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INSTITUTO DE TECNOLOGIA

PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIA E TECNOLOGIA DE ALIMENTOS

TESE

Extração, purificação e aplicação de compostos fenólicos a partir de folhas de olerícolas orgânicas utilizando resinas macroporosas de adsorção

Nathália da Rocha Rodrigues

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UNIVERSIDADE FEDERAL RURAL DO RIO DE JANEIRO
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EXTRAÇÃO, PURIFICAÇÃO E APLICAÇÃO DE COMPOSTOS FENÓLICOS A PARTIR DE FOLHAS DE OLERÍCOLAS ORGÂNICAS UTILIZANDO RESINAS MACROPOROSAS DE ADSORÇÃO

NATHÁLIA DA ROCHA RODRIGUES

Sob a orientação da professora
Maria Ivone Martins Jacintho Barbosa

Tese submetida como requisito parcial para obtenção do grau de **Doutor em Ciência e Tecnologia de Alimentos**, no curso de Pós-Graduação em Ciência e Tecnologia de Alimentos, Área de concentração em Ciência de Alimentos.

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TESE APROVADA EM: 27 de Março de 2018

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RESUMO

RODRIGUES, Nathália da Rocha. **Extração, purificação e aplicação de compostos fenólicos a partir de folhas de olerícolas orgânicas utilizando resinas macroporosas de adsorção.** 2018. 103 p. Tese (Doutorado em Ciência e Tecnologia de Alimentos). Instituto de Tecnologia, Departamento de Tecnologia de Alimentos, Universidade Federal Rural do Rio de Janeiro, Seropédica, RJ, 2018.

Nas últimas décadas, a procura dos consumidores por alimentos com boas características tecnológicas, sensoriais e com propriedades funcionais tem aumentado, devido à busca pela alimentação saudável. Este comportamento culmina com um maior consumo de alimentos de origem vegetal e impacta diretamente no rápido desenvolvimento das indústrias de processamento de alimentos, o que leva a um aumento na geração de resíduos e subprodutos de alimentos, de forma cada vez mais significativa. Por outro lado, o consumo de alimentos não convencionais e subprodutos tem sido alvo de diversos estudos recentes. As folhas de olerícolas, em particular, são materiais de alto valor nutricional e muitas delas são ricas em nutrientes como fibras e compostos fenólicos, além de apresentarem atividade antioxidante mais elevados do que o próprio alimento em si. De forma geral, os vegetais orgânicos apresentam maior conteúdo de compostos fenólicos quando comparados aos convencionais, pois nos primeiros, a produção de metabólitos secundários é maior. Existe uma demanda global pela utilização de técnicas de extração ambientalmente sustentáveis, como as resinas macropososas de adsorção, as quais permitem a separação eficaz e boa recuperação de fitoquímicos de interesse, tais como os compostos fenólicos. Contudo, estudos envolvendo a obtenção de extratos antioxidantes a partir de folhas e sua potencial utilização pela indústria de alimentos ainda são muito incipientes. Sendo assim, este trabalho tem por objetivo realizar a extração e purificação de compostos fenólicos de folhas de diferentes olerícolas orgânicas (batata-doce, mandioca e araruta), utilizando adsorção com resinas macroporosas como uma técnica de "separação verde", com vistas para a aplicação em produtos alimentícios. Foi observado que as folhas de batata-doce de polpa roxa orgânicas foram o material analisado que apresentou resultados mais promissores em relação a alta capacidade antioxidante e conteúdo de compostos fenólicos. Dentre as resinas macroporosas testadas, a XAD 16 e XAD 7HP apresentaram melhor recuperação de fenólicos de folhas de batata-doce de polpa roxa, estando relacionadas à maior capacidade de adsorção e dessorção. No entanto a XAD 7 HP apresentou maior eficiência pois proporcionou menor tempo de processo de adsorção. Os resultados obtidos nas análises de adsorção e dessorção cinética demonstraram que as vazões de 3 mL/ min e 7 mL/ min foram as escolhidas para a adsorção e dessorção cinética, respectivamente. Este estudo demonstrou que a adição de extratos de fenólicos oriundos de folhas de batata-doce de polpa roxa obtidos por separação com resinas macroporosas foi capaz de proporcionar maior estabilidade oxidativa de óleos de soja. A alta capacidade antioxidante dos extratos purificados indicou que a purificação e separação com resinas macroporosas pode ser uma técnica eficiente no desenvolvimento de produtos alimentícios adicionados de compostos de interesse obtidos a partir de subprodutos de vegetais.

Palavras-chave: subprodutos de alimentos, folhas, separação verde.

ABSTRACT

RODRIGUES, Nathália da Rocha. **Extraction, purification and application of phenolic compounds from organic oleraceous leaves using macroporous adsorption resins.** 2018. 103 p. Thesis (PhD in Food Science and Technology). Instituto de Tecnologia, Departamento de Tecnologia de Alimentos, Universidade Federal Rural do Rio de Janeiro, Seropédica, RJ, 2018.

In the last decades, the consumers demand for food with good technological and sensory characteristics and functional properties has increased, due to the search for healthier food. This behavior culminates with a higher consumption of plant food and impacts on the fast development of food processing industries, which increase the wastes and food by-products production. On the other hand, the consumption of non-conventional food and by-products has been studied in the last years. Leaves of vegetable oleraceous present high nutritional value and higher nutrients contents (fibers and phenolic compounds) and antioxidant activity when compared to another parts of the plant. In general, In general, organic vegetables present higher contents of phenolic compounds when compared to conventional ones, because the production of secondary metabolites in organic plants is more expressive. There is a global demand for the use of environmentally sustainable separation techniques, such as microporous adsorption resins, which allows effective separation and good recovery of phytochemicals of interest, as phenolic compounds. However, studies involving the extraction of antioxidant extracts from leaves and their potential use by the food industry are still very incipient. Therefore, the objective of this work is to extract and purify phenolic compounds from leaves of different organic olive groves (sweet potatoes, cassava and arrowroot), using macroporous resins adsorption as a "green separation" technique, with a view to application in food products. It was observed that the organic purple-fleshed sweet potato leaves were the evaluated material that presented more promising results in relation to high antioxidant capacity and content of phenolic compounds. Among the macroporous resins tested, XAD 16 and XAD 7HP presented a better recovery of phenolics from leaves of purple-fleshed sweet potatoes, being related to higher adsorption and desorption capacity. However, XAD 7 HP presented higher efficiency because it provided less time of adsorption process. The results obtained in the adsorption and kinetic desorption assays showed that the flow rates of 3 mL / min and 7 mL / min were chosen for dynamic adsorption and desorption, respectively. This study demonstrated that the addition of phenolic extracts from leaves of purple-fleshed sweet potatoes obtained by separation with macroporous resins was able to provide greater oxidative stability of soybean oils. The high antioxidant capacity of the purified extracts indicated that purification and separation with macroporous resins may be an efficient technique in the development of added food products of compounds of interest obtained from vegetable by-products.

