

UNIVERSIDADE FEDERAL DA PARAÍBA
CENTRO DE TECNOLOGIA
PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIA E
TECNOLOGIA DE ALIMENTOS

JAQUELINE AZEVEDO NASCIMENTO BATISTA

**POTENCIAL ANTIOXIDANTE DOS EXTRATOS DE
MORINGA OLEIFERA LAMARK EM SISTEMAS LIPÍDICOS
DE BAIXA ESTABILIDADE OXIDATIVA**

JOÃO PESSOA – PB

2013

JACQUELINE AZEVEDO NASCIMENTO BATISTA

**POTENCIAL ANTIOXIDANTE DOS EXTRATOS DE
MORINGA OLEIFERA LAMARK EM SISTEMAS LIPÍDICOS
DE BAIXA ESTABILIDADE OXIDATIVA**

JOÃO PESSOA – PB

2013

JACQUELINE AZEVEDO NASCIMENTO BATISTA

**POTENCIAL ANTIOXIDANTE DOS EXTRATOS DE
MORINGA OLEIFERA LAMARK EM SISTEMAS LIPÍDICOS
DE BAIXA ESTABILIDADE OXIDATIVA**

Tese apresentada ao Programa de Pós-Graduação em Ciência e Tecnologia de Alimento, Centro de Tecnologia, Universidade Federal da Paraíba, em cumprimento aos requisitos para obtenção do título de Doutor em Ciência e Tecnologia de Alimentos.

Orientador: Prof. Dr. Antônio Gouveia de Souza

JOÃO PESSOA – PB

2013

B333p Batista, Jaqueline Azevedo Nascimento.

Potencial antioxidante dos extratos de Moringa oleífera Lamark em sistemas lipídicos de baixa estabilidade oxidativa / Jaqueline Azevedo Nascimento Batista.- João Pessoa, 2013.

95f. : il.

Orientador: Antônio Gouveia de Souza

Tese (Doutorado) – UFPB/CT

JAQUELINE AZEVEDO NASCIMENTO BATISTA

**POTENCIAL ANTIOXIDANTE DOS EXTRATOS DE *MORINGA OLEIFERA*
LAMARK EM SISTEMAS LIPÍDICOS DE BAIXA ESTABILIDADE OXIDATIVA**

Tese **APROVADA** em 25/02/2013.

BANCA EXAMINADORA

Prof. Dr. Antônio Gouveia de Souza – DQ/UFPB
Coordenador da Banca Examinadora

Profa. Dra. Marciane Magnani – DEA/UFPB
Examinador Interno

Profa. Dra. Rita de Cássia Ramos do Egypto Queiroga – DN/UFPB
Examinador Interno

Prof. Dr. Raul Rosenhaim – DEQ/UFPB
Examinador Externo

Profa. Dra. Marta Maria da Conceição – CES/UFCG
Examinador Externo

*À Deus, fonte de toda minha força e
inspiração.*

*A Renê e Ramon, meus amados filhos, que me
alegram e motivam para aceitar os desafios.*

*Aos meus pais Elimar e Fátima que sempre
investiram e confiaram em mim.*

*Ao meu amado esposo Renato pelo incentivo e
paciência.*

*A minhas irmãs Janine, Alcerita e Alessandra,
companheiras e conselheiras.*

*Aos meus avos Alcebíades e Aurita, sempre
presentes, dando lições de amor e sabedoria.*

Dedico.

AGRADECIMENTO

Agradeço a **Deus**, princípio de todas as coisas, luz inspiradora de todas as horas, alento nos momentos mais difíceis, esperança de importantes realizações e motivo de louvor e exaltação.

Ao **Prof. Dr. Antônio Gouveia de Souza**, pela receptividade, confiança, incentivo, amizade e pela orientação cercada de seriedade e de rigor científico, que foram imprescindíveis tanto para realização deste trabalho como para construção de um valioso conhecimento, subsídio importante na atuação profissional. Serei eternamente grata...

A **Profa. Dra. Antônio Lúcia de Souza** pela co-orientação deste trabalho, capacidade e competência, além de sua disposição ímpar em ajudar.

A **Profa. Dra. Neide Queiroz**, pela competência, ensinamentos e disponibilização.

A **Profa. Dra. Marciane Magnani**, pela sua importante contribuição na correção dos artigos, orientações e amizade.

A **Profa. Dra. Alessandra Azevedo do Nascimento**, minha querida irmã, pela preciosa contribuição nos ensaios de avaliação da bioatividade.

As amigas e companheiras de todas as horas, **Kassandra, Poliana e Alline** pela amizade e importante ajuda na realização deste trabalho.

As amigas **Ângela, Teta, Andréa e Maristela** pela valorosa contribuição, competência e amizade.

Aos amigos de pós-graduação do Laboratório de Combustíveis e Materiais – LACOM contemporâneos durante o período do meu doutoramento e a todos os amigos de iniciação científica e voluntários pela ajuda e amizade.

A amiga **Ruth Gomes Gadelha**, nestes quase dez anos de convivência desde o mestrado até o Doutorado, sempre disposta a ajudar e repassar seus conhecimentos.

A **Maria Lucia Braga de Carvalho**, pelo excelente trabalho realizado no laboratório de análises térmicas.

A **Humberto Bandeira** (Secretario da Pós-graduação) pelo seu trabalho desempenhado com dedicação na secretaria e pela atenção e amizade em momentos difíceis.

A **Coordenação, funcionários** e a todos os **professores** que compõem o Programa de Pós-Graduação em Ciência e Tecnologia de Alimentos, pela competência, seriedade, honestidade, incentivo e apoio.

A todos que fazem o LACOM;

MUITO OBRIGADA.

“A persistência é o menor caminho do êxito”

Charles Chaplin

RESUMO

A *Moringa oleífera* Lamark é uma planta perene e tolerante a seca cultivada como planta ornamental e medicinal. No presente estudo, os extratos etanólicos das folhas, flores, cascas das vagens e sementes desta planta foram avaliados quanto a sua proteção contra a oxidação dos óleos de soja e de peixe. Para isso, foram determinados o teor de fenólicos extraíveis totais (FET) através do método Folin-Ciocalteu e o potencial antioxidante, empregando-se os métodos de sequestro do radical DPPH (RSA-DPPH), poder de redução do ferro (FRAP) e sistema β -caroteno/ácido linoléico. A estabilidade térmica dos referidos extratos foi avaliada através da análise térmica (TG/DTA) e o efeito antioxidante investigado nos óleos de soja e de peixe através dos métodos Rancimat, calorimetria exploratória diferencial pressurizada (P-DSC) e teste de estocagem acelerada em estufa. A avaliação preliminar da toxicidade dos extratos foi realizada utilizando larvas de *Artemia salina*. Os teores FET nos extratos das folhas, flores, casca das vagens e sementes foram ($53,69 \pm 1,00$); ($45,85 \pm 1,71$); ($41,75 \pm 3,35$) e ($8,06 \pm 0,47$) mg GAE/g de extrato, respectivamente. Nos ensaios de determinação da capacidade antioxidante, o extrato das folhas apresentou melhor RSA-DPPH, FRAP e também maior atividade antioxidante no sistema β -caroteno/ácido linoleico. Na avaliação da estabilidade térmica, o extrato das folhas também se mostrou mais estável do que os demais extratos. Nos ensaios de avaliação do efeito antioxidante dos extratos, verificou-se que o extrato das folhas foi o mais eficiente em ambos os óleos nos métodos Rancimat e PDSC, com efeito protetor equivalente ao antioxidante sintético BHT no óleo de soja na técnica Rancimat. No teste de estocagem acelerada em estufa, foi verificado que após 16 dias de armazenamento o extrato das folhas foi o mais eficaz na inibição da formação dos produtos da oxidação primária e secundária em ambos os óleos, sendo mais eficiente do que o BHT e TBHQ no óleo de peixe. Os resultados da avaliação preliminar de toxicidade indicaram uma baixa toxicidade dos extratos, sugerindo que os mesmos podem ser consumidos sem prejuízo à saúde. Estes resultados sugerem que o extrato etanólico das folhas de moringa possui efeito protetor eficiente quando aplicado a sistemas lipídicos de baixa estabilidade oxidativa, podendo vir a ser uma fonte alternativa de potencial aplicação na indústria de óleos e gorduras.

Palavras-chaves: *Moringa oleífera* Lam., óleo de peixe, óleo de soja, estabilidade oxidativa.

ABSTRACT

The species *Moringa oleifera* Lamarck is a perennial and drought-tolerant plant cultivated as ornamental and medicinal plant. In this study, ethanolic extracts of leaves, flowers, and seed pods of this plant were evaluated for their protective effect against soybean and fish oil oxidation. For this, total extractable phenolics (TEP) were determined by Folin-Ciocalteu method, as well as the antioxidant potential, using the DPPH radical scavenging activity method (RSA-DPPH), iron power reduction (FRAP) and β -carotene/ linoleic acid system. The thermal stability of extracts was measured by thermal analysis (TG / DTA) and the antioxidant effect on soybean and fish oils was investigated by the Rancimat method, pressurized differential scanning calorimetry (P-DSC) and accelerated storage test. The preliminary toxicity assessment of extracts was performed using *Artemia salina* larvae. The TEP levels in extracts of leaves, flowers and seed pods were (53.69 ± 1.00), (45.85 ± 1.71), (41.75 ± 3.35) and (8.06 ± 0.47) mg GAE / g of extract, respectively. In tests to determine the antioxidant capacity, extract from leaves showed better RSA-DPPH and FRAP and also higher antioxidant activity in the β -carotene/ linoleic acid system. In the thermal stability evaluation, extract from leaves was also more stable than the other extracts. In tests to evaluate the antioxidant effect of extracts, it was found that extract from leaves was more efficient in both oils in Rancimat and P-DSC methods with protective effect equivalent to synthetic antioxidant BHT in soybean oil using the Rancimat method. In the accelerated storage test, it was found that after 16 days of storage, extract from leaves was the most effective in inhibiting the formation of oxidation products in both oils, being more effective than BHT and TBHQ in fish oil. The results of the preliminary toxicity assessment showed low toxicity, suggesting that the extracts may be consumed without health damage. These results suggest that the ethanolic extract of moringa leaves has efficient protective effect when applied to lipid systems of low oxidative stability and could be an alternative source of potential application in the oils and fats industry.

Keywords: *Moringa oleifera* Lam., fish oil, soybean oil, oxidative stability.

LISTA DE ILUSTRAÇÕES

Figura 1	<i>Moringa oleífera</i> Lam. com suas folhas, flores, vagens e sementes	18
Figura 2	Mecanismo de ação dos antioxidantes primários	27
Figura 3	Estrutura fenólica de antioxidantes sintéticos	29
Figura 4	Estrutura química dos ácidos benzoicos	31
Figura 5	Estrutura química dos principais ácidos cinâmicos	31
Figura 6	Estrutura química das cumarinas	31
Figura 7	Estrutura química dos flavonoides	32

LISTA DE ABREVIATURAS E SIGLAS

AA	Ácido araquidônico
BHA	Butil hidroxianisol
BHT	Butil hidroxitolueno
BST	Brine shrimp lethality test
CD	Dienos conjugados
CL50	Concentração letal média
DHA	Ácido docosahexaenóico
DPPH	2,2-difenil-1-picrilidrazil
DSC	Calorimetria exploratória diferencial
DTA	Análise Térmica Diferencial
EDTA	Ácido etileno diamino tetra acético
EF	Extrato etanólicos das flores
EL	Extrato etanólicos das folhas
EP	Extrato etanólicos das cascas das vagens
EPA	Ácido eicosapentaenoico
ES	Extrato etanólicos das sementes
FRAP	Poder redutor do ferro
FTIR	Infravermelho com transformada de Fourier
GAE	Equivalente de ácido gálico
IA	Índice de anisidina
OIT	Tempo de indução oxidativa
OSI	Índice de estabilidade oxidativa
P-DSC	Calorimetria Exploratória Diferencial Pressurizada
PG	Propil galato
PI	Período de indução
TBARS	Ácidos tiobarbitúrico-reactivos
TBHQ	<i>terc</i> -butilhidroquinona
TEP	Fenólicos extraíveis totais
TG	Termogravimetria

SUMÁRIO

1 INTRODUÇÃO.....	15
1.1 OBJETIVO GERAL.....	17
1.2 OBJETIVOS ESPECÍFICOS.....	17
2 REVISÃO DE LITERATURA.....	18
2.1 <i>MORINGA OLEIFERA</i> LAM.....	18
2.2 ÓLEOS DE SOJA E DE PEIXE.....	21
2.3 OXIDAÇÃO LIPÍDICA.....	23
2.4 AVALIAÇÃO DA ESTABILIDADE OXIDATIVA DE ÓLEOS.....	24
2.4.1 Teste de estocagem acelerada em estufa ou “Schaal Oven Test”.....	25
2.4.2 Rancimat.....	25
2.4.3 Calorimetria exploratória diferencial pressurizada (P – DSC).....	26
2.5 ANTIOXIDANTES.....	27
2.5.1 Antioxidantes sintéticos.....	28
2.5.2 Antioxidantes naturais.....	30
2.6 BIOATIVIDADE E TOXICIDADE FRENTE À <i>ARTEMIA SALINA</i>	32
3 MATERIAL E MÉTODOS.....	34
3.1 MATERIAL.....	34
3.2 AQUISIÇÃO E PREPARO DAS AMOSTRAS.....	34
3.3 OBTENÇÃO DOS EXTRATOS.....	34
3.4 DETERMINAÇÃO DE FENÓLICOS EXTRAÍVEIS TOTAIS.....	35
3.5 ESTUDO TÉRMICO DOS EXTRATOS.....	35
3.6 DETERMINAÇÃO DA ATIVIDADE ANTIOXIDANTE.....	35
3.6.1 Atividade sequestrante do radical livre DPPH.....	35
3.6.2 Capacidade antioxidante pelo método de redução do ferro (FRAP).....	36
3.6.3 Auto-oxidação do sistema β -caroteno/ácido linoleico.....	36
3.7 BIOENSAIO COM <i>ARTEMIA SALINA</i> LEACH.....	37
3.8 DETERMINAÇÃO DA ESTABILIDADE OXIDATIVA.....	38
3.8.1 Preparação das amostras.....	38
3.8.2 Método Rancimat.....	38
3.8.3 Calorimetria exploratória diferencial pressurizada (P – DSC).....	38
3.8.4 Teste de estocagem acelerada em estufa.....	39

3.8.4.1 Índice de peróxido.....	39
3.8.4.2 Índice de <i>p</i> -anisidina.....	40
3.8.4.3 Determinação do valor de dienos conjugados (CD).....	40
3.8.4.4 Infravermelho com transformada de Fourier (FTIR).....	41
3.8 ANÁLISES ESTATÍSTICAS.....	41
REFERÊNCIAS	42
4 RESULTADOS	50
ARTIGO 1 - Ethanolic extracts of <i>Moringa oleifera</i> Lam.: Evaluation of its potential as an antioxidant additive for fish oil.....	51
ARTIGO 2 - Ethanolics extracts of moringa: antioxidant effect in soybean oil by P-DSC and Rancimat	57
ARTIGO 3 - Oxidative stability of fish oil supplemented with ethanolic extracts of <i>Moringa oleifera</i> Lam. compared with synthetic antioxidants during accelerated test.....	65
ARTIGO 4 - Antioxidant effect of <i>Moringa oleifera</i> Lamark extracts in soybean oil during accelerated storage test.....	76
ARTIGO 5 - Study of antioxidant activity and preliminary bioactivity determination of ethanolic extracts of <i>Moringa oleifera</i> Lam.....	86
5 CONSIDERAÇÕES FINAIS	95

1 INTRODUÇÃO

A *Moringa oleifera* Lamark, é uma planta originária do noroeste da Índia (BEZERRA; MOMENTÉ; MEDEIROS FILHO, 2004) que têm sido referida como fonte de proteínas, β -caroteno, vitamina C, cálcio e potássio, além de compostos fenólicos e carotenóides. A moringa apresenta uma combinação rica e rara de zeatina, quercetina, β - sitosterol, ácido cafeoilquínico e kaempferol, o que atribui também à planta uma potencial atividade antioxidante (ANWAR et al., 2007; BARRETO et al., 2009; IQBAL; BHANGER, 2006; LAKO et al., 2007; REDDY; UROOJ; KUMAR, 2005; SIDDHURAJU; BECKER, 2003). É perfeitamente adaptável ao clima do Nordeste brasileiro, pois, apresenta bom crescimento, mesmo quando submetido a condições de seca e elevadas temperaturas, características desta região (SILVA, et al., 2012).

A importância dos lipídios na nutrição e desenvolvimento humano é reconhecida há muitas décadas, pois os ácidos graxos são constituintes estruturais das membranas celulares, cumprem funções energéticas e de reservas metabólicas, além de formarem hormônios e sais biliares. O homem é capaz de sintetizar todos os ácidos graxos, exceto os essenciais ácido linoléico (ω -6) e o ácido linolênico (ω -3), que devem ser fornecidos pela dieta (KEY et al., 2012). Estudos apontam que a ingestão regular de ácidos graxos poli-insaturados traz benefícios para a saúde humana, prevenindo enfermidades cardiovasculares, câncer de cólon e doenças imunológicas, além de favorecerem o desenvolvimento cerebral e da retina (BALK et al., 2006; KNOTHE, 2008; NEMETS et al., 2006; PIVIK et al., 2009).

O óleo de soja é composto por 12 a 15% de ácidos graxos saturados (na maior parte o palmítico) e 85 a 88% de ácidos graxos insaturados, com destaque para oléico, linoleico e linolênico (RIBEIRO et al., 2009). No Brasil, o óleo de soja responde por cerca de 80,6 % do consumo de óleos vegetais, sendo utilizado preferencialmente como óleo de fritura (USDA, 2011).

O óleo de peixe apresenta um alto teor dos ácidos graxos poli-insaturados, contendo cerca de 18% de ácido eicosapentaenóico (EPA) e 12% de ácido docosahexaenóico (DHA) (TAMJIDI; NASIRPOUR; SHAHEDI, 2012). Tal composição lhe confere uma notável importância, já que é um dos únicos alimentos que aparece como fonte expressiva destes ácidos graxos (BALK et al., 2006).

A presença de alto teor de ácidos graxos poli-insaturados torna esses óleos extremamente susceptíveis a processos oxidativos durante o processamento e estocagem. A

oxidação lipídica compromete a integridade das ligações duplas desses ácidos graxos, alterando sua concentração e funcionalidade, e representando risco à saúde humana, visto que o consumo de produtos da peroxidação lipídica pode induzir a carcinogênese (HALVORSEN; BLOMHOFF, 2011).

Para manter a qualidade e prolongar o tempo de vida útil dos óleos, o processo de oxidação lipídica é usualmente prevenido através do emprego de antioxidantes sintéticos como butilhidroxianisol (BHA), butilhidroxitolueno (BHT) e *terc*-butilhidroquinona (TBHQ). Estas substâncias, entretanto, apesar de serem bastante eficazes como antioxidantes, têm seu uso restrito devido efeitos tóxicos e cancerígenos em seres humanos (SUN-WATERHOUSE; THAKORLAL; ZHOU, 2011). No Brasil, a concentração máxima de TBHQ e BHT permitida é de 200 mg.kg⁻¹ e 100 mg.kg⁻¹, respectivamente (BRASIL, 1988). Neste contexto, antioxidantes provenientes de fontes naturais têm sido estudados, buscando obter produtos mais seguros e eficientes que possam ser usados em alimentos, substituindo total ou parcialmente os antioxidantes sintéticos (BREWER, 2011).

Considerando o potencial antioxidante da moringa, torna-se relevante investigar a sua potencialidade como fonte de aditivo antioxidante em sistemas lipídicos de baixa estabilidade oxidativa, tais como o óleo de soja, óleo mais consumido no Brasil, e o óleo de peixe, fonte nutricional singular.

1.1 OBJETIVO GERAL

Avaliar o potencial antioxidante *in vitro* de extratos etanólicos de *Moringa oleífera* Lam. e analisar seus efeito na estabilidade oxidativa dos óleos de soja e de peixe.

1.2 OBJETIVOS ESPECÍFICOS

Obter os extratos etanólicos das folhas (EL), flores (EF), cascas das vagens (EP) e sementes (ES) de *Moringa oleífera* Lam.;

Avaliar o potencial antioxidante dos extratos etanólicos EL, EF, EP e ES através da determinação do conteúdo de fenólicos extraíveis totais (TEP) pelo método Folin-Ciocalteu;

Determinar a capacidade antioxidante dos extratos EL, EF, EP e ES utilizando os métodos: sequestro do radical livre DPPH, poder redutor do ferro (FRAP) e oxidação acoplada ao sistema β -caroteno/ácido linoleico;

Avaliar através dos métodos acelerados Rancimat, Calorimetria Exploratória Diferencial Pressurizada (P-DSC) e teste de estocagem acelerada em estufa o efeito antioxidante dos extratos que apresentarem boa capacidade antioxidante nos testes de sequestro do radical livre DPPH, poder redutor do ferro (FRAP) e oxidação acoplada ao sistema β -caroteno/ácido linoleico, quando aplicados nos óleos de soja e de peixe.

Avaliar a bioatividade e toxicidade preliminar dos extratos EL, EF, EP e ES através do teste de letalidade da *Artemia salina*.

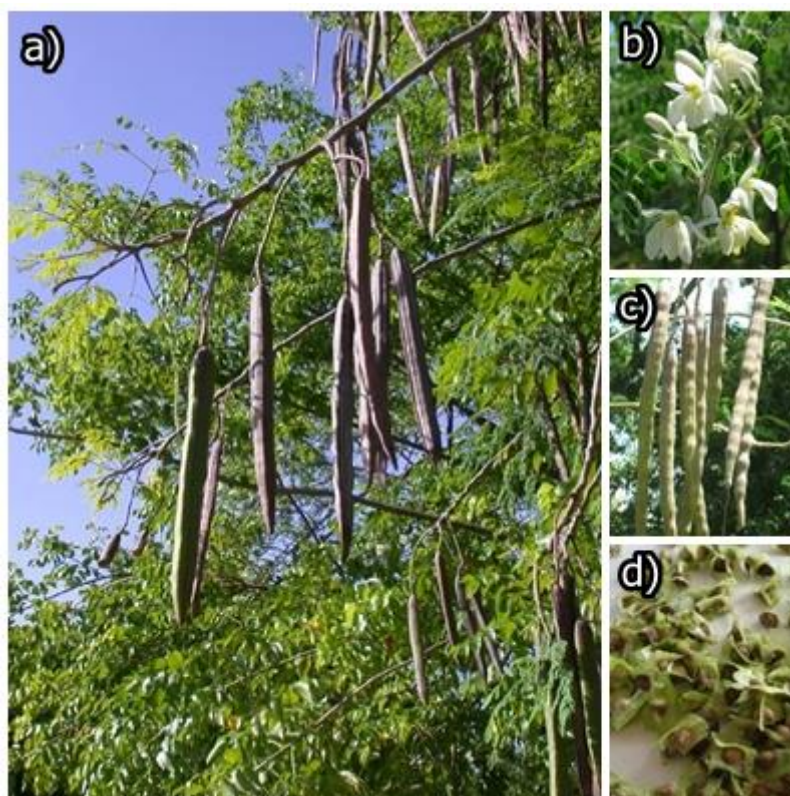
2 REVISÃO DE LITERATURA

2.1 *MORINGA OLEIFERA* LAM

A *Moringa oleífera* Lamarck é uma árvore nativa do noroeste da Índia, sendo cultivada ao longo dos trópicos. Trata-se de uma planta perene, amplamente distribuída nos países da Ásia, Oriente médio e da África, podendo também ser encontrada na América Central e América do Sul (BEZERRA; MOMENTÉ; MEDEIROS FILHO, 2004). É bem tolerante à seca, florescendo e produzindo frutos mesmo com escassez de chuvas (RAMACHANDRAN; PETER; GOPALAKRISHNAN, 1980).

Foi introduzida no Brasil por volta de 1950, sendo cultivada como planta ornamental e medicinal, principalmente na região Nordeste, onde é conhecida como lírio-branco, quiabo de quina ou simplesmente moringa (SILVA, et al., 2012). Pode atingir 10 metros de altura e apresenta folhas grandes e flores perfumadas brancas ou creme, vagens longas, variando de verde a marrom esverdeada, contendo de 10 a 20 sementes globoides (Figura 1). Em condições ideais de cultivo, a moringa começa a frutificar entre o primeiro e o segundo ano (TSAKNIS, et al., 1999).

Figura 1. *Moringa oleífera* Lam. com suas folhas (a), flores (b), vagens (c) e sementes (d)



Todas as partes da moringa podem ser utilizadas para algum fim, sendo seu emprego bastante conhecido na nutrição humana e animal, na agricultura, nas indústrias farmacêutica, cosmética e alimentícia, no tratamento de água e na obtenção de lubrificantes e biodiesel. (DEBNATH et al., 2011; DONGMEZA et al., 2006; RASHID et al., 2008; SHARMA et al., 2009).

Uma das aplicações mais popular da moringa é no tratamento de água para o consumo humano. Suas sementes possuem proteínas coagulantes que tornam a água potável, com desempenho equivalente ao de coagulantes químicos, sendo, entretanto, mais atraente do que estes por ser um produto biodegradável e, portanto, ecologicamente correto. Aliado a isto está o fato de não alterar significativamente o pH e a alcalinidade da água após o tratamento, e não causar problemas de corrosão. A torta das sementes, resultante da extração do óleo, também pode ser usada para o mesmo fim, sem haver diminuição do seu princípio coagulante (PRITCHARD et al., 2010).

É tradicionalmente usada na Índia como erva medicinal para tratar e prevenir inúmeras doenças, agindo como antibiótico, antitripanossomal, hipotensor, antiulcerígeno, antiespasmódico, anti-inflamatório, hipocolesterolêmico e hipoglicemiante. Estas propriedades atribuídas à moringa tem motivado o desenvolvimento de várias pesquisas, objetivando validar cientificamente o seu uso para o tratamento destas patologias. OLUDURO et al. (2010) atestaram a excelente atividade antibacteriana dos extratos brutos das sementes da moringa contra bactérias *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Cladosporium cladosporioides*, *Penicillium* e *sclerotigenum*. Compostos isotiocianatos foram identificados em diferentes partes da moringa, conferindo a esta planta importante atividade antibacteriana contra o microorganismo *Helicobacter pylori*, um dos principais responsáveis pelo surgimento das gastrites e úlceras gástricas e duodenais, classificado como cancerígeno pela Organização Mundial de Saúde (HARISTOY et al., 2005). As folhas e as sementes da moringa possuem excelente atividade antitripanossomal, com ação inibitória da protease, revelando seu potencial no tratamento de infecções com o *Trypanosoma cruzi* (BIJINA et al., 2011). A planta também apresenta efeito hipotensor, atividade atribuída à presença de glicosídeos tiocarbamatos em suas folhas (ABROGOUA et al., 2012; FAIZI et al., 1994). O extrato aquoso das folhas da moringa revelaram atividade protetora contra a formação de úlceras gástricas, apresentando vantagens em relação aos tratamentos clássicos com antiácidos e antihistamínicos (DEBNATH et al., 2011). Infusões utilizando as sementes da moringa mostraram ação antiespasmódica, anti-inflamatória e diurética em ratos (CÁCERES et al., 1992). Coelhos submetidos a uma dieta

hipercolesterolêmica e tratados por 12 semanas com extrato aquoso das folhas da moringa, apresentaram redução nos níveis de colesterol em cerca de 50% e redução na formação de placas ateroscleróticas em cerca de 86%, sendo esses efeitos de grau comparável aos da sinvastatina (CHUMARK et al., 2008). As folhas também demonstraram capacidade em promover uma redução considerável dos níveis de glicose no sangue e na urina, com aumento da proteína sérica total, do peso corporal e da hemoglobina e redução da proteinúria, sendo indicada para o tratamento do *diabetes mellitus* (JAISWAL et al., 2009). Extratos aquosos e alcoólicos da raiz da moringa demonstraram sua eficiência no tratamento de litíase urinária de oxalato de cálcio (KARADI et al., 2006).

A *Moringa oleifera* Lam. é uma boa alternativa na alimentação humana e animal, pois apresenta importantes minerais, como o ferro, além de ser fonte de vitaminas, como o *beta*-caroteno e de aminoácidos como a metionina e cistina, geralmente deficientes em outros alimentos (ANWAR et al., 2007; MAKKAR; BECKER, 1996).

Mohammed et al. (2011), ao utilizarem as folhas frescas da moringa como suplemento alimentar em galinhas, observaram o aumento da taxa de postura de ovos e a melhoria na qualidade dos mesmos, com melhora na conversão alimentar e na cor da gema, quando comparado ao controle, sugerindo que tal planta pode ser usadas como recurso alimentar sustentável para galinhas criadas em áreas tropicais. Já o uso da farinha das sementes desengorduradas da *Moringa oleifera* como aditivo em dietas de ovinos relevou resultados que sugerem que este aditivo tem potencial para melhorar a fermentação no rúmen e a taxa de crescimento dos cordeiros alimentados com feno-SBM (BEN SALEM; MAKKAR, 2009).

Com relação ao óleo extraído das sementes da moringa, o mesmo possui alta resistência à oxidação por conter em sua composição o ácido graxo saturado behênico, apesar de possuir elevados teores de ácidos graxos insaturados, principalmente o oleico, tendo inclusive composição quimicamente semelhante ao azeite de oliva (TSAKNIS, JOHN; LALAS, 2002).

