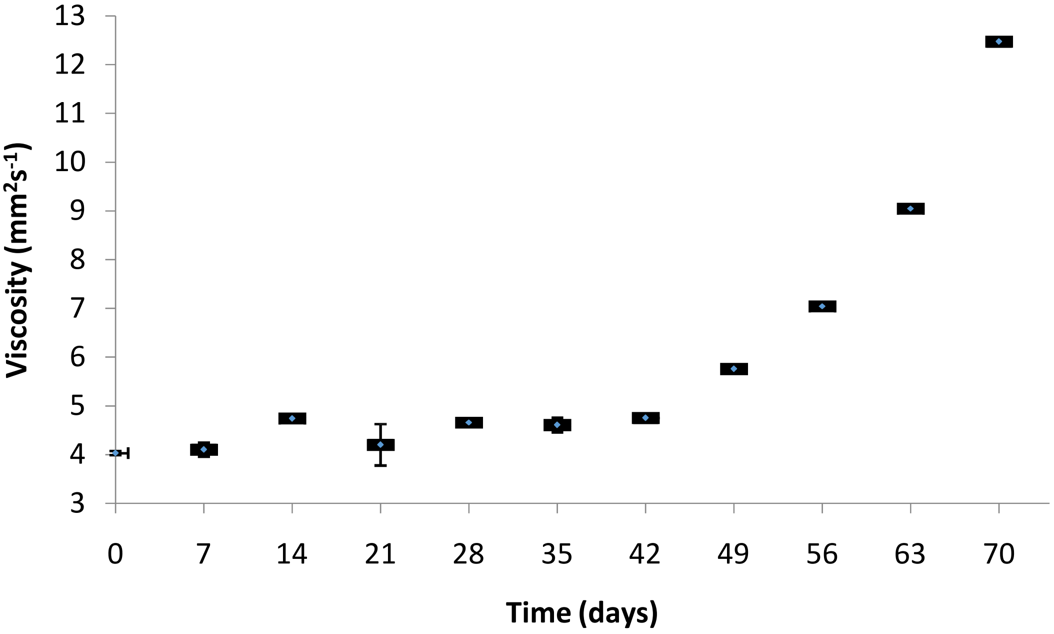


Figure 1: Change in viscosity during the biodiesel storage.

Biodiesel has a predisposition to oxidative processes due to isomerization of the double bond [35], which entails a more efficient packing of the carbon chains [28,29] and favors the production of insoluble polymers [32], causing an increase in the viscosity [36]. The viscosity rate for biodiesel established by ANP is 3 to 6 mm^2s^{-1} [31], and biodiesel values not in accordance with the legislation are not suitable for use. As it can be seen in Figure 1, with storage time, there was an increase in the viscosity of the biodiesel from 4.06 ± 0.04 to 9.09 ± 0.10 mm^2s^{-1} (the deviations of all analyses were below 0.4). However the synthesized biodiesel becomes unfit for use after the 49th day of storage since the viscosity exceeded the limit set by law.

With biodiesel oxidation, the fatty acid esters tend to hydrolyze due to the presence of reactive oxygen species, forming alcohol and acid [33,34] increasing the acid value. The biodiesel acid values studied are shown in Figure 2.

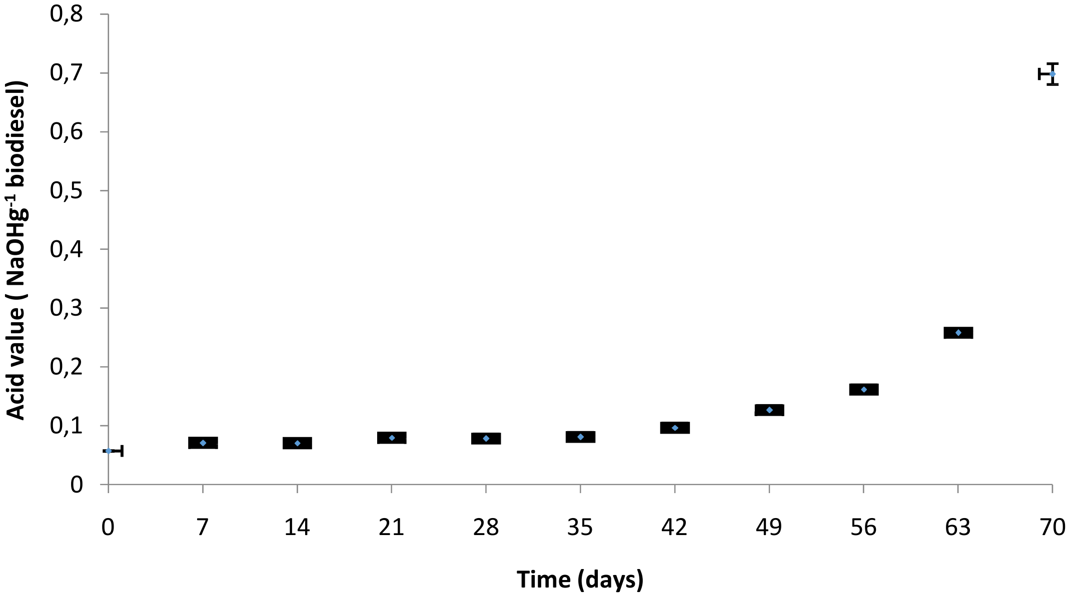


Figure 2: Monitoring of the acid value during storage

The evaluation of the acid value during storage gradually increased (from 0.06 to 0.7 gNaOH^{-1} with lower deviations of 0.02). However, only after 63 days of storage at 50 ° C did the acid value exceed the limit established by the ANP (0.5 mg KOH g^{-1} sample) [31].

Just as the viscosity and acid values, the iodine value can also be used for the quality of biodiesel, since it estimates the degree of unsaturation present in the sample [37]. One can see in Figure 3 that during the time of storage, there was a decrease in iodine value from 180.9 to 94.4 gI$_2$100^{-1} sample (deviations of less than 6 %) showing a decrease in unsaturation in the sample.

The results demonstrated herein for the evaluation of iodine value are in agreement with those obtained by Bouaidet al., (2007) who observed the decrease of iodine value after storage (30 months) of biodiesel from cooking oil [6]. Although there is no legislation on the control values set for iodine index in biodiesel, it is known that the compounds from the oxidation affect the performance of automobile engines that make use of biodiesel [6, 38]. Thus, the results shown in Figure 3 are important as they show the accelerated oxidation of biodiesel with storage time.

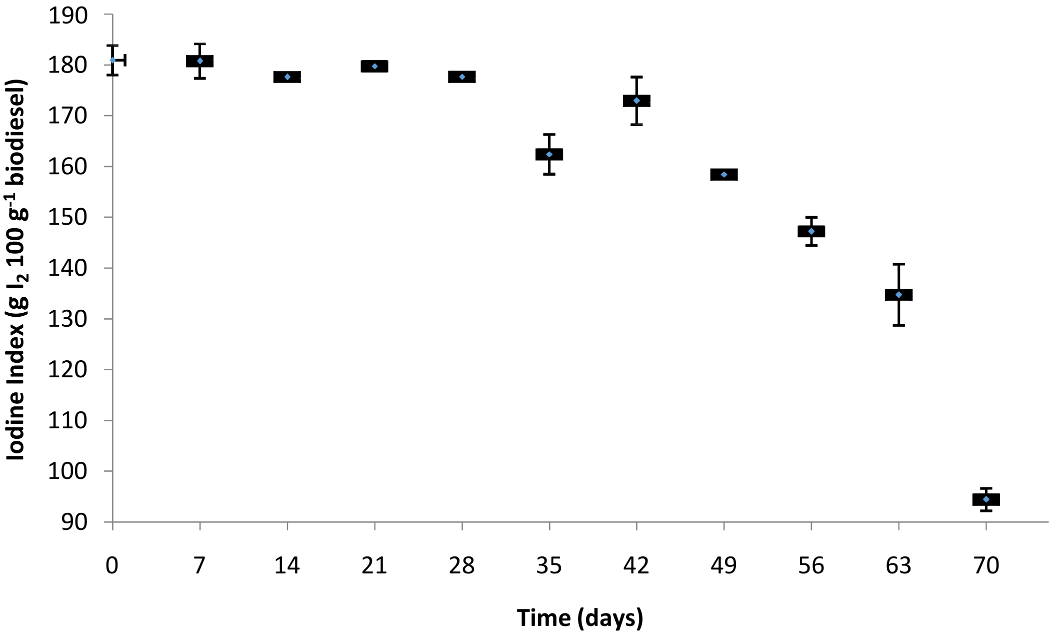


Figure 3: Variation of iodine value during storage

Additionally, the evaluation of the lipid profile of the sample was performed before and after the storage period. Table 2 shows the percentages of fatty acids present in sunflower biodiesel before and after storage.

It can be seen in Table 2 that after 70 days of storage in an oven at 50 °C, a variation was observed in all the studied concentrations of fatty ester. The concentration of polyunsaturated fatty acids decreased after storage. There was a decrease of approximately 14 and 1% of linoleate acid (C18: 2n6c) and alpha-linoleate (C18: 3n3), respectively. This decrease can be explained because these compounds present methylenes groups adjacent (allylic position) that are susceptible to oxidation due to free radical attack [40]. Furthermore, linoleic acid showed more degradation after storage, it has a methylene group between two double bonds (bis-allylic sites) thus becoming more vulnerable to oxidation [6, 8].