Keywords: food by-products, leaves, green separation.

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LISTA DE ABREVIATURAS

AC	antioxidant capacity
ACE	Angiotensin Converting Enzyme
BV	bed volume
CQA	caffeoilquinic acid
DW	dry weight
FRAP	ferric reducing antioxidant power
FRS	free radical scavenging
GAE	galic acid equivalent
g	Gram
HCA	Hierarquical Cluster Analysis
HPLC	High performance liquid chromatography
h	Hour
IAPS	Integrated Agroecological Production
LDS	least signifficant difference
LEA	Leaf extracts from arrowroot
LECC	Leaf extracts from cassava cultivar *Cachoeira*
LECI	Leaf extracts from cassava cultivar *IAC*
LECS	Leaf extracts from cassava cultivar *Saracura*
LESB	Leaf extracts from sweet potatoes cultivar *Beterraba*
LESCa	Leaf extracts from sweet potatoes cultivar *Capivara*
LESCe	Leaf extracts from sweet potatoes cultivar *Cenoura*
LESN	Leaf extracts from sweet potatoes cultivar *Nusay*
LESR	Leaf extracts from sweet potatoes cultivar *Rosinha de Verdan*
MARs	macroporous adsorbent resins
mL	Mililiter
mM	Milimolar
μM	Micromolar
PCA	Principal component analysis
PFSPL	purple-fleshed sweet potatoes leaves
ppm	parts per million
RMSE	root mean square error
SD	standard deviation
SPLE	Sweet potatoes leaves extract
TBHQ	Tert-butyl hydroquinone
TE	Trolox equivalent
TPC	total phenolics contents

SUMÁRIO

ESTRUTURA DA TESE

A tese está estruturada conforme descrito a seguir: Inicialmente no Capítulo I, é apresentada uma revisão de literatura acerca dos principais artigos científicos abordando as propriedades funcionais e medicinais da utilização de folhas de olerícolas, bem como suas principais aplicações na tecnologia de alimentos.

No Capítulo II está apresentada a avaliação da capacidade antioxidante, teores de compostos fenólicos totais e clorofilas de diferentes folhas de olerícolas orgânicas (folhas de batata-doce, mandioca e araruta), com o objetivo de selecionar as matérias-primas com resultados mais promissores. Além disso, foi realizada a avaliação do potencial inibitório da enzima conversora da angiotensina, o qual tem sido associado à capacidade dos compostos fenólicos atuarem na redução da pressão arterial, como forma de determinar a bioatividade dos compostos fenólicos nas matrizes alimentícias estudadas.

No Capítulo III são apresentados resultados concernentes ao estudo da extração e purificação de compostos fenólicos a partir de folhas das olerícolas selecionadas (folhas de batata-doce de polpa roxa) utilizando 6 diferentes resinas macroporosas de adsorção, onde foram realizados testes de adsorção e dessorção estáticos, avaliando-se as diferentes resinas e diferentes pHs de adsorção; cinéticas de adsorção; isotermas de adsorção, avaliando-se diferentes temperaturas de adsorção e diferentes concentrações dos extratos; testes de adsorção e dessorção dinâmicos; e por fim, comparação do perfil de compostos fenólicos das amostras secas e dos extratos purificados.

No Capítulo IV são apresentados os resultados da avaliação da estabilidade oxidativa de óleo de soja adicionado de extratos purificados de folhas de batata-doce de polpa roxa como antioxidantes naturais obtidos por adsorção e dessorção com resinas macroporosas, avaliando a aplicabilidade dos extratos purificados na indústria de alimentos.

Por fim, são apresentadas as considerações finais, sugestões para estudos futuros e os anexos.

Cada capítulo está apresentado na forma de artigo e, portanto, está formatado de acordo com as normas exigidas por cada revista à qual foi submetido.

1. INTRODUÇÃO GERAL

Nas últimas décadas, as preocupações acerca da segurança alimentar, impactos ambientais e saúde humana têm contribuído para o aumento do interesse por sistemas agrícolas alternativos, tais como a agricultura orgânica (SUJA et al., 2017). A demanda crescente por alimentos orgânicos no mundo tem gerado um mercado atraente para produtores e distribuidores e o Brasil apresenta grande potencial frente à conquista deste mercado, pois se destaca como um dos grandes produtores em área plantada (MOOZ; SILVA, 2014). Neste contexto, destaca-se como um cenário promissor para a produção de alimentos orgânicos (ORGANICS BRASIL, 2012).

A agricultura orgânica é um dos setores de mais rápido crescimento na produção de alimentos mundial (SEUFERT et al., 2017), constituindo uma alternativa para produção de alimentos de forma sustentável e segura, e preservação do solo e o meio ambiente (SUJA et al., 2017).

De acordo com a Sociedade Nacional de Agricultura (SNA), a produção orgânica nacional em 2017 estaria ultrapassando a marca dos 750 mil hectares registrados em 2016. Segundo a Coordenação de Agroecologia (Coagre) da Secretaria de Desenvolvimento Agropecuário e Cooperativismo (SDC), esse tipo de cultivo no campo poderia ser registrado em 22,5% dos municípios brasileiros. De acordo com dados reportados pela Coagre, houve um salto no número de propriedades adotando o cultivo orgânico, de 6.700 mil unidades, em 2013, para aproximadamente 15.700, em 2016 e o Sudeste possuía a maior concentração destas unidades, totalizando 333 mil hectares (SNA, 2017). Com base neste panorama, o mercado de alimentos orgânicos foi, em 2011, considerado um dos ramos do *agribusiness* de maior crescimento de demanda no contexto do mercado internacional (OTA, 2011).