A moringa possui excelente potencial como antioxidante, pois apresenta entre seus constituintes químicos os ácidos fenólicos (ácido gálico, ácido clorogênico, ácido elágico e ácido ferúlico), além dos flavonoides (campferol, quercetina e rutina), substâncias com atividade antioxidante relevante atribuída à capacidade de sequestrarem radicais, além de serem excelentes quelantes de metais (VERMA et al., 2009).

A atividade antioxidante *in vitro* de extratos aquosos de folhas da moringa em dois estágios de maturação foi estudada, e os resultados mostraram que os extratos são potentes fontes de substâncias sequestradoras de radicais livres, com ação comparável a de

antioxidantes de referência, sugerindo que esses extratos são capazes de prevenir os danos oxidativos no organismo humano (SREELATHA; PADMA, 2009).

A presença de ácido gálico, ácido clorogénico, ácido elágico, ácido ferúlico, kaempferol, quercetina e vanilina em extratos aquosos das folhas, frutos e sementes da *Moringa oleifera* foi revelada através de análises em HPLC e MS, sendo o extrato da folha o mais rico em compostos fenólicos totais, flavonóides e ácido ascórbico, com consequente melhor atividade antioxidante (SINGH et al., 2009). A moringa é também uma excelente fonte de tocoferóis (SÁNCHEZ-MACHADO; LÓPEZ-CERVANTES; VÁZQUEZ, 2006).

Hazra et al. (2012), avaliando o efeito dos extratos brutos das folhas de *Moringa oleifera* sob a carne cozida de búfalo, evidenciou efeito antioxidante e antimicrobiano, além de melhoria na qualidade da carne, aumentando a maciez, suculência e prevenindo a descoloração.

Arabshahi-D; Vishalakshi Devi; Urooj (2007), avaliaram a atividade antioxidante de extratos etanólicos de folhas de moringa (*Moringa oleifera* Lam.), folhas de hortelã (*Mentha spicata*) e tubérculo de cenoura (*Daucus carota*), bem como seu pH e estabilidade durante o armazenamento e concluíram que estes vegetais são fontes potenciais de compostos antioxidantes, apresentando atividade antioxidante potente em sistemas lipídicos diferentes, e que a atividade antioxidante dos extratos variou de acordo com o pH, tratamento térmico e armazenamento. Tais descobertas confirmam que as propriedades anti e pró-oxidantes de vegetais são fortemente influenciadas por uma série de fatores de processamento e pelas condições de reação, sendo importante considerar as ótimas condições tecnológicas e os fatores de processamento que influenciam a atividade e biodisponibilidade de antioxidantes vegetais para utilização em alimentos e sistemas biológicos.

2.2 ÓLEOS DE SOJA E DE PEIXE

Por definição, óleos e gorduras são substâncias insolúveis em água e solúveis em solventes orgânicos. Um óleo se diferencia de uma gordura pelo estado físico a temperatura de 20 °C, apresentando os óleos a forma líquida, enquanto que as gorduras apresentam forma sólida nessa temperatura.

São compostos constituídos por uma mistura de tri, di e monoacilgliceróis, ácidos graxos livres, glicolipídeos, fosfolipídeos, esteróis e outras substâncias, que apresentam oxidação distinta, sendo que os ácidos graxos insaturados são os componentes mais

susceptíveis ao processo oxidativo (RAMALHO, V.; JORGE, 2006). Podem ser originários de fontes animais, vegetais ou mesmo microbianas.

Estas substâncias exercem papel fundamental na alimentação humana, pois além de fornecem calorias, agem como veículos de vitaminas lipossolúveis (A, E, D e K) e de ácidos graxos essenciais como o linoleico e o linolênico e contribuem para melhorar a palatabilidade dos alimentos (CASTRO; MENDES; SANTOS, 2004).

Fisiologicamente, atuam na estrutura, composição e permeabilidade das membranas e das paredes celulares, e são os componentes majoritários do tecido adiposo, isolando o organismo, protegendo os órgãos internos e contribuindo para a configuração do corpo.

Os óleos e gorduras também desempenham importantes papéis tecnológicos, podendo ser empregados como emulsificantes, texturizantes, aromatizantes, umectantes e transmissores de calor a alta temperatura. Segundo a ABIOVE (2012), são processados diariamente cerca de 173.441 toneladas de óleos vegetais no Brasil, incluindo óleo de soja, algodão, canola, girassol, linhaça e babaçu.

O óleo de soja surgiu como um subproduto do processamento do farelo de soja, e tornou-se um dos líderes mundiais no mercado de óleos devido as suas qualidades nutricionais, abundância, valor econômico e larga aplicabilidade na formulação de outros produtos. Destaca-se pelo alto teor de ácido graxos insaturados, cerca de 85 a 88% (principalmente oleico, linoleico e linolênico) e apenas 12 a 15% de ácidos graxos saturados (na maior parte palmítico) (RIBEIRO et al., 2009). Caracteriza-se também por ter vários componentes menores que podem ser recuperados durante o processo de refino. Estes incluem os fosfolipídios recuperados como lecitina, esteróis mistos, que servem como matéria-prima para a produção de produtos farmacêuticos valiosos, além dos tocoferóis (vitamina E) (FERRARI et al., 1996).

A composição e o teor de cada ácido graxo do óleo de soja podem ser afetados pelas diferenças de variedade e pelos vários fatores geográficos e do meio ambiente, principalmente das condições climáticas (DAHMER et al., 1989). O teor elevado de ácidos graxos insaturados e o teor relativamente elevado de ácido linolênico, torna o óleo de soja muito suscetível às reações de oxidação (RAMALHO; JORGE, 2006).

Os óleos de peixes, em especial os de origem marinha, são ricos em ácidos graxos poli-insaturados de cadeia longa eicosapentaenoico (EPA) e docosahexaenoico (DHA), sendo estes conhecidos por seu importante papel na nutrição humana. Esses ácidos graxos, contidos no óleo de peixe, competem com o ácido araquidônico (AA), nas vias enzimáticas, reduzindo a formação de mediadores pró-inflamatórios e favorecendo a produção de mediadores com

reduzida atividade biológica (FAIZI et al., 1994). Dessa forma o consumo regular de óleos de peixes trazem inúmeros benefícios ao organismo humano, dentre os quais a proteção contra doenças como o mal de Alzheimer (LUKIW; BAZAN, 2008), depressão (NEMETS et al., 2006), declínio cognitivo (MORRIS et al., 2005), artrite reumatoide (BYKERK; KEYSTONE, 2005), doenças cardiovasculares (KRIS-ETHERTON et al., 2002), além de ajudar no desenvolvimento do sistema nervoso e no crescimento (PIVIK et al., 2009).

O perfil de ácidos graxos dos peixes varia em função de vários fatores, como a temperatura do meio ambiente, idade, sexo, espécie, tipo de peixe e principalmente em função dos perfis de ácidos graxos dos componentes da cadeia alimentar, característicos do ecossistema das espécies selvagens, ou do perfil de ácidos graxos da ração nos peixes cultivados (AVERINA; KUTYREV, 2011).

Considera-se que o perfil lipídico constitui a principal diferença entre os peixes de água doce e os peixes marinhos, mas existem algumas espécies de peixes de água doce que são especialmente ricas em ácidos graxos ω -3 (AVERINA; KUTYREV, 2011).

2.3 OXIDAÇÃO LIPÍDICA

A oxidação lipídica é um processo afetado por diversos fatores como a sua composição em ácidos graxos, a temperatura, a luz incidente, a presença de oxigênio, e a ação de íons metálicos, dentre outros, sendo o fator primordial, a presença de oxigênio (NAZ et al., 2005; REGITANO-D'ARCE, 2006). Tal processo pode ocorrer por várias vias, dependendo do meio e dos agentes catalisadores, resultando em uma oxidação enzimática, uma fotoxidação e/ ou em uma autooxidação, sendo esta última o principal mecanismo de deterioração lipídica (SCHAICH, 2012).

A oxidação enzimática é resultante da ação das enzimas lipoxigenases sobre os ácidos graxos poli-insaturados, catalisando a adição de oxigênio à cadeia hidrocarbonada poli-insaturada, com formação de peróxidos e hidroperóxidos, com duplas ligações conjugadas que podem envolver-se em diferentes reações degradativas, originando diversos produtos secundários, semelhantes ao processo de autooxidação (RAMALHO; JORGE, 2006).

A fotoxidação ocorre através de reações fotoquímicas e/ou fotossensibilizadoras e envolve a interação entre a dupla ligação e o oxigênio singlete, produzido pelo efeito da luz sobre o oxigênio tripleto, na presença de sensibilizadores como a clorofila, sendo a reação de fotoxidação 1500 vezes mais rápida que a autooxidação (GUPTA, 2000). Assim, o produto

primário da fotoxidação é o hidroperóxido e sua velocidade de formação é de 10 a 30 vezes maior que na autoxidação (KIM; DECKER; LEE, 2012).

A autoxidação lipídica ocorre segundo um mecanismo de reação em cadeia de radicais livres, e divide-se em três fases: iniciação, propagação e terminação. Na fase de iniciação, a molécula de ácido graxo sofre a retirada de um hidrogênio adjacente à dupla ligação, em condições favorecidas por catalisadores como íons metálicos, enzimas, meio alcalino, calor, luz e disponibilidade de oxigênio para reação, entre outros, levando a formação de um radical livre. Na fase de propagação, o radical livre reage com molécula de oxigênio, formando radical livre peróxido, que por sua vez, reage com outra molécula insaturada para formar um hidroperóxido e um radical livre capaz de dar continuidade à reação, resultando em um processo autocatalítico. A fase de terminação caracteriza-se pelas reações dos radicais livres entre si, formando produtos estáveis (OSTROWSKA-LIGEZA et al., 2010).

Aldeídos, cetonas, álcoois e hidrocarbonetos também são formados, os quais são voláteis e contribuem para as alterações de sabor, odor e cor dos óleos, gorduras e alimentos oxidados, comprometendo a qualidade nutricional e a segurança alimentar (ZHANG et al., 2010).

2.4 AVALIAÇÃO DA ESTABILIDADE OXIDATIVA DE ÓLEOS

Os métodos acelerados de determinação da estabilidade oxidativa surgiram a partir de uma tentativa de prever a vida útil de óleos e gorduras, pois o acompanhamento das alterações ocorridas nestes produtos, nas condições de armazenamento, é lento e pode consumir grande quantidade de reagentes (GORDON, 2003).

Neste contexto, estes métodos utilizam condições padronizadas de oxidação acelerada, tais como oxigenação intensiva, tratamento térmico e/ou catálise metálica. Estes testes são empregados na indústria, pois permitem estimar de forma rápida a estabilidade oxidativa de uma matéria graxa ou a eficácia 'teórica' de um antioxidante, isolado ou em associação (TAN et al., 2002). Dentre os testes de oxidação acelerada mais utilizados industrialmente, destacam-se o teste de estocagem acelerada em estufa, o Rancimat e a calorimetria exploratória diferencial pressurizada (P-DSC), métodos que serão descritos a seguir.

2.4.1 Teste de estocagem acelerado em estufa ou “Schaal Oven Test”

O teste de estocagem acelerada em estufa ou “Schaal oven test” é um método que reconhecidamente fornece uma boa correlação com o armazenamento ao ambiente (REGITANO-D’ARCE, 2006). Este procedimento inicialmente envolvia a exposição de um béquer contendo 100 g de amostra em uma estufa a temperatura de 70 °C ou 63 °C até o desenvolvimento do ranço. A amostra era examinada em intervalos diários ou semanais e o ponto final era determinado através de uma avaliação organoléptica do odor e índice de peróxido (GRAY, 1978).

Atualmente, para a realização deste teste são utilizadas temperaturas variadas com amostras com e sem aditivos, e a evolução do processo oxidativo é monitorado através do acompanhado do índice de peróxido, como também por outras análises como o índice de anisidina, conteúdo de ácidos graxos livres, ácido tiobarbitúrico-substâncias reativas (TBARS), valor de dienos e trienos conjugados e determinação dos ácidos graxos por CG-MS, entre outras.

Wang et al. (2011), compararam os efeitos da aditivização do óleo de peixe com ácido carnósico em diferentes concentrações (0,1, 0,2, e 0,3 mg.g⁻¹) e com antioxidantes sintéticos, durante 66 dias nas temperaturas de 30 °C e 4 °C. Eles observaram o aumento dos valores de peróxido e dos valores de dienos conjugados, assim como os ácidos graxos livres e os ácidos tiobarbitúrico-reactivos (TBARS). A mensuração dos ácidos graxos *trans* e do conteúdo de compostos aldeídos foram investigadas por espectroscopia de infravermelho com transformada de Fourier, enquanto que as mudanças no conteúdo de ácido graxos poli-insaturados foram monitoradas por cromatografia gasosa acoplada a espectrometria de massa.

Os resultados mostram que as três concentrações de ácido carnósico foram eficazes na contenção da oxidação de óleo de peixe, sendo mais eficaz que a vitamina E, porém, não tão eficiente quando o TBHQ.

2.4.2 Rancimat

O método Rancimat permite que a estabilidade oxidativa de uma matriz lipídica seja determinada automaticamente, sob condições padronizadas. É muito utilizado na indústria de óleos e gorduras, sendo um dos parâmetros mais importantes para a avaliação da qualidade (KOWALSKI et al., 2004). O método baseia-se no aumento da condutividade elétrica da água deionizada pelos produtos voláteis gerados de amostras de óleo aquecidas em altas

temperaturas em aeração constante. Os softwares dos aparelhos Rancimat® fornecem uma curva de condutividade elétrica ($\mu\text{s.cm}^{-1}$) em função do tempo (min). As projeções de retas passam pela linha de base e pela tangente, a partir do ponto de inflexão da curva se interceptam num ponto que corresponde na escala de tempo ao período de indução (PI) (VELASCO; ANDERSEN; SKIBSTED, 2004).

2.4.3 Calorimetria exploratória diferencial pressurizada (P-DSC)

A Calorimetria exploratória diferencial pressurizada (P-DSC) surgiu da evolução da calorimetria exploratória diferencial (DSC) utilizando-se uma célula de pressão acoplada ao equipamento de análise. As altas pressões utilizadas pela P-DSC inibem a taxa de volatilização da amostra, elevando o seu ponto de ebulição, como também eleva a saturação da fase líquida com o oxigênio, aumentando a interação do gás oxidante com a amostra; permitindo, assim, o uso de baixas temperaturas de teste ou tempos de testes mais curtos às mesmas temperaturas. Os resultados obtidos pela P-DSC são mais precisos que os obtidos por DSC. Trata-se de uma técnica rápida, precisa e que utiliza uma pequena quantidade de amostra em comparação com as outras técnicas de análise de oxidação de óleos (KODALI, 2005).

A curva P-DSC registra o fluxo calor (mW/mg) em função do tempo (min). Transições endotérmicas ou exotérmicas são caracterizadas como picos e sua área é proporcional à entalpia (ΔH), expressa em Joule por grama (J/g). As curvas isotérmicas da P-DSC são úteis para determinar o tempo de indução oxidativa (OIT), que é observado como um pico súbito nas curvas P-DSC referente ao processo exotérmico gerado pela oxidação da amostra (MOTHE; AZEVEDO, 2009).

Os métodos acelerados determinam estágios diferenciados da auto-oxidação. As técnicas P-DSC e Rancimat apresentam um comportamento distinto, pois o P-DSC é medido na etapa inicial da propagação oxidativa, relativa às reações de formação de peróxidos, que levam ao pico exotérmico, representado pelo valor de OIT, enquanto que o Rancimat é determinado entre a etapa final de propagação e o início da terminação do processo oxidativo, caracterizado pelo aumento nos voláteis, representado pelo índice de estabilidade oxidativa (OSI) (RAMALHO et al., 2011).

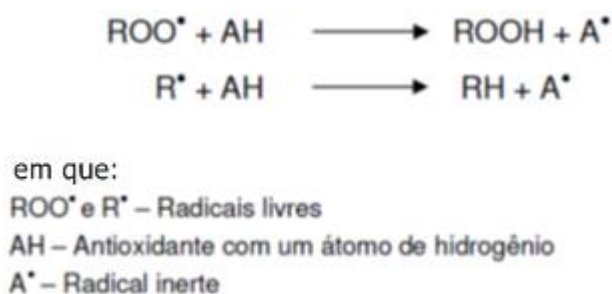
2.5 ANTIOXIDANTES

De acordo com Halliwell (2000), antioxidante é qualquer substância que, quando presente em baixa concentração comparada à do substrato oxidável, regenera o substrato ou previne significativamente a oxidação do mesmo.

Os antioxidantes, segundo o mecanismo de ação, são classificados em antioxidantes primários e secundários. Os primários são compostos de estrutura fenólica que promovem a remoção ou inativação dos radicais livres formados durante a iniciação ou propagação da reação, através da doação de átomos de hidrogênio a estas moléculas, interrompendo a reação em cadeia (SIMIC; JAVANOVIC, 1994). O mecanismo de ação para os antioxidantes primários, é representado na Figura 2.

Figura 2. Mecanismo de ação dos antioxidantes primários.

FONTE: (RAMALHO; JORGE, 2006)



O átomo de hidrogênio ativo do antioxidante é sequestrado pelos radicais livres $\text{R}^\bullet$ e $\text{ROO}^\bullet$ com maior facilidade que os hidrogênios alílicos das moléculas insaturadas. Assim, formam-se espécies inativas para a reação em cadeia e um radical inerte ($\text{A}^\bullet$) procedente do antioxidante. Este radical, estabilizado por ressonância, não tem a capacidade de iniciar ou propagar as reações oxidativas.

Os principais antioxidantes primários são: butil-hidroxianisol (BHA), butil-hidroxitolueno (BHT), propil galato (PG), terc-butilhidroquinona (TBHQ), e tocoferóis (DUBINSKY, 2000).

Os antioxidantes secundários contribuem para retardar a autooxidação por mecanismos diferentes aos dos antioxidantes primários (DUBINSKY, 2000). Nesta categoria encontram-se:

- Agentes quelantes – complexam íons metálicos, principalmente cobre e ferro, que catalisam a oxidação lipídica. Um par de elétrons não compartilhado na sua estrutura

molecular promove ação de complexação. Os mais comuns são ácido cítrico e seus sais, fosfatos e sais de ácido etileno diamino tetra acético (EDTA).

- Removedores de oxigênio – atuam capturando o oxigênio presente no meio, através de reações químicas estáveis, tornando-os, conseqüentemente, indisponíveis para atuar como propagadores da autoxidação. Ácido ascórbico e palmitato de ascorbila são os melhores exemplos deste grupo.

- Compostos que decompõem os hidroperóxidos – formam produtos finais estáveis, como os fosfolipídios em determinadas condições.

- Compostos que regeneram os antioxidantes primários – como o ácido ascórbico, que regenera o α -tocoferol.

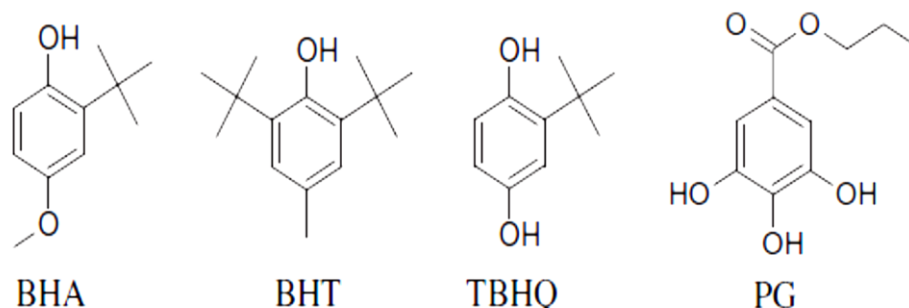
Na seleção de antioxidantes para a indústria alimentícia, devem ser levados em consideração alguns aspectos: eficácia em baixas concentrações (0,001% a 0,01%); ausência de interferências indesejáveis nos parâmetros sensoriais característicos do alimento; compatibilidade com a matriz alimentar a qual será aplicado e facilidade de aplicação; estabilidade nas condições de processo e armazenamento e segurança de ingestão do composto e seus produtos de oxidação mesmo se ingeridos em doses maiores do que as geralmente ingeridas nos alimentos. Além disso, é prudente considerar a legislação vigente, custo de aplicação e preferência de mercado por antioxidantes sintéticos ou naturais (RAMALHO; JORGE, 2006). Antioxidantes sintéticos como o BHT (butil hidroxitolueno), BHA (butil hidroxianisol), PG (propil galato) e TBHQ (terc-butil hidroquinona) são amplamente usados como aditivos alimentares a fim de aumentar a vida útil dos alimentos, especialmente os ricos em óleos e gorduras, por meio do retardo do processo de peroxidação lipídica (BAYDAR; OZKAN; YASAR, 2007).

2.5.1 Antioxidantes sintéticos

Os antioxidantes sintéticos de estrutura fenólica (Figura 3) como BHA, BHT, PG e TBHQ são os mais utilizados na indústria de alimentos por diminuírem a fase de propagação da reação de oxidação. Entretanto, apresentam o inconveniente de serem voláteis e facilmente decompostos em altas temperaturas (MARTÍNEZ-TOMÉ et al., 2001).

Figura 3. Estrutura fenólica dos antioxidantes sintéticos.

FONTE: (SANTOS et al., 2012)



O BHA é muito estável em gorduras animais e em óleos vegetais, sendo um dos antioxidantes mais eficazes. Apresenta, porém, o inconveniente de ser ligeiramente volátil e, por isso, pode evaporar e perder-se parcialmente nos processos de desidratação e destilação; ainda assim, o BHA residual pode revelar-se como um ativo antioxidante.

O BHT, assim como o BHA, é solúvel em gorduras e resiste bem ao calor. É mais volátil que o BHA e, por essa razão, no caso do preparo de alimentos desidratados, é utilizado em combinação com ele. Esse antioxidante é mais eficaz em gorduras animais do que em óleos vegetais. Seu odor é um pouco desagradável. O BHA e o BHT são sinergistas entre si. O BHA age como sequestrante de radicais peróxidos, enquanto o BHT age como sinergista, ou regenerador de radicais BHA (RAMALHO; JORGE, 2006).

GP é mais solúvel em água do que nas gorduras, e pouco resistente ao calor, não suportando tratamentos de cocção. Junto com o ferro, dá origem a sais de cor azul-escuro que podem provocar efeitos adversos durante o armazenamento dos óleos. Tem uma concentração ótima de atividade como antioxidante, e quando usado em níveis elevados pode atuar como pró-oxidantes.

TBHQ é um pó cristalino branco e brilhante, moderadamente solúvel em óleos e gorduras e não se complexa com íons de cobre e ferro como o galato. É considerado, em geral, mais eficaz em óleos vegetais que BHA ou BHT; em relação a gordura animal, é tão efetivo quanto o BHA e mais efetivo que o BHT ou o PG. O TBHQ é considerado também o melhor antioxidante para óleos de fritura, pois resiste ao calor e proporciona uma excelente estabilidade para os produtos acabados. Ácido cítrico e TBHQ apresentam excelente sinergismo em óleos vegetais (RAMALHO; JORGE, 2006).

Entretanto, estudos toxicológicos associam estes compostos à carcinogênese, entre outras doenças (SUN-WATERHOUSE; THAKORLAL; ZHOU, 2011). Tais evidências têm restringido a utilização destes antioxidantes sintéticos, controlando seu emprego em alguns países como Canadá e também na Comunidade Europeia, onde o uso de TBHQ não é permitido e o Codex Alimentar limita a 200 mg.kg^{-1} o uso de BHT em óleos vegetais. No Brasil, o uso de antioxidantes é controlado pelo Ministério da Saúde, sendo os limites máximos permitidos de 200 mg.kg^{-1} para BHA e TBHQ e 100 mg.kg^{-1} para BHT (SILVA; JORGE, 2012).

Nesse sentido, muitas pesquisas têm sido dirigidas com a finalidade de encontrar produtos naturais com atividade antioxidante, porque estes são presumidamente seguros e permitirão substituir os sintéticos ou fazer associações entre eles, com o intuito de diminuir sua quantidade nos alimentos (SOARES, 2002).

2.5.2 Antioxidantes naturais

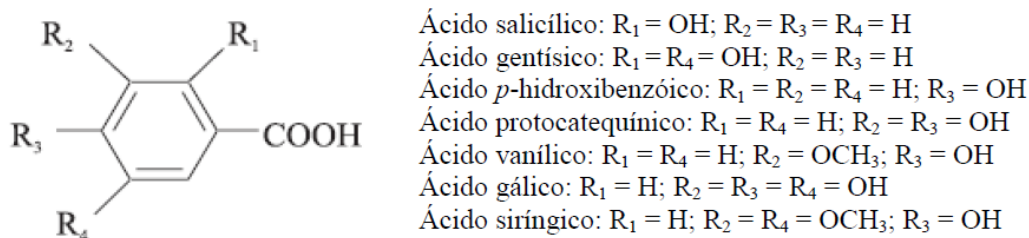
Compostos fenólicos e outros bioativos, como carotenoides, tocoferóis e ácido ascórbico, são exemplos de substâncias naturalmente presentes em algumas plantas, capazes de interceptar radicais livres e evitar processos oxidativos (BREWER, 2011).

Entre os bioativos presentes nos vegetais, encontram-se os compostos fenólicos, que são quimicamente definidos como substâncias que possuem anel aromático com um ou mais substituintes hidroxílicos, incluindo seus grupos funcionais. Possuindo, assim, estrutura variável e com isso apresentando características multifuncionais. Existem cerca de cinco mil fenólicos divididos nas suas diversas classes, dentre estes se sobressaem os ácidos fenólicos, os flavonoides e as cumarinas, que constituem a família dos compostos fenólicos largamente distribuídos na natureza (ANGELO; JORGE, 2007).

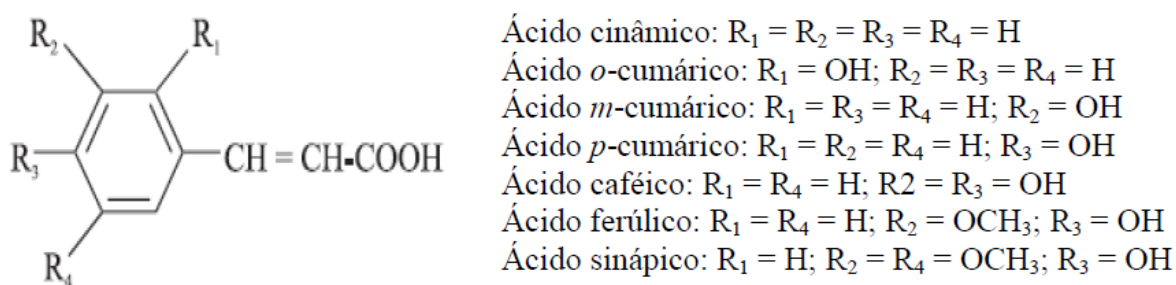
Os ácidos fenólicos caracterizam-se pela presença de um anel benzênico, um grupamento carboxílico e um ou mais grupamentos de hidroxila e/ou metoxila na molécula, conferindo-lhes propriedades antioxidantes (SOARES, 2002). Consistem em três grupos: os ácidos benzoicos, com fórmulas gerais e denominações representadas na Figura 4; os ácidos cinâmicos, sendo sete os mais comumente encontrados na natureza, com fórmulas gerais e denominações representadas na Figura 5; e as cumarinas que são derivadas do ácido cinâmico por ciclização da cadeia lateral do ácido *o*-cumárico descrito na Figura 6 (RAMALHO; JORGE, 2006).

Figura 4. Estrutura química dos ácidos benzoicos.

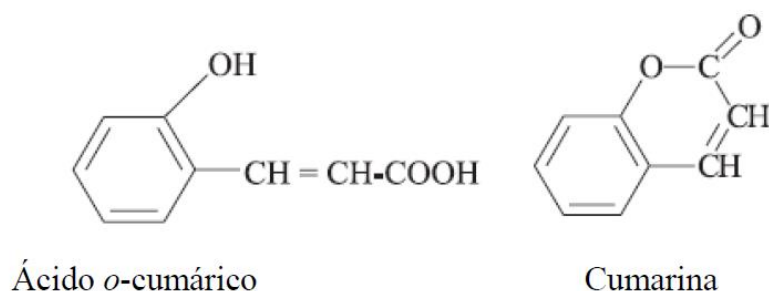
FONTE: (RAMALHO; JORGE, 2006)

**Figura 5.** Estrutura química dos principais ácidos cinâmicos.

FONTE: (RAMALHO; JORGE, 2006)

**Figura 6.** Estrutura química das cumarinas.

FONTE: (RAMALHO; JORGE, 2006)

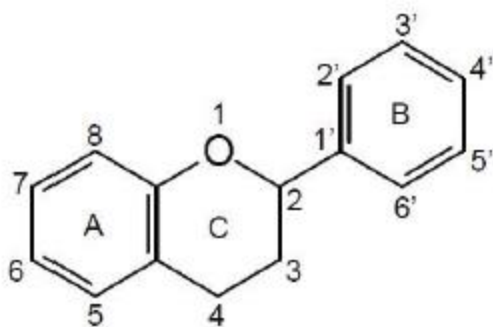


Os flavonoides são compostos largamente distribuídos no reino vegetal, encontram-se presentes em frutas, folhas, sementes e em outras partes da planta na forma de glicosídeos ou agliconas. São compostos de baixo peso molecular, consistindo em 15 átomos de carbono, organizados na configuração $\text{C}_6\text{-C}_3\text{-C}_6$. Caracterizam-se pela presença de dois anéis aromáticos, denominados anel A e B, unidos por três carbonos que formam um anel

heterocíclico, denominado anel C, como representado na Figura 7. Variações em substituição do anel C padrão resultam em importantes classes de flavonoides, como flavonóis, flavonas, flavanonas, flavanóis (ou catequinas), isoflavonas e antocianidinas. Substituições dos anéis A e B originam diferentes compostos dentro de cada classe de flavonoides (RAMALHO; JORGE, 2006).