However, one can observe an increased concentration of oleate (C18:1n9c) which also have two adjacent carbons. This is due to the degradation of polyunsaturated fatty acids (C18: 2n6c, C18: 3n6c and C18: 3n3) that can form C16: 1 and C18: 1n9c [40]. However, oleate is formed in greater amounts for having the same number of carbon as polyunsaturated compounds [41].

Table 2: Lipid composition of biodiesel before and after storage

Fatty Ester	[%] Fatty Ester		
	Before storage	42 days storage	After storage
Methyl Palmitate (C16:0)	7.38±1.2	7,81±0.9	10.70±1.0
Methyl Stearate (C18:0)	4.69±0.8	4,81±1.1	6.71±0.9
Methyl Oleate (C18:1n9c)	26.97±1.9	29,27±1.7	36.78±1.6
Methyl Linoleate (C18:2n6c)	56.07±2.5	53,31±1.9	41.64±2.0
Methyl Alpha-Linoleate (C18:3n3)	1.74±0.5	1,47±0.3	0.78±0.3

The oxidation products of double bonds are unstable allylic hydroperoxides that can form a variety of secondary oxidation products [42]. This includes the product of the rearrangement of similar molecular weights forming compounds such as aldehydes and short-chain saturated fatty acids [43, 44]. This corroborates the results shown here because there was an increase in the concentration of the saturated palmitate (C16:0), stearate (C18:0). Figure 4 shows sunflower oil Biodiesel chromatogram. The results found in the performed analysis showed that biodiesel basically kept their properties for 42 days of storage.

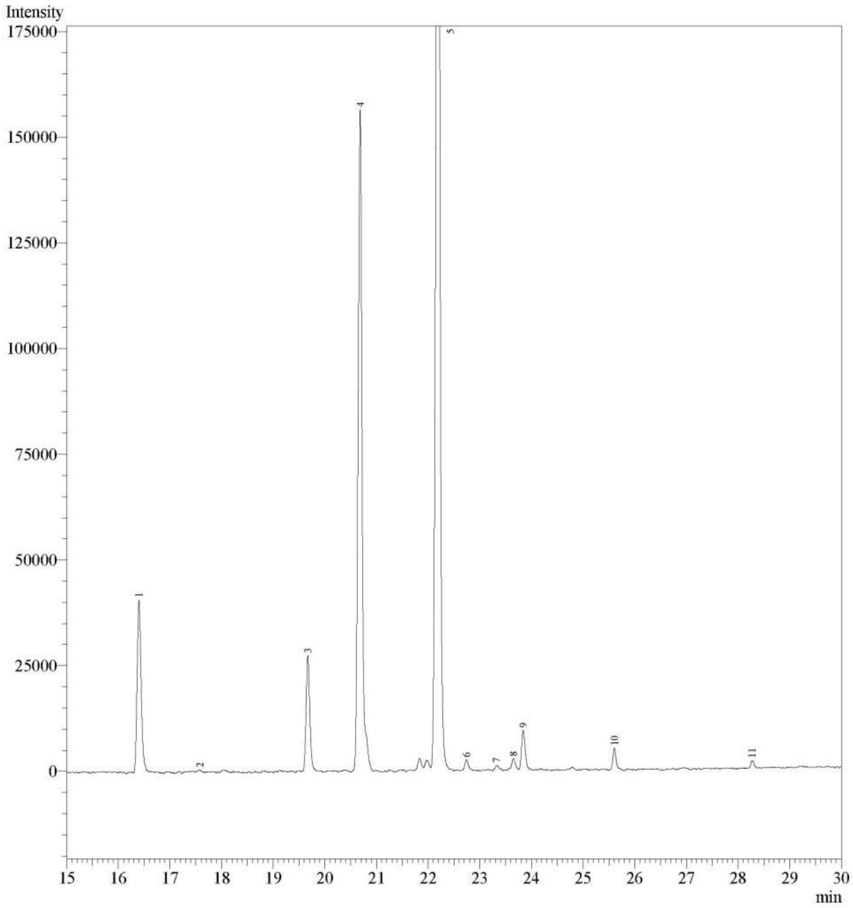


Figure 4: Chromatogram of fatty acid profile of sunflower biodiesel subjected to transesterification via ultrasound. (1) Methyl Palmitate, (2) Methyl Palmitoleate, (3) Methyl Stearate (4) Methyl Oleate (5) Methyl Linoleate (6) Methyl Arachidate, (7) Methyl Linolenate (8) Methyl Gadolenate (9) Methyl Alpha-Linolenate (10) Methyl Behenate, (11) Methyl Lignocerate.

It is known that a Biodiesel of high oleic acid content has good flow properties at low temperatures [40], and the higher the palmitic acid content, the higher the stability of biodiesel against oxidation [47] as this compound only has saturated bonds in its carbon chain. However, the saturation of palmitic acid gives it poor flow properties at low temperatures [39] unlike oleic acid [1].

Thus, evaluating the results of physicochemical parameters (viscosity, iodine index and acidity) and the fatty acid profile, it can be concluded that there was oxidation of biodiesel during storage. However this biofuel remained within the parameters analyzed during the 49 days, since the viscosity of the biodiesel analyzed only passed the limits established by ANP after this storage period.

4. Conclusions

This study investigated different conditions for biodiesel synthesis from refined sunflower oil using an ultrasonic probe. One may infer that the use of ultrasound as a means for promoting synthesis proved effective since with 0.15 g of KOH, 10 minutes reaction time, with 30 % amplitude in 99 % conversion of biodiesel was reached, being in accordance with the current legislation. By monitoring and evaluation of physicochemical properties of this biodiesel, it can be stated that the product should be discarded only after 49 days of storage because it exceeds the viscosity limit set by the ANP. However, the acid value exceeded the recommended limit only after 63 days. The gas chromatography (GC) has proved a valuable tool because through fatty acid profile analysis the degradation of unsaturated fatty acids can be confirmed, thus demonstrating the oxidation of biodiesel with storage. In consideration with chemistry analysis, the sunflower oil is good starting material to biodiesel and the ultrasound was confirmed as an efficient promoter to transesterification reaction.

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Capítulo 2

5 ARTIGO CIENTÍFICO 2

EVALUATION OF CHALCONES IN THE BIODIESEL OXIDATIVE PROCESS

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Abstract:

The main idea of this study is to evaluate different chalcones as antioxidant for sunflower biodiesel. Sunflower oil transesterification was carried out by ultrasound-assisted basic catalysis, 98.5 % conversion was obtained. Physicochemical parameters (viscosity, acid, iodine and saponification values) were evaluated to determine the biodiesel quality. The fatty acid profile was determined by gas chromatography with flame ionization detection (GC-FID). Eleven chalcones having different substituents were synthesized, characterized by Fourier transform infrared (FT-IR) and gas chromatography coupled to mass spectrometry (GC-MS), and their purities were determined by GC-MS and melting point. The evaluation of the antioxidant activity of these chalcones was performed using the methods of DPPH and Chemiluminescence, the chalcones that showed the highest antioxidant activity in *in vitro* tests were added to biodiesel, wich had its oxidative stability measured in Rancimat

Keywords: Biodiesel, oxidation and chalcone

1 Introduction

The use of biodiesel has grown since fuels from renewable sources are important for the imminent possibility of scarcity and pollution caused by fossil fuels. Biodiesel is typically obtained from oleaginous composed of different fatty acids, which may affect the quality of the final product since samples with large amounts of unsaturated chains are more prone to oxidation, thus committing the physicochemical characteristics and therefore product quality, often making the product unfit for use [1].

The most important factors that influence lipid oxidation are the presence of light (photo-oxidation), high temperature (thermo-oxidation), unsaturated fatty acids, the presence of catalytic enzymes and storage conditions [2, 3]. Another significant cause of lipid oxidation is the position of the double bonds, since *bis*-allylic methylenes are more susceptible to oxidation [4, 5]. Oxidative stability is explained as the resistance of a substance to be oxidized [6] and may be expressed by induction time (hours) which represents how much a given sample has degraded at a given time thus allowing comparison of the characteristics of the product before and after the formation of degradation products [7].

In this sense, during storage, biodiesel can be exposed to high temperatures and in contact with air, which enhances the radical reactions, increasing oxidation making it unsuitable to use [8]. To avoid this situation, one can make use of antioxidant compounds that are molecules that undergo oxidation in place of the component to be protected, thus reducing or in some cases even inhibiting the oxidation of the same [9]. These substances may be natural or synthetic [9]. Among the substances with the highest concentration and more frequently present in vegetable oils, tocopherols can be cited [10, 11]. Come in the synthetic compounds, phenolics such as chalcones, that have antioxidant properties attributed to the aryl structure, α, β-unsaturation, and substituents of the aromatic ring [12] stand out. In this sense, the hydroxyl substituent is regarded as the enhancer of antioxidant activity of the chalcones, for being easily converted into a phenoxy radical through the hydrogen atom transfer mechanism [13] .The antioxidants may be classified according to their action and may be free radical sequestrants, chelating agents of ions involved in the catalysis of lipid oxidation or sequestrants that react with oxygen in closed systems [9].

Since the biodiesel oxidation is one of the main problems affecting the biorefinery, the search for synthetic compounds that stabilize this oxidation has grown in recent years [14], and therefore the study of these substances has been carried out in different areas of science and technology, covering a wide range of applications [15]. Thus, in continuation of our research with focus on chalcones and also research of fatty acids [16-18] the objective of this study includes the evaluation of the antioxidant potential of different chalcones and further evaluation as antioxidants for biodiesel.