Dentre as culturas orgânicas de interesse para a agricultura orgânica, diversas olerícolas apresentam longos ciclos, possibilidade de armazenamento, capacidade de plantio em grandes áreas e uso de mecanização (SENAR, 2012). As culturas de raízes e tubérculos tais como mandioca, batata-doce e inhame, foram consideradas como o terceiro grupo mais importante de culturas alimentares, após os cereais e leguminosas. Estes grupos de alimentos contribuem para a segurança alimentar e são consumidos principalmente por populações pobres dos países em desenvolvimento (SUJA et al., 2009; SUJA et al., 2010). Algumas classes de tubérculos e raízes são consideradas importantes fontes de compostos bioativos (TIERNO et al., 2015). As folhas de olerícolas têm sido reconhecidas como potenciais fontes de compostos fenólicos com alta biodisponibilidade, comparados a diversos vegetais folhosos comercializáveis. Contudo, sua biodisponibilidade pode ser afetada por etapas de processamento e/ou cozimento (CARVALHO et al., 2010). Algumas folhas têm sido consideradas fonte de outros nutrientes, tais como proteínas, fibra dietética, carotenoides, vitaminas e minerais, apresentando a vantagem adicional de colheita em diversas épocas do ano e grande rendimento quando comparados a outros folhosos (HUANG et al., 2013).

Nas últimas décadas, o uso de fitoquímicos presentes em fontes naturais como antioxidantes e ingredientes funcionais, encontrados em vegetais, vêm sendo investigados pela comunidade científica (CARVALHO et al., 2010; HOSSAIN et al., 2011). O processamento de alimentos de origem vegetal pode resultar na produção de coprodutos que são ricas fontes de substâncias bioativas, incluindo os polifenóis (LAGHA-BENAMROUCHE; MADANI, 2013), os quais são reportados por inúmeros estudos como efetivos sequestradores de radicais livres em vegetais (CHOUDHARY; SWARNKAR, 2011; ZHANG et al., 2011).

Estudos demonstram que o setor agrícola brasileiro atua como uma das mais importantes âncoras da economia e apresenta recordes de safras, mas em contrapartida tem sido consagrado como um campeão em perdas pós-colheita e desperdícios (VILELA et al., 2003). Cerca de 35% de toda a produção agrícola vai para o lixo, o que significa que mais de 10 milhões de toneladas de alimentos poderiam ser destinados ao consumo ou outras aplicações (CARVALHO, 2009).

De acordo com Lima et al. (2013), como consequência do aumento do processamento de vegetais, são gerados cerca de 40% dos resíduos agroindustriais, composto de restos de polpa, casca, caroços, folhas ou sementes.

O aproveitamento integral dos alimentos permite que as partes não convencionais dos mesmos sejam aproveitadas, visando, muitas vezes, a agregação de valor nutricional às preparações, além de reduzir custos e evitar o desperdício (STEFANELLO; ROSA, 2012). As partes não aproveitáveis dos alimentos poderiam ser utilizadas enfatizando o enriquecimento alimentar e aumentando o valor nutricional das refeições, pois talos e folhas podem ser mais nutritivos do que a parte nobre do vegetal como é o caso das folhas verdes da couve-flor que, mesmo sendo mais duras, contêm mais ferro que a couve manteiga e são mais nutritivas que a própria couve-flor (SOUZA et al., 2007).

As folhas de diversos alimentos são fontes alternativas de folhosos em períodos de escassez, pois, comparadas a outros vegetais folhosos, são mais tolerantes a pestes, doenças e condições de alta umidade e apresentam rendimentos maiores que outros vegetais verdes, pois podem ser colhidas em diversas épocas do ano (XU et al. 2010; HUANG et al., 2013; TAIRA et al., 2013; NKONGHO et al., 2014). Além disso, são recursos residuais na agricultura e sua obtenção é mais ampla e mais econômica, o que torna necessário o seu uso pleno (LI et al., 2011).

Alguns estudos demonstram que as folhas de batata-doce apresentam elevado conteúdo de compostos fenólicos, disponíveis para absorção (CARVALHO et al., 2010). Um estudo realizado por XU et al. (2010) demonstrou que extratos de folhas de 53 diferentes cultivares de batata-doce apresentaram potencial antioxidante e que os principais compostos bioativos responsáveis por esta atividade foram os polifenóis, especialmente os derivados de ácido cafeoilquínico. Algumas propriedades benéficas têm sido atribuídas às folhas de batata *yacon*, principalmente quando utilizadas tradicionalmente como chá (AYBAR et al., 2001). Estudos diversos têm demonstrado que folhas de *yacon* apresetam uma variedade de atividades fitoquímicas (OLIVEIRA et al., 2013; BARCELLONA et al., 2012). Um trabalho realizado por Andrade et al. (2014) revelou que todos os extratos obtidos a partir de folhas desta batata apresentaram conteúdo considerável de compostos fenólicos totais, cujos valores foram mais elevados do que os extratos obtidos a partir das flores destes tubérculos. Trombini & Leonel (2014) afirmaram que a farinha de folhas de mandioca é fonte de proteínas, fibras, vitamina C e β-caroteno. No entanto, sua toxicidade restringe seu uso *in natura* devido ao alto conteúdo de cianeto, sendo necessária a realização de um processamento prévio.

Os extratos crus de polifenóis a partir de folhas apresentam clorofila, proteínas, polissacarídeos e outras impurezas em sua composição, as quais limitam a aplicação dos polifenóis de folhas. Por esta razão, um eficiente método de purificação é necessário para obter polifenóis de alta pureza a partir de folhas (XI; MU; SUN, 2015).

O interesse em compostos naturais para potencial aplicação como antioxidantes alimentares e como suplementos dietéticos tem aumentado nos últimos anos (LIN et al., 2012). A alta pureza dos flavonoides é necessária no âmbito farmacêutico e da tecnologia de alimentos, e sua separação e purificação têm sido extensivamente estudados (WU et al., 2015). A extração de compostos polifenólicos depende principalmente da polaridade do solvente, método e tempo de extração, os quais determinam tanto a composição qualitativa quanto a quantitativa destes compostos. As polaridades dos fenólicos variam significativamente, sendo difícil desenvolver um método específico para a extração ótima de todos os compostos fenólicos (RODRÍGUEZ-PÉREZ et al., 2015).