Figura 7. Estrutura química dos flavonoides.

FONTE: (RAMALHO; JORGE, 2006)



Os flavonoides, assim como os ácidos fenólicos, funcionam como sequestradores de radicais, e algumas vezes, como quelantes de metais, agindo tanto na etapa de iniciação como na propagação do processo oxidativo (ANGELO; JORGE, 2007).

2.6 BIOATIVIDADE E TOXICIDADE FRENTE À *ARTEMIA SALINA*

Os metabólitos secundários presentes nas plantas são os responsáveis por atividades biológicas tais como as toxicológicas, alelopática, antibacteriana, antioxidante, antiparasitária, analgésica, anti-inflamatória, diurética, anticonvulsivante, miorelaxante e antiespasmódica entre outras.

Compostos bioativos presentes nas plantas medicinais são quase sempre tóxicos em altas doses. Desta maneira, a avaliação da letalidade em um organismo animal menos complexo pode ser usada para um monitoramento simples e rápido. O ensaio de letalidade para larvas de *Artemia salina* tem sido introduzido na rotina de muitos grupos de pesquisa envolvidos com isolamento, purificação e elucidação estrutural, devido sua simplicidade (KANWAR, 2007).

A *Artemia salina* é um crustáceo da classe Anostracea, de água salgada que é utilizado como alimento vivo para peixes, sendo seus ovos facilmente encontrados em lojas de aquaristas, permanecendo viáveis por anos no estado seco. Possui 4 estágios de

desenvolvimento (ovo, náuplio, metanáuplio e adulto) e alguns mecanismos de adaptação que as tornam cosmopolitas, como a osmorregulação, a presença de pigmentos respiratórios como a hemoglobina e a disponibilidade de alternativas reprodutivas que facilitam a dispersão e a perpetuação dessa espécie (KANWAR, 2007).

Utilizando-se a concentração letal média (CL50) é possível determinar e avaliar a atividade biológica (toxicidade) de um composto ou extrato natural. Diversos trabalhos correlacionam a toxicidade sobre *Artemia salina* com atividades biológicas de extratos, como pode ser visto no estudo desenvolvido por Kumbhare et al.(2012), onde extratos da casca do caule da *Moringa oleífera* foram testados quanto à atividade antioxidante e citotoxicidade. Um outro estudo desenvolvido por Kannan et al. (2013), testou extratos de seis ervas marinhas quanto a sua atividade antibacteriana, citotóxica e hemolítica, e seus resultados revelaram uma boa atividade antibacteriana aliada a baixa toxicidade, o que sugere seu uso como aditivos alimentares.

3 MATERIAL E MÉTODOS

Os experimentos foram realizados no Laboratório de Combustíveis e Materiais do Departamento de Química do Centro de Ciências Exatas e da Natureza da Universidade Federal da Paraíba (LACOM/DQ/CCEN/UFPB)

3.1 MATERIAL

O óleo de soja refinado sem adição de antioxidante foi adquirido da indústria Cargill Agrícola S. A., Brasil. O óleo de peixe refinado sem adição de antioxidante e os reagentes butil-hidroxitolueno (BHT), terc-butil-hidroquinona (TBHQ), 2,2-difenil-1-picrilidrazil (DPPH) foram adquiridos na Sigma-Aldrich (Steinheim, Germany) e o reagente de Folin-Ciocalteu, ácido gálico e todos os outros reagentes foram adquiridos na Merck (Dusseldorf, Germany).

3.2 AQUISIÇÃO E PREPARO DAS AMOSTRAS

As folhas, flores, casca das vagens e sementes da moringa (*Moringa oleífera* Lam.) foram coletadas durante o mês de abril de 2010, no Campus I da Universidade Federal da Paraíba, João Pessoa/PB, Brasil e submetidas à secagem, em estufa com circulação de ar a 45 °C (Modelo MA 035, Marconi) durante 24 h. Após desidratação, cada parte foi triturada separadamente, em moinho de facas, e posteriormente acondicionada em sacos de polietileno a vácuo, sendo mantidas, à temperatura de -18 °C durante a execução dos experimentos. Um exemplar da exsicata foi depositado no Herbário do Departamento de Botânica da UFPB com o registro– J.A.N. Batista 01 (JPB).

3.3 OBTENÇÃO DOS EXTRATOS

Os extratos foram obtidos através de agitação de 50 g do material vegetal em pó e 500 mL de etanol em banho termostatizado por 2 horas a 25 °C, seguido de filtração à vácuo. Os extratos das folhas (EL), das flores (EF), da casca das vagens (EP) e das sementes (ES) foram concentrados em evaporador rotativo (Modelo TE-221 Tecnal) a 60 °C até a retirada total do

solvente. Em seguida acondicionados em frascos âmbar em atmosfera inerte (N_2) sob refrigeração (aproximadamente 8 °C) até o momento de sua utilização.

3.4 DETERMINAÇÃO DE FENÓLICOS EXTRAÍVEIS TOTAIS

Os teores de fenólicos extraíveis totais (FET) foram determinados colorimetricamente pelo método de Folin-Ciocalteu (SLINKARD; SINGLETON, 1977). Uma alíquota de 0,05 mL das amostras diluídas em 3,95 mL de água foi adicionada de 0,25 mL de reagente de Folin-Ciocalteu e posteriormente de 0,75 mL de solução de carbonato de sódio 20%. A mistura foi agitada e mantida no escuro por 2 h. A absorbância foi medida a 765 nm em espectrofotômetro UV-vis (modelo UV-2550, Shimadzu), juntamente com o controle, que continha somente os reagentes e água. A concentração de compostos fenólicos foi estimada, usando curva de calibração de ácido gálico (50-500 mg.L⁻¹). Os resultados foram expressos como média $\pm$ desvio-padrão de mg equivalente de ácido gálico (GAE) em cada grama de extrato.

3.5 ESTUDO TÉRMICO DOS EXTRATOS

A curva Termogravimétrica (TG) e a Análise Térmica Diferencial (DTA) dos extratos desidratados foram obtidas em Analisador Térmico Simultâneo DTA-TG, DTG modelo H-60 da Shimadzu, utilizando aproximadamente 10 mg de amostra em cadinho de alumina, atmosfera de ar sintético com fluxo de 50 mL/min, razão de aquecimento de 10 °C.min⁻¹ e temperatura de 25 - 600 °C.

3.6 DETERMINAÇÃO DA ATIVIDADE ANTIOXIDANTE

3.6.1 Atividade sequestrante do radical livre DPPH

A capacidade dos extratos etanólicos e dos antioxidantes sintéticos BHT e TBHQ em sequestrar o radical livre DPPH foi analisada através de metodologia proposta por Blois (1958) com modificações de Brand-Williams et al. (1995). Alíquotas de 3,0 mL das amostras nas diluições de 20, 40 e 80 µg/mL foram adicionadas a 0,1 mL de solução etanólica de DPPH• 0,1 mol.L⁻¹. O controle das amostras consistiu de 3,0 mL de cada amostra e 0,1 mL de etanol e o controle negativo de 3,0 mL de etanol e 0,1 mL de solução de DPPH•. O

decréscimo da absorbância a 515 nm foi mensurado após 120 min. A atividade sequestrante do radical DPPH foi calculada usando a seguinte fórmula:

$$\text{Atividade de sequestro do radical DPPH (\%)} = \left[\left(1 - \frac{A_a - A_b}{A_c} \right) \times 100 \right] \quad \text{EQUAÇÃO 1}$$

em que A_a é a absorbância das amostras, A_b é a absorbância do controle das amostras de cada extrato e A_c é a absorbância do controle negativo.

A partir dos resultados, foi construído um gráfico para o percentual de atividade sequestradora do radical DPPH em cada concentração de extrato ($\mu\text{g.mL}^{-1}$) testada. Para o cálculo do EC_{50} (concentração do extrato com capacidade de reduzir 50% do $\text{DPPH}^\bullet$ inicial), foi utilizada a equação da reta, substituindo o valor de y por 50. A eficiência antirradical foi definida como a relação inversa do EC_{50} , ou seja, $(1/\text{EC}_{50})$.

3.6.2 Capacidade antioxidante pelo método de redução do ferro (FRAP)

A capacidade antioxidante foi estimada pelo ensaio do poder antioxidante de redução do ferro (FRAP), seguindo o procedimento descrito por Benzie e Strain (1996) com as modificações de Pulido et al. (2000). Uma alíquota de 2,7 mL do reagente FRAP recém preparado (TPTZ 10 mM, FeCl_3 20 mM e tampão de acetato) foi adicionado a 90 μL de cada extrato e 270 μL de água destilada. A mistura foi homogeneizada em banho termostatizado a 37 °C por 30 min e em seguida a leitura da absorbância a 595 nm foi mensurada usando o reagente FRAP como controle negativo para calibrar o espectrofotômetro. Concentrações de 500-2000 $\mu\text{mol.L}^{-1}$ de sulfato ferroso ($\text{FeSO}_4 \bullet 7\text{H}_2\text{O}$) foram utilizadas para a determinação da curva-padrão. Os resultados foram expressos como média $\pm$ desvio-padrão de μM sulfato ferroso/g de extrato.

3.6.3 Auto-oxidação do sistema β -caroteno/ácido linoleico

A atividade antioxidante pelo sistema modelo β -caroteno/ácido linoleico foi determinado conforme método descrito por Marco (1968) com modificações de Miller (1971). O ensaio baseia-se na oxidação do β -caroteno induzida pelos produtos de degradação oxidativa do ácido linoleico. As soluções foram preparadas pela mistura de 5 mL da solução sistema de β -caroteno/ácido linoleico e 0,4 mL de cada extrato ou solução de trolox (200

$\mu\text{g.mL}^{-1}$). Após leitura inicial da absorbância a 470 nm, a mistura foi mantida em banho termostaticado a 40 °C, e a absorbância medida em intervalos de 15 min até 120 min. A amostra controle negativo consistiu de 5 mL da solução sistema de β -caroteno/ácido linoleico e 0,4 mL de etanol. Os resultados foram expressos como percentagens de inibição da oxidação, usando a seguinte fórmula:

$$\text{Inibição da oxidação (\%)} = \left[\left(1 - \frac{A_i - A_f}{C_i - C_f} \right) \times 100 \right] \quad \text{EQUAÇÃO 2}$$

em que A_i é a absorbância inicial da amostra, A_f é a absorbância final da amostra, C_i é a absorbância inicial do controle e C_f é a absorbância final do controle negativo.

3.7 BIOENSAIO COM *ARTEMIA SALINA* LEACH

Para a avaliação preliminar da bioatividade e toxicidade dos extratos etanólicos das folhas, flores, cascas das vagens e sementes de *Moringa oleifera* procedeu-se o Brine shrimp lethality test (BST) com *Artemia salina*, de acordo com a metodologia segundo (MEYER et al., 1982), com algumas modificações.

Os cistos de *Artemia salina* (25 mg) foram obtidos comercialmente e incubados em solução marinha artificial (pH 8,5 e 29 °C) em recipiente protegido da luminosidade, porém dotado de uma barreira perfurada com orifícios para permitir a migração de larvas recém eclodida em direção a luz (lâmpada de 40 watt), graças ao fototropismo positivo destas. Em 24 horas após a incubação as larvas se encontram na forma de nauplii. Cerca de 10-13 larvas foram transferidas para tubos testes contendo o volume final de 1mL, cada, e diferentes concentrações dos extratos (1, 3, 10, 30, 100, 300, 500, 750 e 1000 $\mu\text{g.mL}^{-1}$). Um tubo controle foi obtido usando apenas o veículo (solução salina e solução de cremofor a 5%) e as larvas. Cada concentração foi testada em triplicata e o experimento repetido por duas vezes. Após 24 horas de exposição das larvas aos extratos foram contabilizados os números de larvas vivas e mortas para determinação da CL50.

3.8 DETERMINAÇÃO DA ESTABILIDADE OXIDATIVA

3.8.1 Preparação das amostras

Alíquotas de 50 mL dos óleos de soja e de peixe foram adicionadas dos extratos etanólicos das folhas (EL), flores (EF) e cascas das vagens (EP) na concentração de 100 mg.kg⁻¹ em relação ao seu conteúdo de fenólicos extraíveis totais (TEP) ou de BHT e TBHQ na concentração de 100 mg.kg⁻¹. As misturas foram homogeneizadas em agitador magnético durante 30 min, acondicionadas em frascos âmbar em atmosfera inerte a -18 °C até o momento das análises. Os óleos de soja e de peixe sem adição de antioxidantes foram submetidos ao mesmo procedimento e utilizados como controle.

3.8.2 Método Rancimat

A estabilidade oxidativa dos óleos de soja e de peixe adicionado dos extratos ou dos antioxidantes sintéticos foi analisada pelo emprego do método proposto pela AOCS CD 12b-92 (AOCS, 2003) em equipamento Rancimat (873 Biodiesel, Metrohm). Um total de 2 g de cada amostra foram submetidos a 110 °C sob fluxo constante de ar (10 L.h⁻¹). O resultado foi determinado a partir do ponto de inflexão da curva tempo em função da condutividade. Os cálculos dos períodos de indução foram realizados com o auxílio do Software 873-Rancimat®, sendo os resultados expressos como índice de estabilidade oxidativa (OSI) em h e como percentual de aumento do OSI.

3.8.3 Calorimetria exploratória diferencial pressurizada (P-DSC)

As curvas de P-DSC das amostras foram obtidas através de um calorímetro exploratório diferencial acoplado a uma célula de pressão da TA Instruments, modelo DSC Q1000.

A curva dinâmica do óleo de peixe puro foi realizada utilizando cadinho de alumínio com aproximadamente 10 mg da amostra e as condições de análise no calorímetro foram atmosfera de oxigênio, pressão de 1400 kPa e razão de aquecimento de 10 °C.min⁻¹ no intervalo de 25 a 500 °C. As curvas isotermas foram realizadas a 100 °C, nas mesmas condições descritas.

As análises no modo isotérmico (110 °C) do óleo de soja foram processadas utilizando cadinho de alumínio com aproximadamente 10 mg da amostra, sob atmosfera de oxigênio com pressão de 1400 kPa.

Os valores do OIT foram determinados pela diferença entre os tempos *onset* e do inicial e os resultados expressos como tempo de indução oxidativa (OIT) em minutos e como percentual de aumento da OIT.

3.8.4 Teste de estocagem acelerada em estufa

Os extratos de moringa na concentração de 100 mg.kg⁻¹ em relação ao seu conteúdo de fenólicos extraíveis totais (TEP), o BHT e o TBHQ nesta mesma concentração foram adicionadas em 15 g de óleo de soja e de peixe e homogeneizadas em agitador magnético durante 30 min. O teste de estocagem acelerada em estufa do óleo de soja e do óleo de peixe foi conduzido em estufa com circulação de ar durante 16 dias a temperatura de 60 ± 2 °C. A cada quatro dias, foram realizadas as análises dos índices de peróxido, índice de *p*-anisidina, valor de dienos conjugados e espectroscopia de infravermelho por transformada de Fourier. Os óleos de soja e de peixe sem adição de antioxidantes submetidos ao teste de estocagem foram utilizados como controle.

3.8.4.1 Índice de peróxido

O índice de peróxido foi determinado pelo método Cd 8-53 da AOCS (2003). Alíquotas de 2 g dos óleos foram dissolvidas em 30 mL de uma solução de ácido acético-clorofórmio (3:2 v/v), e adicionadas de 0,5 mL de solução saturada de iodeto de potássio. Após 1 min em repouso foram adicionados 30 mL de água e 0,5 mL de uma solução de amido a 1 %. Na sequência foi realizada titulação com solução de tiosulfato de sódio 0,01 N, até o desaparecimento da coloração azulada. Uma prova em branco foi conduzida nas mesmas condições, sem a presença da amostra. Os cálculos foram realizados de acordo com Equação:

$$\text{Índice de peróxido (meq / kg)} = \frac{N \times (V_A - V_B) \times 1000}{P \text{ da amostra (g)}}$$

EQUAÇÃO 3

em que N é a normalidade da solução de $\text{Na}_2\text{S}_2\text{O}_3$, V_a é o volume da solução de $\text{Na}_2\text{S}_2\text{O}_3$ consumido pela amostra (mL), V_b é o volume da solução de $\text{Na}_2\text{S}_2\text{O}_3$ consumido pelo branco (mL) e m é a massa da amostra (g).

3.8.4.2 Índice de p-anisidina

O índice de anisidina foi determinado seguindo metodologia Cd 18-90 da AOCS (2003). Alíquotas de 0,5 g dos óleos foram dissolvidas em 25 mL de isooctano, com posterior medida da absorbância a 350 nm, utilizando como branco somente o isooctano. Posteriormente 5 mL da amostra foi adicionada de 1 mL de solução de anisidina (0,25 % de p-anisidina em ácido acético glacial) e após 10 minutos lidos a 350 nm em espectrofotômetro UV-vis. O índice de anisidina (IA) foi calculado de acordo com a equação:

$$\text{Índice de Anisidina} = \frac{25 (1,2A_s - A_b)}{P} \quad \text{EQUAÇÃO 4}$$

em que A_s é medida de absorbância da solução gordura-anisidina, A_b é a medida de absorbância da solução de gordura inicial e m é massa da amostra em gramas.

3.8.4.3 Determinação do valor de dienos conjugados (CD)

O método proposto por (LECLERC et al., 2007) foi modificado e usado para a determinação do conteúdo de CD no óleo. As amostras (0,02 g) foram diluídas com isooctano, e a absorbância da solução foi determinada usando o isooctano como branco em 232 nm. O valor de CD calculado foi a partir do valor de absorbância e da concentração final da amostra (g/100 mL). Os resultados são expressos como valores de CD, calculado como se segue:

$$CD = \frac{A_{232}}{C \times P} \quad \text{EQUAÇÃO 5}$$

em que A é a absorvância da amostra a 232 nm; C denota a concentração final da diluição da amostra ($\text{g} \cdot 100\text{mL}^{-1}$), e P representa o comprimento da célula de medição (cm).

3.8.4.4 *Infravermelho com transformada de Fourier (FTIR)*

Os espectros de absorção na região do infravermelho foram obtidos em espectrofotômetro de IV com transformada de Fourier modelo IR Prestige-21, Class 1, Laser Product da marca Shimadzu. A análise foi realizada na região de 4.000 a 650 cm^{-1} utilizando acessório ATR de uma reflexão com prisma de ZnSe, na resolução de 4 cm^{-1} .

3.9 ANÁLISES ESTATÍSTICAS

Os ensaios foram conduzidos em triplicata e os resultados expressos como média $\pm$ desvio-padrão. Os resultados foram analisados no Statistica 7.0 (Statsoft®) utilizando ANOVA e teste de Tukey considerando $P < 0,05$.

Os resultados da bioatividade foram expressos como média de análises em triplicata, sendo os ensaios repetidos para obtenção de nova média. A CL50 foi estabelecida com um intervalo de confiança de 95% para todos os experimentos, sendo os resultados considerados significativos quando $P < 0,05$, de acordo com a análise estatística de Probit, segundo Finney (1971), e usando o programa GraphPad Prism 4.03.

REFERÊNCIAS

- ABIOVE. *Pesquisa de Capacidade Instalada da Indústria de Óleos Vegetais - 2012*. Disponível em: <<http://www.abiove.org.br>>. Acesso em: 4 jan. 2013.
- ABROGOUA, D. P.; DANO, D. S.; MANDA, P.; ADEPO, A. J. B.; KABLAN, B. J.; GOZE, N. B.; EHABNTOULÉ, K. Effect on blood pressure of a dietary supplement containing traditional medicinal plants of Côte d'Ivoire. **Journal of Ethnopharmacology**, v. 141, p. 840-847, 2012.
- ANGELO, P. M.; JORGE, N. Compostos fenólicos em alimentos – Uma breve revisão. **Revista do Instituto Adolfo Lutz**, v. 66, n. 1, p. 232-240, 2007.
- ANWAR, F.; LATIF, S.; ASHRAF, M.; GILANI, A. H. *Moringa oleifera* : A Food Plant with Multiple Medicinal Uses. **Phytotherapy Research**, v. 21, p. 17-25, 2007.
- AOCS. Fats, Oils and Lipid Related Analytical Methods. In: FIRESTONE, D. (Org.). *Official Methods and Recommended Practices of the AOCS*. 6. ed. [S.l.]: AOCS, 2003. p. Method Cd 12b-92.
- ARABSHAHI-D, S.; VISHALAKSHI DEVI, D.; UROOJ, A. Evaluation of antioxidant activity of some plant extracts and their heat, pH and storage stability. **Food Chemistry**, v. 100, n. 3, p. 1100-1105, 2007.
- AVERINA, E. S.; KUTYREV, I. A. Perspectives on the use of marine and freshwater hydrobiont oils for development of drug delivery systems. **Biotechnology Advances**, v. 29, n. 5, p. 548-557, 2011.
- BALK, E. M.; LICHTENSTEIN, A. H.; CHUNG, M.; KUPELNICK, B.; CHEW, P.; LAU, J. Effects of omega-3 fatty acids on serum markers of cardiovascular disease risk: a systematic review. **Atherosclerosis**, v. 189, n. 1, p. 19-30, 2006.
- BARRETO, M. B.; FREITAS, J. V. B.; SILVEIRA, E. R.; BEZERRA, A. M. E.; NUNES, E. P.; GRAMOSA, N. V. Constituintes químicos voláteis e não-voláteis de *Moringa oleifera* Lam., Moringaceae. **Brazilian Journal of Pharmacognosy**, v. 19, n. 4, p. 893-897, 2009.
- BAYDAR, N. G.; OZKAN, G.; YASAR, S. Evaluation of the antiradical and antioxidant potential of grape extracts. **Food Control**, v. 18, p. 1131-1136, 2007.
- BEN SALEM, H.; MAKKAR, H. P. S. Defatted *Moringa oleifera* seed meal as a feed additive for sheep. **Animal Feed Science and Technology**, v. 150, n. 1-2, p. 27-33, 2009.
- BENZIE, I. F.; STRAIN, J. J. The ferric reducing ability of plasma (FRAP) as a measure of “antioxidant power”: the FRAP assay. **Analytical Biochemistry**, v. 239, p. 70-76, 1996.
- BEZERRA, A. M. E.; MOMENTÉ, V. G.; MEDEIROS FILHO, S. Germinação de sementes e desenvolvimento de plântulas de moringa (*Moringa oleifera* Lam.) em função do peso da semente e do tipo de substrato. **Horticultura Brasileira**, v. 22, n. 2, p. 295-299, 2004.

BIJINA, B.; CHELLAPPAN, S.; KRISHNA, J. G.; BASHEER, S. M.; ELYAS, K. K.; BAHKALI, A. H.; CHANDRASEKARAN, M. Protease inhibitor from *Moringa oleifera* with potential for use as therapeutic drug and as seafood preservative. **Saudi Journal of Biological Sciences**, v. 18, p. 273-281, 2011.

BLOIS, M. S. Antioxidant Determinations by the Use of a Stable Free Radical. **Nature**, v. 181, p. 1199-1200, 1958.

BRAND-WILLIAMS, W.; CUVELIER, M. E.; BERSET, C. Use of a free radical method to evaluate antioxidant activity. **LWT - Food Science and Technology**, v. 28, n. 1, p. 25-30, 1995.

BRASIL. *Resolução nº 04, de 24 de novembro de 1988*. Brasília: Ministério da Saúde, 1988.

BREWER, M. S. Natural Antioxidants: Sources, Compounds, Mechanisms of Action, and Potential Applications. **Comprehensive Reviews in Food Science and Food Safety**, v. 10, n. 4, p. 221-247, 2011.

BYKERK, V. P.; KEYSTONE, E. C. What are the goals and principles of management in the early treatment of rheumatoid arthritis? **Best practice & research. Clinical rheumatology**, v. 19, n. 1, p. 147-61, 2005.

CASTRO, H. F.; MENDES, A. A.; SANTOS, J. C. Modificação de óleos e gorduras por biotransformação. **Química Nova**, p. 146-156, 2004.

CHUMARK, P.; KHUNAWAT, P.; SANVARINDA, Y.; PHORNCHIRASILP, S.; MORALES, N. P.; PHIVTHONG-NGAM, L.; RATANACHAMNONG, P.; SRISAWAT, S.; PONGRAPEEPORN, K. U. S. The in vitro and ex vivo antioxidant properties, hypolipidaemic and antiatherosclerotic activities of water extract of *Moringa oleifera* Lam. leaves. **Journal of Ethnopharmacology**, v. 116, n. 3, p. 439-46, 2008.

CÁCERES, A.; SARAIVA, A.; RIZZO, S.; ZABALA, L.; DE LEON, E.; NAVE, F. Pharmacologic properties of *Moringa oleifera*. 2: Screening for antispasmodic, antiinflammatory and diuretic activity. **Journal of Ethnopharmacology**, v. 36, p. 233-237, 1992.

DAHMER, M. L.; FLEMING, P. D.; COLLINS, G. B.; HILDEBRAND, D. F. A rapid screening technique for determining the lipid composition of soybean seeds. **Journal of the American Oil Chemists' Society**, v. 66, n. 4, p. 543-548, 1989.

DEBNATH, S.; BISWAS, D.; RAY, K.; GUHA, D. *Moringa oleifera* induced potentiation of serotonin release by 5-HT(3) receptors in experimental ulcer model. **Phytomedicine**, v. 18, p. 91-95, 2011.

DONGMEZA, E.; SIDDHURAJU, P.; FRANCIS, G.; BECKER, K. Effects of dehydrated methanol extracts of moringa (*Moringa oleifera* Lam.) leaves and three of its fractions on growth performance and feed nutrient assimilation in Nile tilapia (*Oreochromis niloticus* (L.)). **Aquaculture**, v. 261, n. 1, p. 407-422, 2006.

DUBINSKY, E. Utilización de antioxidantes en aceites y grasas. **Aceites y Grasas**, v. 12, n. 1, p. 191-199, 2000.

FAIZI, S.; SIDDIQUI, B. S.; SALEEM, R.; SIDDIQUI, S.; AFTAB, K.; GILANI, A. H. Isolation and structure elucidation of new nitrile and mustard oil glycosides from *Moringa oleifera* and their effect on blood pressure. **Journal of Natural Products**, v. 57, n. 9, p. 1256-1261, 1994.

FERRARI, R. A.; SCHULTE, E.; ESTEVES, W.; BRIIHL, L.; MUKHERJEE, K. D. Minor Constituents of Vegetable Oils During Industrial Processing 1. **Journal of the American Oil Chemists` Society**, v. 73, n. 5, p. 587-592, 1996.

FINNEY, D. J. *Probit analysis*. Cambridge: Cambridge University, 1971. p. 333

GORDON, M. Measuring antioxidant activity practical applications. In: POKORNY, J.; YANISHLIEVA, N.; GORDON, M. (Org.). *Antioxidants in food: practical applications*. Cambridge: Woodhead Publishing, 2003. p. 71-84.

GRAY, J. I. Measurement of lipid oxidation: a review. **Journal of the American Oil Chemists` Society**, v. 55, n. 6, p. 539-546, 1978.

GUPTA, M. K. Oil quality improvement through processing. In: O'BRIEN, R. D.; FARR, W. E.; WAN, P. J. (Org.). *Introduction to Fats and Oils Technology*. 2^a. ed. [S.l.]: Champaign :AOCS press, 2000.

HALLIWELL, B. Lipid peroxidation, antioxidants and cardiovascular disease: how should we move forward? **Cardiovascular Research**, v. 47, n. 3, p. 410-418, 2000.

HALVORSEN, B. L.; BLOMHOFF, R. Determination of lipid oxidation products in vegetable oils and marine omega-3 supplements. **Food & Nutrition Research**, v. 55, p. 5792-801, 2011.

HARISTOY, X. W.; FAHEY, J. W.; SCHOLTUS, I. W.; LOZNIEWSKI, A. W. Adicionar ao Meu Espaço Evaluation of the Antimicrobial Effects of Several Isothiocyanates on *Helicobacter pylori*. **Planta Medica**, v. 71, n. 4, p. 326-330, 2005.

HAZRA, S.; BISWAS, S.; BHATTACHARYYA, D.; DAS, S. K.; KHAN, A. Quality of cooked ground buffalo meat treated with the crude extracts of *Moringa oleifera* (Lam.) leaves. **Journal of Food Science and Technology**, v. 49, n. 2, p. 240-245, 2012.

IQBAL, S.; BHANGER, M. I. Effect of season and production location on antioxidant activity of *Moringa oleifera* leaves grown in Pakistan. **Journal of Food Composition and Analysis**, v. 19, n. 6-7, p. 544-551, 2006.

JAISWAL, D.; RAI, P. K.; KUMAR, A.; MEHTA, S.; WATAL, G. Effect of *Moringa oleifera* Lam. leaves aqueous extract therapy on hyperglycemic rats. **Journal of Ethnopharmacology**, v. 123, p. 392-396, 2009.

KANNAN, R. R. R.; ARUMUGAM, R.; IYAPPARAJ, P.; THANGARADJOU, T.; ANANTHARAMAN, P. In vitro antibacterial, cytotoxicity and haemolytic activities and

phytochemical analysis of seagrasses from the Gulf of Mannar, South India. **Food chemistry**, v. 136, p. 1484-9, 2013.