The composition of biodiesel was determined by gas chromatography and the physicochemical parameters evaluated were viscosity, iodine, acidity and saponification values. The synthesized chalcones were purified by recrystallization and characterized by FT-IR and GC-MS. The antioxidant activity of chalcones was evaluated by the methods of DPPH and chemiluminescence. The chalcones that showed best antioxidant activities were added to the biodiesel and the estimated induction period.

2 Methods and materials

Biodiesel Synthesis and evaluation of its physicochemical properties

The sunflower oil transesterification was promoted by ultrasound (Sonics model VC 500, serial 480 357, 500 Watts, frequency 20 KHz, 220 V, 30 % amplitude) for 10 minutes. The procedure consisted of adding 0.15 g of KOH (Sigma-Aldrich) and 13 mL of MeOH (Sigma-Aldrich) in 25 mL of sunflower oil (extracted from sunflower seed with petroleum ether (Vetec) – via Soxhlet extraction). Subsequently, the mixture was transferred to a separatory funnel and the glycerin was separated. The biodiesel was washed with 15 mL of aq. HCl (5 % v / v - Sigma-Aldrich) and 15 mL of saturated NaCl solution (Sigma-Aldrich). Finally the methanol residue was evaporated under vacuum and the biodiesel was obtained [16].

Determination of the Fatty Acids

Esters of converted fatty acids were determined according to EN 14103:2003, by gas chromatography (GC-17A - Shimadzu / Class GC 10) equipped with split/splitless injector, flame ionization detector (FID) and RTX- Wax (30 m x 0.32 mm x 0.25 microns) capillary column. The following conditions were used: The carrier gas was hydrogen, flow rate of 1.2 ml min^{-1} Split1:50, 1 µL sample volume, programmed oven temperature: initial temperature of 100 °C kept at this temperature for 0.5 min with a heating ramp at 7 °C min $^{-1}$ until 175 °C and later with heating of 5 °C min $^{-1}$ until 190 °C, keeping this temperature for 1 min. The heating rate was 1.20 °C min^{-1} until 230 °C kept at this temperature for 12 minutes with a total time of 60 min. The temperature of the injector and detector was 250 °C [19].

Viscosity

A Saybolt viscometer (Q288SR) was used to determine the viscosity at 40 °C according to ASTM-D-88 and ABNT-MB326 standards (method for determining fuel viscosity). The procedure was performed using 60 mL sample, which was inserted into the Saybolt tube with temperature control. When the temperature (40 °C) was reached, the tube was opened and reading of the flow time of the sample volume through a standard orifice was taken. The flow time of the 60 mL sample in the standard test conditions is the Saybolt viscosity at the temperature of thermal equilibrium [20].

Iodine index

0.25 g of biodiesel, 10 mL of cyclohexane (Synth) and 25 mL of Wijis solution (Synth) was added in a 500 mL flask. The mixture was left under agitation in a closed system until it was homogeneous. Next, the flask was left to stand in the dark for 30 minutes. Then, 10 mL of 15% KI (Sigma-Aldrich) and 100 mL of ultrapure water were added and the obtained mixture was titrated with 0.1 mol L^{-1} $Na_2S_2O_3$ (Sigma-Aldrich) until the appearance of a faint yellow color. Subsequently, 2 mL of starch indicator solution (1 %) were added and the solution was titrated again until the disappearance of the blue color [20].

Acid value

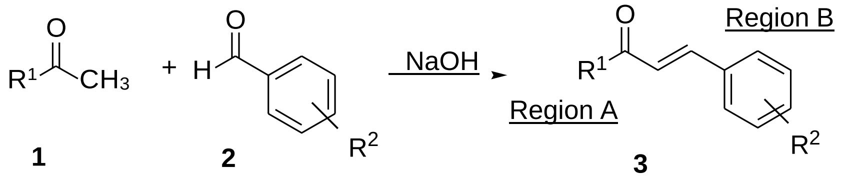
25 mL of a solution of ether-ethanol (2:1, Synth) was added in 4 g of biodiesel contained in a 125 mL Erlenmeyer flask. The mixture was titrated with 0.01 mol.L^{-1} NaOH (Sigma-Aldrich) untila pinkish coloration was evident [20] using phenolphthalein (1 %) as indicator.

Saponification index

A mass of 4.5 g of biodiesel was added to 50 ml of a KOH (4 %) methanol solution. The mixture was kept at 90 °C under stirring for 1 hour. After cooling to room temperature, 1 mL of phenolphthalein solution (1 %, Synth) was added and proceeded to titration with HCl 0.5 mol.L^{-1} until the disappearance of the pink color [20].

Chalcone synthesis

6 mmol NaOH was diluted in 3 mL H_2O/ethanol solution (1:2), 5 mmol of acetophenone and 5 mmol of differentsubstituted benzaldehydes were added to this solution, as shown in the scheme. The mixture was stirred for 4 hours. Thereafter, 0.5 % HCl solution was added until neutral pH was reached and then the reaction was cooled and filtered under vacuum. Purification took place by recrystallization in hexane and ethyl acetate [21].



R^1 = Phenyl
Tien-2-il
2-Hydroxyphenyl

R^2 = H 3-OH
4-Br 2-NO_2 4-NO_2

Scheme: Synthesis of chalcones

One of the objectives of this study was to study antioxidant activity of chalcones*2.3 Chalcone characterization*

Chromatographic Analysis

The substances were qualitatively analyzed in a gas chromatograph coupled to a mass detector, model GC-MS QP-2010SE (Shimadzu, Japan) equipped with an AOC-20i autoinjector. An RTX-5MS capillary column 30 m x 0.25 mm x 0.25 µM (PerkinElmer, USA) was used under the following chromatographic conditions: initial temperature of 50 °C increasing by 10 °C/min until 280 °C and kept at this temperature for 10 min; injected volume: 1 µL; Interface: 280 °C; injector temperature: 280 °C; carrier gas: helium; linear gas flow: 1.22 mL / min; split: 1:50; run in scan mode; mass range of 40 to 700 m/z; filament voltage of 70 eV [22].

Fourier transform infrared (FT-IR)

The infrared analyses were performed by attenuated total reflectance in a Cary 600 series equipment from Agilent Technologies.

Melting point

To determine the melting point, was used a capillary tube into the melting point determiner (Fisaton, Brazil).

Antioxidant tests

DPPH (2,2-diphenyl-1-picrylhydrazyl)

Determination of antioxidant activity of synthesized chalcones were performed according to Gressler et al (2011). Solutions with concentrations of 4000, 2000, 1000, 500, 250, 125 and 62.5 µg mL^{-1} were prepared in DMSO (Vetec). For comparison purposes, Vitamin E (Sigma-Aldrich) and *N*-

acetyl-L-cysteine (Sigma-Aldrich) were used as reference standards. For each 100 µL of chalcone, 10 µL of methanol solution of 100 µM DPPH (Sigma-Aldrich) was added, and this mixture remained under protection from light for 30 minutes at 25 °C and then the absorbance was measured by means of a spectrophotometer (Biotek, Epoch model) at wavelength of 517 nm [23, 24]. For each sample concentration, the blank was discounted (containing the same amount of DMSO without DPPH). The tests were performed in triplicate for each concentration. The antioxidant activity was calculated using Equation 1.

$$\%\, inhibition = \frac{(\AE bs_{DPPH} - \AE BS_{sample})}{\AE bs_{DPPH}}\, x\ 100 \qquad (1)$$

Where: $Abs_{DPPH}$is the blank absorption (MeOH + DPPH);

$ABS_{sample}$is the absorption of the test solution + DPPH (t = 30 min).

Chemiluminescence Method

175 µL solution of each chalcona was added in every proficiently of a white flat bottom plate containing 96 wells (3912 Splabor). 25 µL of the solution of 1.13 10^{-3} molL^{-1} luminol (Sigma-Aldrich) was then added in DMSO (Sigma-Aldrich) and 25 µL of hydrogen peroxide (Synth) 5.10^{-4} molL^{-1}. The plate was incubated for 3 minutes at 30 °C in the dark. A reaction was started by adding the enzyme HRP (EC1.11.1.7; Sigma-Aldrich) at a final concentration of 0.2 IU/mL in phosphate buffer (pH 7.4). The final volume was 250 µL and the sample concentrations varied according to the solubility the same (the chalconas **3b, 3d** and **3K** were examined at concentrations from 160 to 2.5 µg mL^{-1}; chalcone **3c** was studied between 20 and 0.3 µg L^{-1}; and chalcone **3j** in concentrations between 10 and 0.3 µg L^{-1}). As maximum light emission control, 175 µL phosphate buffer/5 % DMSO was used to replace the sample. The emission luminescence of the chalcone in the absence of the enzyme was also measured and the value was deducted from the final value. From the addition of the enzyme, the light emission was monitored for 15 minutes at 30 °C. The measurement of luminance was plotted versus time and the area under the [23] curve calculated. These area values were used to calculate the inhibition percentage as shown in Equation 2.

$$\%\, inhibition = \frac{(\AE_{control} - \AE_{sample})}{\AE_{control}}\, x100 \qquad (2)$$

Where: $A_{sample}$is the area under curve of the blank (Buffer + H2O2+ Luminol + HPR) for 15 minutes;

$A_{sample}$is the area under the curve of the sample (sample + H_2O_2 + luminol + HPR) for 15 minutes.