Os métodos de separação e purificação de polifenóis a partir de plantas são basicamente extração com solventes orgânicos, separação por membranas e extração por fluido supercrítico (DAI; MUMPER, 2010; FARÍAS-CAMPOMANES; ROSTAGNO; MEIRELES, 2013), por exemplo. No entanto, estes métodos apresentam algumas desvantagens, tais como produção de

longos ciclos, requerendo muito tempo para realização, alto custo, consumo de grande quantidade de solventes e pouca recuperação, o que gera desvantagens para uso em escala industrial (FU et al., 2006).

As resinas macroporosas são polímeros duráveis polares, apolares ou levemente hidrofílicos com alta capacidade de adsorção por moléculas orgânicas (FU et al., 2006). Seu uso tem recebido atenção significativa na separação e purificação de compostos bioativos de vários produtos naturais devido a suas características e vantagens, incluindo adsorção favorável, baixo consumo de solventes, alta eficiência (ZOU et al., 2017; WU et al., 2015), estabilidade físico-química, alta seletividade de adsorção e fácil reciclagem (WAN et al., 2014), o que as torna mais atrativas que outros adsorventes comuns, e também pelas suas características de alta taxa de adsorção, forte capacidade de adsorção, fácil eluição e conveniente processo de regeneração (DU et al., 2012; LI et al., 2011; LIU et al., 2011). Sua alta capacidade de adsorção se deve não apenas à similaridade de sua polaridade em relação aos compostos de interesse, mas também devido à sua grande área superficial (LIU et al., 2010a, 2010b, 2010c).

Recentemente, as resinas macroporosas têm sido utilizadas na purificação de constituintes bioativos de extratos naturais, devido a sua alta eficiência (JIA & LU, 2008; LIU et al., 2010a; 2010b; 2010c). No entanto, poucos trabalhos têm sido desenvolvidos utilizando resinas para extração de componentes ativos de extratos naturais, como pigmentos (ZHANG et al., 2011), licopeno (LIU et al., 2010c), antocianinas (D'ALESSANDRO et al., 2013) e fenólicos (LIN et al, 2012).

Firdaous et al. (2017) estudaram os efeitos das resinas como forma de remoção de polifenóis das proteínas de alfalfa (*Medicago sativa*). Zou et al. (2017) avaliaram o isolamento de antioxidantes provenientes de extrato de alho negro (*Allium nigrum*), estudando 3 diferentes resinas e suas características. Leyton et al. (2017) avaliaram os efeitos de 6 resinas macroporosas de adsorção na eficiência de purificação de compostos fenólicos de algas marinhas. Lin e colaboradores (2012) utilizaram resinas macroporosas para obter compostos fenólicos e ácido rosmarínico de extratos de folhas de *Rabdosia serra*. Xi, Mu & Sun (2015) obtiveram produtos purificados de forte atividade antioxidante a partir de folhas de batata-doce (*Ipomoea batata),* utilizando resinas macroporosas AB-8 (resinas fracamente polares), demonstrando que o método apresentou alta eficiência, sendo econômico e ambientalmente favorável, além de apresentar grande potencial de produção a nível industrial. Wang et al. (2013), extraíram compostos fenólicos de folhas de romã (*Punica granatum*) utilizando cromatografia em resina macroporosa. Wan et al. (2014) utilizaram cinco diferentes tipos de resinas macroporosas, de diferentes propriedades físicas e químicas para extrair e purificar flavonoides totais de extratos de *FlosPopuli*. Zhao et al. (2011) obtiveram rutina e quercetina purificadas com resina macroporosa AB-8 a partir de *Euonymus alatus*, observando alta capacidade de adsorção e dessorção. Kuhn et al. (2014) realizaram purificação de flavonoides de resíduos do processamento de cebolas (*Allium cepa* L.) através de resina acrílica macroporosa, obtendo resultados eficientes, sugerindo que este processo contribui para a agregação de valor aos materiais residuais de vegetais. Buran et al. (2014) avaliaram características de adsorção e dessorção e separação de antocianinas e polifenóis de mirtilos (*Cyanococcus*) utilizando resinas adsorventes macroporosas, com vistas a obtenção de extratos com potencial aplicação como corantes naturais, suplementos dietéticos, ingredientes antioxidantes para alimentos funcionais, e/ou como matérias-primas em preparações do setor cosmético e farmacêutico.

Apesar das potencialidades destas matrizes na alimentação humana e como agentes para serem empregados na indústria alimentícia e farmacêutica, ainda existe uma escassez de dados e estudos sobre a composição química, propriedades funcionais, presença de fitoquímicos e eficientes métodos de extração de compostos bioativos a partir de folhas provenientes de

vegetais orgânicos, sobretudo no Brasil, pois estes materiais ainda apresentam uso negligenciado, sendo descartados ou utilizados na alimentação animal. Portanto, é importante o desenvolvimento de métodos simples, eficientes e ambientalmente sustentáveis, para garantir a separação e purificação de compostos bioativos de interesse a partir destas matrizes, possibilitando o uso de seus fitoquímicos como forma de forticar produtos alimentícios e incentivando o aproveitamento destes produtos de alto valor agregado, os quais ainda são comumente descartados.

2. OBJETIVOS

2.1. Objetivo Geral

O objetivo deste estudo foi avaliar as melhores condições de extração e purificação de compostos fenólicos a partir de folhas de diferentes olerícolas orgânicas, utilizando diferentes resinas macroporosas de adsorção como uma técnica de "separação verde", com vistas para a obtenção de um extrato purificado para futura aplicação na indústria alimentícia e/ou farmacêutica.

2.2. Objetivos Específicos

-Avaliar a capacidade antioxidante, teor de compostos fenólicos totais, teor de clorofilas e a atividade inibidora da enzima conversora de angiotensina de folhas de olerícolas orgânicas (batata-doce, mandioca e araruta), a fim de selecionar amostras com resultados mais promissores para prosseguimento dos estudos posteriores;

-Determinar as melhores condições da extração dos compostos fenólicos, selecionando os melhores parâmetros de extração em relação ao teor de compostos fenólicos totais das amostras selecionadas;

-Realizar a extração e a purificação dos compostos fenólicos totais das amostras selecionadas utilizando resinas macroporosas de adsorção, avaliando efeitos do pH, temperatura, concentração de extrato e tipo de resina sobre a capacidade de adsorção;

-Obter um extrato purificado de compostos fenólicos, utilizando uma metodologia ambientalmente sustentável, com vistas à aplicação em produtos alimentícios;

-Avaliar o efeito dos extratos purificados de fenólicos de folhas de batata-doce de polpa roxa como antioxidantes naturais na estabilidade oxidativa de óleo de soja, utilizando sistemas-modelo.