KANWAR, A. S. Brine shrimp (*Artemia salina*) – a marine animal for simple and rapid biological assays. **Journal of Chinese Clinical Medicine**, v. 2, n. 4, p. 236-240, 2007.

KARADI, R. V; GADGE, N. B.; ALAGAWADI, K. R.; SAVADI, R. V. Effect of *Moringa oleifera* Lam. root-wood on ethylene glycol induced urolithiasis in rats. **Journal of Ethnopharmacology**, v. 105, n. 1-2, p. 306-11, 2006.

KEY, T. J.; APPLEBY, P. N.; MASSET, G.; BRUNNER, E. J.; CADE, J. E.; GREENWOOD, D. C.; STEPHEN, A. M.; KUH, D.; BHANIANI, A.; POWELL, N.; KHAW, K.-T. Vitamins, minerals, essential fatty acids and colorectal cancer risk in the United Kingdom Dietary Cohort Consortium. **International journal of cancer**, v. 131, n. 3, p. 320-325, 2012.

KIM, T. S.; DECKER, E. A.; LEE, J. Effects of chlorophyll photosensitisation on the oxidative stability in oil-in-water emulsions. **Food Chemistry**, v. 133, n. 4, p. 1449-1455, 2012.

KNOTHE, G. “ Designer ” Biodiesel : Optimizing Fatty Ester Composition to Improve Fuel Properties. **Energy & Fuels**, v. 22, p. 1358-1364, 2008.

KODALI, D. R. Oxidative stability measurement of high-stability oils by pressure differential scanning calorimeter (PDSC). **Journal agricultural and food chemistry**, v. 53, p. 7649-7653, 2005.

KOWALSKI, B.; RATUSZ, K.; KOWALSKA, D.; BEKAS, W. Determination of the oxidative stability of vegetable oils by Differential Scanning Calorimetry and Rancimat measurements. **European Journal of Lipid Science and Technology**, v. 106, n. 3, p. 165-169, 2004.

KRIS-ETHERTON, P. M.; HECKER, K. D.; BONANOME, A.; COVAL, S. M.; BINKOSKI, A. E.; HILPERT, K. F.; GRIEL, A. E.; ETHERTON, T. D. Bioactive Compounds in Foods : Their Role in the Prevention of Cardiovascular Disease and Cancer. **The American Journal of Medicine**, v. 113, n. 9, p. 71-88, 2002.

KUMBHARE, M.; GULEHA, V.; SIVAKUMAR, T. Estimation of total phenolic content, cytotoxicity and in-vitro antioxidant activity of stem bark of *Moringa oleifera*. **Asian Pacific Journal of Tropical Disease**, v. 2, n. 2, p. 144-150, 2012.

LAKO, J.; TRENNERY, V.; WAHLQVIST, M.; WATTANAPENPAIBOON, N.; SOTHEESWARAN, S.; PREMIER, R. Phytochemical flavonols, carotenoids and the antioxidant properties of a wide selection of Fijian fruit, vegetables and other readily available foods. **Food Chemistry**, v. 101, n. 4, p. 1727-1741, 2007.

LECLERC, L.; ZHAO, X.; SIMPSON, B. K.; ZUTA, P. C. The effect of α -tocopherol on the oxidation of mackerel oil.pdf. **Food Chemistry**, v. 100, p. 800-807, 2007.

LUKIW, W. J.; BAZAN, N. G. Docosahexaenoic Acid and the Aging Brain. **American Society for Nutrition**, v. 138, n. 12, p. 2510-2514, 2008.

MAKKAR, H. P. S.; BECKER, K. Nutritional value and antinutritional components of whole and ethanol extracted *Moringa oleifera* leaves. **Animal Feed Science and Technology**, v. 63, n. 1-4, p. 211-228, 1996.

MARCO, G. A rapid method for evaluation of antioxidants. **Journal of the American Oil Chemists' Society**, v. 45, p. 594-598, 1968.

MARTÍNEZ-TOMÉ, M.; JIMÉNEZ, A. M.; RUGGIER, S.; FREGA, N.; STRABBIOLI, R.; M.A., M. Antioxidant properties of mediterranean spices compared to common food additives. **Journal of Food Protection**, v. 64, n. 9, p. 1412-1419, 2001.

MEYER, B. N.; FERRIGNI, N. R.; PUTNAM, J. E.; JACOBSEN, L. B.; NICHOLS, D. E.; MCLAUGHLIN, J. L. Brine shrimp: A convenient general bioassay for active plant constituents. **Planta Medica**, v. 45, p. 31-34, 1982.

MILLER, H. E. A simplified method for the evaluation of antioxidants. **Journal of the American Oil Chemists' Society**, v. 48, p. 91, 1971.

MOHAMMED, K. A. E. F.; SARMIENTO-FRANCO, L.; SANTOS-RICALDE, R.; SOLORIO-SANCHEZ, J. F. The nutritional effect of *Moringa oleifera* fresh leaves as feed supplement on Rhode Island Red hen egg production and quality. **Tropical animal health and production**, 2011.

MORRIS, M. C.; EVANS, D.; TANGNEY, C. C.; BIENIAS, J. L.; WILSON, R. S. Fish consumption and cognitive decline with age in a large community study. **Archives of neurology**, v. 62, n. 12, p. 1849-53, 2005.

MOTHE, C. G.; AZEVEDO, A. D. *Análise Termica de Materiais*. 1^o. ed. [S.l: s.n.], 2009.

NAZ, S.; SIDDIQI, R.; SHEIKH, H.; SAYEED, S. Deterioration of olive, corn and soybean oils due to air, light, heat and deep-frying. **Food Research International**, v. 38, n. 2, p. 127-134, 2005.

NEMETS, H.; NEMETS, B.; APTER, A.; BRACHA, Z.; BELMAKER, R. H. Omega-3 treatment of childhood depression: a controlled, double-blind pilot study. **The American journal of psychiatry**, v. 163, n. 6, p. 1098-100, 2006.

OSTROWSKA-LIGEZA, E.; BEKAS, W.; KOWALSKA, D.; LOBACZ, M.; WRONIAK, M. Kinetics of commercial olive oil oxidation : Dynamic differential scanning calorimetry and Rancimat studies. **European Journal Lipid Science Technology**, v. 112, p. 268-274, 2010.

PIVIK, R. T.; DYKMAN, R. A.; JING, H.; GILCHRIST, J. M.; BADGER, T. M. Early Infant Diet and the Omega 3 Fatty Acid DHA: Effects on Resting Cardiovascular Activity and Behavioral Development During the First Half-Year of Life. **Developmental Neuropsychology**, v. 34, n. 2, p. 139-158, 2009.

PRITCHARD, M.; CRAVEN, T.; MKANDAWIRE, T.; EDMONDSON, A. S.; O'NEILL, J. G. A study of the parameters affecting the effectiveness of *Moringa oleifera* in drinking water purification. **Physics and Chemistry of the Earth, Parts A/B/C**, v. 35, n. 13-14, p. 791-797, 2010.

PULIDO, R.; BRAVO, L.; FULGENCIO, S. C. Antioxidant Activity of Dietary Polyphenols As Determined by a Modified Ferric Reducing/Antioxidant Power Assay. **Journal of Agricultural and Food Chemistry**, v. 48, p. 3396-3402, 2000.

RAMACHANDRAN, C.; PETER, K. V; GOPALAKRISHNAN, P. K. Drumstick (*Moringa oleifera*) a multipurpose Indian vegetable. **Economic Botany**, v. 34, n. 3, p. 276-283, 1980.

RAMALHO, E. F. S. M.; ALBUQUERQUE, A. R.; SOUZA, A. L.; BARRO, A. K.; MAIA, A. S.; SANTOS, I. M. G.; SOUZA, A. G. Use of different techniques in the evaluation of the oxidative stability of poultry fat biodiesel. **Journal of Thermal Analysis and Calorimetry**, v. 106, n. 3, p. 787-791, 2011.

RAMALHO, V.; JORGE, N. Antioxidantes utilizados em óleos, gorduras e alimentos gordurosos. **Química Nova**, v. 29, n. 4, p. 755-760, 2006.

RASHID, U.; ANWAR, F.; MOSER, B. R.; KNOTHE, G. *Moringa oleifera* oil: a possible source of biodiesel. **Bioresource technology**, v. 99, n. 17, p. 8175-9, 2008.

REDDY, V.; UROOJ, A.; KUMAR, A. Evaluation of antioxidant activity of some plant extracts and their application in biscuits. **Food Chemistry**, v. 90, p. 317-321, 2005.

REGITANO-D'ARCE, M. A. B. Deterioração de lipídeos – ranço. In: OETTERER, M.; REGITANO-D'ARCE, M. A. B.; SPOTTO, M. H. F. (Org.). *Fundamentos de ciência e tecnologia de alimentos*. Barueri: Manole, 2006. p. 243-270.

RIBEIRO, A. P. B.; GRIMALDI, R.; GIOIELLI, L. A.; GONÇALVES, L. A. G. Zero trans fats from soybean oil and fully hydrogenated soybean oil: Physico-chemical properties and food applications. **Food Research International**, v. 42, n. 3, p. 401-410, 2009.

SANTOS, N. A.; CORDEIRO, A. M. T. M.; DAMASCENO, S. S.; AGUIAR, R. T.; ROSENHAIM, R.; CARVALHO FILHO, J. R.; SANTOS, I. M. G.; MAIA, A. S.; SOUZA, A. G. Commercial antioxidants and thermal stability evaluations. **Fuel**, v. 97, p. 638-643, 2012.

SCHAICH, K. M. Thinking outside the classical chain reaction box of lipid oxidation. **Lipid Technology**, v. 24, n. 3, p. 55-58, 2012.

SHARMA, B. K.; RASHID, U.; ANWAR, F.; ERHAN, S. Z. Lubricant properties of Moringa oil using thermal and tribological techniques. **Journal of Thermal Analysis and Calorimetry**, v. 96, n. 3, p. 999-1008, 2009.

SIDDHURAJU, P.; BECKER, K. Antioxidant properties of various solvent extracts of total phenolic constituents from three different agroclimatic origins of drumstick tree (*Moringa oleifera* Lam.) leaves. **Journal of Agricultural and Food Chemistry**, v. 51, p. 2144-2155, 2003.

SILVA, A. C.; JORGE, N. Influence of *Lentinus edodes* and *Agaricus blazei* extracts on the prevention of oxidation and retention of tocopherols in soybean oil in an accelerated storage test. **Journal of Food Science and Technology**, 2012.

SILVA, A. V. C.; SANTOS, A. R. F.; LÉDO, A. S.; ALMEIDA, R. B. F. C. S.; SILVA, G. M.; RANGEL, M. S. A. Moringa genetic diversity from germplasm bank using RAPD markers. **Tropical and Subtropical Agroecosystems**, v. 15, n. 1, p. 31-39, 2012.

SIMIC, M. G.; JAVANOVIC, S. V. Inactivation of oxygen radicals by dietary phenolic compounds in anticarcinogenesis. In: HO, C. T. et al. (Org.). *Food phytochemicals for cancer prevention*. Washington: American Chemical Society, 1994. p. 20-33.

SINGH, B. N.; SINGH, B. R.; SINGH, R. L.; PRAKASH, D.; DHAKAREY, R.; UPADHYAY, G.; SINGH, H. B. Oxidative DNA damage protective activity, antioxidant and anti-quorum sensing potentials of *Moringa oleifera*. **Food and Chemical Toxicology**, v. 47, p. 1109-16, 2009.

SLINKARD, K.; SINGLETON, V. L. Total Phenol Analysis: Automation and Comparison with Manual Methods. **American Journal of Enology and Viticulture**, v. 28, n. 1, p. 49-55, 1977.

SOARES, S. E. Ácidos fenólicos como antioxidantes. **Revista de Nutrição**, v. 15, n. 1, p. 71-81, 2002.

SREELATHA, S.; PADMA, P. R. Antioxidant activity and total phenolic content of *Moringa oleifera* leaves in two stages of maturity. **Plant foods for human nutrition**, v. 64, n. 4, p. 303-11, 2009.

SUN-WATERHOUSE, D.; THAKORLAL, J.; ZHOU, J. Effects of added phenolics on the storage stability of avocado and coconut oils. **International Journal of Food Science & Technology**, v. 46, n. 8, p. 1575-1585, 2011.

SÁNCHEZ-MACHADO, D. I.; LÓPEZ-CERVANTES, J.; VÁZQUEZ, N. J. R. High-performance liquid chromatography method to measure alpha- and gamma-tocopherol in leaves, flowers and fresh beans from *Moringa oleifera*. **Journal of chromatography. A**, v. 1105, n. 1-2, p. 111-4, 2006.

TAMJIDI, F.; NASIRPOUR, A.; SHAHEDI, M. Physicochemical and sensory properties of yogurt enriched with microencapsulated fish oil. **Food science and technology international**, From Duplicate 1 (Physicochemical and sensory properties of yogurt enriched with microencapsulated fish oil. - Tamjidi, F; Nasirpour, A; Shahedi, M) , v. 18, n. 4, p. 381-90, 2012.

TAN, C. P.; CHE MAN, Y. B.; SELAMAT, J.; YUSOFF, M. S. A. Comparative studies of oxidative stability of edible oils by differential scanning calorimetry and oxidative stability index methods. **Food Chemistry**, v. 76, n. 3, p. 385-389, 2002.

TSAKNIS, J; LALAS, S.; GERGIS, V.; DOURTOGLOU, V.; SPILIOTIS, V. Characterisation of *Moringa oleifera* variety Mbololo seed oil of Kenya. **Journal of agricultural and food chemistry**, v. 47, p. 4495-4499, 1999.

TSAKNIS, JOHN; LALAS, S. Stability During Frying of *Moringa oleifera* Seed Oil Variety “Periyakulam 1”. **Journal of Food Composition and Analysis**, v. 15, n. 1, p. 79-101, 2002.

USDA, U. S. D. O. A. *Foreign Agricultural Service. Global Agricultural Information Network: Brazil, Oilseeds and Products Annual, 2010.*

VELASCO, J.; ANDERSEN, M. L.; SKIBSTED, L. H. Evaluation of oxidative stability of vegetable oils by monitoring the tendency to radical formation. A comparison of electron spin resonance spectroscopy with the Rancimat method and differential scanning calorimetry. **Food Chemistry**, v. 85, n. 4, p. 623-632, 2004.

VERMA, A. R.; VIJAYAKUMAR, M.; MATHELA, C. S.; RAO, C. V. In vitro and in vivo antioxidant properties of different fractions of *Moringa oleifera* leaves. **Food and Chemical Toxicology**, v. 47, n. 9, p. 2196-2201, 2009.

WANG, H.; LIU, F.; YANG, L.; ZU, Y.; WANG, H.; QU, S.; ZHANG, Y. Oxidative stability of fish oil supplemented with carnosic acid compared with synthetic antioxidants during long-term storage. **Food Chemistry**, v. 128, n. 1, p. 93-99, 2011.

ZHANG, Y.; YANG, L.; ZU, Y.; CHEN, X.; WANG, F.; LIU, F. Oxidative stability of sunflower oil supplemented with carnosic acid compared with synthetic antioxidants during accelerated storage. **Food Chemistry**, v. 118, n. 3, p. 656-662, 2010.

4 RESULTADOS

Os resultados do presente estudo estão apresentados na forma de artigos. São eles:

Artigo 1	Ethanollic extracts of <i>Moringa oleifera</i> Lam.: evaluation of its potential as an antioxidant additive for fish oil <i>Publicado em: Journal of Thermal Analysis and Calorimetry</i>
Artigo 2	Ethanolics extracts of moringa: antioxidant effect in soybean oil by P-DSC and Rancimat <i>Submetido em: Journal of Thermal Analysis and Calorimetry</i>
Artigo 3	Oxidative stability of fish oil supplemented with ethanolic extracts of <i>Moringa oleifera</i> Lam. compared with synthetic antioxidants during accelerated test <i>Submetido em: Journal of Agricultural and Food Chemistry</i>
Artigo 4	Antioxidant effect of <i>Moringa oleifera</i> Lamark extracts in soybean oil during accelerated storage test <i>Submetido em: Journal of the American Oil Chemists' Society</i>
Artigo 5	Study of antioxidant activity and preliminary bioactivity determination of ethanolic extracts of <i>Moringa oleifera</i> Lam. <i>Submetido em: Natural Product Communications</i>

Ethanollic extracts of *Moringa oleifera* Lam.

Evaluation of its potential as an antioxidant additive for fish oil

Jaqueline A. Nascimento · Kassandra L. G. V. Araújo · Poliana S. Epaminondas ·
Alline S. Souza · Marciane Magnani · Antonia L. Souza · Luiz E. B. Soledade ·
Neide Queiroz · Antonio G. Souza

Received: 18 September 2012 / Accepted: 5 February 2013
© Akadémiai Kiadó, Budapest, Hungary 2013

Abstract This paper reports the evaluation of the antioxidant potential of the ethanollic extracts of the leaves (EL), flowers (EF), seed pods (EP) and seeds (ES) from *Moringa oleifera* Lam. The antioxidant potential was assessed, upon the addition of the extracts to fish oil, by means of the total extractable phenol content (TEP), the DPPH[•] free radical scavenging efficiency and using pressurized differential scanning calorimetry (P-DSC). The results of TEP and DPPH[•] showed that the ES extract does not present a potential to be used as an antioxidant additive, and it was thus discarded from the remaining analyses. Thus, the following treatments were prepared: pure fish oil, fish oil with BHT (100 mg kg⁻¹), fish oil with TBHQ (100 mg kg⁻¹) and fish oil with EL, EF and EP—all at the concentration of 100 mg kg⁻¹, in relation to the total extractable phenolics contained in each one of the extracts. The leaf and flower extracts displayed a protecting effect,

with an increase in about 20 and 11 % of the OIT values, respectively. However, such protection was smaller than that conferred by the synthetic antioxidants utilized. As for the thermal analysis results, it was noticed that EL presented the highest thermal stability among the extracts.

Keywords Fish oil · *Moringa oleifera* Lam. · Antioxidant · Thermal oxidation · Thermal analysis

Introduction

The regular consumption of marine fish oil brings countless benefits to the human organism, among which is the protection against diseases such as Alzheimer's disease [1], depression [2], cognitive impairment [3], rheumatoid arthritis [4] and cardiovascular diseases [5], besides helping in the development and growth of the nervous system [6]. Such properties are attributed to the presence of long-chain polyunsaturated fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). These are essential acids and compete with arachidonic acid (AA) in the enzymatic pathways, promoting a reduction in the formation of proinflammatory mediators [7]. Nevertheless, the presence of these acids makes fish oil highly susceptible to oxidation, a reaction catalyzed by light, heat, metal traces or enzymes [8]. The oxidative process that degrades the lipids includes three steps: initial step, propagation step and termination step. In the first two steps, the formation of free radicals and neutral molecules such as the hydroperoxides takes place. Without the interruption, by means of antioxidants, the reaction continues with the consequent formation of secondary oxidation products, such as small-chain carboxylic acids, aldehydes and ketones. The secondary oxidation products impair the colour, smell and flavour of the oil [9]. Besides, modifications

J. A. Nascimento (✉) · K. L. G. V. Araújo ·
P. S. Epaminondas · A. S. Souza · M. Magnani
Programa de Pós-Graduação em Ciência e Tecnologia de
Alimentos, CT, Campus I, Universidade Federal da Paraíba,
João Pessoa, Paraíba 58051-970, Brazil
e-mail: jaquelinejp@yahoo.com.br

P. S. Epaminondas
Setor de Agroindústria, Instituto Federal de Educação,
Ciência e Tecnologia (IFPB), Campus Sousa, Sousa,
Paraíba 58800-970, Brazil

A. L. Souza · N. Queiroz · A. G. Souza
Laboratório de Combustíveis e Materiais—LACOM,
Departamento de Química, CCEN, Campus I, Universidade
Federal da Paraíba, João Pessoa, Paraíba 58059-900, Brazil

L. E. B. Soledade
Campus de Pinheiro, Universidade Federal do Maranhão,
Pinheiro, Maranhão 65200-000, Brazil

can occur in the nutritional characteristics resulting in functionality loss and toxicity [10].

Industrially, the fish oil stability is improved by the addition of synthetic antioxidants, which are compounds that delay the oxidative deterioration and consequently extend its useful life. However, the synthetic antioxidants have a restricted use in several countries, as they may present harmful effects to the human organism [11]. In Brazil, the maximum limit for TBHQ is 200 mg kg⁻¹ and for BHT it is 100 mg kg⁻¹ [12]. Therefore, antioxidants provenient from natural sources have been studied aiming at obtaining safer and more efficient products that may be used in foods, thus substituting partially or completely the synthetic antioxidants [13].

Among the several sources of natural antioxidants studied, the *Moringa oleifera* Lam. should be emphasized. It is a plant widely cultivated in tropical regions, due to the low requirements of climate and soil and whose leaves, flowers, green fruits and roots display excellent antioxidant activity, which was already proved in the literature [14–16].

Considering *M. oleifera* Lam. as a natural source of antioxidant products, the present work was developed with the purpose of evaluating the ethanolic extracts of its leaves, flowers, seed pods and seeds in maintaining the oxidative stability of fish oil, in comparison with the synthetic antioxidants TBHQ and BHT employed for this purpose.

Experimental

Refined fish oil and the reagents 2,2-diphenyl-1-picrylhydrazyl (DPPH), butylated hydroxytoluene (BHT) and *tert*-butylhydroquinone (TBHQ) were purchased from Sigma-Aldrich (Steinheim, Germany) and Folin–Ciocalteu reagent; 3,4,5-trihydroxybenzoic acid (gallic acid) and all other analytical reagents were acquired from Merck (Düsseldorf, Germany).

The leaves, flowers, seed pods and seeds from *M. oleifera* Lam. were collected during the month of April 2010, in Campus I from Universidade Federal da Paraíba, João Pessoa, Paraíba State, Brazil and submitted to drying in an oven with air circulation at 45 °C (Marconi, Model MA 035) for 24 h. After complete drying, each part was separately comminuted in a knife mill and subsequently conditioned in polyethylene bags in vacuum and kept at the temperature of –18 °C during the experiments. A dried specimen exemplar was deposited in the Herbarium of the Botany Department of UFPB with the register—J.A.N. Batista 01 (JPB).

The extracts were separately obtained by means of using 50 g of the powdered plant material and 500 mL of ethanol. The blend was agitated in a metabolic water bath (Model MA-093 Dubnoff, Marconi, Brazil), for 2 h, at

25 °C, followed by vacuum filtering. The extracts of leaves (EL), flowers (EF), seed pods (EP) and seeds (ES) were concentrated in a rotary evaporator (Tecnal, Model TE-221) at 60 °C up to complete solvent removal. Afterwards the extracts are conditioned in amber flasks at refrigeration (about 8 °C) until the moment of being used.

The total extractable phenol contents (TEP) were colorimetrically determined by the Folin–Ciocalteu method [17] with volume reductions. Aliquots of 4 mL of each ethanolic extract, previously diluted in water, received the addition of 0.25 mL of the Folin–Ciocalteu reagent and 0.75 mL of a saturated sodium carbonate solution. The blend was initially stirred and later was kept in the dark for 2 h. Afterwards the absorbance measurement was taken at 765 nm in a UV–Vis spectrophotometer (model UV-2550, Shimadzu, Japan). Different lectures were made, for each sample and for a control sample, containing only water and the reagents. The concentration of the phenolic compounds was estimated by a gallic acid calibration curve at the 50–500 mg L⁻¹ concentration range. The results were expressed as average ± standard deviation of gallic acid equivalent (GAE) mg g⁻¹ of extract.

The capacity of the EL, EF, EP and ES extracts in scavenging the radical DPPH [18, 19] was compared with the same capacity for the synthetic antioxidants BHT and TBHQ. Aliquots of 3.0 mL each of phenolic extract and of the synthetic antioxidants, at the dilutions of 20, 40 and 80 µg mL⁻¹ added to 0.1 mL of DPPH 0.1 mM ethanolic solution. The control consisted of 3.0 mL ethanol and 0.1 mL of the DPPH solution. For each dilution of each different sample, a blank test was prepared, composed of 3.0 mL of the sample dilution and 0.1 mL of ethanol. The absorbance decrease was measured at 515 nm in a UV–Vis spectrophotometer (model UV-2550, Shimadzu, Japan) after a time span of 120 min. The scavenging activity of the DPPH radical was calculated using the following formula:

$$\text{Scavenging activity of the DPPH}^{\bullet}\text{radical}(\%) = \left[\left(1 - \frac{A_a - A_b}{A_c} \right) \times 100 \right]$$

In which A_a is the sample absorbance, A_b is the blank absorbance and A_c is the negative control absorbance.

With the values obtained by the formula, a graph was built for the scavenging activity of the DPPH radical against the concentration of the extracts in µg mL⁻¹. For the calculation of EC₅₀ (extract concentration with the capacity of reducing 50 % of the initial DPPH concentration) the line equation was used, replacing the value by 50. The antiradical efficiency as defined as the inverse of EC₅₀ (1/EC₅₀).

Fish oil received the addition of the extracts EL, EF or EP at the concentration of 100 mg kg⁻¹ in relation of their

TEP, or BHT, or TBHQ both at 100 mg kg^{-1} . Simultaneous thermogravimetry and differential thermal analysis (TG/DTA) of extracts EL, EF and EP were obtained in a Thermal Analyzer from TA instruments SDT 2960, at the 25 and $600 \text{ }^{\circ}\text{C}$ temperature range, $10 \text{ }^{\circ}\text{C min}^{-1}$ heating rate in an oxygen atmosphere with the flow rate of 100 mL min^{-1} .

The pressurized differential scanning calorimetry (P-DSC) calorimetric curves of the fish oil, with and without additives were obtained in a differential scanning calorimeter, model DSC Q1000 from TA instruments. The non isothermal curve for pure fish oil was performed in an aluminium crucible and the calorimeter analysis conditions were oxygen atmosphere with a 100 mL min^{-1} flow and a pressure of $1,400 \text{ kPa}$, heating rate of $10 \text{ }^{\circ}\text{C min}^{-1}$ and temperature range from 25 to $500 \text{ }^{\circ}\text{C}$. The isothermal curves were carried out at $100 \text{ }^{\circ}\text{C}$, at the same conditions previously described. Oxidation induction time (OIT) values were determined by the difference of the onset time and the initial time.

The results were analyzed with the aid of the Statistica 7.0 (Statsoft®) software and were expressed as average $\pm$ standard deviation and compared by analysis of variance (ANOVA) and by the Tukey test, considering $p < 0.05$.

Results and discussions

The analyses of the TEP contents present in the extracts showed that the highest values were observed for EL and the smallest for ES, and that no significant difference ($p < 0.05$) was found in relation to the amount of phenolics determined for EF and EP (Table 1). The highest value of PET for the EL extracts agrees with the data available in the literature, that report the presence of the flavonoids: gallic acid, ellagic acid, ferulic acid, chlorogenic acid, kaempferol, quercetin, vanillin and rutin in the leaves of *M. oleifera* Lam. [20].

The antioxidant activities of the natural and synthetic additives, measured by the DPPH[•] radical tests, are presented

in Table 1. The data show that, among the *M. oleifera* Lam. extracts, EL was the most efficient in relation to the DPPH[•] radical, even when compared to the synthetic antioxidant BHT, whereas the ES extract exhibited a very discrete antioxidant efficiency. These data clearly reveal a correlation between the amount of phenolic compounds and antioxidant capacity of the extracts.

Phenolic compounds are structurally characterized by the presence of hydroxyl groups bound to aromatic rings. These hydroxyl groups are able to neutralize the free radicals by means of the donation of a hydrogen radical, leading to the formation of the chemically stable phenoxide radical, interrupting the progress of the auto-oxidation reaction [19, 21, 22]. A comparison made between the *M. oleifera* Lam. extracts and the synthetic antioxidants BHT and TBHQ showed that, in relation to the synthetic antioxidants, the plant extracts presented a less expressive response upon being compared to TBHQ and a more expressive response than BHT. The superiority verified for TBHQ can be attributed to the presence of two hydroxyl groups in its molecule, which confers to this antioxidant a higher power for donating hydrogen to the free radicals, thus more easily interrupting the chain reaction of oxidation [23].

The thermal stability of the substances used as antioxidants is extremely important, once a substance with a very low degradation temperature can easily undergo degradation and lose the effect as antioxidant or even act as a pro-oxidant [24]. Aiming at evaluating the thermal stability of the *M. oleifera* Lam. extracts, thermal analyses experiments were performed for EP, EF and EL. A similar experiment was not run for ES, as this extract displayed a low TEP content and a scarcely effective antioxidant activity towards the DPPH[•] radical.

The thermogravimetric curves (TG) and the DTA curves of the *M. oleifera* Lam. extracts are presented in Fig. 1.