Induction Period

The standard that determines the biodiesel oxidative stability is EN 14112 (European Standard) [20]. This methodology uses the Rancimat at 110 °C, where 3 grams of biodiesel (with and without antioxidants) were added in the Rancimat tube under flow of pure oxygen 10 Lh^{-1}. Antioxidants were added at a concentration of 2000 μgmL^{-1} [6]. The induction time experiments were performed in triplicate.

3 Results and discussion

Biodiesel

According to the ANP the minimum rate of conversion of esters acceptable for biodiesel production is 96.5% [20].

The ultrasound-assisted reaction yielded an average conversion of 98.5 % (n = 3 with relative standard deviation of 1 %), according to the European Standard 14103. The results show that the sonocatalysis process for biodiesel production is a promising technology because it usually obtains greater conversion compared to conventional processes at lower time rates [25]. Table 1 shows the results for the physicochemical analysis of sunflower oil biodiesel.

Table1. Physicochemical properties of sunflower oil biodiesel

Analysis	**Biodiesel of sunflower oil**	**Values stipulated by the ANP ***
Saponification (mg KOH g^{-1}sample)	190±4.3	Not specified
Acid value (mg KOHg^{-1}sample)	0.07±0.01	Up to 0.5
Iodine value (g $I_2$100 g^{-1} sample)	175±5.2	There is no limit
Viscosity (mm^2s^{-1})	4.2±0.5	Between 3.0 and 6.0.

* Value defined according to ANP 14/2012

The saponification value is essential for evaluating the quality of biodiesel, as it indicates mean molecular weight of fatty esters [26]. High saponification values indicate the formation of low molecular weight compounds that can originate from biodiesel oxidation. There is no limit to the saponification value for biodiesel by the ANP, however studies have reported that the saponification value sunflower materials are generally around 190 mg KOHg^{-1} sample [27]. Taking into consideration the standard deviation of the value found for the saponification index shown in Table 1 (190mg

KOH.g^{-1} sample), it can be seen that the value is very close to that found in literature, thus demonstrating the effectiveness of the methodology.

The acid value can be used to monitor the degradation of biodiesel, since higher acid values are due to higher concentrations of free fatty acids, decreasing the quality of biodiesel [28]. The biodiesel obtained from sunflower oil presented an acidity value of 0.07 mg KOHg^{-1} sample, lower than that found in the literature for the same matrix (0.15 mg sample KOHg-^{1} sample) [29] and within the limits established by the ANP (0.5 mg KOHg^{-1} sample) [20].

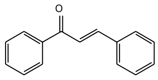
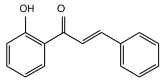
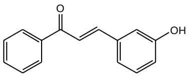
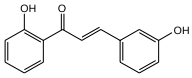
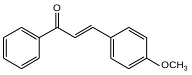
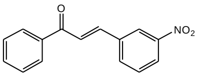
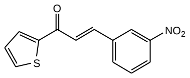
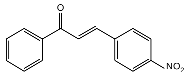
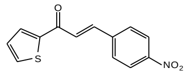
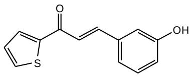
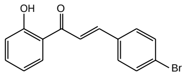
The degree of unsaturation of the fatty esters present in biodiesel is dependent on the triglyceride matrix used, directly affecting the iodine value and oxidative stability of biodiesel [30]. The iodine value of the sample analyzed in this research was 175 g $I_2$100 g^{-1} sample, however there are no limits established by legislation.

Note that high viscosity values indicate the presence of soap [31] that affects combustion and increases the level of unwanted emissions [32]. In the analysis performed in the sunflower biodiesel sample a viscosity of 4.2 mm^2 s^{-1} was observed thus demonstrating the application viability of this matrix for use as biodiesel, because this parameter is within the limits established by legislation (3 to 6mm^2s^{-1}) [20]. In accordance with previous studies by Ferrari et al. (2009) who found 4.5 mm^2 s^{-1} for sunflower biodiesel [33].

Chalcones

The synthesized chalcones were purified by recrystallization, presenting good yields (59-98 %). Table 2 shows the results obtained by mass spectrometry analysis (exact mass of the compound), melting point analysis (obtained in this work and that described in literature), and the yields of the reactions.

Table 2: Synthesized Chalcones

	Product	Exact Mass	M.p. Found(°C)	M.p. Lit (°C)	Yield (%)
3a	 (*E*)-chalcone $C_{15}H_{12}O$	208.089	52-54	52-54 [34]	80
3b	 (*E*)-1-(2-hydroxyphenyl)-3-phenylprop-2-en-1-one $C_{15}H_{12}O_2$	224.084	72-74	74-75 [35]	71
3c	 (*E*)-3-(3-hydroxyphenyl)-1-phenylprop-2-en-1-one $C_{15}H_{12}O_2$	224.084	147-150	150-153[36]	70
3d	 (*E*)-1-(2-hydroxyphenyl)-3-(3-hydroxyphenyl)prop-2-en-1-one $C_{15}H_{12}O_3$	240.079	149-151	150-153[39]	65
3e	 (*E*)-3-(4-methoxyphenyl)-1-phenylprop-2-en-1-one $C_{16}H_{14}O_2$	238.099	68-70	65-67 [38]	61
3f	 (*E*)-3-(3-nitrophenyl)-1-phenylprop-2-en-1-one $C_{15}H_{11}NO_3$	253.074	141-143	142-144[40]	81
3g	 (*E*)-3-(3-nitrophenyl)-1-(thiophen-2-yl)prop-2-en-1-one $C_{30}H_{22}N_2O_6$	259.03	133-134	131-132[42]	98
3h	 (*E*)-3-(4-nitrophenyl)-1-phenylprop-2-en-1-one $C_{30}H_{22}N_2O_6$	253.074	155-158	157-158[41]	89
3i	 (*E*)-3-(4-nitrophenyl)-1-(thiophen-2-yl)prop-2-en-1-one $C_{13}H_9NO_3S$	259.03	217-219	218-219[43]	92
3j	 (*E*)-3-(3-hydroxyphenyl)-1-(thiophen-2-yl)prop-2-en-1-one $C_{13}H_{10}O_2S$	230.04	142-144	141-143[37]	59
3k	 (*E*)-3-(4-bromophenyl)-1-(2-hydroxyphenyl)prop-2-en-1-one $C_{15}H_{11}BrO_2$	301.994	122-124	124-125[44]	86

As it can be seen in Table 2, experimental melting points of these chalconas are also reported. Each compound has its characteristic melting point, which varies depending on the structure and the intermolecular forces between them. Comparing the experimental melting points with those described in the literature, a similarity between them is observed in most cases. Furthermore, the melting point range is found experimentally has a narrow variation.

In the analysis by FT-IR, bands corresponding to carbonyl functional groups (C=O) were found in all chalcones, due to axial strain vibrations[45], with the band between 1820 and 1600 cm^{-1}[46], double bond (C=C), that presents the band at 1600 cm^{-1}[47], and the aromatic ring with a strong band between 1650 and 1450 cm^{-1} [48] . It appears with a slightly lower than expected frequency, as is conjugated with the phenyl group [49]. Figure 1 shows an infrared spectrum of chalcone **3a**.

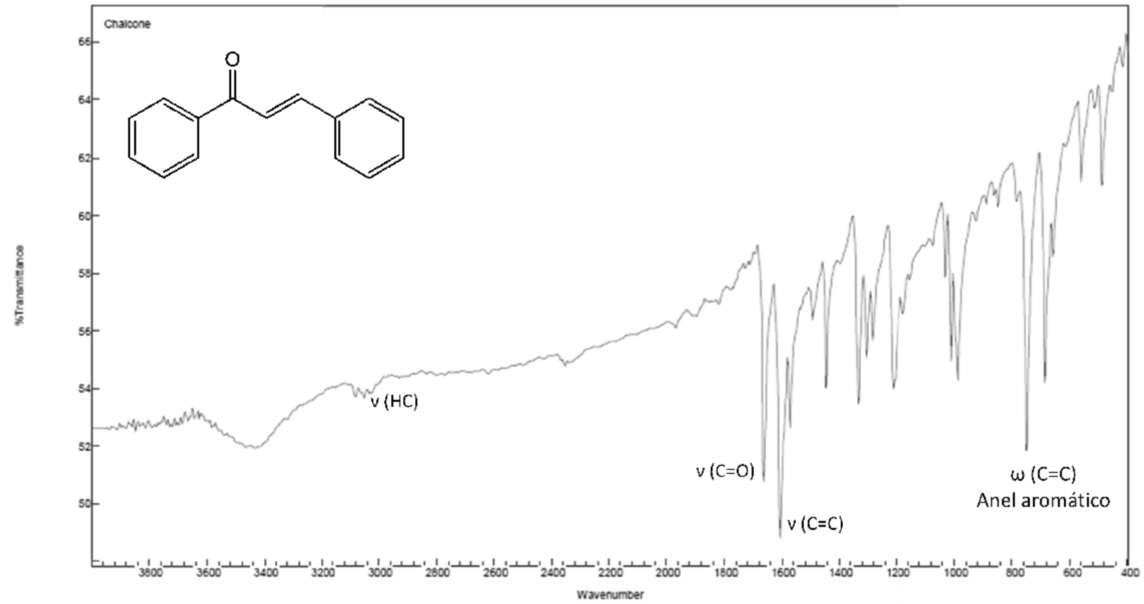


Figure 1 - Infrared spectrum with absorption bands of chalcone **3a**.