3. JUSTIFICATIVA DO TRABALHO

A promoção da alimentação saudável e a segurança alimentar e nutricional são essenciais para a saúde, para a qualidade de vida e para o desenvolvimento pleno dos indivíduos (RODRIGUES et al., 2011). O alimento, anteriormente considerado apenas fonte de nutrientes essenciais à manutenção da vida, tornou-se objeto de estudos que o relacionam à melhoria das funções metabólicas e prevenção de enfermidades (SILVA et al., 2014).

A transição nutricional verificada nas últimas décadas tem desencadeado um maior consumo de alimentos de origem vegetal pela população, o que impactou no rápido desenvolvimento das indústrias de processamento de alimentos vegetais em todo o mundo (CLEMENTE et al., 2015). O Brasil é um dos maiores produtores agrícolas, sendo detentor de uma produção média anual de 183,9 milhões de toneladas (IBGE, 2016) de frutas e hortaliças. No entanto, dados alarmantes acerca do desperdício verificado apontam que, em um total de dez toneladas de alimentos produzidos, apenas quatro chegam à mesa do consumidor, o que representa que cerca de 39 mil toneladas de alimentos são desperdiçados por dia (ECOD, 2013).

De acordo com dados da Organização das Nações Unidas para Agricultura e Alimentação (FAO/ONU), as consequências econômicas do desperdício de alimentos, totalizam cerca de 750 bilhões de dólares por ano. Desse montante, 54% ocorre na fase inicial da produção, na manipulação, pós-colheita e armazenagem. O restante (46%) acontece nas etapas de processamento, distribuição e consumo (Sociedade Nacional de Agricultura, 2017).

As perdas e desperdícios de alimentos impactam diretamente os sistemas de produção em diversos cenários da cadeia alimentar, contribuindo para a redução da disponibilidade local e global de alimentos, perdas de renda para produtores, ascensão de preços para consumidores e gerando danos ao meio ambiente, devido ao uso insustentável dos recursos naturais (FAO, 2014).

O incentivo ao aproveitamento de partes comestíveis não convencionais de vegetais na alimentação e como recursos para se extraírem nutrientes e compostos bioativos é uma alternativa viável e rentável para interferir neste panorama, uma vez que os recursos vegetais apresentam ampla disponibilidade e são ricas fontes de diversos compostos de interesse. De acordo com Badawi (2011), utilizar o alimento em sua totalidade significa mais do que economia. Significa usar os recursos disponíveis sem desperdício, reciclar, respeitar a natureza e alimentar-se bem, com prazer e dignidade.

As folhas representam uma rica fonte de proteínas que em grande parte é inexplorada. As proteínas foliares representam uma fonte potencial de uso humano por causa de seu valor nutritivo e seus altos rendimentos por hectare (PIRIE, 1972). Além disso, folhas de diversos vegetais podem ser fontes potenciais de compostos bioativos, tais como compostos fenólicos (RANA et al., 2016).

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CAPÍTULO I: Functional properties and technological applications of vegetables leaves

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Functional properties and technological applications of vegetables leaves

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Abstract
Recently, there has been a global trend for natural sources of phytochemicals as antioxidants and functional ingredients. Phenolic compounds is a large group of secondary metabolites and the presence of these compounds in several vegetables leaves has been related to sensory and health properties. Considering that most part of the vegetables leaves are discarded although they present high nutritional value, this literature review aimed to group the most recent studies, which have evaluated the therapeutic and functional aspects and the technological applications of leaves. Moreover, the role of phenolic compounds in the functional properties and on the sensory characteristics are also discussed. Studies evaluating the main technologies in the reuse of vegetables leaves are presented as well. Finally, it was discussed the main challenges of the use of the leaves in the food industry. The search for new applications of byproducts is an alternative to valorize these materials, in order to provide new opportunities of use for the food and pharmaceutical industry.

Keywords: bioactive compounds, by-products, leaves, technological applications, toxicological aspects of leaves

1. Introduction

Phenolic compounds is a large group of bioactive chemicals of different biological functions. These compounds are secondary metabolite of plants, and comprise a wide variety of molecules that have a polyphenol structure (i.e. several hydroxyl groups on aromatic rings) (BALASUNDRAN; SUNDRAM; SAMMAN, 2006; VOGT, 2010).

Vegetables leaves have been recognized as potential sources of phenolic compounds, with high bioavailability, compared to many marketable leafy vegetables, such as brassicas and lettuce (CARVALHO et al., 2010). These compounds present antioxidant properties, acting as reducing or metal chelating agents, hydrogen donors and singlet oxygen quenchers. For this reason, they are related to healthy diets and to the prevention of several chronic diseases (TSAO; YANG, 2003).

Leaves are considered alternative sources of vegetables due to their resistance, tolerance and production yield (NKONGHO et al., 2014; XU et al., 2010). Moreover, they are used in order to increase the nutritional value and help in the malnutrition treatment (LATIF; MULLER, 2015). In addition to being used dehydrated in forms of teas (dried) (AYBAR et al., 2001) leaves have also been used as flours (dried and powdered) (MADRONA et al., 2011), composing bakery products (GAWLIK-DZIKI et al., 2015), beverages (RANA et al., 2016), and even as matrices for bioactive compounds extraction (RANA et al., 2016; ROMERO-GARCIA et al., 2016). The phytochemicals isolated from leaves have been used in

pharmaceutical industry and also as natural additives to preserve and / or enrich food products at food industry (NOWAK et al., 2016).