The decomposition process involved several mass loss events, of which the first ones are endothermic and the

Table 1 Total extractable phenol content (TEP), antioxidant capacity (EC_{50}) and antiradical efficiency measured in the DPPH[•] radical tests for EL, EF, EP, ES, BHT and TBHQ

Samples	TEP/mg GAE/g of extract	$\text{EC}_{50}/\mu\text{g mL}^{-1}$	Antiradical efficiency
EL	53.69 ± 1.00^a	15.59 ± 1.92^c	64.84 ± 8.47^b
EF	45.85 ± 1.71^b	63.22 ± 17.60^b	16.68 ± 4.68^c
EP	41.75 ± 3.35^b	80.89 ± 7.70^b	12.43 ± 1.12^c
ES	8.06 ± 0.47^c	816.61 ± 50.00^a	1.23 ± 0.07^d
BHT	N.A.	74.57 ± 9.37^b	13.55 ± 1.73^c
TBHQ	N.A.	1.33 ± 0.02^d	753.84 ± 8.71^a

Values are reported as mean $\pm$ standard deviation, $n = 3$

Letters (a, b, c, d) within a column shows that the differences between mean values were statistically significant ($p < 0.05$)

NA not applicable, as it is a pure compound

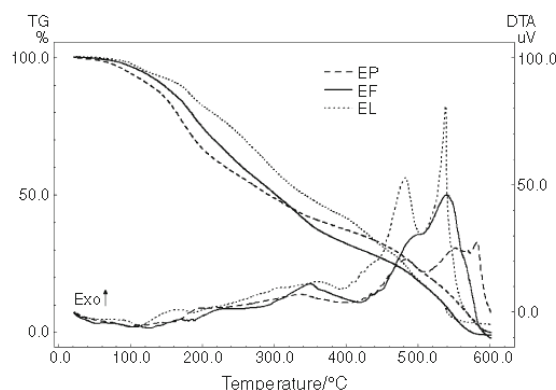


Fig. 1 TG/DTA curve and the EL, EF and EP extracts

remaining are exothermic. In order to evaluate the thermal stability, the onset temperature (T_{onset}) obtained from the TG curve of each extract was used as parameter. The mass loss T_{onset} values ranged from 66 to 83 °C and pointed out the highest stability for the extract EL, about 20 % higher than the least stable extract (EP). In the temperature range from 22 to 259 °C, the samples EF and EP showed mass loss values of 40 and 43 %, respectively, for the endothermic events. The extract EL showed a smaller mass loss (29 %), which can be attributed to a possible smaller amount of volatiles present in the sample. The remaining mass losses occurred as exothermic events, being ascribed to the processes of decomposition and combustion of the more thermally stable compounds.

The thermal analysis results suggest the highest stability for EL, the extract with the highest value of TEP, which displays a better antioxidant activity towards the DPPH radical than the synthetic antioxidant BHT.

The P-DSC analyses of the fish oil without additives were carried out in the non isothermal and isothermal modes, whereas for the samples of fish oil with different additives, only the isothermal mode was employed.

The non isothermal P-DSC curve (Fig. 2a) showed an example for the calculation of the value of oxidation temperature (OT) of 120 °C. The non isothermal P-DSC curves

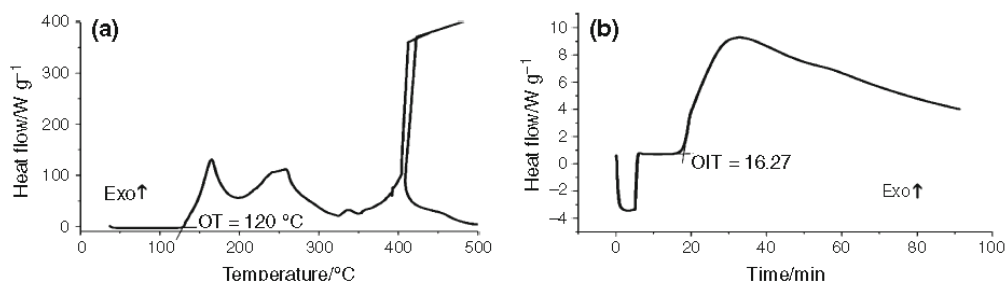


Fig. 2 Non isothermal (a) and isothermal (b) P-DSC curves of pure fish oil

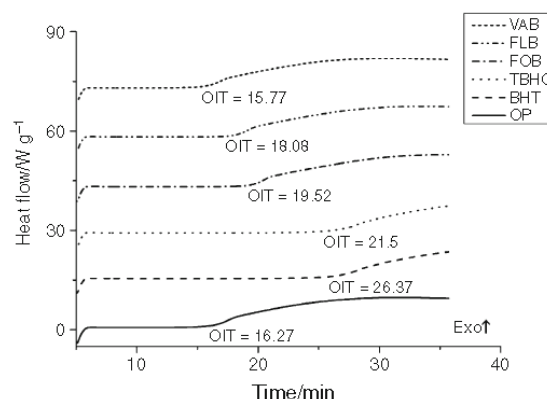


Fig. 3 Effect of the addition of the antioxidants TBHQ, BHT and the extracts EL, EF and EP on the OIT value of fish oil

are useful to analyze the heat flow at different temperatures of a sample and determine an adequate temperature to perform the isothermal P-DSC. Based on such information, the oxidative induction time (OIT) of the samples of fish oils with the addition or not of the extracts and/or the synthetic antioxidants was determined at 100 °C.

The OIT value (Fig. 2b) of the sample of fish oil without antioxidants was of 16.27 min, a result which is compatible with the fatty acid profile of fish oils. Araújo et al., studying the refined fish oil, found a value of 53 min, determined at the temperature of 100 °C and pressure of 3,450 kPa. Although such value was 3.26 times higher than the one reported in the present paper, the authors showed by means of other analyses that this oil was suffering an oxidation process [25]. The P-DSC technique analyzes the oxidative stability of a sample in the initial step and at the oxidation propagation step, in which the formation of peroxides occurs [26]. An oil in an advanced stage of oxidation can yield a very high OIT value, leading to a false interpretation of the result, in the case of being evaluated separately.

Figure 3 shows the effect of the synthetic antioxidants TBHQ and BHT, as well as the extracts EL, EP and EF on the oxidative stability of fish oil. Based on the OIT values, the extracts EL and EF increased the oxidative stability of fish oil

in 19.98 and 11.12 %, respectively. The extract EP, although presenting a remarkable amount of PET and an antioxidant capacity towards the DPPH[•] radical, was shown to be pro-oxidant, diminishing the OIT control value in 3 %. This result can be attributed to the presence of glycosylated structures widely distributed in all parts of *M. oleifera* Lam. [27]. The presence of a sugar unit condensed to the flavonoid molecule, as well as to the phenolic acid, decreases their affinity by the oil molecules and thus hampers the antioxidant action of these substances in a purely lipid environment.

Among the synthetic antioxidants, BHT, surprisingly, displayed a higher protecting effect than TBHQ, although the latter presented a much superior antioxidant activity in the DPPH[•] radical test. However, it should be taken into account that P-DSC utilizes elevated pressures. When high pressures are applied in polar molecules such as TBH, intermolecular forces are intensified. In the case of TBHQ such forces are of the hydrogen bond-type. The result of the intensification of these forces is to decrease the affinity of TBHQ by the oil molecules, thus diminishing its antioxidant effect. In the case of BHT molecules, these forces do not act as effectively, once they only present one hydroxyl group and it is sterically hampered by the vicinity of two *tert*-butyl groups.

Conclusions

The results showed that higher amounts of phenolic compounds exist in the leaves and in the flowers of *M. oleifera* Lam., justifying a higher antioxidant capacity of these extracts towards the DPPH[•] radical. As far as the antioxidant effect on the fish oil is concerned, the efficiency of EL and EF were verified by means of the increase in the OIT value of 19.98 and 11.12 %, respectively. In relation to the feasibility of the usage of the extracts as antioxidant additives in oils, the potential of such usage was observed. However, due to the great amount of glycosylated substances, the best application of the extracts would be in the form of emulsions. An interesting topic for investigation would be to assess the synergetic effect of blends composed of these extracts and antioxidants. In terms of the use of P-DSC technique for the evaluation of antioxidant activity of the studied substances, it was noticed that for the case of TBHQ, P-DSC was not adequate to assess its efficiency as antioxidant. It is believed that pressure intensifies the intermolecular forces, hydrogen bonds in this case, and diminishes the availability of the hydrogen from the phenolic hydroxyl for the recovery of the fatty acid molecule.

Acknowledgments The authors acknowledge the Brazilian agencies Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for the financial support.

References

1. Lukiw WJ, Bazan NG. Docosahexaenoic acid and the aging brain. *Am Soc Nutr.* 2008;138:2510–4.
2. Nemets H, Nemets B, Apter A, Bracha Z, Belmaker RH. Omega-3 treatment of childhood depression: a controlled, double-blind pilot study. *Am J Psychiatr.* 2006;163:1098–100.
3. Morris MC, Evans D, Tangney CC, Bienias JL, Wilson RS. Fish consumption and cognitive decline with age in a large community study. *Arch Neurol.* 2005;62:1849–53.
4. Bykerk VP, Keystone EC. What are the goals and principles of management in the early treatment of rheumatoid arthritis? Best practice and research. *Clinic Rheumat.* 2005;19:147–61.
5. Kris-etherton PM, Hecker KD, Bonanome A, Coval SM, Binkoski AE, Hilpert KF, Griell AE, Etherton TD. Bioactive compounds in foods : their role in the prevention of cardiovascular disease and cancer. *Am J Med.* 2002;113:71–88.
6. Pivik RT, Dykman RA, Jing H, Gilchrist JM, Badger TM. Early infant diet and the omega 3 fatty acid DHA: effects on resting cardiovascular activity and behavioral development during the first half-year of life. *Dev Neuropsychol.* 2009;34:139–58.
7. Balk EM, Lichtenstein AH, Chung M, Kupelnick B, Chew P, Lau J. Effects of omega-3 fatty acids on serum markers of cardiovascular disease risk: a systematic review. *Atherosclerosis.* 2006;189:19–30.
8. Mothé CG, Castro BCS, Mothé MG. Characterization by TG/DTG/DSC and FTIR of frying and fish oil residues to obtain biodiesel. *J Therm Anal Calorim.* 2011;106:811–7.
9. Barriuso B, Astiasarán I, Ansorena D. A review of analytical methods measuring lipid oxidation status in foods: a challenging task. *Eur Food Res Technol.* 2013;236:1–15.
10. Siriwardhana N, Lee KW, Kim SH, Há JH, Park GT, Jeon YJ. Lipid peroxidation inhibitory effects of *Hizikia fusiformis* methanolic extract on fish oil and linoleic acid. *Food Sci Technol Int.* 2004;10:65–72.
11. Hras AR, Hadolin M, Knez Z, Bauman D. Comparison of anti-oxidative and synergistic effects of rosemary extract with α -tocopherol, ascorbyl palmitate and citric acid in sunflower oil. *Food Chem.* 2000;71:229–33.
12. Brasil. Ministério da Saúde. Comissão Nacional de Normas e Padrões para Alimentos. Resolução no. 04/88. In: Associação Brasileira das Indústrias de Alimentação, *Compêndio da Legislação de Alimentos*. São Paulo: ABIA, 2001, v. 1, p. 3.26.
13. Brewer MS. Natural Antioxidants: sources, Compounds, Mechanisms of Action, and Potential Applications. *Compr Rev Food Sci F.* 2011;10:221–47.
14. Iqbal S, Bhanger MI. Effect of season and production location on antioxidant activity of *Moringa oleifera* leaves grown in Pakistan. *J Food Compos Anal.* 2006;19:544–51.
15. Lako J, Trenerry V, Wahlqvist M, Wattanapenpaiboon N, Sotheeswaran S, Premier R. Phytochemical flavonols, carotenoids and the antioxidant properties of a wide selection of Fijian fruit, vegetables and other readily available foods. *Food Chem.* 2007;101:1727–41.
16. Reddy V, Urooj A, Kumar A. Evaluation of antioxidant activity of some plant extracts and their application in biscuits. *Food Chem.* 2005;90:317–21.
17. Slinkard K, Singleton VL. Total phenol analysis: automation and comparison with manual methods. *Am J Enol Vitic.* 1977;28:49–55.
18. Blois MS. Antioxidant determinations by the use of a stable free radical. *Nature.* 1958;181:1199–200.
19. Fresco P, Borges F, Diniz C, Marques MPM. New insights on the anticancer properties of dietary polyphenols. *Medic Res Rev.* 2006;26:747–66.
20. Singh BN, Singh BR, Singh RL, Prakash D, Dhakarey R, Upadhyay G, Singh HB. Oxidative DNA damage protective activity, antioxidant and anti-quorum sensing potentials of *Moringa oleifera*. *Food Chem Toxicol.* 2009;47:1109–16.

21. Angelo PM, Jorge N. Evaluation of stored sunflower oil with the addition of antioxidants. *Ciênc Tecnol Aliment*. 2008;28:498–502.
22. Contreras-Calderón J, Calderón-Jaimes L, Guerra-Hernández E, García-Villanova B. Antioxidant capacity, phenolic content and vitamin C in pulp, peel and seed from 24 exotic fruits from Colombia. *Food Res Int*. 2011;44:2047–53.
23. Santos NA, Cordeiro AMTM, Damasceno SS, Aguiar RT, Rosenhaim R, Carvalho Filho JR, Santos IMG, Maia AS, Souza AG. Commercial antioxidants and thermal stability evaluations. *Fuel*. 2012;97:638–43.
24. Fernandes FHA, Santana CP, Santos RL, Correia LP, Conceição MM, Macêdo RO, Medeiros ACD. Thermal characterization of dried extract of medicinal plant by DSC and analytical techniques. *J Therm Anal Calorim*. 2012;. doi:[10.1007/s10973-012-2807-3](https://doi.org/10.1007/s10973-012-2807-3).
25. Araújo KLG, Epaminondas PS, Silva MCD, Lima AEA, Rosenhaim R, Maia AS, Soledade LEB, Souza AL, Santos IMG, Souza AG, Queiroz N. Influence of thermal degradation in the physicochemical properties of fish oil. *J Therm Anal Calorim*. 2011;106:557–61.
26. Leonardo RS, Murta Valle ML, Dweck J. An alternative method by pressurized DSC to evaluate biodiesel antioxidants efficiency. *J Therm Anal Calorim*. 2012;108:751–9.
27. Bennett RN, Mellon FA, Foidl N, Pratt JH, Dupont MS, Perkins L, Kroon PA. Profiling glucosinolates and phenolics in vegetative and reproductive tissues of the multi-purpose trees *Moringa oleifera* L. (horseradish tree) and *Moringa stenopetala* L. *J Agric Food Chem*. 2003;51:3546–53.

ETHANOLICS EXTRACTS OF MORINGA: ANTIOXIDANT EFFECT IN SOYBEAN OIL BY PDSC AND RANCIMAT

Jaqueline A. Nascimento^{1*}, Kassandra L. G. V. Araújo¹, Poliana S. Epaminondas^{1,3}, Alline L. S. Pontes¹, Antonia L. Souza², Neide Queiroz², Antonio G. Souza²

¹Programa de Pós-Graduação em Ciência e Tecnologia de Alimentos, CT, Campus I, Universidade Federal da Paraíba, 58051-970, João Pessoa, Paraíba, Brazil.

²Laboratório de Combustíveis e Materiais – LACOM, Departamento de Química, CCEN, Universidade Federal da Paraíba, 58059-900, Campus I, João Pessoa, Paraíba, Brazil.

³Setor de Agroindústria, Instituto Federal de Educação, Ciência e Tecnologia (IFPB), 58800-970, Campus Sousa, Sousa - Paraíba, Brasil.

*Corresponding author: antonia_lucia@yahoo.com.br

Fax: +55-83-32167441; Phone: +55-83-32167441

Abstract

The use of natural antioxidants has been widely promoted in the food industry because it is economically viable and very attractive with consumers. In this study we determined the extractable total phenolic content (FET) of crude ethanol extracts of the leaves (EL), flowers (EF) and seed pods (EP) of *Moringa oleifera* Lam. We also evaluated the antioxidant effect of the extracts on oxidative stability of soybean oil, through the accelerated PDSC and Rancimat® techniques using the synthetic antioxidants butylatedhydroxytoluene (BHT) and *tert*-Butylhydroquinone (TBHQ) as positive control. The values of FET in the extracts ranged from 41.75 ± 3.35 to 53.69 ± 1.00 mg GAE/g, and EL extract exhibited best result. The results of oxidative stability in both techniques showed that the EL extract provided greater protection to the oil, indicating a correlation between the amount of FET and the protective effect. Comparison made between the synthetic antioxidants and extracts revealed that in the Rancimat technique, extracts were less effective than the synthetic antioxidants TBHQ and BHT, however, in the PDSC technique the EL extract was more effective than BHT, proving to be a good alternative for applications in the soybean oil, replacing this synthetic antioxidant.

Keywords: Oxidative stability, *Moringa oleifera* Lam., accelerated techniques, PDSC, Racimat

Introduction

Soybean is a culture of great commercial value in the world and is used mainly for the production of oil, which comprises 12 to 15% of saturated fatty acids (mostly palmitic acid) and 85 to 88% acid unsaturated fatty acids, mainly oleic, linoleic and linolenic acids [1]. The high percentage of unsaturated fatty acids makes the oil highly susceptible to oxidative processes [2], leading to its rancidity and impaired quality [3].

To maintain the quality and prolong the shelf life of the oils, the lipid oxidation process is usually prevented by employing synthetic antioxidants as butylated hydroxyanisole (BHA), the Butylated hydroxytoluene (BHT) and tert-butyl hydroquinone (TBHQ). These compounds, although very satisfactory as antioxidants, have its use restricted for having assigned to them carcinogenic and toxic effects in humans [4]. In Brazil, the maximum concentration of BHT and TBHQ allowed is 200 mg kg⁻¹ and 100 mg kg⁻¹, respectively [5].

Given the above, antioxidants from natural sources have been studied in the search for safer and more effective products for use in foods, replacing all or part of synthetic antioxidants [6].

Moringa oleifera Lam, is a plant that has, among its chemical constituent, high content of phenolic compounds distributed in leaves, flowers, roots and unripe fruits [7–9]. The plant is native to northern India, but widely cultivated in tropical regions around the world. It is perfectly adapted to the climate of northeastern Brazil, because it presents good growth, even when subjected to drought conditions and high temperatures, characteristic of this region.

Considering the *Moringa* as a natural source of antioxidant compounds, the present work was to evaluate the antioxidant potential of crude ethanol extracts of leaves, flowers and seed pods on the oxidative stability of soybean oil compared to synthetic antioxidant TBHQ and BHT, by employing the technique of pressurized differential scanning calorimetry (PDSC) and Rancimat ®.

Experimental

Refined soybean oil was acquired from the local market. According to the manufacturer specifications, the soybean oil was free from antioxidant. Reagents butylatedhydroxytoluene (BHT) and tert-Butylhydroquinone (TBHQ) were purchased from Sigma-Aldrich (Steinheim, Germany). Folin-Ciocalteu reagent, 3,4,5-trihydroxybenzoic acid (gallic acid) and all other reagents and solvents of analytical grade were purchased from Merck (Düsseldorf, Germany).

The leaves, flowers and seed pods from moringa (*Moringa oleifera* Lam.) were collected at Campus I, Federal University of Paraíba, in the city of João Pessoa/PB, Brazil, during the month of April 2010. The plant

material was submitted to drying in an oven air circulation at 45 °C (Marconi, Model MA 035) for 24 h. After complete drying, each part was separately comminuted in a knife mill and subsequently conditioned in polyethylene bags in vacuum and kept at the temperature of -18 °C during the experiments. A copy of exsiccate was deposited in the Herbarium of the Botany of UFPB with the register – J.A.N. 01 Batista (JPB).

The extracts were separately obtained by means of using 50 g of the powdered plant material and 500 mL of ethanol. The blend was agitated in a metabolic water bath (Dubnoff Model MA-093, Marconi, Piracicaba, Brazil) for 2 hours at 25 °C, followed by vacuum filtering. The extracts of leaves (EL), flowers (EF) and seed pods (EP) were concentrated in a rotary evaporator (Model TE-221 Tecnal Piracicaba, Brazil) at 60 °C up to complete solvent removal. Afterwards the extracts are conditioned in amber at refrigeration (about 8 °C) until the moment of being used.

The level of total extractable phenolics (FET) was colorimetrically determined by the Folin-Ciocalteu method [10] with volume reductions. Aliquots of 4 mL of each ethanolic extract diluted in water, received the addition of 0.25 mL of Folin-Ciocalteu reagent and 0.75 mL of saturated sodium carbonate. The mixture was stirred and later was kept in the dark for 2 hours. The absorbance was measurement at 765 nm in a UV-vis spectrophotometer (model UV-2550, Shimadzu, Japan). A sample containing only water and the reagents (white) was used as a reference. The concentration of the phenolic compounds was estimated by using a gallic acid calibration curve at the 50-500 mg L⁻¹ concentration range. The results were expressed as mean ± standard deviation of gallic acid equivalents mg (GAE)/g of extract.

Soybean oil (SO) received the addition of the extracts EL, EF and EP at the concentration of 100 mg kg⁻¹, in relation of their extractable total phenolic contents of each extract. The synthetic antioxidant BHT and TBHQ were also used at a concentration of 100 mg kg⁻¹.

The PDSC curves of the soybean oil, with and without additives were obtained by a model DSC Q1000, TA Instruments, pressurized differential scanning calorimeter. The isothermal curves were carried out at 110 °C, utilizing an aluminum crucible with approximately 10 mg sample, under oxygen atmosphere with initial pressure of 1,400 kPa and heating rate of 10 °C min⁻¹. Oxidation induction time (OIT) values were determined by the difference of the onset time and the initial time.

The oxidative stability of soybean oil without and with antioxidant additives was determined employing the method proposed by AOCS CD 12b-92 [11], in 873 Biodiesel Rancimat equipment from Metrohm, utilizing a sample of 2 grams, temperature of 110 °C, under a constant air flow (10 L h⁻¹). The result, expressed as

induction period (IP), is automatically determined from the inflection point of the curve by the software that accompanies the equipment.

Results and Discussion

From the determination of the levels of total extractable phenolics (FET) it was possible to determine the necessary amount of each extract equivalent to one additive 100 mg kg⁻¹ GAE in oil. In Table 1, we can see the total extractable phenolic content (FET) expressed as gallic acid equivalents (GAE), of the extracts studied. The content of FET in the extracts ranged from 41.75 to 53.69 ± 3.35 ± 1.00 mg GAE g⁻¹, with EL extract higher levels. EF extracts and EP showed no significant difference in the content of FET.

Table 1 FET Values of EL, EF and EP extracts

Samples	mg GAE g ⁻¹ of extract
EL	53.69 ± 1.00 ^a
EF	45.85 ± 1.71 ^b
EP	41.75 ± 3.35 ^b

Values are mean ± standard deviation, n=9

Mean followed by different letters in the same column differs significantly ($P \leq 0.05$)

Analyzing the calorimetric curves (PDSC) of soybean oil with and without additives by the isothermal method (110 °C) in oxygen atmosphere, there were obtained the values of the oxidation induction time (OIT) of each sample (Figure 1).

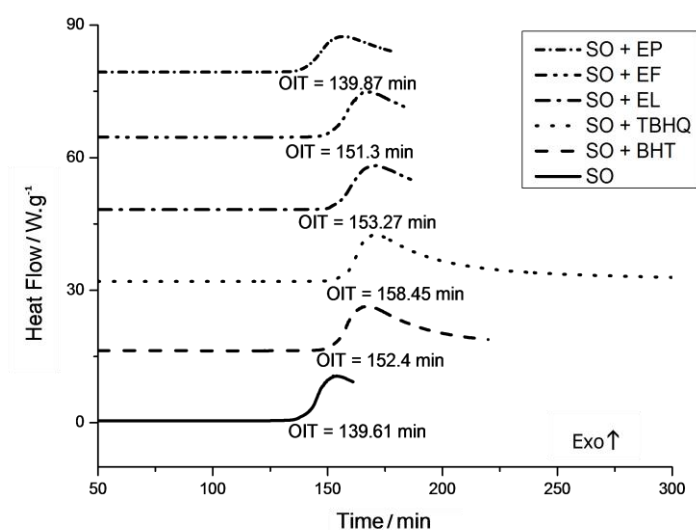


Fig. 1 PDSC Curves of all samples with their respective OIT

According to the OIT values, it is found that addition of the antioxidants BHT and TBHQ, as well as extracts of *Moringa* promoted increased oxidative stability of soybean oil. The percentages increase of OIT additives oils, figure, 2, was calculated according formula:

$$\% \text{ increase percentage} = (\text{OIT sample} - \text{OIT control}) \times 100 / \text{OIT control}$$

According to the results the EL extract provided better protective effect, increasing 9.78% on the value of the OIT in relation to oil without additive. This result, although seemingly small, was superior to the synthetic antioxidant BHT, which increased 9.16% in the value of OIT oil without additives. The extracts EF and EP were increased by 8.37% and 0.19% respectively. Although not having shown significant difference in the content of FET, it was observed that EF and EP exhibited differentiated antioxidant effects when added to soybean oil. This fact may be related to the type of antioxidant present in the extracts, which can not differentiate quantitatively, but structurally [6].

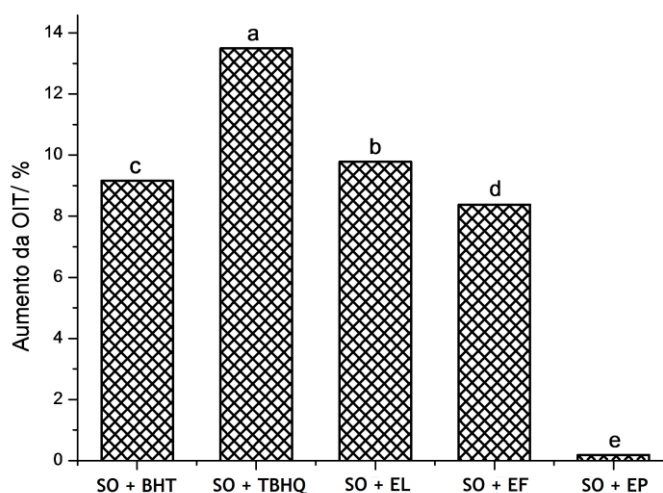


Fig. 2 Percentage increase in OIT of BHT, TBHQ, EL, EF and EP compared to soybean oil without additive.

Different letters differs significantly ($P \leq 0.05$)

The results of oxidative stability of soybean oil with and without additives by the Rancimat® Method are shown in Table 2. Extracts of *Moringa oleifera* Lam showed protective effect against oxidation of soybean oil, with emphasis on the EL. The synthetic antioxidant TBHQ showed the best protective effect among

additives, corroborating the results obtained by the method PDSC. Unlike calorimetric test results, however, it was observed that BHT protects more soybean oil against oxidation than EL. These conflicting results EL and BHT for the two assessment methods of oxidative stability leads us to suggest that EL would promote a greater protective effect of soybean oil in the early spread of rust, while BHT would cause greater protection in the step of lipid oxidation termination, and this enhances the antioxidant effect of the natural extract. Thus, the increase percentage sequence of IP value was TBHQ> BHT> EL> EF> EP. Study with basil and thyme oleoresin additives in soybean oil at a concentration of 500 mg kg⁻¹ showed an increase of IP of 10.44 to 11.32 and 11.38 respectively, results similar to those obtained in this work with EL extract at a concentration of 100 mg kg⁻¹ [12].

Table 2 Oxidative Stability of samples soybean oil with additives of the extracts of Moringa, BHT and TBHQ (IP) expressed in hours (h) and as percentage of increase (%)

Samples	IP / h	% increase
SO	10.56 ^f	N.A.
SO + BHT	12.97 ^b	22.78
SO + TBHQ	19.40 ^a	83.73
SO + EL	11.42 ^c	8.18
SO + EF	11.06 ^d	4.75
SO + EP	10.99 ^e	4.10

N.A. = not applicable because it is the control

Values IP Followed by different letters in the same column Differs Significantly ($P \leq 0.05$)

The comparison between the oxidative stability of the samples determined by both technique shows that not always there a correlation between these. In PDSC technique, the extrat EL was more effective than BHT antioxidant synthetic. In Rancimat method, however, does not show the same efficacy, 14.6% less effective than BHT. The difference in the effectiveness of the antioxidants can be attributed to different variables used in each technique, and the principles evaluated. In the PDSC exothermic events are measured and the Rancimat, the conductivity of the volatiles formed during oxidation [13]. According to data reported in the literature the

techniques determine different stages of lipid autoxidation. The technique PDSC evaluates the beginning of the propagation of the oxidation step in which peroxides are formed, leading to an exothermic peak which is expressed by the value OIT [14-15]. The Rancimat method, evaluates the final stage of oxidation, where volatile compounds, such as carboxylic acids, ketones and aldehydes, increase the electrical conductivity of water, expressed by OSI value [16]. By correlating the results obtained by these two techniques into fatty acid esters that showed OSI less than 40 hours, R^2 0.5852 was found, a result close to that found in this study [17].

Conclusions

The ethanol extracts of leaf, flower and of *Moringa oleifera* Lam husks increased the oxidative stability of soybean oil, demonstrating that its application as an antioxidant in polyunsaturated lipid systems is viable. However, it is necessary to develop complementary research with investigations of a possible synergism between extracts and synthetic antioxidants in order to obtain a more potent antioxidant composition of the extracts used individually. An antioxidant composition involving the extracts of Moringa, besides being economically viable for the industry, since the costs of production are low, is attractive for the consumer, for being a natural product, with low toxicity, and therefore a safer intake when compared to synthetic antioxidants.

Acknowledgement

The authors would like to thank the Brazilian agencies National Council for Scientific and Technological Development (CNPq) and Coordination for the Improvement of Higher Education Personnel (CAPES) for the financial support.