Thus, one may infer the formation of a chalcone, based on the detection of infrared functional groups present in the molecule and absence of the band of one of the starting reagents, the aldehyde [50].

As regards the analysis in a mass spectrometer, all molecular ions corresponded to the respective chalcones, and the fragments shown in the spectra corresponded to breaks in the molecules. In Figures 2, the spectrum of chalcone **3a** is presented.

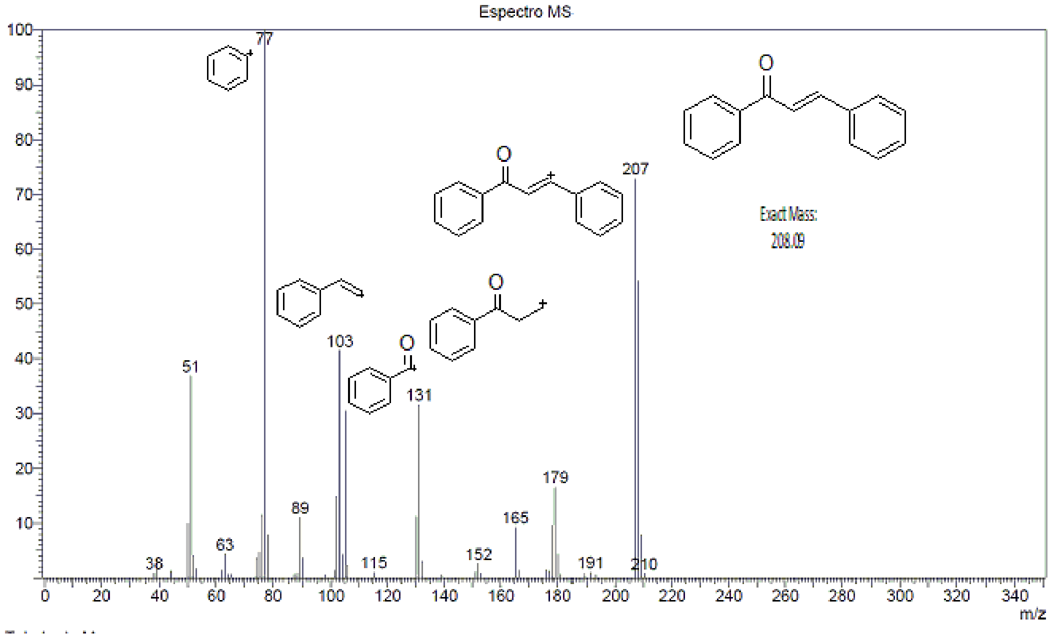


Figure 2 - Mass spectrum of the fragments with the chalcone **3a**

In figure 2, the molecular ion of m/z 208 corresponds to the mass of chalcone **2a**. The second peak is m/z 207, which shows a rapid loss of hydrogen during the electron impact. The peak at m/z 131 corresponds to the loss of an aromatic ring in the molecule, the same ring is identified as the spectrum base peak (m/z 77), being the most stable fragment [45]. The peaks at m/z 103 and 105 show the break between the carbons of the carbonyl and the unsaturation. The remaining spectra of chalcones from acetophenone follow the profile, differing by summing the substituents in the main structure.

The spectra of the other chalcones (available in the supplementary data) are similar to those shown in Figure 2, differing slightly in relation to intensity since peaks were produced by different substituted fragments. The FT-IR analysis, melting point and GC-MS were sufficient to characterize the synthesized chalcones.

Antioxidant studies

The synthesis of chalcones from different reagents and with another substituents has been extensively explored [51], because these compounds can confer diverse pharmacological activities to samples [52-54]. Thus, the choice of starting reagents studied in this work (Benzaldehyde, 3-hydroxybenzaldehyde, 4-bromobenzaldehyde, 2-nitrobenzaldehyde, 4-nitrobenzaldehyde and ketones: acetophenone, 2-hydroxyacetophenone, acetylthiophene) was based on important considerations regarding molecules. It is known that the resonance behavior of a chalcone with or without substituents can influence its antioxidant capacity, since hydroxyl stabilize electrons for having an electron-donor resonance activation effect [55]. Additionally, it is known that the acetylthiophene is known the sulfur has a pair of free electrons, thus giving aromaticity to the structure [56]. Phenolic compounds with nitro substituents are known to exhibit biological activity, since the nitro substituent has a strong electron-withdrawing induction effect [57]. The abovementioned pharmacological effects are influenced by the α, β-unsaturated chalcone structure [58].

Thus, the tests used to evaluate the antioxidant activity of chalcones were DPPH and Chemiluminescence. Against the DPPH, chalcones **3b**, **3d** and **3k** showed higher antioxidant activity (Figure 3). However, for the analyses carried out by chemiluminescence, it was observed that each than these, other samples stood out, with the **3b**, **3c**, **3d**, **3j** and 3**k** showing higher antioxidant activity than the another synthesized chalcones (Figure 4). The chemiluminescence assay is mostly used for being a sensitive assay due to high light emission produced by the H_2O / luminol / HRP system [59], something that can be confirmed with the results shown in Figure 4 since more molecules showed greater antioxidant activity at lower concentration levels in studies performed with DPPH.

Another study was carried out with different hydroxylated chalcones, the best results against the DPPH assay occurred when the chalcone had two or more hydroxyl groups, just as in the present study the chalcone **3b** presented [60, 61] activity, however these chalcones have higher polarity [61] not solubilizing in biodiesel. A study of chalcones similar to chalcone **3d** showed antioxidant activity when the molecule had thiophene and hydroxyl [63].