Therefore, the knowledge on several phenolic compounds and other nutrients may contribute to the development of functional foods or pharmaceutical products using leaves from different oleraceous as natural ingredients (SWIECA, 2014). Functional properties of these materials can be evaluated by the determination of some parameters such as antioxidant capacity, phenolic compounds contents, and antihypertensive and antihyperglycemic activities (GOGNA; HAMID; DORAI, 2015; JAISWAL et al., 2009; MATSUO et al., 2010). This work conducts an overview about the main phenolic compounds present in vegetables leaves, and discusses the healthy promoting and technological properties of these compounds in leaves. For this purpose, the main therapeutic effects, functional properties and the technological feasibility are discussed, as well as the challenges for the use of leaves in the food industry and in the human diet.

2. Phenolic compounds in vegetables

The consumption of leaves have been showed as an alternative for combating malnutrition in the human diet and in the animal feed (FERRARI; LEONEL; MISCHAN, 2014). Furthermore, these materials are sustainable sources of bioactive compounds (i.e. polyphenolic compounds) that could be used for different applications such as food additives, functional ingredients and nutraceuticals (MARTIN et al., 2013).

Polyphenolic compounds are a large group of secondary metabolites widely distributed in plants, divided into two major subgroups: flavonoids and phenolic acids (PATEL, 2008). Flavonoids (Figure 1) comprise a group of over 4000 aromatic plant compounds, in which anthocyanins, proanthocyanidins, flavonols and catechins are the major ones (Figure 2). They are low molecular weight compounds, which chemical structure consists of two aromatic rings, called ring A and B, joined by three carbons, forming a heterocyclic ring, called ring C (MERKEN; BEECHER, 2000).

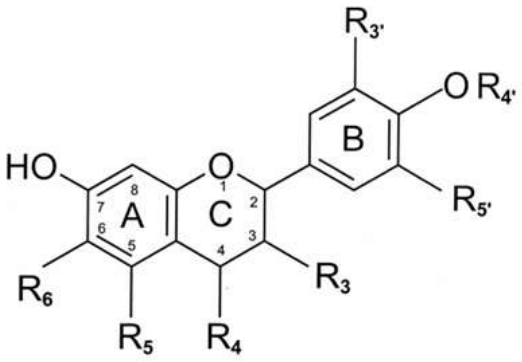

Figure 1. Basic structure of flavonoids (PATEL, 2008).

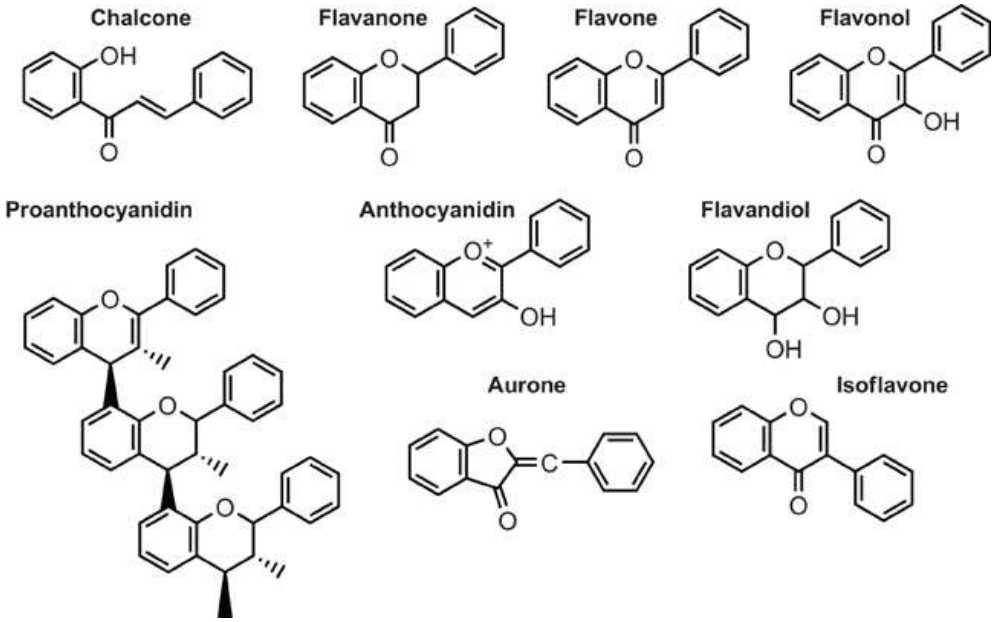

Figure 2. Structure of the main flavonoids (FERREYRA; RIUS, CASATI, 2012).

Phenolic acids (Figure 3) are characterized by a benzene ring, a carboxylic grouping and one or more groups of hydroxyl and/ or methoxyl in the molecule, responsible for antioxidant capacity in vegetables (SOARES, 2002); and include hydroxycinnamic acids (e.g. caffeic or ferulic acid conjugates, sinapic acid) and hydroxybenzoic acids (e.g. benzoic, gentisic or p-anisic acids) (CARVALHO et al., 2010; LARBAT et al., 2014; LI et al., 2007). These compounds are important in pharmaceutical and food industries and provide health benefits for human, acting as antioxidants in vegetables (EL GHARRAS, 2009; FU et al., 2016). This activity is associated with high adsorption and neutralization ability of free radicals, which extinguishes singlet and triplet oxygen, or peroxides in decomposition (UTHURRY; GÓMEZ-CORDOVÉZ, 2015).

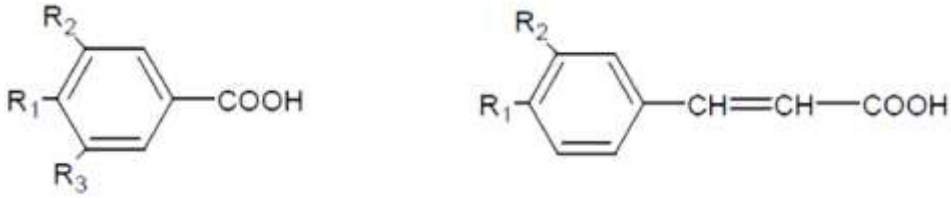

Figure 3. Basic structures of phenolic acids (BRAVO; COPAJA; LAMBOROT, 2013).

Furthermore, phenolics are important and decisive for sensory and nutritional quality of fruit, vegetables and other plants (QUIDEAU et al., 2011), and may also affect positively or negatively the characteristics of food with impacts on colour, flavour and astringency (OLIVEIRA; CARVALHO; MELO, 2014). For example, polyphenolic compounds (mainly tannins) form complexes with salivary proteins, and this interaction plays an important role in the sensation of astringency, due to delubrication of oral surfaces (CONDELLI et al., 2006; EL GHARRAS, 2009). This interaction may form a layer acting as a water barrier and produces a mouth-drying sensation (FONTOIN et al., 2008).