References

- 1 Ribeiro APB, Grimaldi R, Gioielli LA, Gonçalves LAG. Zero trans fats from soybean oil and fully hydrogenated soybean oil: Physico-chemical properties and food applications. Food Res Int.2009;42:401–10.
- 2 Hou JC, Jiang LZ, ZhangCW. Effects of frying on the trans-fatty acid formation in soybean oils. Eur J Lipid Sci Technol.2012;114:287–93.
- 3 Lee KW, Hong Z, Piao F, Kim YW, Chung KW. Bond reactivity and antioxidant effect on the autoxidation of soybean oil. J Ind Eng Chem.2010;16:419–24.
- 4 Sun-Waterhouse D, Thakorlal J, Zhou J. Effects of added phenolics on the storage stability of avocado and coconut oils. Int J Food Sci Technol.2011;46:1575–85.
- 5 Brasil. Ministério da Saúde. Comissão Nacional de Normas e Padrões para Alimentos. Resolução nº 04/88. Brasília.1988.

- 6 Brewer MS. Natural Antioxidants: Sources, Compounds, Mechanisms of Action, and Potential Applications. *Compr Rev Food Sci F*.2011;10:221–47.
- 7 Iqbal S, Bhanger MI. Effect of season and production location on antioxidant activity of *Moringa oleifera* leaves grown in Pakistan. *J Food Compos Anal*. 2006;19:544–51.
- 8 Lako J, Trenerry V, Wahlqvist M, Wattanapenpaiboon N, Sotheeswaran S, Premier R. Phytochemical flavonols, carotenoids and the antioxidant properties of a wide selection of Fijian fruit, vegetables and other readily available foods. *Food Chem*.2007;101:1727–41.
- 9 Reddy V, Urooj A, Kumar A. Evaluation of antioxidant activity of some plant extracts and their application in biscuits. *Food Chem*.2005;90:317–21.
- 10 Slinkard K, Singleton VL. Total Phenol Analysis: Automation and Comparison with Manual Methods. *Am. J. Enol. Vitic*. 1977;28:49–55.
- 11 American Oil Chemists Society. Official methods and recommended practices of the American Oil Chemists Society. 4th ed. Champaign:AOCS Press;1993.
- 12 Del Ré PV, Jorge N. Antioxidant potential of oregano (*Oreganum vulgare* L.), basil (*Ocimum basilicum* L.) and thyme (*Thymus vulgaris* L.): application of oleoresins in vegetable oil. *Cien Tecnol Aliment*. 2011;31:955–59.
- 13 Ramalho EFSM, Albuquerque AR, Souza AL, Barro AK, Maia AS, Santos IMG, Souza AG. Use of different techniques in the evaluation of the oxidative stability of poultry fat biodiesel. *J Therm Anal Calorim*. 2011;106:787–91.
- 14 Diniz ZN, Bora PS, NetoVQ, Cavaleiro JMO. *Sterculia striata* seed kernel oil: Characterization and thermal stability. *Gras Aceit*, vol. 59, no. 2, pp. 160–165, Jun. 2008.
- 15 Candeia RA, Sinfrônio FSM, Bicudo TC, Queiroz N, Barros Filho AKD, Soledade LEB, Santos IMG, Souza AL, Souza AG. Influence of the storage on the thermo-oxidative stability of methyl and ethyl esters by PDSC. *J Therm Anal Calorim*.2011;106:581–6.
- 16 Leonardo RS, Valle MLM, Dweck J. An alternative method by pressurized DSC to evaluate biodiesel antioxidants efficiency. *J Therm Anal Calorim*.2012;108:751–9.
- 17 Moser BR. Comparative Oxidative Stability of Fatty Acid Alkyl Esters by Accelerated Methods. *J Am Oil Chem Soc*.2009;86:699–706.

Oxidative stability of fish oil supplemented with ethanolic extracts of *Moringa oleifera* Lam. compared with synthetic antioxidants during accelerated test

Jaqueline A. Nascimento^{1*}, Kassandra L. G. V. Araújo¹, Poliana S. Epaminondas^{1,3}, Alline S. Souza¹, Antônia L. Souza², M. C. D. Silva⁴, Antônio G. Souza²

¹Graduate Program in Food Science and Technology, CT, Campus I, Federal University of Paraíba, CEP 58051-970, João Pessoa, Paraíba, Brazil.

² Laboratory of Fuels and Materials - LACOM, Department of Chemistry, CCEN, Campus I, Federal University of Paraíba, CEP 58059-900, João Pessoa, Paraíba, Brazil.

³ Department of Agribusiness, Federal Institute of Education, Science and Technology (IFPB), 58800-970, Campus Sousa, Sousa - Paraíba, Brazil.

⁴ Center for Social Science, Health and Technology, Federal University of Maranhão, Campus Imperatriz

*Corresponding author: jaquelinejp@yahoo.com.br

Fax: +55-83-32167441; Phone: +55-83-32167441

Abstract

The effects of ethanolic extracts of leaves, flowers and seed pods from *Moringa oleifera* Lam and synthetic antioxidants TBHQ and BHT on the oxidative stability of fish oil stored for 16 days at 60°C were compared. The investigation focused on the increased peroxide, anisidine and conjugated diene contents. Changes in trans fatty acid and aldehyde contents were investigated by Fourier transform infrared spectroscopy. Results showed that extracts were effective in delaying oxidation and among these, extracts of leaves was the most efficient, being more protective than TBHQ and BHT in controlling the formation of peroxides and hydroperoxides. In controlling secondary oxidation, synthetic antioxidants were more effective; however, extracts of leaves preserved better PUFAS than TBHQ. Results showed that the extracts of leaves is an antioxidant as effective as BHT and TBHQ for use in fish oil and its natural origin possibly makes it a safe additive for use in foods.

Keywords: Fish oil, *Moringa oleifera* Lam, Oxidative stability

1. Introduction

Marine fish oils are composed of long-chain polyunsaturated fatty acids, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). These fatty acids have beneficial properties for human health, protecting against diseases such as Alzheimer's disease (Lukiw & Bazan, 2008), depression (Nemets, Nemets, Apter, Bracha, & Belmaker, 2006), cognitive decline (Morris, Evans, Tangney, Bienias, & Wilson, 2005), rheumatoid arthritis (Bykerk & Keystone, 2005) and cardiovascular disease (Kris-Etherton et al., 2002), besides contributing in the development of the nervous system and growth (Pivik, Dykman, Jing, Gilchrist, & Badger, 2009). However, the presence of such acids in fish oil makes it highly susceptible to oxidation (Leclerc, Zhao, Simpson & Zuta, 2007).

Autoxidation is a process that occurs in three stages. In the initial and propagation stage, there is the formation of free radicals and neutral molecules such as hydroperoxides, which without interruption by antioxidants, the reaction proceeds resulting in the formation of secondary oxidation products, small-chain carboxylic acids, aldehydes and ketones. Secondary oxidation products compromise not only color, odor and flavor but also the nutritional characteristics of the oil, resulting in loss of functionality and toxicity (Rehman & Salariya, 2006; Siriwardhana, Lee, Kim, Ha, Park & Jeonl, 2004).

In industry, the stability of fish oil is improved by the use of synthetic antioxidants, capable of delaying oxidative deterioration and, consequently, extending its shelf life. However, the use of synthetic antioxidants is restricted in many countries due to possible adverse effects on human health (Hras, Hadolin, Knez, & Bauman, 2000). In Brazil, the use of TBHQ is limited to 200 mg.kg⁻¹ and BHT to 100 mg.kg⁻¹ (Brasil, Saúde, & Conselho Nacional de Saúde, 1988). In this context, anti-oxidants from natural sources have been studied in the attempt to obtain safer and more effective products which can be used in foods, fully or partially replacing synthetic antioxidants (Brewer, 2011).

Among the various sources of natural antioxidants studied, *Moringa oleifera* Lam. stands out, a plant originating from northwest India and widely cultivated in tropical regions due to the low demands of climate and soil and whose leaves, flowers, unripe fruits and roots have a rich and rare combination of zeatin, quercetin, β - sitosterol, caffeoylquinic acid and kaempferol, giving the plant a potential antioxidant activity (Iqbal & Bhanger, 2006; Lako, Trenerry, Wahlqvist, Wattanapenpaiboon, Sotheeswaran & Premier, 2007; Reddy, Urooj, & Kumar, 2005; Siddhuraju & Becker, 2003). In southern India, its fresh leaves are traditionally used in the preparation of foods rich in fat in order to increase their shelf life (Siddhuraju & Becker, 2003). Considering *Moringa* as a natural source of antioxidant compounds, the objective of this study was to evaluate the ethanol extracts of its leaves, flowers and seed pods, to assess the technical feasibility of its constituents as antioxidant additives in fish oil and to compare its efficacy compared with synthetic antioxidants industrially used for this purpose.

2. Material and methods

2.1. Materials

Refined fish oil and reagents butylatedhydroxytoluene (BHT) and *tert*-butylhydroquinone (TBHQ) were purchased from Sigma-Aldrich (Steinheim, Germany) and Folin-Ciocalteu reagent, 3,4,5-trihydroxybenzoic acid (gallic acid) and all other analytical reagents were acquired from Merck (Düsseldorf, Germany).

2.2. Samples

Leaves, flowers and seed pods of moringa (*Moringa oleifera* Lam) were collected during the month of April 2010 at Campus I, Federal University of Paraíba, João Pessoa / PB, Brazil and dried in an oven with circulating air at 45°C (Model MA 035, Marconi) for 24 h. After thorough drying, each piece was crushed separately in mill, vacuum-packed in polyethylene bags and stored at temperature of -18°C until the preparation of extracts. An exsiccata specimen was deposited in the Herbarium, Department of Botany at UFPB under registration number –J.A.N. Batista 01 (JPB).

2.3. Preparation of extracts

To obtain each extract, 50 g of powdered plant material and 500 ml ethanol were used. The mixture was stirred in a metabolic water bath (Model MA-093 Marconi) for 2 hours at 25 ° C followed by vacuum filtration. The extracts of leaves (EL), flowers (EF) and seed pods (EP) were concentrated on a rotary evaporator (Model TE-221 Tecnal) at 60 ° C until complete solvent removal. Then, the material was packed in amber vials under refrigeration until the time of application as antioxidant additive for fish oil.

2.4. Determination of total extractable phenolics

The levels of total extractable phenolics (TEP) were colorimetrically measured by the Folin-Ciocalteu method, according to Slinkard & Singleton, 1977, with the aid of a UV-vis spectrophotometer (model UV-2550, Shimadzu). Results were expressed as mean $\pm$ standard deviation mg of gallic acid equivalent (GAE) / g extract.

2.5. Determination of the oxidative stability

2.5.1. Sample preparation

Fish oil (FO) was added of EL, EF and EP extracts in the concentration of 100 mg kg⁻¹, with respect to total extractable phenolic contents of each extract. Synthetic antioxidants BHT and TBHQ were also used at concentration of 100 mg kg⁻¹. Fish oil (with or without additives) was submitted to accelerated storage test in an oven with air circulation (Marconi model MA 035) for 16 days at temperature of 60°C. Every four days, sample fractions were collected for the determination of the peroxide value (PV), anisidine value (AV), conjugated diene (CD) and Fourier transform infrared spectroscopy value (FTIR).

2.5.2. Peroxide value (PV)

The peroxide value was determined by the Cd 8-53 method of AOCS (AOCS, 2003). Fish oil samples (2g) were dissolved in 30 ml of acetic acid: chloroform solution (3:2 v / v), followed by the addition of 0.5 ml of saturated potassium iodide solution. The mixture was allowed to stand for exactly one minute and then added of 30 ml of freshly boiled water and 0.5 mL of 1% starch. The iodine released was titrated with sodium thiosulfate solution 0.01 N until the disappearance of blue coloration. A blank test was performed under the same conditions, but without the sample. The calculations were performed according to Equation:

$$PV \text{ (meq/kg)} = N \times (V_A - V_B) \times 1000 / W \text{ sample (g)}$$

Where: N is the normality of the $\text{Na}_2\text{S}_2\text{O}_3$ solution, V_A is the $\text{Na}_2\text{S}_2\text{O}_3$ solution volume consumed by the sample (mL), V_B is the $\text{Na}_2\text{S}_2\text{O}_3$ solution volume consumed by the blank (mL), and W is the sample weight (g).

2.5.3. Anisidine value (AV)

The anisidine value was determined according to Cd 8-53 method of AOCS (AOCS, 2003). Fish oil sample (0.5g) was dissolved in 25 mL of isooctane and its absorbance was measured at 350 nm using isooctane with blank. About 5 ml of fat and isooctane solution was added of 1 mL of reagent anisidine (0.25% p-anisidine solution in glacial acetic acid) and after 10 minutes in the dark, the absorbance of the fat-anisidine solution against solvent anisidine solution was measured at 350 nm in UV-vis spectrophotometer (model UV-2550, Shimadzu). The anisidine value (AV) was calculated according to the equation:

$$AV = 25 (1.2 A_S - A_B) / P$$

Where A_S is absorbance of the fat-anisidine solution, A_B is the absorbance of the initial fat solution and P is the sample weight in grams.

2.5.4. Conjugated diene (CD) value

The method Proposed by Leclerc, Zhao, Simpson & Zuta (2007) was slightly modified and used for the measurement of the CD content in the oil. Samples (0.02 g) were diluted with isooctane, and the solution absorbance was determined against a blank made from isooctane at 232 nm. The CD value was calculated from the absorbance value and the final sample concentration (g/100 mL). The results are expressed as CD values, computed as follows:

$$CD = A_{232} / C \times P$$

where A is the sample absorbance at 232 nm, C denotes the final dilution of the sample concentration (g 100 mL), and P represents the length of the measuring cell (cm).

2.5.5. Fourier transform infrared spectroscopy (FTIR)

The absorption spectra in the infrared region were obtained from IR spectrophotometer with Fourier transform model IR Prestige-21, Class 1 Laser Product from Shimadzu. The analysis was performed in the region from 4000 to 650 nm using a reflection ATR accessory with ZnSe prism, at resolution of 4 cm^{-1} .

2.5.6. Statistical analysis

Results were expressed as mean $\pm$ standard deviation, compared by analysis of variance (ANOVA) and Duncan test, considering $p < 0.05$, using the Statistica 7.0 software (Statsoft[®]).

3. Results and discussion

3.1. Total extractable phenolics (TEP)

From the determination of total extractable phenolics (TEP), it was possible to determine the necessary amount of each extract equivalent to addition of 100 mg kg⁻¹ GAE in the oil. The TEP content expressed as gallic acid equivalents (GAE) of extracts studied was 41.75 ± 3.35 , 45.85 ± 1.71 and 53.69 ± 1.00 mg GAE / g, respectively for EP, EF and EL extracts.

3.2. Peroxide value (PV)

Primary oxidation products (peroxides and hydroperoxides) from fish oil samples with and without additives (100 mg kg⁻¹) were determined by measuring the PV, which is one of the most widely used tests for evaluating the initial oxidation in oils and fats. Figure 1 shows the influence of different additives on the increase in PV in fish oil during the storage period. The results indicated that all samples showed increasing trend in the peroxide value and the control sample (FO) showed greater increase in PV compared to other treatments, demonstrating that all additives provide protection to fish oil against oxidation. Samples added of EL showed the smallest increase in PV, revealing a greater antioxidant effect of this extract in the initial oxidation phase, exceeding synthetic antioxidants BHT and TBHQ, which did not differ significantly from each other ($p > 0.5$). It was observed that in the control sample (FO) and in samples added of EL, EF and EP extracts, the maximum PV was reached at about the 12th day of storage, and that after this day, the PV began to fall, which means that the primary oxidation products (hydroperoxides) began to decompose, yielding stable products such as aldehydes, ketones, small-chain carboxylic acids, among others (Rehman & Salariya, 2006; Siriwardhana et al. 2004). Thus, the effectiveness sequence as antioxidant in the initial oxidation stages was $EL > BHT = TBHQ > EP > EF$. Wang et al. (2011) evaluated the oxidative stability of fish oil supplemented with carnosic acid compared with synthetic antioxidants during storage at different temperatures and observed that the synthetic antioxidant TBHQ was more effective than carnosic acid, since TBHQ showed lower PV.

3.3. Anisidine value (AV)

The anisidine value is useful in assessing secondary lipid oxidation. It determines the aldehyde levels in animal fats and vegetable oils. According to Figure 2, AV values increased in all samples during the storage period. The increase in AV in control samples (FO) was significantly higher ($p < 0.05$) than that obtained in samples with additives. Samples containing antioxidants TBHQ and BHT did not differ significantly from each other ($p >$

0.05), and showed the lowest increase in AV, followed by samples with EL. The highest AV was observed in samples containing EF and EP, which showed no significant difference from each other ($p > 0.05$). The AV data corroborate the PV results, indicating that all additives studied provided oxidative stability to fish oil; however, the most effective were synthetic antioxidants BHT and TBHQ, followed EL extract.

3.4. Conjugated diene (CD) value

The conjugated diene value is often used to measure hydroperoxides and together with PV, evaluates primary oxidation products. The peroxidation of unsaturated fatty acids leads to the displacement of the double bond, forming a conjugated diene system which strongly absorbs ultraviolet light at wavelength between 232 and 234 nm. The CD determination is a strong indicator of the oxidative status of oils, and can be used to evaluate the effectiveness of antioxidants (Iqbal & Bhanger, 2007). The CD values of fish oil samples with and without additives increased during the storage period, and from the 12th day of storage, the control sample (FO), showed a higher increase compared to samples with additives (Fig. 3). On the 16th day of storage, CD values of FO sample increased by 55.63%, while samples added of TBHQ, BHT and EL showed the smallest increase in the CD value, with 26.40%, 27.00% and 27.09%, respectively, with no significant difference ($p > 0.05$) between the results of these samples. Samples added of EF and EP showed increased in CD value by 40.24% and 40.98%, differing significantly ($p < 0.05$) from value shown by the control sample, but with no significant difference from each other. These results show that all extracts used in the experiment exhibited effective inhibition of the CD form in the fish oil, especially EL, which was as effective as synthetic antioxidants BHT and TBHQ, which is consistent with the results obtained for PV and AV tests previously shown. Studies using carnosic acid as fish oil additive also exhibited effective inhibition of the CD form (Wang et al. 2011).

3.5. Fourier Transform Infrared Spectroscopy (FTIR)

In the evaluation of the fish oil degradation by FTIR, the following spectral regions were observed: (1) absorption peaks around 982 cm^{-1} corresponding to absorption of *trans* fatty acids (TFA) relating to the C-H vibration outside the *trans* configuration plane of the double bond, (2) absorption peaks around 1734 cm^{-1} corresponding to carbonyl absorption, (3) absorption peaks around 3015 cm^{-1} , corresponding to *cis* double bonds which are related to the PUFA contents present in the fish oil, and (4) absorption peaks at 3500 cm^{-1} , corresponding to carbonyl frequency multiplication. Since the absorption peak of the carbonyl frequency multiplication was broad and the noise was greater nearby, quantification was difficult. Content changes in the carbonyl *cis* double bond, and *trans* double bond of fish oil during storage at $60\text{ }^{\circ}\text{C}$ are shown in Fig 4 (AC). The initial stage of the oxidation process of fish oil results in a reduction in the *cis* double bonds of polyunsaturated fatty acids. Figure 4 – A shows that on the 16th day of storage, the control sample (FO) showed a decrease in the absorption peak at 3015 cm^{-1} of 15.60%. The sample added of EF showed identical reduction, indicating that this extract did not preserve PUFAS present in the sample. On the other hand, EL and EP extracts decreased by only 7.72% and 11.87% respectively. Samples added of synthetic antioxidants BHT and TBHQ showed a decrease of 3.29% and 11.82%,

respectively, showing that EL was more effective than TBHQ in the preservation of PUFAs. While the content of *cis* double bonds decreases throughout the storage time, Figure 4 – C shows that the content of *trans* double bonds relating to trans fatty acids (TFA) increased by 49.04% in the control sample (FO), 27.41% for sample added of EL, 20.43% for sample added of BHT and 14.94% for sample added of TBHQ. Samples added of EF showed an increase of 38.76%, while samples added of EP increased by 50.68%, higher than values observed in the control sample. The carbonyl content is related to the oxidation degree, increasing during the storage time with the formation of products arising from the oxidation of DHA, EPA or other constituents present in fish oil. Figure 4 – B shows that the carbonyl content increased by 11.50% in the control samples. Samples added of EL (8.71%), EP (10.10%), BHT (6.29%) and TBHQ (5.56%) showed increase in carbonyl content lower than the control, while samples added of EF increased by 13.13%.

4. Conclusions

The ethanol extracts of moringa showed inhibitory effect against fish oil oxidation, and EL extract was the most effective, whose protection was more effective than that performed by synthetic antioxidants BHT and TBHQ in the formation of peroxides and hydroperoxides. The effect of the EL extract on fish oil was also significant in reducing secondary oxidation products and in protecting polyunsaturated fatty acids, reducing the formation of *trans* fatty acids during storage at 60°C, which demonstrates its potential use in edible oils, as an alternative to replace synthetic antioxidants.

5. Acknowledgement

The authors would like to thank the Brazilian agencies National Council for Scientific and Technological Development (CNPq) and Coordination for the Improvement of Higher Education Personnel (CAPES) for the financial support.

6. References

- AOCS. (2003). *Official Methods and Recommended Practices of the American Oil Chemists Society*. Champaign Illinois, USA:AOCS.
- Brasil (1988). Resolução CNS/MS no 04 de 24 de novembro de 1988. Diário Oficial da União, 19 de dezembro de 1988.
- Brewer, M. S. (2011). Natural Antioxidants: Sources, Compounds, Mechanisms of Action, and Potential Applications. *Comprehensive Reviews in Food Science and Food Safety*, 10(4), 221–247.

- 258 Bykerk, V. P., & Keystone, E. C. (2005). What are the goals and principles of management in
259 the early treatment of rheumatoid arthritis? *Best practice & research. Clinical*
260 *rheumatology*, 19(1), 147–61.
- 261 Hras, A. R., Hadolin, M., Knez, Z., & Bauman, D. (2000). Comparison of antioxidative and
262 synergistic effects of rosemary extract with α -tocopherol, ascorbyl palmitate and citric
263 acid in sunflower oil. *Food Chemistry*, 71, 229–233.
- 264 Iqbal, S., & Bhanger, M. I. (2006). Effect of season and production location on antioxidant
265 activity of Moringa oleifera leaves grown in Pakistan. *Journal of Food Composition and*
266 *Analysis*, 19(6-7), 544–551.
- 267 Iqbal, S., & Bhanger, M. I. (2007). Stabilization of sunflower oil by garlic extract during
268 accelerated storage. *Food Chemistry*, 100(1), 246–254.
- 269 Kris-etherton, P. M., Hecker, K. D., Bonanome, A., Coval, S. M., Binkoski, A. E., Hilpert, K.
270 F., Griel, A. E., Etherton, T. D. (2002). Bioactive Compounds in Foods: Their Role in
271 the Prevention of Cardiovascular Disease and Cancer. *The American Journal of*
272 *Medicine*, 113(9), 71–88.
- 273 Lako, J., Trenerry, V., Wahlqvist, M., Wattanapenpaiboon, N., Sotheeswaran, S., & Premier,
274 R. (2007). Phytochemical flavonols, carotenoids and the antioxidant properties of a wide
275 selection of Fijian fruit, vegetables and other readily available foods. *Food Chemistry*,
276 101(4), 1727–1741.
- 277 Leclerc, L., Zhao, X., Simpson, B. K., & Zuta, P. C. (2007). The effect of α -tocopherol on the
278 oxidation of mackerel oil.pdf. *Food Chemistry*, 100, 800–807.
- 279 Lukiw, W. J., & Bazan, N. G. (2008). Docosahexaenoic Acid and the Aging Brain. *American*
280 *Society for Nutrition*, 138(12), 2510–2514.
- 281 Morris, M. C., Evans, D., Tangney, C. C., Bienias, J. L., & Wilson, R. S. (2005). Fish
282 consumption and cognitive decline with age in a large community study. *Archives of*
283 *neurology*, 62(12), 1849–53.
- 284 Nemets, H., Nemets, B., Apter, A., Bracha, Z., & Belmaker, R. H. (2006). Omega-3 treatment
285 of childhood depression: a controlled, double-blind pilot study. *The American journal of*
286 *psychiatry*, 163(6), 1098–100.
- 287 Pivik, R. T., Dykman, R. A., Jing, H., Gilchrist, J. M., & Badger, T. M. (2009). Early Infant
288 Diet and the Omega 3 Fatty Acid DHA: Effects on Resting Cardiovascular Activity and
289 Behavioral Development During the First Half-Year of Life. *Developmental*
290 *Neuropsychology*, 34(2), 139–158.

- Reddy, V., Urooj, a, & Kumar, a. (2005). Evaluation of antioxidant activity of some plant extracts and their application in biscuits. *Food Chemistry*, 90(1-2), 317–321.
- Rehman, Z., & Salariya, A. (2006). Effect of synthetic antioxidants on storage stability of Khoa – a semi-solid concentrated milk product. *Food Chemistry*, 96(1), 122–125.
- Siddhuraju, P., & Becker, K. (2003). Antioxidant properties of various solvent extracts of total phenolic constituents from three different agroclimatic origins of drumstick tree (*Moringa oleifera* Lam.) leaves. *Journal of agricultural and food chemistry*, 51(8), 2144–55.
- Siriwardhana, N., Lee, K.W., Kim, S.H., Ha, J.H., Park, G.T., & Jeon, Y.J. (2004). Lipid Peroxidation Inhibitory Effects of Hizikia Fusiformis Methanolic Extract on Fish Oil and Linoleic Acid. *Food Science and Technology International*, 10(2), 65–72.
- Slinkard, K., & Singleton, V. L. (1977). Total Phenol Analysis: Automation and Comparison with Manual Methods. *American Journal of Enology and Viticulture*, 28(1), 49–55.
- Wang, H., Liu, F., Yang, L., Zu, Y., Wang, H., Qu, S., & Zhang, Y. (2011). Oxidative stability of fish oil supplemented with carnosic acid compared with synthetic antioxidants during long-term storage. *Food Chemistry*, 128(1), 93–99.

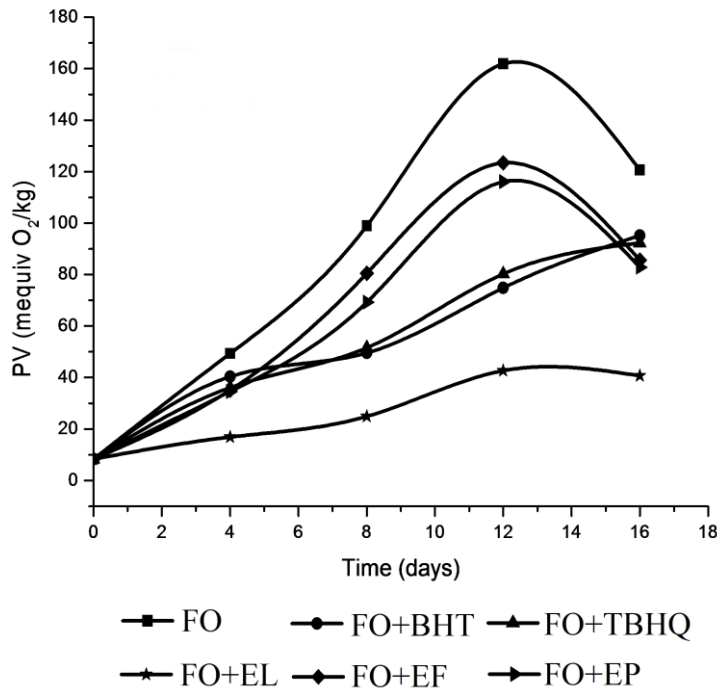


Fig 1. Changes on peroxide values (PV) of fish oil samples with and without additives (100 ppm), stored at temperature of 60°C for 16 days.

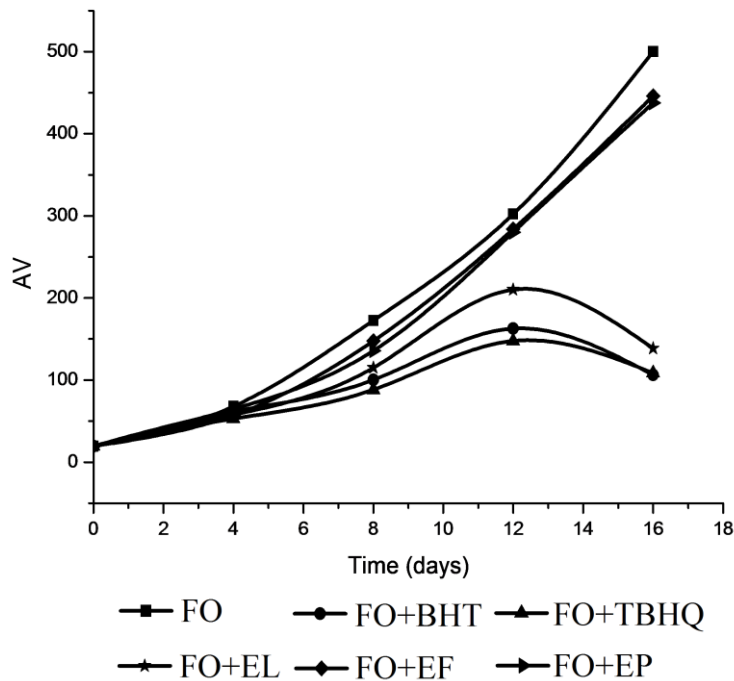


Fig 2. Changes on anisidine values (AV) of fish oil samples with and without additives (100 ppm), stored at temperature of 60°C for 16 days.