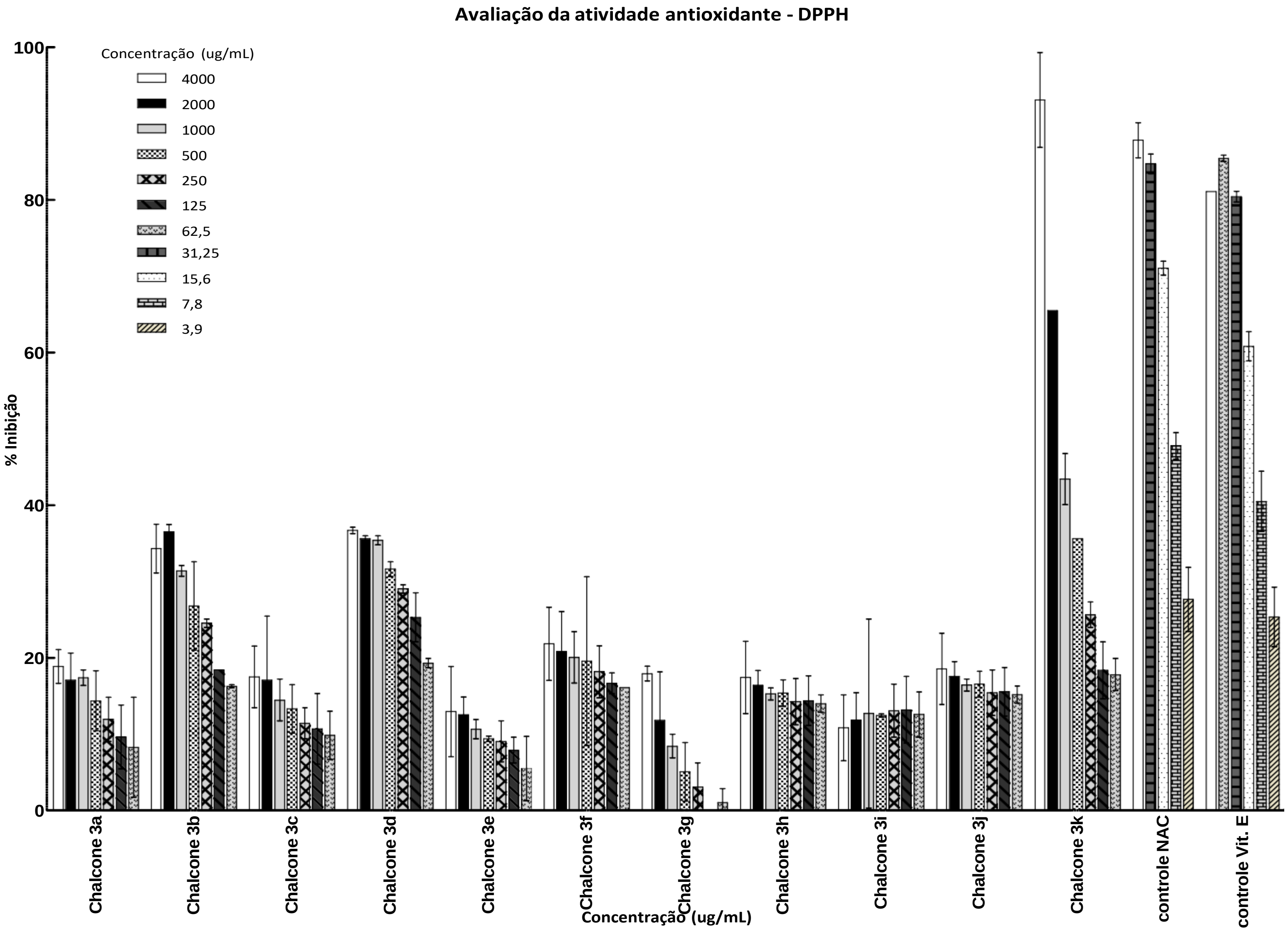


Figure 3 - Antioxidant Activity by DPPH

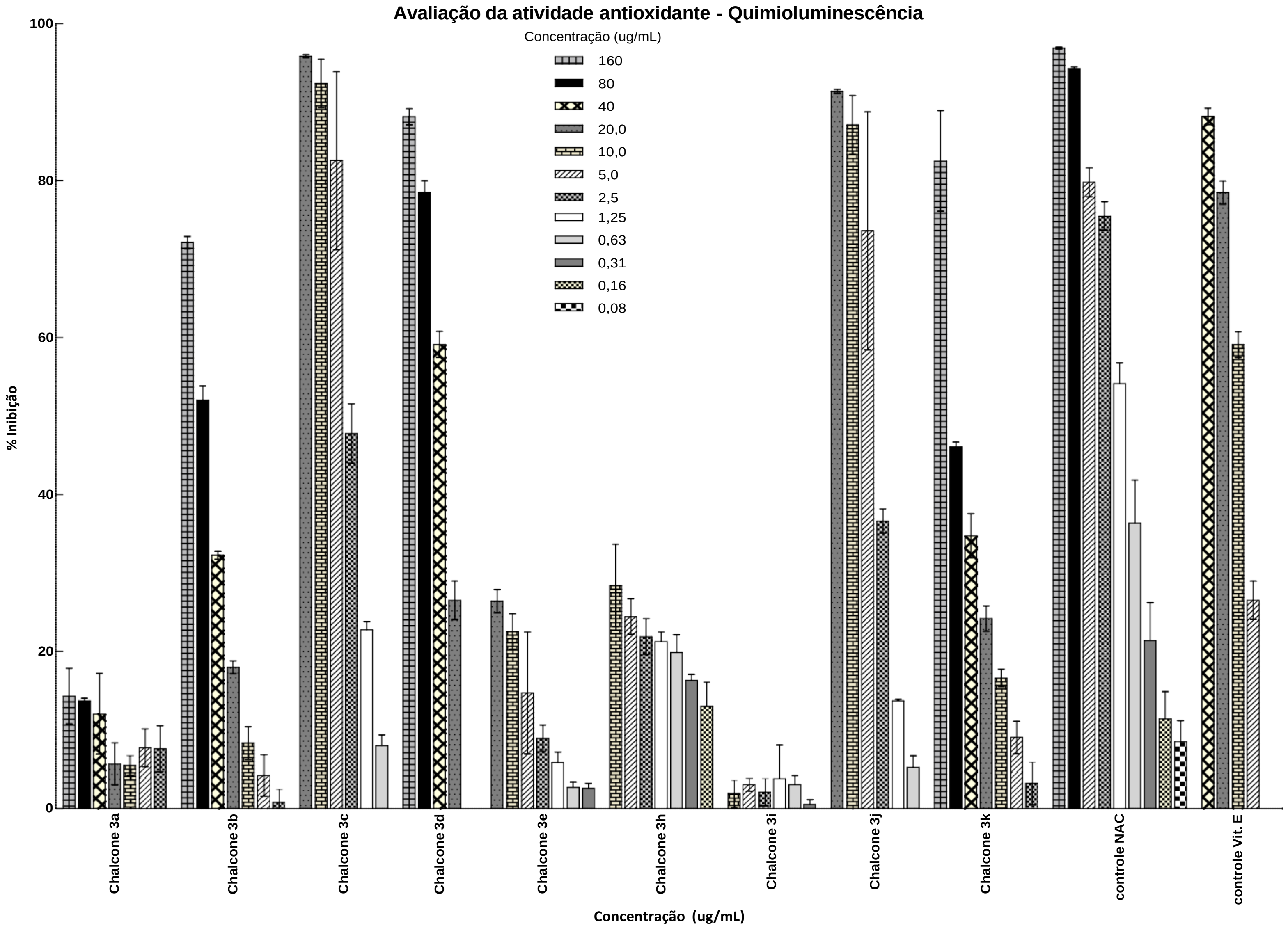


Figure 4 - Antioxidant Activity by Chemiluminescence

Most chalcones showed better percentage of antioxidant activity against the DPPH test in the concentration of 2000 µgmL^{-1}, thus this concentration was used for the induction period. With the *in vitro* assay results, the chalcones selected to perform the induction period were **3c, 3d, 3j** and **3k** because they have higher antioxidant activity and are soluble in biodiesel. As a reference standard, vitamin E was used. Table 4 shows the results of the induction period (oxidative stability time) of pure biodiesel and biodiesel with the addition of an antioxidant.

Table 4: Induction period pure biodiesel and different antioxidants*

Sample Composition	Period (hours)
Sunflower Biodiesel	2.52±0.08
Sunflower Biodiesel and chalcona **3c**	1.46±0.02
Sunflower Biodiesel and chalcona **3d**	1.28±0.13
Sunflower Biodiesel and chalcona **3j**	0.99±0.11
Sunflower Biodiesel and chalcona **3k**	0.79±0.04
Sunflower Biodiesel and vitamina E	3.01±0.10

* Chalcone **3b** and NAC whose results were not included in the table proved to be insoluble in biodiesel.

According to the data presented in Table 4 it can be seen that from 2.52 hours sunflower biodiesel oxidation starts and when vitamin E was added to it the start of the oxidation was delayed (3.01 hours) thus proving the antioxidant action of this vitamin. However, when different chalcones were added to biodiesel it can be observed that the induction period even faster. As stated previously, the chalcones studied here have ability to give and/or receive electrons, giving them the ability to oxidize and/or reduce. With the results shown in Figures 3 and 4, it can be inferred that chalcones have antioxidant activity, observations also corroborated by literature [13, 61, 64-66]. However, when they were added to the biodiesel they had pro-oxidant activity. This antagonistic behavior can be explained by the high complexity of biodiesel that can have different redox pairs, behaving like a rich redox system Dias et al. [67] investigated the action of ethanethiol (as oxidant or antioxidant) in a natural redox system (wine) and noted that the compound itself acts as an antioxidant, but when added to the sample, it acts as oxidant. The authors explain that the redox capacity of ethanethiol in complex systems makes it behave differently depending on the medium that it is present in. The same relationship can be made in our studies, since chalcones have redox properties [68] and biodiesel is a high complexity sample [69, 70]. Rahman et al. evaluated the redox potential of different chalcones by cyclic voltammetry and noted that the substitutions of the same are directly related to its reduction potential. They noted that Br and NO_2 substituents confer high reduction potential (-1.74 V and -1.26, respectively) to chalcone demonstrating that these molecules can act as oxidants. In the present studies, the chalcone **3k** (3-(4-bromophenyl)-1-(2-hydroxyphenyl)prop-2-en-1-one) showed a higher effect as a pro-oxidant in relation to chalcone **3j** (3-(4--nitrophenyl)-1-(thiophen-2-yl)prop-2-en-1-one) and this may be due to greater oxidizing effect of Br (Region B) compared to NO_2 (Region B) [68]. It is worth noting that chalcone **3j** also has a heteroatom S in the ring (Region A) and knowing that the compound S has the ability to oxidize and/or reduce [67], it can also enhance the oxidizing effect of the molecule. Comparing the structure of chalcones **3f** (1-(2-hydroxyphenyl)-3-(3-hydroxyphenyl) prop-2-en-1-one) and **3d** (3- (3-hydroxyphenyl)-1-(thiophen-2-yl) prop-2-en-1-one), what differs between the two is that the second grouping does not have the NO_2 and this gives it less effect against oxidative chalcone **3j**. One can also infer that chalcone **3c** showed less pro-oxidant effect because it has not heteroatoms or Br groupings and presents NO_2 (Region B).

With the explanations discussed herein, despite the chalcones presenting antioxidant potential, it can be inferred that the chalcones **3k**, **3j**, **3c** and **3d** showed higher pro-oxidant action (respectively) compared to biodiesel since their molecules consist of elements which have redox capacity. This antagonistic behavior can be explained because of the ability of chalcones to be found in reduced and oxidized forms as well as because of the complexity of the real samples investigated. There are several redox pairs that can promote the oxidation or reduction of chalcones.

4 Conclusions

From the analysis of the results, it can be inferred that the matrix used to obtain biodiesel is promising, because the physicochemical parameters evaluated showed results within the limits established by the ANP. In addition, the synthesized chalcones also showed antioxidant activity against DPPH and Chemiluminescence tests, however because biodiesel is a complex matrix such chalcones showed no antioxidant activity when added in biodiesel. Given the synthetic versatility and possibility of chemical variation of the chalcones, the possibility to investigate these molecules in the application in the productive sectoris amplified, since the present study as well as recent research show the activity of various chalcones as antioxidant molecules.