Particular structures including pigments (yellow, orange, red and blue), as anthocyanins, yellow flavanols and flavones are involved in food flavour (EL GHARRAS, 2009). In food processing, the conversion of anthocyanins to other compounds may decrease the stabilization

of colour and increases their variety. Besides these characteristics, there are volatile polyphenols, as vanillin and eugenol, which provide strong odour to foods and are related to bitterness and adstringency (EL GHARRAS, 2009).

3. Different vegetables leaves and their functional properties and therapeutic effects

Several studies suggest therapeutic and functional effects of leaves from fruits and vegetables (Table 1). The health promoting properties of leaves are related to the presence of phenolic compounds, which have called attention due to their antioxidant properties (OLIVEIRA; CARVALHO; MELO, 2014). Epidemiological studies have revealed that these compounds provide a significant protection against the development of several oxidative stress associated to cardiovascular diseases, cancer, diabetes, infections, aging and asthma. Their antioxidant function is due to different mechanisms of action including inhibition of free radical formation, inhibition of free radical chain reaction, chelating free radical producing metal ions and reducing the localized O^2 concentration by quenching O^{2-} radicals (BEN AHMED et al., 2017; NAWAZ; SHAD; RAUF, 2017).

Table 1. Studies on functional properties and therapeutic effects of leaves.

Material	Product	Concentration or contente	Functional properties and therapeutic effects	Authors
Smallantus sonchifolius (yacon) leaves	Aqueous, leaf rinse and polar extracts	0.25 - 1.00 $\mu g \cdot ml^{-1}$	*In vitro* anti-inflammatory activity	OLIVEIRA et al. (2014)
Ficus glumosa leaves	Aqueous extracts of fresh samples	225.3 - 375.0 $mg \cdot kg^{-1}$	Hypolipidemic and anti-atherosclerotic effects, wich could explain the use of the plant by traditional healers in the treatment of hypertension, cardiovascular diseases and diabetes.	NTCHAPDA et al. (2015)
Ribes nigrum L. (blackurrant) leaves	Extracts of fresh samples	0.05 - 0.50 $\mu g \cdot ml^{-1}$	Protection of the organism against oxidative stress, preventing the development of dangerous diseases.	CYBORAN et al. (2013)
Punica granatum (pomegranate) leaves	Extracts of four different solvents	19.50 - 669.35 $\mu g \cdot ml^{-1}$	Antioxidant activities, related to anti-parasitic and antimicrobial effects.	WANG et al. (2013)
Psidium guajava L. (guava) leaves	Extracts of dried samples	100 $\mu g \cdot ml^{-1}$ 30 $\mu g \cdot ml^{-1}$	Antioxidant mechanisms of extracts attributed to their free radical-scavenging ability. Decrease in prostraglandin E_2 (PGE_2) production in LPS-induced RAW264.7, providing a crude extract with high anti-inflammatory activity.	CHEN; YEN (2007) JANG et al. (2014)
Moringa oleifera (moringa) leaves	Extracts of dried samples	600 $mg \cdot kg^{-1}$ daily for 12 weeks	Amelioration of genes expression., paralleled by a reduction in body weight and improvement of the atherogenic and coronary artery index, as well as glucose level and insulin resistance of obese rats.	METWALLY et al. (2017) VONGSAK et al. (2013)
Chenopodium quinoa (quinoa) leaves	Different aqueous extracts obtained by squeezing, decoction of fresh and dried leaves, maceration of fresh and dried leaves, percolation of dried material and Soxhlet extraction of dried leaves.	100 $\mu g \cdot ml^{-1}$	Significative reduction of intracellular reactive oxygen species (ROS), which means that these leaves present high antioxidant capacity.	GAWLIK-DZIKI et al. (2013)

Sweet potato leaves have been reported to have high content of total phenolic compounds, compared to major commercial leafy vegetables (CARVALHO et al., 2010) (Table 2), with emphasis on derivatives of caffeoylquinic (XU et al., 2010) and chlorogenic acids (LI et al., 2007) (Figure 4). Yacon leaves have been related to several phytochemical activities, such as anti-microbial and anti-inflammatory actions (OLIVEIRA et al., 2013) and are associated with considerable content of phenolic compounds, among which caffeic acid and gallic acid (Figure 4) stand out as major phenolics (ANDRADE et al., 2014).

Table 2. Content of phenolic compounds in vegetables leaves.

Leaves	Compounds	Content [g·kg^{-1}]	Authors
Ipomoea batatas (sweet potatoes)	Total phenolic compounds	49 - 250	CARVALHO et al. (2010)
Smallantus sonchifolius (yacon)	Total phenolic compounds	42	ANDRADE et al. (2014)
Eugenia uniflora (pitanga)	Total polyphenols	26.0 - 233.8	TAHA et al. (2015)
	Total flavonoids	15 – 153	
Prunus cerasus L. (cherries)	Total phenolic compounds	93.97	OSZMIANSKY; WOJDYO (2014)
	Polymeric procyanidins	41.80	
	Chlorogenic acid	18.96	
Vaccinium ashei (blueberries)	Total phenolic compounds	11.3	MATSUO et al. (2010)
Citrus sinensis L. and *Citrus aurantium* L. (oranges)	Total phenolic compounds	12.54 - 44.41	LAGHA-BENANROUCHE; MADANI (2013)
Punica granatum L. (pomegranate)	Total phenolic compounds	19.50 - 669.35	WANG et al. (2013)
Carica papaya L. (papaya)	Flavonoids	2.31 - 2.63	CANINI et al. (2007)
	Phenolic acids	2.81 - 3.18	
	Flavonoids	59.3 - 112.0	SAKANAKA; TACHIBANA; OKADA (2005)
Moringa oleifera (moringa)	Phenolics	80.3	MOYO et al. (2012)
	Flavonoids	170	
	Flavonols	75.4	
Olea europaea (olives)	Polyphenols	2.88	BRAHMI et al. (2013)
Brassica oleracea (cabbage)	Polyphenols	0.44 - 5.71	NILNAKARA; CHIEWCHAN; DEVAHASTIN (2009)
Schinus terebinthifolius (spices)	Phenolics	69.6 - 221.6	ULIANA et al. (2016)
	Flavonoids	69.7 - 243.1	