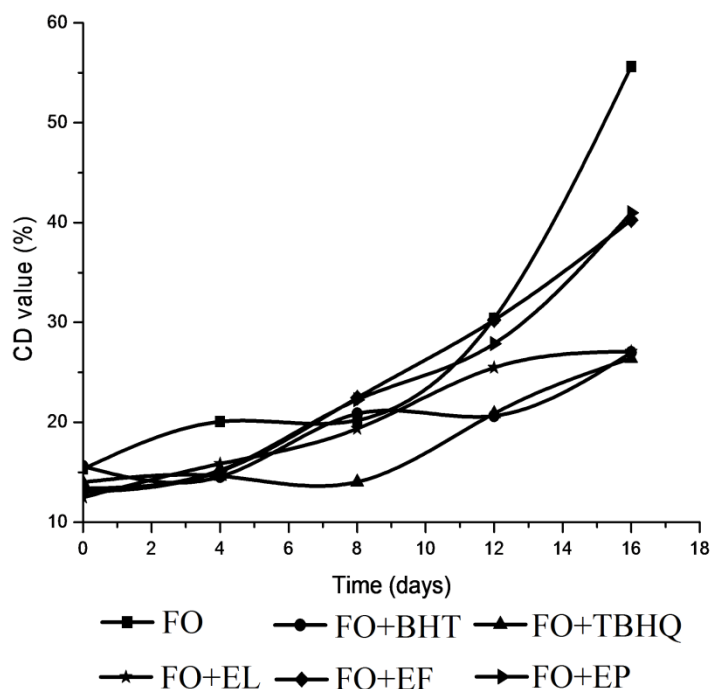


Fig 3. Changes on conjugated diene (CD) values of fish oil samples with and without additives (100 ppm), stored at temperature of 60°C for 16 days.

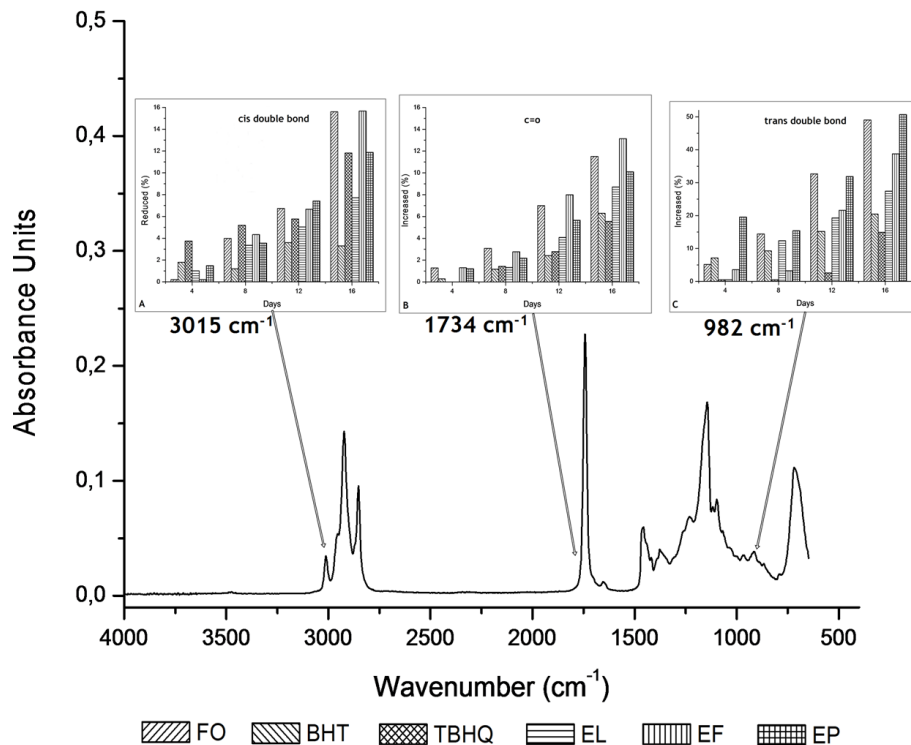


Fig. 4 Changes of the main spectral peaks of fish oil by FTIR. The spectrogram was fish oil at the time 0 with no antioxidant analysed by FTIR. (A) cis double bond, (B) C=O vibration, (C) trans double bond. All the samples for analyse were stored at the temperature of 60°C.

Antioxidant effect of *Moringa oleifera* Lamark extracts in soybean oil during accelerated storage test

Jaqueline A. Nascimento^{1*}, Kassandra L. G. V. Araújo¹, Poliana S. Epaminondas^{1,3}, Alline S. Souza¹, Antônia L. Souza², Marciane Magnani¹, Neide Queiroz², Antônio G. Souza²

¹Graduate Program in Food Science and Technology, CT, Campus I, Federal University of Paraíba, CEP 58051-970, João Pessoa, Paraíba, Brazil.

² Laboratory of Fuels and Materials - LACOM, Department of Chemistry, CCEN, Campus I, Federal University of Paraíba, CEP 58059-900, João Pessoa, Paraíba, Brazil.

³ Department of Agribusiness, Federal Institute of Education, Science and Technology (IFPB), 58800-970, Campus Sousa, Sousa - Paraíba, Brazil.

*Corresponding author: jaquelinejp@yahoo.com.br

Fax: +55-83-32167441; Phone: +55-83-32167441

Abstract

The effects of ethanolic extracts of leaves (EL), flowers (EF) and seed pods (EP) from *Moringaoleifera*Lamark on the oxidative stability of soybean oil stored for 16 days at 60°C were evaluated. The investigation was focused on the increase in peroxide, anisidine and conjugated diene values. The changes in *trans* fatty acid and aldehyde contents were investigated by Fourier transform infrared spectroscopy. The results show that the moringa extracts tested have protective effects against soybean oil oxidation, showing higher activity compared to synthetic antioxidant BHT in inhibiting peroxide formation and controlling secondary oxidation. The most efficient extract was EL, with protective effects equal to or better than BHT against oxidation of PUFAs, and the formation of carbonyl and *trans* fatty acids, respectively. In addition, this extract was more effective than TBHQ in the inhibition of *trans* fatty acids. The results suggest that EL extract has potential to be used as an antioxidant for preserving soybean oil.

Keywords: Soybean oil, *Moringa oleifera* Lam., Oxidative stability

1. Introduction

The species *Moringa oleifera* Lamarck is a perennial and drought-tolerant plant cultivated as ornamental and medicinal plant (Sánchez-Machado, Núñez-Gastélum, Reyes-Moreno, Ramirez-Wong, & Lopez-Cervantes, 2009). In Brazil, it is found mainly in the Northeastern region, where it is known as lily-white or simply moringa (Silva et al., 2012). All parts of this plant can be used for some purpose, and its use is known in human and animal nutrition, agriculture, pharmaceuticals, cosmetics and food, in water treatment and in the production of biodiesel and lubricant. (Debnath, Biswas, Ray, & Guha, 2011; Rashid Anwar, Moser, & Knothe, 2008; Sharma, Rashid Anwar, & Erhan, 2009). This plant has a high content of phenolic acids and carotenoids, and its constitution has a rare combination of

zeatin, quercetin, β -sitosterol, kaempferol caffeoylquinic acid, which provides moringa a high antioxidant power (Anwar Hussain, Iqbal, & Bhanger, 2007; Iqbal & Bhanger, 2006; Lako et al., 2007).

Antioxidants from natural sources have been studied with the goal of identifying effective compounds that can fully or partially replace synthetic antioxidants in foods, such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and *tert*-butyl hydroquinone (TBHQ) (Brewer, 2011), which have restricted use due to their toxicity and potential carcinogenic effects to humans (Sun-Waterhouse, Thakorlal, & Zhou, 2011).

Since moringa is a natural source of compounds with antioxidant potential, and that soybean oil is the most consumed edible oil worldwide, the present work aimed to evaluate the oxidative stability of soybean oil added to ethanolic extracts of leaves, flowers and seed pods of moringa during accelerated storage test.

2. Material and methods

2.1. Material

Refined soybean oil without additives were purchased from Cargill Agricultural S.A., Brazil. Reagents butylatedhydroxytoluene (BHT) e *tert*-Butylhydroquinone (TBHQ) were purchased from Sigma-Aldrich (Steinheim, Germany) and Folin-Ciocalteau reagent, 3,4,5-trihydroxybenzoic acid (gallic acid) and all other analytical reagents were acquired from Merck (Düsseldorf, Germany).

2.2. Samples

Leaves, flowers and seed pods of moringa (*Moringa oleifera* Lam) were collected during the month of April 2010 at Campus I, Federal University of Paraíba, João Pessoa / PB, Brazil and dried in an oven with circulating air at 45°C (Model MA 035, Marconi) for 24 h. After thorough drying, each piece was crushed separately in mill, vacuum-packed in polyethylene bags and stored at temperature of -18°C until the preparation of extracts.

2.3. Extracts

To obtain each extract, 50 g of powdered plant material and 500 ml ethanol were used. The mixture was stirred in a metabolic water bath (Model MA-093 Marconi) for 2 hours at 25 ° C followed by vacuum filtration. The extracts of leaves (EL), flowers (EF) and seed pods (EP) were concentrated on a rotary evaporator (Model TE-221 Tecnal) at 60 ° C until complete solvent removal. Then, the material was packed in amber vials under refrigeration until the time of application as antioxidant additive for soybean oil.

2.4. Determination of total extractable phenolics

The levels of total extractable phenolics (TEP) were colorimetrically measured by the Folin-Ciocalteu method, according to Slinkard & Singleton, 1977, with the aid of a UV-vis spectrophotometer (model UV-2550, Shimadzu). Results were expressed as mean $\pm$ standard deviation mg of gallic acid equivalent (GAE) / g extract.

2.5. Determination of the oxidative stability

2.5.1. Sample preparation

Soybean oil (SO) was added of EL, EF and EP extracts in the concentration of 100 mg kg⁻¹, with respect to total extractable phenolic contents of each extract. Synthetic antioxidants BHT and TBHQ were also used at concentration of 100 mg kg⁻¹. Soybean oil (with or without additives) was submitted to accelerated storage test in an oven with air circulation (Marconi model MA 035) for 16 days at temperature of 60°C. Every four days, sample fractions were collected for the determination of the peroxide value (PV), anisidine value (AV), conjugated diene (CD) and Fourier transform infrared spectroscopy value (FTIR).

2.5.2. Peroxide value (PV)

The peroxide value was determined by the Cd 8-53 method of AOCS (AOCS, 2003). Soybean oil samples (2g) were dissolved in 30 ml of acetic acid: chloroform solution (3:2 v / v), followed by the addition of 0.5 ml of saturated potassium iodide solution. The mixture was allowed to stand for exactly one minute and then added of 30 ml of freshly boiled water and 0.5 mL of 1% starch. The iodine released was titrated with sodium thiosulfate solution 0.01 N until the disappearance of blue coloration. A blank test was performed under the same conditions, but without the sample. The calculations were performed according to Equation:

$$PV \text{ (meq/kg)} = N \times (V_A - V_B) \times 1000 / W \text{ sample (g)}$$

In which: N is the normality of the Na₂S₂O₃ solution, V_A is the Na₂S₂O₃ solution volume consumed by the sample (ml), V_B is the Na₂S₂O₃ solution volume consumed by the blank (mL), and W is the sample weight (g).

2.5.3. Anisidine value (AV)

The anisidine value was determined according to Cd 8-53 method of AOCS (AOCS, 2003). Soybean oil sample (0.5g) was dissolved in 25 ml of isooctane and its absorbance was measured at 350 nm using isooctane with blank. About 5 ml of fat and isooctane solution was added of 1 mL of reagent anisidine (0.25% p-anisidine solution in glacial acetic acid) and after 10 minutes in the dark, the absorbance of the fat-anisidine solution against solvent anisidine solution was measured at 350 nm in UV-vis spectrophotometer (model UV-2550, Shimadzu). The anisidine value (AV) was calculated according to the equation:

$$AV = 25 (1.2 A_S - A_B) / P$$

In which A_S is absorbance of the fat-anisidine solution, A_B is the absorbance of the initial fat solution and P is the sample weight in grams.

2.5.4. Conjugated diene (CD) value

The method proposed by Leclerc et al. (2007) was slightly modified and used for the measurement of CD content in the oil. Samples (0.02 g) were diluted with isooctane, and the solution absorbance was determined against a blank made from isooctane at 232 nm. The CD value was calculated from the absorbance value and the final sample concentration (g / 100 mL). The results are expressed as CD values, computed as follows:

$$CD = A_{232} / C \times P$$

In which A_{232} is the absorbance of the sample at 232 nm; C denotes the final dilution of the sample concentration (g / 100 mL); and P represents the length of the measuring cell (cm).

2.5.5. Fourier transform infrared spectroscopy (FTIR)

The absorption spectra in the infrared region were obtained from IR spectrophotometer with Fourier transform model IR Prestige-21, Class 1 Laser Product from Shimadzu. The analysis was performed in the region from 4000 to 650 nm using a reflection ATR accessory with ZnSe prism, at resolution of 4 cm^{-1} .

2.5.6. Statistical analysis

Results were expressed as mean $\pm$ standard deviation, compared by analysis of variance (ANOVA) and Tukey test, considering $p < 0.05$, using the Statistica 7.0 software (Statsoft[®]).

3. Results and discussion

3.1. Total extractable phenolics (TEP)

The TEP content expressed as gallic acid equivalents (GAE) of extracts studied was 41.75 ± 3.35 , 45.85 ± 1.71 and 53.69 ± 1.00 mg GAE / g, respectively for EP, EF and EL extracts.

3.2. Peroxide value (PV)

The results showed that all soybean oil samples showed increasing PV trend, which measures the primary oxidation of products, whereas the control sample (SO) showed higher increase in PV in relation to the other samples (Figure 1). Among samples, the one containing TBHQ showed the lowest increase in PV, followed by sample added with EL extract, indicating greater antioxidant effect in the early oxidation stage. The activities of EF and EP extracts did not differ from each other and were higher than synthetic antioxidant BHT. The high effectiveness of TBHQ is probably related to the configuration of hydroxyls in its molecule, which gives it greater power to donate hydrogen to free radicals more easily

interrupting the oxidation chain reaction (Ai-li & Chang-hai, 2006). Similar result was reported by Azeredo, Faria, & Silva (2004) in a study with soybean oil using different antioxidants, where TBHQ stood out due to its efficiency. Although the concentration of phenolics in samples added of extracts was the same, a distinct behavior between extracts was observed, probably due to the variation of compounds present in each extract (Brewer, 2011).

3.3. Anisidine value (AV)

The AV is useful in assessing compounds formed in the second oxidation stage, since it determines the dialdehyde content present in animal fats and vegetable oils such as soybean (Mohdaly, Smetanska, Ramadan, Sarhan, & Mahmoud, 2011). AV increased in all samples during the storage period, with no difference between samples added of BHT and control (SO), demonstrating its ineffectiveness in the secondary oxidation phase (Figure 2). The other samples showed differences compared to control, and that added of TBHQ showed the lowest increase in AV, followed by samples containing EP, EF and EL. Results of AV and PV measurement indicate efficiency of all moringa extracts in controlling soybean oil oxidation, with higher activity compared to BHT.

The efficiency of extracts in the second oxidation stage was not observed in the first oxidation stage, which is believed to be due to the predominance of compounds with phenolic structure that promotes the removal or inactivation of free radicals formed during the onset or spread of the reaction by donating hydrogen atoms to these molecules, stopping the chain reaction (Simic & Javanovic, 1994). However, these compounds do not act as efficiently in preventing the formation of aldehydes and ketones from peroxides and hydroperoxides previously formed.

3.4. Conjugated diene (CD) value

The peroxidation of unsaturated fatty acids leads to displacement of the double bond, forming a conjugated diene system that strongly absorbs ultraviolet light at wavelength between 232 and 234 nm (Iqbal & Bhanger, 2007). The CD determination is a strong indicator of the oxidative status of oils, and can be used to evaluate the effectiveness of antioxidants (Iqbal & Bhanger, 2007). The CD values of soybean oil samples with or without additives increased during the storage period (Fig. 3). After the 16th day of storage at 60°C, the CD values of soybean oil samples supplemented with EL, BHT, EF and EP, as well as SO (control), increased by around 9% with no significant difference between each other. Since there was significant difference between oil samples added of moringa extracts and control in the PV values, the divergence of results regarding the CD values may be due to the inclusion of conjugated dienes that are not necessarily hydroperoxides (Dobarganes & Velasco, 2002; Grau, Guardiola Boatella, Baucells, & Codony, 2000). The oil added of TBHQ showed increase of 4.84% in the CD values, confirming the improved efficiency also observed in other trials.

3.5. Fourier Transform Infrared Spectroscopy (FTIR)

In assessing soybean oil degradation by FTIR, the following spectral regions were observed: (1) absorption peaks around 982 cm^{-1} , corresponding to absorption of *trans* fatty acids (TFA) related to the C-H vibration outside of plane in the *trans* configuration of the double bond, (2) absorption peaks around 1734 cm^{-1} , corresponding to absorption of carbonyl, (3) absorption peaks around 3015 cm^{-1} , corresponding to *cis* double bonds, which are related to the PUFA content in the soybean oil (Wang et al. May 2011). Content changes in the *cis* double bond, carbonyl and *trans* double bond of soybean oil during storage at $60\text{ }^{\circ}\text{C}$ are shown in Fig 4 (A-C). The initial stage of the oxidation process of soybean oil leads to a reduction in the *cis* double bonds of polyunsaturated fatty acids. Figure 4 – A shows that after the 16th day of storage, the control sample (SO) showed a decrease in the absorption peak at 3015 cm^{-1} of 14.97%. All samples added of moringa extracts and synthetic antioxidants showed differences in peak reducing compared to control sample (SO), revealing preservation of PUFAs in samples with additives. EL and BHT extracts showed reduction of 10.76% and 10.71% respectively, not significantly differing from each other. Samples added of EF and EP extracts differed from each other, showing a reduction of 11.70% and 12.87% respectively. Synthetic antioxidant TBHQ was the most effective in preserving PUFAS, with a 9% reduction. While the content of *cis* double bonds decreased throughout the storage time, Figure 4 – C shows that the content of *trans* double bonds related to *trans* fatty acids (TFA) was increased by 26.68% in control (SO) and 26.75% the sample added of EP, with no significant difference between samples. In sample added of EF, EL, BHT and TBHQ, the increase was 19.82%, 20.16%, 23.27% and 24.49% respectively, differing from control samples. The carbonyl content is related to the oxidation degree, increasing during the storage time with the formation of products from DHA, EPA oxidation or other constituents present in the soybean oil. Figure 4 – B shows that the control sample and those added of PE and BHT had exactly the same increase in the carbonyl content (7.59%), while samples added of EL, EF and TBHQ showed smaller increases, differing significantly from the control sample.

4. Conclusions

Ethanollic extracts of leaves, flowers and seed pods of *Moringa oleífera* Lamark showed a protective effect against soybean oil oxidation, and the extract from leaves (EL) was the most effective, with antioxidant activity higher than that of synthetic antioxidant BHT. The action of this extract on soybean oil was also significant in reducing secondary oxidation products and in protecting polyunsaturated fatty acids, reducing the formation of *trans* fatty acids during storage at 60°C , suggesting their potential for use in the edible oils industry, as an alternative to replace synthetic antioxidants.

5. Acknowledgement

The authors would like to thank the Brazilian agencies National Council for Scientific and Technological Development (CNPq) and Coordination for the Improvement of Higher Education Personnel (CAPES) for the financial support.

6. References

- Ai-li, J., & Chang-hai, W. (2006). Antioxidant properties of natural components from *Salvia plebeia* on oxidative stability of ascidian oil. *Process Biochemistry*, 41(5), 1111–1116.
- Anwar, F., Hussain, A. I., Iqbal, S., & Bhanger, M. I. (2007). Enhancement of the oxidative stability of some vegetable oils by blending with *Moringa oleifera* oil. *Food Chemistry*, 103(4), 1181–1191.
- Azeredo, H. M. C., Faria, J. A. F., & Silva, M. A. A. P. (2004). Minimization of peroxide formation rate in soybean oil by antioxidant combinations. *Food Research International*, 37(7), 689–694.
- Brewer, M. S. (2011). Natural Antioxidants: Sources, Compounds, Mechanisms of Action, and Potential Applications. *Comprehensive Reviews in Food Science and Food Safety*, 10(4), 221–247.
- Debnath, S., Biswas, D., Ray, K., & Guha, D. (2011). *Moringa oleifera* induced potentiation of serotonin release by 5-HT(3) receptors in experimental ulcer model. *International Journal of Phytotherapy and Phytopharmacology*, 18(2-3), 91–5.
- Dobarganes, M. C., & Velasco, J. (2002). Analysis of lipid hydroperoxides. *European Journal of Lipid Science and Technology*, 104, 420–428.
- Grau, A., Guardiola, F., Boatella, J., Baucells, M. D., & Codony, R. (2000). Evaluation of lipid ultraviolet absorption as a parameter to measure lipid oxidation in dark chicken meat. *Journal of Agricultural and Food Chemistry*, 48(9), 4128–4135.
- Iqbal, S., & Bhanger, M. I. (2006). Effect of season and production location on antioxidant activity of *Moringa oleifera* leaves grown in Pakistan. *Journal of Food Composition and Analysis*, 19(6-7), 544–551.
- Iqbal, S., & Bhanger, M. I. (2007). Stabilization of sunflower oil by garlic extract during accelerated storage. *Food Chemistry*, 100(1), 246–254.
- Lako, J., Trenerry, V., Wahlqvist, M., Wattanapenpaiboon, N., Sotheeswaran, S., & Premier, R. (2007). Phytochemical flavonols, carotenoids and the antioxidant properties of a wide selection of Fijian fruit, vegetables and other readily available foods. *Food Chemistry*, 101(4), 1727–1741.
- Leclerc, L., Zhao, X., Simpson, B. K., & Zuta, P. C. (2007). The effect of α -tocopherol on the oxidation of mackerel oil.pdf. *Food Chemistry*, 100, 800–807.
- Mohdaly, A. a. a., Smetanska, I., Ramadan, M. F., Sarhan, M. a., & Mahmoud, A. (2011). Antioxidant potential of sesame (*Sesamum indicum*) cake extract in stabilization of sunflower and soybean oils. *Industrial Crops and Products*, 34(1), 952–959.
- Rashid, U., Anwar, F., Moser, B. R., & Knothe, G. (2008). *Moringa oleifera* oil: a possible source of biodiesel. *Bioresource technology*, 99(17), 8175–9.
- Sharma, B. K., Rashid, U., Anwar, F., & Erhan, S. Z. (2009). Lubricant properties of *Moringa* oil using thermal and tribological techniques. *Journal of Thermal Analysis and Calorimetry*, 96(3), 999–1008.
- Silva, A. V. C., Santos, A. R. F., Léo, A. S., Almeida, R. B. F. C. S., Silva, G. M., & Rangel, M. S. A. (2012). *Moringa* genetic diversity from germplasm bank using RAPD markers. *Tropical and Subtropical Agroecosystems*, 15(1), 31–39.

- Simic, M. G., & Javanovic, S. V. (1994). Inactivation of oxygen radicals by dietary phenolic compounds in anticarcinogenesis. In C. T. HO, T. OSAWA, T. M. HUANG, & R. T. ROSEN (Eds.), *Food phytochemicals for cancer prevention* (pp. 20–33). Washington: American Chemical Society.
- Sun-Waterhouse, D., Thakorlal, J., & Zhou, J. (2011). Effects of added phenolics on the storage stability of avocado and coconut oils. *International Journal of Food Science & Technology*, 46(8), 1575–1585.
- Sánchez-Machado, D. I., Núñez-Gastélum, J. a., Reyes-Moreno, C., Ramírez-Wong, B., & López-Cervantes, J. (2009). Nutritional Quality of Edible Parts of *Moringa oleifera*. *Food Analytical Methods*, 3(3), 175–180.
- Wang, H., Liu, F., Yang, L., Zu, Y., Wang, H., Qu, S., & Zhang, Y. (2011). Oxidative stability of fish oil supplemented with carnosic acid compared with synthetic antioxidants during long-term storage. *Food Chemistry*, 128(1), 93–99.

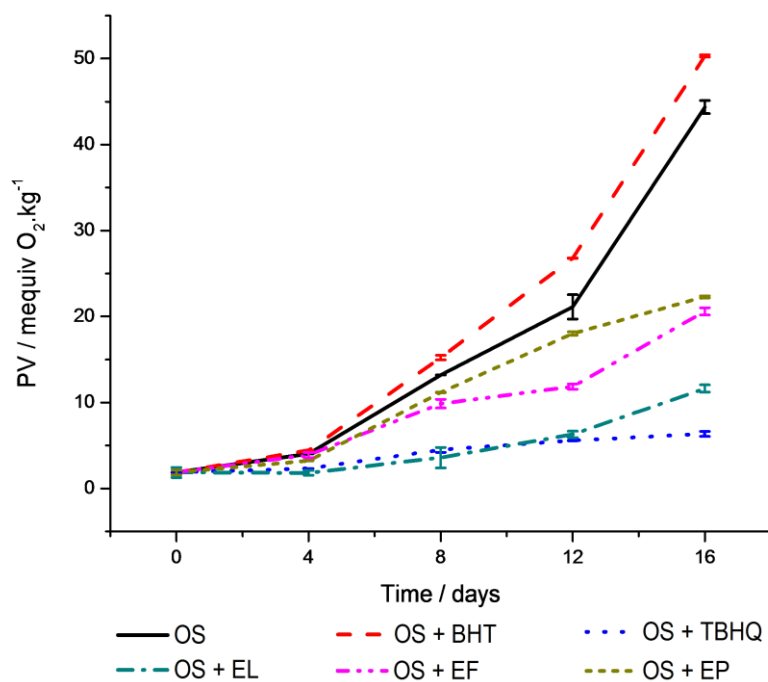


Fig 1. Changes on peroxide values (PV) of soybean oil samples with and without additives (100 ppm), stored at temperature of 60°C for 16 days.

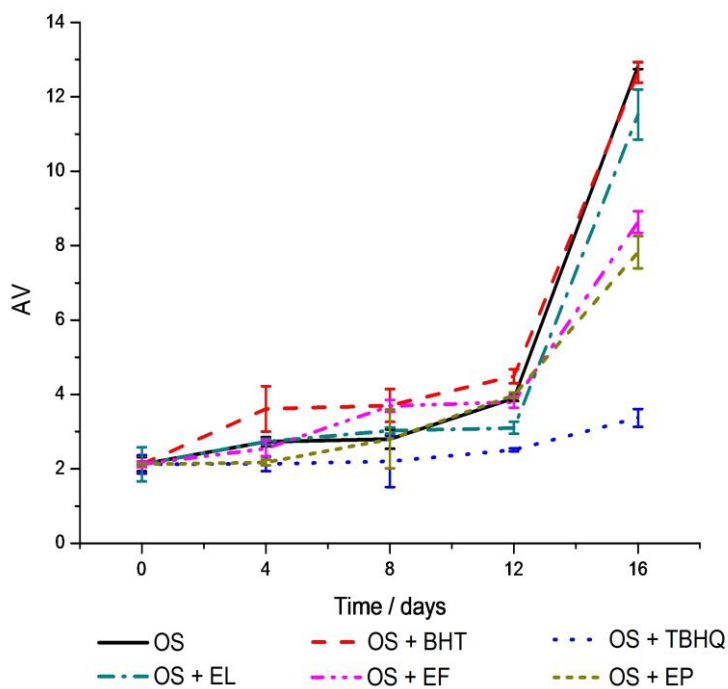


Fig 2. Changes on anisidine values (AV) of soybean oil samples with and without additives (100 ppm), stored at temperature of 60°C for 16 days.

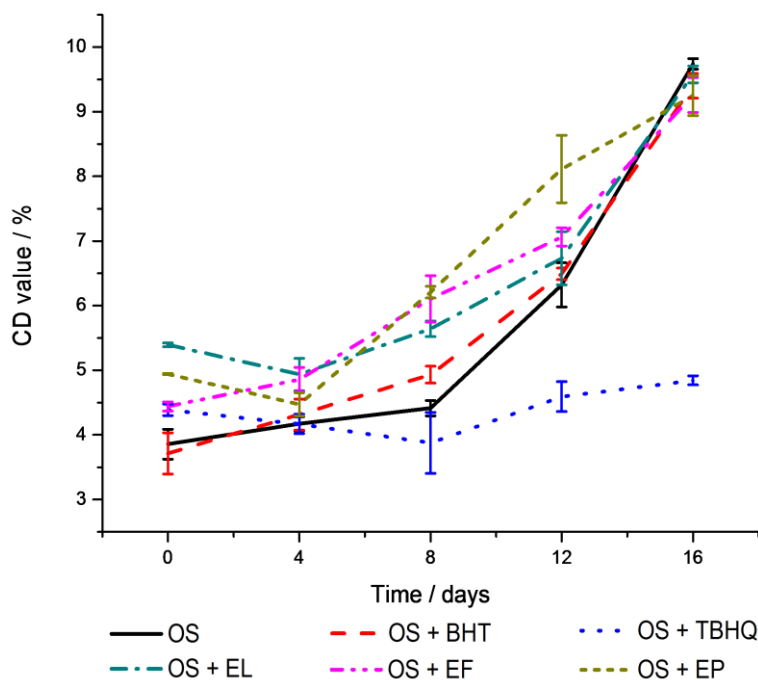


Fig 3. Changes on conjugated diene (CD) values of soybean oil samples with and without additives (100 ppm), stored at temperature of 60°C for 16 days.