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Supplementary Data

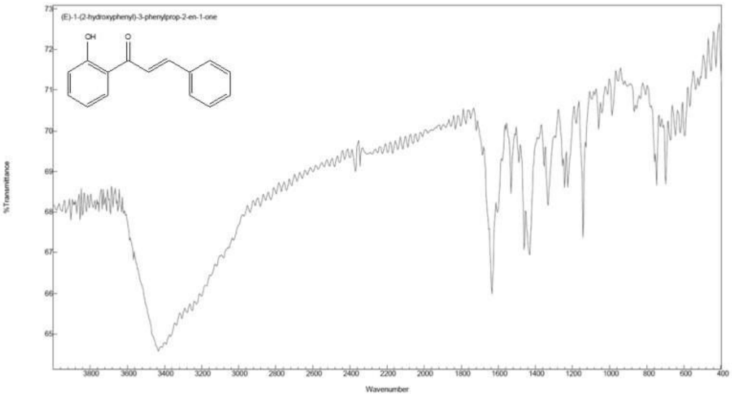

Figure 1a - Infrared spectrum with absorption bands of chalcone **3b**

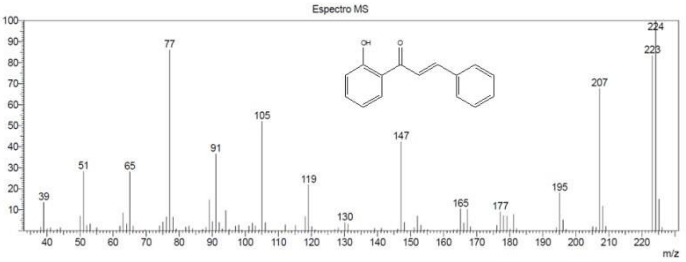

Figure 2a - Mass spectrum of the fragments with the chalcone **3b**

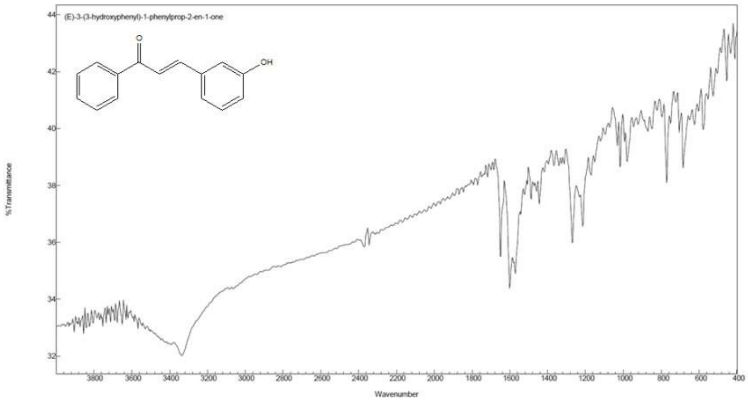

Figure 1b - Infrared spectrum with absorption bands of chalcone **3c**

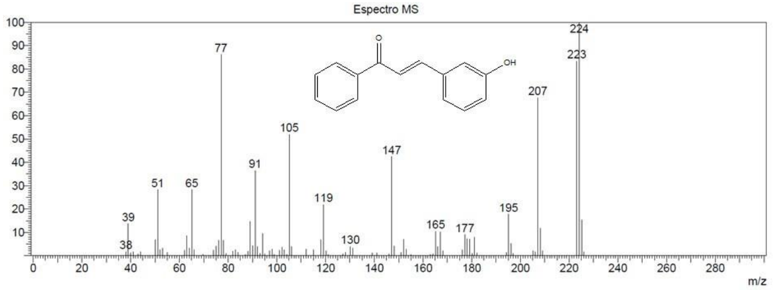

Figure 2b - Mass spectrum of the fragments with the chalcone **3c**

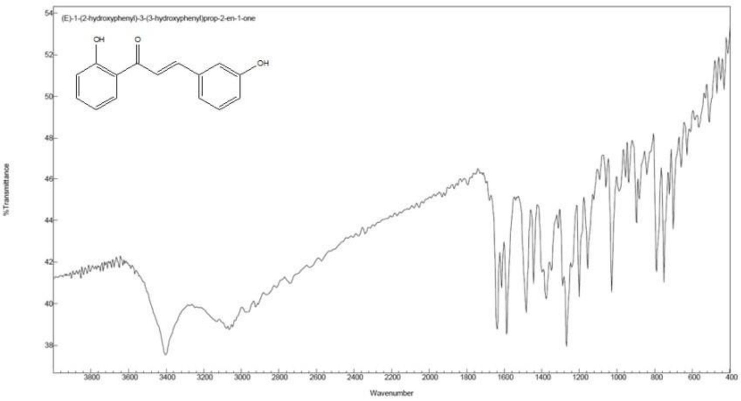

Figure 1c - Infrared spectrum with absorption bands of chalcone **3d**

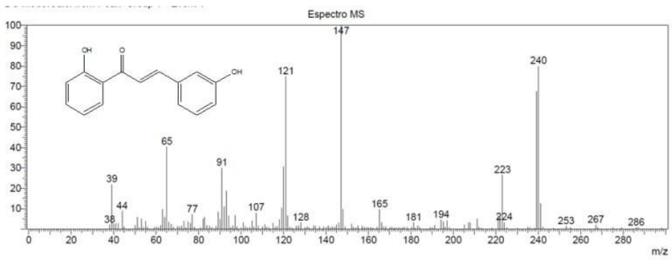

Figure 2c - Mass spectrum of the fragments with the chalcone **3d**

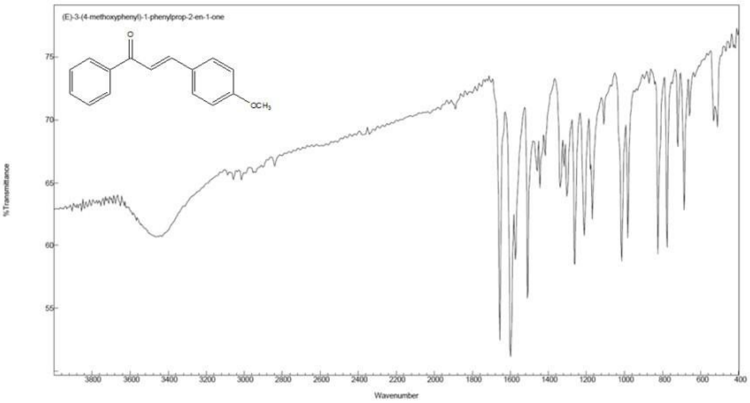

Figure 1d - Infrared spectrum with absorption bands of chalcone **3e**

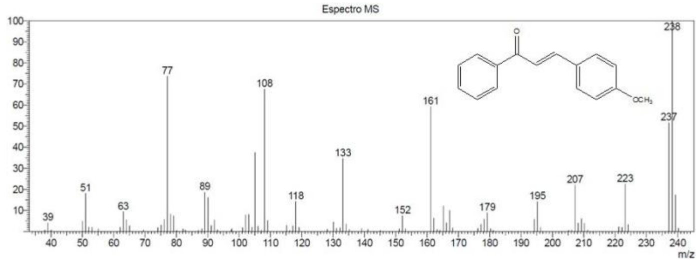

Figure 2d - Mass spectrum of the fragments with the chalcone **3e**

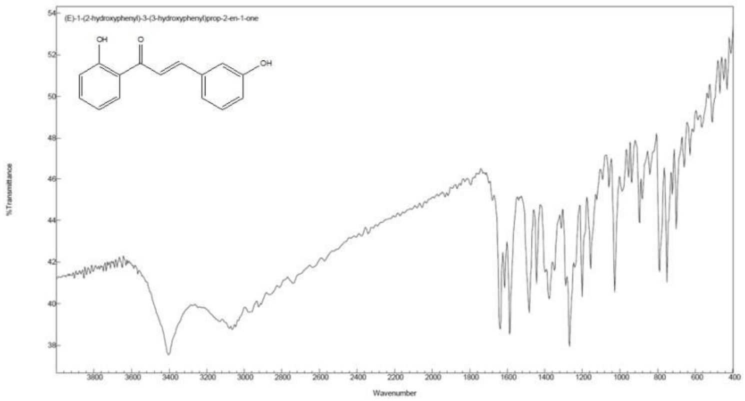

Figure 1e -Infrared spectrum with absorption bands of chalcone **3f**

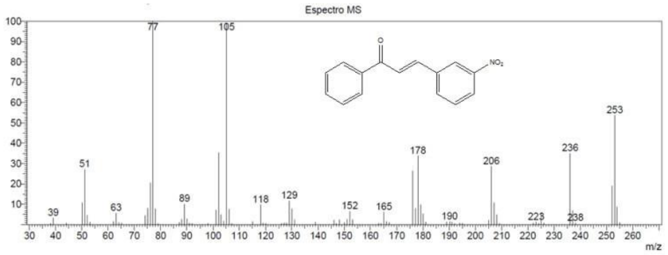

Figure 2e - Mass spectrum of the fragments with the chalcone **3f**

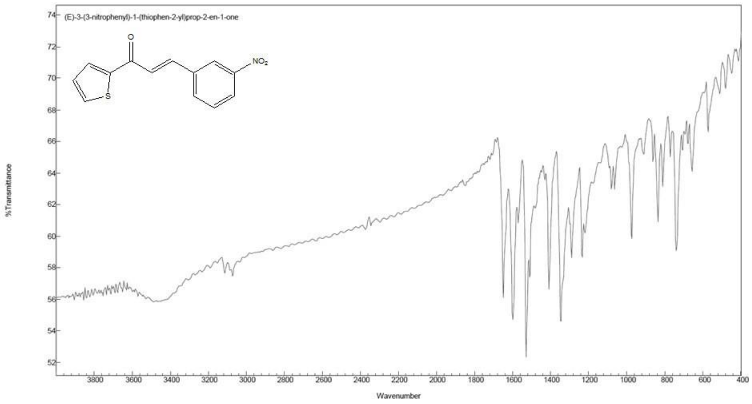

Figure 1f - Infrared spectrum with absorption bands of chalcone **3g**

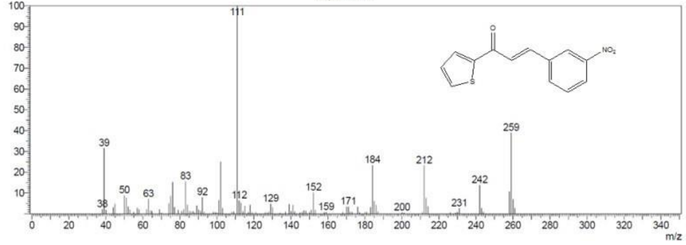

Figure 2f - Mass spectrum of the fragments with the chalcone **3g**

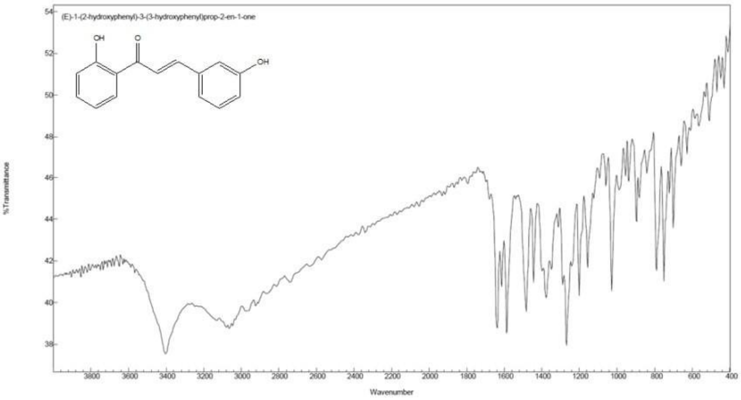

Figure 1g - Infrared spectrum with absorption bands of chalcone **3h**

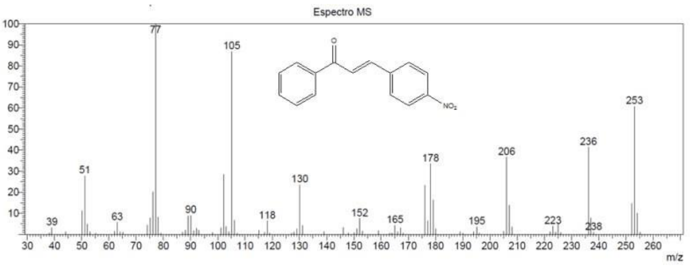

Figure 2g - Mass spectrum of the fragments with the chalcone **3h**

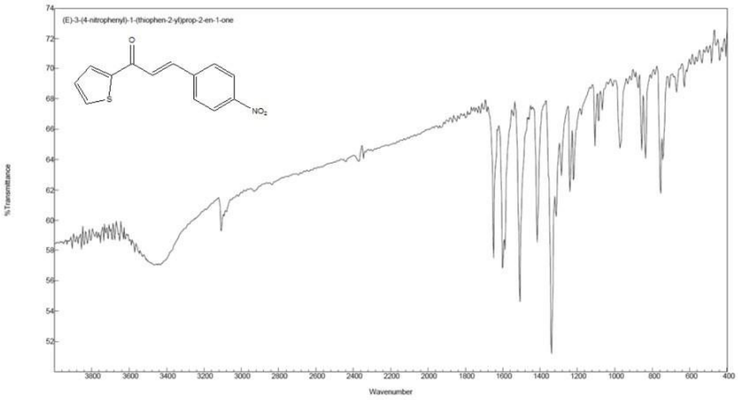

Figure 1h - Infrared spectrum with absorption bands of chalcone **3i**

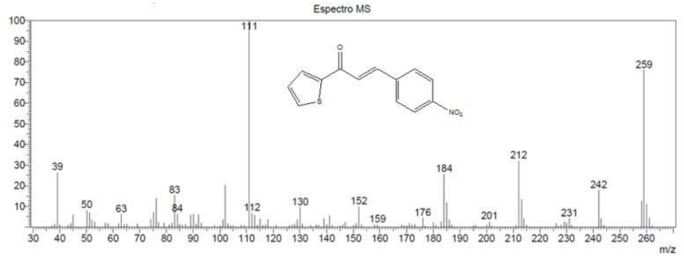

Figure 2h - Mass spectrum of the fragments with the chalcone **3i**

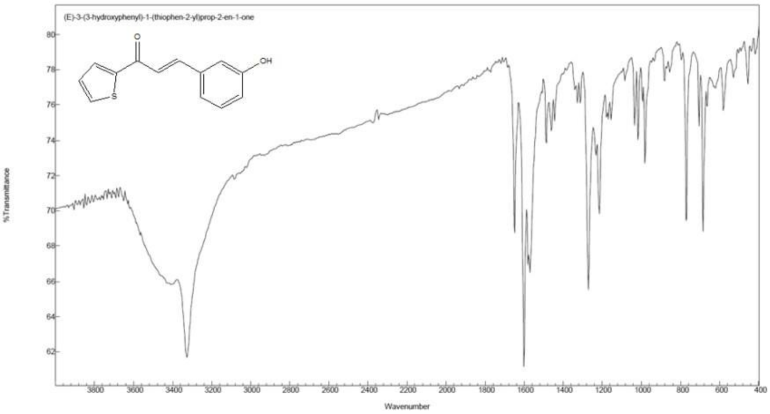

Figure 1i - Infrared spectrum with absorption bands of chalcone **3j**