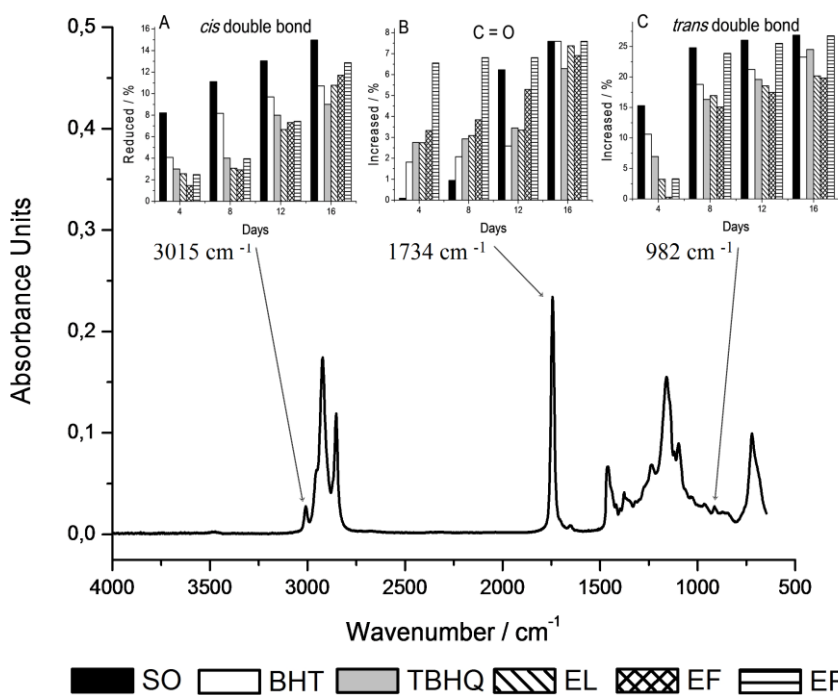


Fig. 4 Changes of the main spectral peaks of soybean oil by FTIR. The spectrogram was soybean oil at the time 0 with no antioxidant analysed by FTIR. (A) cis double bond, (B) C=O vibration, (C) trans double bond. All the samples for analyse were stored at the temperature of 60°C.

Study of antioxidant activity and preliminary bioactivity determination of ethanolic extracts of *Moringa oleifera* Lam.

Jaqueline A. Nascimento^{1*}, Kassandra L. G. V. Araújo¹, Poliana S. Epaminondas^{1,3}, Alline S. Souza¹, Antonia L. Souza², Antonio G. Souza², André M. Santos⁴, Alessandra A. Nascimento⁴

¹Graduate Program in Food Science and Technology, CT, Campus I, Federal University of Paraíba, CEP 58051-970, João Pessoa, Paraíba, Brazil.

² Laboratory of Fuels and Materials - LACOM, Department of Chemistry, CCEN, Campus I, Federal University of Paraíba, CEP 58059-900, João Pessoa, Paraíba, Brazil.

³ Department of Agribusiness, Federal Institute of Education, Science and Technology (IFPB), 58800-970, Campus Sousa, Sousa - Paraíba, Brazil.

⁴Laboratory of Animal Experimentation, Federal University of Amapá, 68906-970, Campus I, Macapá, Amapá, Brazil.

*Corresponding author: jaquelinejp@yahoo.com.br

Fax: +55-83-32167441; Phone: +55-83-32167441

Abstract

Moringa oleifera Lam is an Indian plant popularly known by its pharmacological properties still little explored in Brazil. In the present study, it was determined the levels of totals extractables phenolics (TEP) and antioxidant activity of ethanolic extracts from leaf, flowers, seed pods and seeds of *Moringa oleifera* Lam. A preliminary assessment of the toxicity of above extracts was also performed using the bioassay with *Artemia salina*. The content of TEP expressed as gallic acid equivalents (GAE) showed that “moringa” leaves are excellent sources of phenolic compounds. The assays Ferric Reducing Antioxidant Power (FRAP) and β -Carotene bleaching showed promising antioxidant activity for the extracts, being of the leaves extracts the most efficient in the two methods, although the extracts were evaluated in the same concentration of TEP. All etanolic extracts from *M. oleifera* showed bioactivity against *Artemia salina*, however, unrelated toxicity according to determined LC50 (394.07, 349.82, 391.97 e 444.83, respectively). These results suggest the viability of using *M. oleifera* Lam. as source of antioxidant and alternative food.

Keywords: *Moringa oleifera* Lam., Antioxidant, *Artemia salina*, Toxicity

1. Introduction

Moringa oleifera Lamarck is a tree native to northwestern India and widely cultivated throughout the tropics. It is a perennial and drought tolerant plant. It was introduced in Brazil by 1950, where it is cultivated as an ornamental and medicinal plant. It is found mostly in the Northeast, where it is known as “lírio-branco”, “quiabo de quina” or simply “moringa”. All parts of *Moringa* have applicability, being well known its use in nutrition due to its important minerals and because it is a good source of vitamins, beta-carotene and proteins (Anwar et al., 2007; Makkar and Becker, 1996). It is traditionally used in India as a medicinal herb to treat and prevent numerous diseases, acting as antibiotic, antitripanosomal, hypotensive, anti-ulcer, antispasmodic, anti-inflammatory, hypocholesterolemic and hypoglycemic (Abrogoua et al., 2012; Bijina et al., 2011; Debnath et al., 2011; Oluduro et al., 2010). *Moringa* also stands out due to carotenoids and phenolic compounds and provides a rich and rare combination of zeatin, quercetin, β -sitosterol, caffeoylquinic acid and kaempferol which also gives the plant a potential antioxidant activity (Barreto et al., 2009; Iqbal and Bhanger, 2006; Lako et al., 2007; Reddy et al., 2005; Siddhuraju and Becker, 2003).

Given the rich constitution of compounds found in “moringa”, it should also be emphasized the need to demonstrate the safe use of the plant, ie, the guarantee that it has no ability to produce toxic effects to the consumer.

A bioassay which has been employed for evaluating the pharmacological activity of plant extracts is the brine shrimp lethality test (BST). It uses *Artemia salina* to determine the LC₅₀ (median lethal concentration) due to the fact this microcrustacean be sensitive to a number of substances and also the tendency to limit the use of laboratory animals in toxicity testing (Ghisalberti, 2008; Kanwar, 2007; Solis et al., 1993).

Given the excellent nutritional and pharmacological characteristics of *Moringa* and considering its antioxidant potential, this study investigated the in vitro antioxidant activity of ethanol extracts of its leaves, flowers and string beans as well as the bioactivity of ethanolic extracts of leaves, flowers, string beans and seeds against *Artemia salina* Leach.

2. Materials and methods

2.1. Materials

Reagents *tert*-Butylhydroquinone (TBHQ), 6-hydroxy-2,5,7,8-tetramethylchoman-2-carboxylic acid (Trolox), 2,4,6,tris(2-pyridyl)-s-triazina (TPTZ), linoleic acid, were purchased from Sigma-Aldrich (Steinheim, Germany) while β -carotene, ferric chloride hexahydrate ($\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$), ferrous sulphate heptahydrate ($\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$), Folin-Ciocalteu reagent, 3,4,5-trihydroxybenzoic acid (gallic acid) and all other analytical reagents were acquired from Merck (Düsseldorf, Germany).

2.2. Samples

Leaves, flowers, seed pods and seeds of “moringa” (*Moringa oleifera* Lam.) were collected during April, 2010, in Campus I of the Federal University of Paraíba (UFPB), João Pessoa/PB, Brazil, and subsequently dried in air circulating oven at 45 °C (model MA 035, Marconi) for 24 hours. After complete drying, each part was ground separately in mill knives and subsequently packed in polyethylene vacuum bags and kept at -18 °C during the experiments.

2.3. Preparation of extracts

It was used 50 g of powdered plant material and 500 mL of ethanol to obtain each extract. The mixture was stirred in metabolic water bath, for 2 hours, at 25 °C, followed by vacuum filtration. The extracts of leaves (EL), flowers (EF) and shell of seed pods (EP) were concentrated on a rotatory evaporator at 60 °C until complete removal of the solvent. Each one was packed in amber vials under refrigeration until the time of analysis.

2.4. Determination of total extractable phenolics

The levels of total extractable phenolics (TEP) were measured colorimetrically by the Folin-Ciocalteu according to Slinkard and Singleton (1977), with the assistance of a UV-vis spectrophotometer (model UV-2550, Shimadzu). The results were expressed as mean $\pm$ standard deviation mg of gallic acid equivalents (GAE) / g extract.

2.5. Ferric reducing antioxidant power (FRAP) assay

The antioxidant activity of extracts of “moringa” was estimated by the assay of ferric reducing antioxidant power (FRAP), following the procedure described in literature (Benzie and Strain, 1996) with modifications previously reported (Pulido et al., 2000). Initially a 2.7 mL aliquot of the freshly prepared FRAP reagent (TPTZ 10 μ M, FeCl₃ 20 μ M and acetate buffer) was homogenized with 90 μ L of extract and 270 μ L of distilled water in bath water at 37 °C for 30 minutes. The absorbance at 595 nm of the mixture was measured in UV-vis spectrophotometer (model UV-2550, Shimadzu). The FRAP reagent was used as blank to calibrate the spectrophotometer. Concentrations of 500-2000 μ M of ferrous sulfate (FeSO₄·7H₂O) were used to determine the standard curve. The results were expressed as mean $\pm$ standard deviation μ M ferrous sulfate / g extract.

2.6. β -carotene bleaching method

The antioxidant activity of the extracts of “moringa” by β -carotene / linoleic acid model system was determined as described in the literature by Marco (1968) with modifications by Miller (1971). The spectrophotometric assay is based on oxidation (bleaching) of β -carotene induced by products of the oxidative degradation of linoleic acid. The solutions were prepared by mixing 5 mL of system solution of β -carotene/linoleic acid and 0.4 mL of “moringa” extracts or Trolox solution. The mixture was kept in water bath at 40 °C. Readings were taken in UV-vis spectrophotometer (model UV-2550, Shimadzu) at 470 nm 2 minutes after mixing and then at intervals of 15-120 minutes. The control sample consisted of 5 mL of system solution of β -carotene/linoleic acid and 0.4 mL of solvent in place of the extracts. The reference substance (Trolox) was used at a concentration of 200 μ g.mL⁻¹. The results were expressed as percentages of inhibition of oxidation by using the following formula:

$$\text{Oxidation inhibition (\%)} = [(1 - (\text{Abs } A_i - \text{Abs } A_f) / (\text{Abs } C_i - \text{Abs } C_f)) \times 100]$$

where Abs A_i is the initial absorbance of the sample, Abs A_f is the final absorbance of the sample, Abs C_i is the initial absorbance of the control and Abs C_f is the final absorbance of the control.

2.7. Brine shrimp lethality test (BST) with *Artemia salina*

For assessing the bioactivity of extracts of leaves, flowers, seed pods and seeds of *Moringa oleifera* it was performed the Brine shrimp lethality test (BST) with *Artemia salina* according to Meyer et al. (1982).

Artemia salina cysts (25 mg) were obtained in Laboratory of Animal Experimentation, Amapá, Brazil and were incubated in artificial sea solution (pH 8.5 and 29 °C) under light (40 watt lamp) for 24 hours to hatching the nauplii. Approximately 10-13 larvae were transferred to test tubes containing final volume of 1 mL each and different concentrations of extracts (1, 3, 10, 30, 100, 300, 500, 750 and 1000 μ g.mL⁻¹). A control tube was obtained using 1 mL of vehicle (cremophor solution 5%) and larvae. Each concentration was tested in triplicate and repeated in two experiments. It was counted the number of living and dead larvae to determine the LC50.

2.8. Statistical analysis

The results were expressed as mean $\pm$ standard deviation, compared by analysis of variance (ANOVA) and Duncan test, considering $p < 0.05$, assisted by software Statistica 7.0 (Statsoft®).

In the assessment of bioactivity, the results were expressed as mean of triplicate analysis and the experiment was repeated to obtain a new mean. CL50 was established with a 95% confidence interval for all experiments, being significant when $p < 0.05$ according to Probit analysis (Finney, 1971) and assisted by software GraphPad Prism 4.03.

3. Results and discussion

3.1. Total extractable phenolics (TEP)

From the determination of the levels of total extractable phenolics (TEP) it was possible to determine the necessary amount of each extract equivalent to one addition of 200 mg.kg⁻¹ of GAE for the assay in the system β -carotene / linoleic acid. The analyses of the TEP contents present in the extracts showed that the highest values were observed for EL and the smallest for ES, and that it was not found a significant difference ($p < 0.05$) in relation the amount of phenolics determined for EF and EP (Table 1). The highest value of TEP for the EL extracts agrees with the data available in the literature, that report the presence of the flavonoids: gallic acid, ellagic acid, ferulic acid, chlorogenic acid, kaempferol, quercetin, vanillin and rutin in the leaves of *Moringa oleifera* Lam (Singh et al., 2009).

3.2. Ferric reducing antioxidant Power (FRAP) assay

The FRAP assay is based on the ability of an antioxidant to reduce Fe^{3+} to Fe^{2+} with maximum absorption at 593 nm. This assay provides rapid and reproducible results, however, it cannot measure all the antioxidants present in a complex matrix, as in the case of extracts. For this reason, many studies have used this method in conjunction with other assays (Niki, 2010).

The results of the FRAP assay for the “moringa” extracts and TBHQ and Trolox standards are summarized in Table 1. According to these data all samples exhibited ferric reducing activity. Among the extracts, EL showed better activity, followed by EF and EP. These data clearly reveal a correlation between the amount of phenolic compounds and antioxidant capacity of the extracts. TBHQ was more active than the extracts.

3.3. β -Carotene bleaching method

In the coupled oxidation assay of β -carotene / linoleic acid, the system model when submitted to oxidizing conditions generates a free radical from the oxidation of linoleic acid, which will abstract the hydrogen from the molecule of unsaturated β -carotene. As a result of the oxidation of this molecule, the system loses its characteristic orange color which is monitored spectrophotometrically in order to quantify the degree of inhibition of oxidation by the antioxidant to be tested (Abidille et al., 2005).

The kinetic curve of the potential antioxidant of the ethanolic extracts of “moringa” in the β -carotene/linoleic acid system is shown in Figure 1. According to that, it is possible to observe the kinetic of the degradation of β -carotene for 120 minutes using the extracts of leave (EL), flowers (EF) and seed pods (EP) at concentration of 200 $\mu\text{g.mL}^{-1}$ compared to total phenolic content. Trolox was used for comparison at concentration of 200 $\mu\text{g.mL}^{-1}$. The extracts inhibited the oxidation of β -carotene throughout the degradation curve in both the beginning of oxidation (within 15-45 minutes) as the ending of oxidation (between 75-105 minutes). EL extract obtained better antioxidant activity compared to Trolox and it was more efficient than EF and EP.

3.4. *Artemia salina* survival trials

According to Meyer et al. (1982) when the determined LC50 is less than 1000 $\mu\text{g.mL}^{-1}$, it indicates that the compound presents evidence of biological activity. Dolabela (1997) has also established a correlation between LC50 value and the level of toxicity against *Artemia salina*, namely: $\text{CL}_{50} < 80 \mu\text{g.mL}^{-1}$, highly toxic, LC_{50} between 80 $\mu\text{g.mL}^{-1}$ and 250 $\mu\text{g.mL}^{-1}$, moderately toxic, and $\text{LC}_{50} > 250 \mu\text{g.mL}^{-1}$, nontoxic or low toxicity.

Table 2 shows values of LC50 determined for the ethanolic extracts of leaves, flowers, seed pods and seeds of “moringa” through bioassay with *Artemia salina*. The respective values show that such compounds has pharmacological activity (Meyer et al., 1982), however, it allows to infer that such activity does not rank as

toxic, since for each tested part of the plant, the LC50 value found was greater than 250 $\mu\text{g.mL}^{-1}$, which classifies these extracts as nontoxic (Dolabela, 1997).

4. Conclusions

Moringa oleifera showed a promising antioxidant activity for all the tested extracts, which was evidenced *in vitro* in different methods. The content of phenolic compounds is higher in extract EL, which stood out for its greater effectiveness in producing antioxidant effect independently of the determination method employed. The results of brine shrimp lethality test of the ethanolic extracts from *M. oleifera* (leaves, flowers, seed pods and seeds) shows that in all tested parts of the plant it was found bioactive compounds, which corroborate the findings of medicinal properties related to this species. Those results do not indicate toxicity in the extracts, suggesting safety in the use of possible products formulated from this species for human consumption, or even as alternative food and source of natural antioxidants.

5. Acknowledgement

The authors acknowledge the Brazilian agencies *Conselho Nacional de Desenvolvimento Científico e Tecnológico* (CNPq) and *Coordenação de Aperfeiçoamento de Pessoal de Nível Superior* (CAPES) for financial support.

6. References

- Abidille, M.H., Singh, R.P., Jayaprakasha, G.K., Jena, B.S., 2005. Antioxidant activity of the extracts from *Dillenia indica* fruits. *Food Chemistry* 90, 891–896.
- Abrogoua, D.P., Dano, D.S., Manda, P., Adepo, A.J.B., Kablan, B.J., Goze, N.B., Ehoulé, K., 2012. Effect on blood pressure of a dietary supplement containing traditional medicinal plants of Côte d'Ivoire. *Journal of Ethnopharmacology* 141, 840–847.
- Anwar, F., Latif, S., Ashraf, M., Gilani, A.H., 2007. *Moringa oleifera* : A Food Plant with Multiple Medicinal Uses. *Phytother. Res.* 21, 17–25.
- Barreto, M.B., Freitas, J.V.B., Silveira, E.R., Bezerra, A.M.E., Nunes, E.P., Gramosa, N. V., 2009. Constituintes químicos voláteis e não-voláteis de *Moringa oleifera* Lam., *Moringaceae*. *Brazilian Journal of Pharmacognosy* 19, 893–897.
- Benzie, I.F., Strain, J.J., 1996. The ferric reducing ability of plasma (FRAP) as a measure of “antioxidant power”: the FRAP assay. *Analytical Biochemistry* 239, 70–76.
- Bijina, B., Chellappan, S., Krishna, J.G., Basheer, S.M., Elyas, K.K., Bahkali, A.H., Chandrasekaran, M., 2011. Protease inhibitor from *Moringa oleifera* with potential for use as therapeutic drug and as seafood preservative. *Saudi Journal of Biological Sciences* 18, 273–281.
- Chumark, P., Khunawat, P., Sanvarinda, Y., Phornchirasilp, S., Morales, N.P., Phivthong-Ngam, L., Ratanachamnong, P., Srisawat, S., Pongrapeeporn, K.U.S., 2008. The *in vitro* and *ex vivo* antioxidant properties, hypolipidaemic and antiatherosclerotic activities of water extract of *Moringa oleifera* Lam. leaves. *Journal of Ethnopharmacology* 116, 439–46.
- Cáceres, A., Saravia, A., Rizzo, S., Zabala, L., De Leon, E., Nave, F., 1992. Pharmacologic properties of *Moringa oleifera*. 2: Screening for antispasmodic, antiinflammatory and diuretic activity. *Journal of Ethnopharmacology* 36, 233–237.

- Debnath, S., Biswas, D., Ray, K., Guha, D., 2011. *Moringa oleifera* induced potentiation of serotonin release by 5-HT(3) receptors in experimental ulcer model. *International Journal of Phytotherapy and Phytopharmacology* 18, 91–5.
- Dolabela, M.F., 1997. Triagem in vitro para atividade antitumoral e anti-Tripanossoma cruzi de extratos vegetais, produtos naturais e substâncias sintéticas.
- Faizi, S., Siddiqui, B.S., Saleem, R., Siddiqui, S., Aftab, K., Gilani, A.H., 1994. Isolation and structure elucidation of new nitrile and mustard oil glycosides from *Moringa oleifera* and their effect on blood pressure. *Journal of Natural Products* 57, 1256–1261.
- Finney, D.J., 1971. Probit analysis. Cambridge University, Cambridge.
- Ghisalberti, E.L., 2008. Detection and isolation of bioactive natural products, in: COLEGATE, S.M., MOLYNEUX, R.J. (Eds.), *Bioactive Natural Products – Detection, Isolation, and Structural Determination*. CRC Press, Boca Raton, pp. 11–76.
- Haristoy, X.W., Fahey, J.W., Scholtus, I.W., Lozniewski, A.W., 2005. Adicionar ao Meu Espaço Evaluation of the Antimicrobial Effects of Several Isothiocyanates on *Helicobacter pylori*. *Planta Medica* 71, 326–330.
- Iqbal, S., Bhanger, M.I., 2006. Effect of season and production location on antioxidant activity of *Moringa oleifera* leaves grown in Pakistan. *Journal of Food Composition and Analysis* 19, 544–551.
- Jaiswal, D., Rai, P.K., Kumar, A., Mehta, S., Watal, G., 2009. Effect of *Moringa oleifera* Lam. leaves aqueous extract therapy on hyperglycemic rats. *Journal of Ethnopharmacology* 123, 392–396.
- Kanwar, A.S., 2007. Brine shrimp (*Artemia salina*) – a marine animal for simple and rapid biological assays. *Journal of Chinese Clinical Medicine* 2, 236–240.
- Karadi, R. V, Gadge, N.B., Alagawadi, K.R., Savadi, R. V, 2006. Effect of *Moringa oleifera* Lam. root-wood on ethylene glycol induced urolithiasis in rats. *Journal of Ethnopharmacology* 105, 306–11.
- Lako, J., Trenerry, V., Wahlqvist, M., Wattanapenpaiboon, N., Sotheeswaran, S., Premier, R., 2007. Phytochemical flavonols, carotenoids and the antioxidant properties of a wide selection of Fijian fruit, vegetables and other readily available foods. *Food Chemistry* 101, 1727–1741.
- Makkar, H.P.S., Becker, K., 1996. Nutritional value and antinutritional components of whole and ethanol extracted *Moringa oleifera* leaves. *Animal Feed Science and Technology* 63, 211–228.
- Marco, G., 1968. A rapid method for evaluation of antioxidants. *Journal of the American Oil Chemists' Society* 45, 594–598.
- Meyer, B.N., Ferrigni, N.R., Putnam, J.E., Jacobsen, L.B., Nichols, D.E., McLaughlin, J.L., 1982. Brine shrimp: A convenient general bioassay for active plant constituents. *Planta Medica* 45, 31–34.
- Miller, H.E., 1971. A simplified method for the evaluation of antioxidants. *Journal of the American Oil Chemists' Society* 48, 91.
- Niki, E., 2010. Assessment of antioxidant capacity in vitro and in vivo. *Free Radical Biology & Medicine* 49, 503–515.

- Oluduro, O.A., Aderiye, B.I., Connolly, J.D., Akintayo, E.T., Famurewa, O., 2010. Characterization and Antimicrobial Activity benzyl thiocarboxamide ; a Novel Bioactive Compound from *Moringa oleifera* Seed Extract. *Folia Microbiol* 55, 422–426.
- Pulido, R., Bravo, L., Fulgencio, S.C., 2000. Antioxidant Activity of Dietary Polyphenols As Determined by a Modified Ferric Reducing/Antioxidant Power Assay. *Journal of Agricultural and Food Chemistry* 48, 3396–3402.
- Reddy, V., Urooj, A., Kumar, A., 2005. Evaluation of antioxidant activity of some plant extracts and their application in biscuits. *Food Chemistry* 90, 317–321.
- Siddhuraju, P., Becker, K., 2003. Antioxidant properties of various solvent extracts of total phenolic constituents from three different agroclimatic origins of drumstick tree (*Moringa oleifera* Lam.) leaves. *Journal of Agricultural and Food Chemistry* 51, 2144–2155.
- Singh, B.N., Singh, B.R., Singh, R.L., Prakash, D., Dhakarey, R., Upadhyay, G., Singh, H.B., 2009. Oxidative DNA damage protective activity, antioxidant and anti-quorum sensing potentials of *Moringa oleifera*. *Food and chemical toxicology : an international journal published for the British Industrial Biological Research Association* 47, 1109–16.
- Slinkard, K., Singleton, V.L., 1977. Total Phenol Analysis: Automation and Comparison with Manual Methods. *Am. J. Enol. Vitic* 28, 49–55.
- Solis, P.N., Wright, C.W., Anderson, M.M., Gupta, M.P., Phillipson, D., 1993. A microwell cytotoxicity assay using *Artemia salina* (Brine shrimp). *Planta Medica* 59, 250–252.

Table 1

Antioxidant capacity determined by the FRAP method and expressed as $\text{mmol Fe}^{2+} \cdot \text{g}^{-1}$ of extract and % of oxidation inhibition (OI) by the β -carotene method.

Samples	FRAP / $\text{mmol Fe}^{2+} \cdot \text{g}^{-1}$	OI β -carotene* %
EL	19.82 ± 0.69^b	82.80 ± 0.88^a
EF	11.61 ± 0.65^c	73.15 ± 0.52^c
EP	10.40 ± 0.59^d	61.25 ± 0.74^d
TBHQ	30.29 ± 0.10^a	---
TROLOX	---	81.35 ± 0.79^b

Values are mean $\pm$ standard deviation, $n=3$.

Mean followed by different letters in the same column differs significantly ($p \leq 0.05$).

* Extracts at concentration of $200 \mu\text{g} \cdot \text{mL}^{-1}$ / mL compared to total phenolic content. Trolox was used at concentration of $200 \mu\text{g} \cdot \text{mL}^{-1}$.

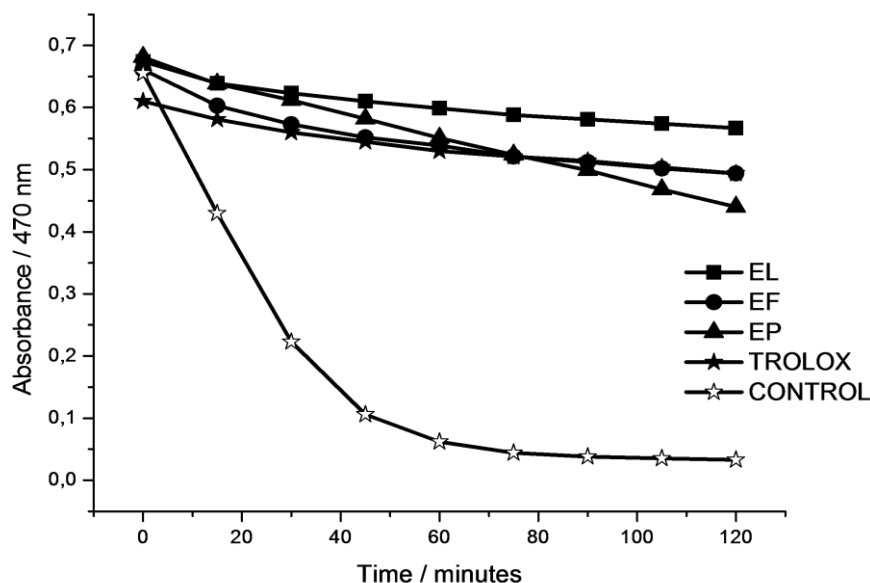


Fig. 1. Kinetic curve of the potential antioxidant of ethanolic extracts of leaves (EL), flowers (EF) and seed pods (EP) of “moringa” with phenolic concentration of $200 \mu\text{g} \cdot \text{mL}^{-1}$ compared to the control (no antioxidant) and with Trolox at concentration of $200 \mu\text{g} \cdot \text{mL}^{-1}$ through β -carotene/linoleic acid system.

Table 2Brine shrimp toxicity results of ethanolic extracts of parts of *Moringa oleifera*

Tested part	LC50/ $\mu\text{g.mL}^{-1}$ ^a	p value	95 % CI / $\mu\text{g.mL}^{-1}$ ^b	r^2
Leaves	394.07	< 0.0001	185.51 – 512.37	0.9216
Flowers	349.82	0.0003	187.19 – 512.36	0.8653
Seed pods	391.97	0.0003	192.54 – 514.03	0.8681
Seeds	444.83	< 0.0001	385.87 – 503.32	0.9800

^a median lethal concentration^b confidence interval

5 CONSIDERAÇÕES FINAIS

- Os extratos etanólicos das folhas, flores e casca das vagens da moringa mostraram que são fontes em potencial de compostos fenólicos, tendo exibido resultados expressivos no sequestro de radicais livres pelo método DPPH, tendo sido, também, eficientes na redução do ferro e no sistema β -caroteno/ácido linoleico.
- Os extratos estudados exibiram ação protetora superior ao antioxidante sintético BHT, quando aplicados aos óleos de peixe e de soja e avaliados pelos métodos Rancimat e P-DSC, indicando que, do ponto de vista químico, os mesmos podem vir a serem fontes alternativas de aditivos antioxidantes para uso na indústria de alimentos.
- A avaliação do efeito antioxidante dos extratos pelo teste acelerado de estufa revelou que os extratos das folhas e das flores são eficientes no controle da oxidação primária, reduzindo consideravelmente os teores de peróxidos e de dienos conjugados, quando comparados ao controle negativo de ambos os óleos.
- Os resultados da avaliação preliminar da bioatividade e toxicidade sugerem que os extratos testados possuem compostos bioativos supostamente atóxicos, o que é um indicativo da viabilidade de utilização dos referidos extratos como fonte alternativa de alimentos e antioxidantes naturais.
- Estes resultados sugerem que o extrato etanólico das folhas de moringa possui efeito protetor eficiente quando aplicado a sistemas lipídicos de baixa estabilidade oxidativa, podendo vir a ser uma fonte alternativa de potencial aplicação na indústria de óleos e gorduras.