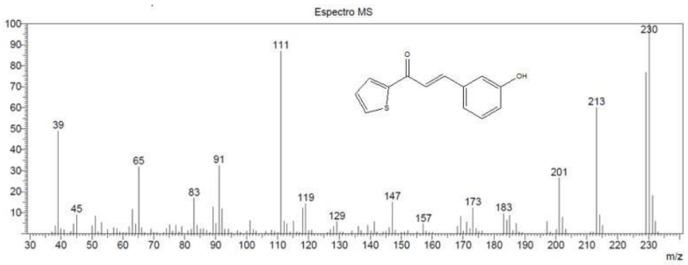

Figure 2i - Mass spectrum of the fragments with the chalcone **3j**

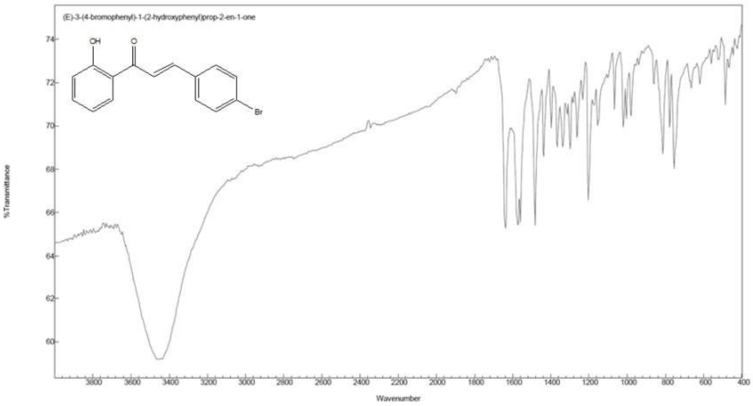

Figure 1j - Infrared spectrum with absorption bands of chalcone **3k.**

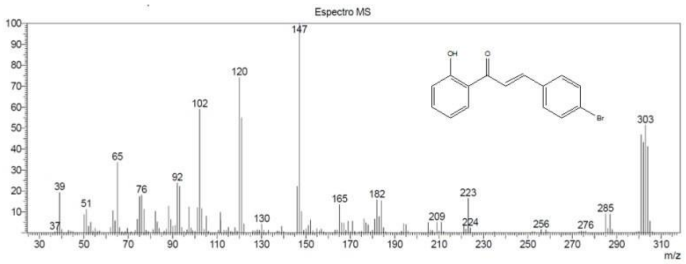

Figure 2j - Mass spectrum of the fragments with the chalcone **3k**

PARTE IV

6 CONCLUSÃO GERAL

O presente estudo avaliou diferentes condições para a síntese de biodiesel a partir de óleo refinado de girassol utilizando uma sonda de ultrassom. Pode-se inferir que a utilização do ultrassom como meio promotor para síntese se mostrou eficiente uma vez com 0.15 g de KOH, 10 minutos de reação com 30 % de amplitude, obteve-se 99 % de conversão em biodiesel estando em acordo com a legislação vigente. Através do monitoramento e avaliação de propriedades físico-químicas deste biodiesel pode-se afirmar que somente após 49 dias de armazenamento o produto deve ser descartado, pois excede o limite de viscosidade estabelecido pela ANP. Entretanto, o índice de acidez excedeu o limite preconizado somente após 63dias. A cromatografia se mostrou uma ferramenta importante, pois através da análise do perfil ácido graxo pode-se confirmar a degradação dos ácidos graxos insaturados, demonstrando desta forma a oxidação do biodiesel com o armazenamento.

Adicionalmente, as chalconas sintetizadas também apresentaram atividade antioxidante frentes aos testes de DPPH e Quimioluminescência, entretanto pelo fato de o biodiesel ser uma matriz complexa estas chalconas não apresentaram atividade antioxidante quando adicionadas no biodiesel. Tendo em vista a versatilidade sintética e possibilidade de variação química das chalconas, se amplia a possibilidade na investigação destas moléculas na aplicação no setor produtivo, uma vez que o presente estudo assim como pesquisas recentes, revela a atividade de varias chalconas como moléculas antioxidantes.

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