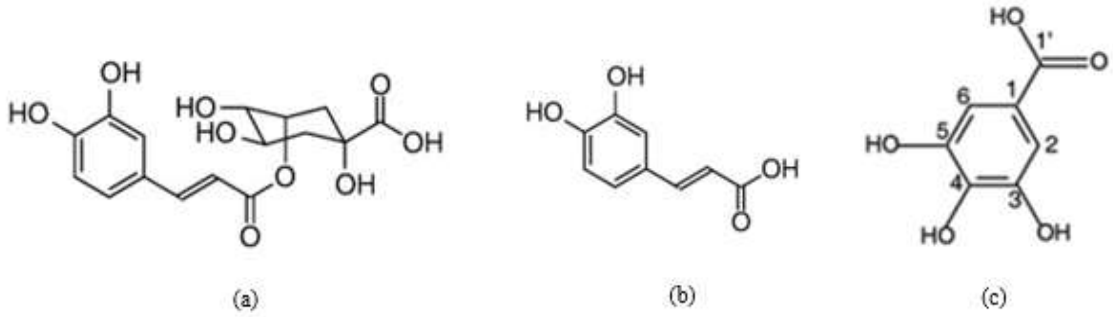

Figure 4. Chemical structures of some phenolic compounds found in foods.
(a) chlorogenic acid; (b) caffeic acid; (c) galic acid.
(SOUZA FILHO et al., 2006; TSUDA et al., 2012)

The therapeutic benefits attributed to pitanga leaves are related to the presence of total polyphenols and total flavonoids (GARMUS et al., 2014). Oszmiansky and Wojdylo (2014) have classified green and yellow leaves of cherries as phenolic sources, especially polymeric procyanidins and chlorogenic acid. Extracts of strawberry leaves showed to be dominated by gallic acid derivatives and flavonol derivatives, which seem to be quite promising as antioxidants and anti-proliferative agents (TAVARES et al., 2010). High phenolic compounds contents have been identified in leaves from blueberries (MATSUO et al., 2010), oranges (CANINI et al., 2007) and pomegranate (WANG et al., 2013). Papaya leaves are also known to contain a large number of biologically active metabolites (GOGNA; HAMID; DORAI, 2015) such as flavonoids and phenolic acids (CANINI et al., 2007). Rana et al. (2016) reported that phenolics from apple leaves can be used for the treatment of inflammatory diseases and pathogenic infections. Persimmon leaves present quercetin, kaempferol and their glycosides as the main flavonoids (ZHANG et al., 2016b), and have been traditionally used for increasing cognitive function associated with the regulation of the antioxidative defense system (HUANG et al., 2016), and for preventing chronic diseases (SAKANAKA; TACHIBANA; OKADA, 2005). Quercetin have been showed as an important polyphenol in guava leaves (CHEN; YEN, 2007; LAILY et al., 2015; WANG et al., 2016), and are related to antioxidant, anti-bacterial (RANILLA et al., 2010) and antifungal activities (MORAIS-BRAGA et al., 2017).

Moringa oleifera leaves have been considered as excellent sources of natural antioxidants due to the presence of several kinds of polyphenolic contents (TAHA, 2015) such as phenolics, flavonoids and flavonols (MOYO et al., 2012). Phenolic compounds such as chlorogenic acid, isoquercetin and astragalin are identified as majority phytochemicals, and at the concentration of 100 $\mu g \cdot ml^{-1}$, aqueous and alcoholic extracts of *Moringa oleifera* leaves could significantly reduce relative amount of intracellular reactive oxygen species assay (VONGSAK; SITHISARN; GRITSANAPAN, 2013). Leaves of moringa have been also related to antifungal properties (MAQSOOD et al., 2017). Four different polar extracts of soybean leaves have been also reported notably due to the presence of seven phenolic compounds which coumestrol was related as the major one and correlated to α-glucosidase inhibitory properties (YUK et al., 2011). Leaves from olives (BRAHMI et al., 2013) and cabbage (NILNAKARA; CHIEWCHAN; DEVAHASTIN, 2009) have been reported as antioxidant and polyphenols potential. Some studies have also revealed the significant potential of eucalyptus leaves as a source of phenolics and antioxidants and as an antifungal agent (BHUYAN et al., 2017).

Moreover, leaves from several vegetable crops provide other important nutrients for human health. For example, leaves of sweet potatoes contain relevant levels of dietary fibre,

carotenoids, vitamins and minerals (HUANG et al., 2013). Furthermore, they are important in the prevention of hypertension and atherosclerosis prevention (SUN et al., 2015).

In general, the study of polyphenols from plant materials can be an alternative of underutilized vegetables (YANG; LIN; KUO, 2008). These compounds stand out due to different functions (ZHANG et al., 2016). Several pharmaceutical studies report that polyphenols from sweet potato leaves present health- promoting biological activities (FU et al., 2016) such as anti-carcinogenesis (determined by growth suppression of cancer cells by caffeoylquinic acids), anti-atherogenic and cardiovascular (related to antioxidant mechanisms) and anti-microbial (KURATA et al., 2007; TAIRA et al., 2013).

Spices leaves have been also related to several functional properties. Uliana et al. (2016) described Brazilian rose pepper leaves as holders of anti-bacterial substances, as phenolics, according to solvent extraction.

In addition to all related functional properties of leaves, recent studies have been conducted in order to evaluate the antihyperglycemic and antihypertensive activity of leaves. These properties are justified by the abundance of phenolic compounds in leaves, in general. (CARVALHO et al., 2010). These substances exhibit an expressive association with the inhibition mechanisms of enzymes responsible for increasing of glucose contents and blood pressure by mechanisms still poorly understood (MAZZA et al., 2007).

Several authors, such as Altan (2003), have reported that diabetes mellitus is a chronic metabolic disorder that is characterized by hyperglycemia, which is resulted from the deficiency in the insulin production by the pancreas or its action.

Despite the effectiveness of various substances used for diabetes control, there is a growing demand to promote the use of natural products to the management of this disorder and its complications (VISNAGRI et al., 2014). Recently, it has been showed that polyphenols have potential to contribute in the management of type 2 diabetes (McCUE; SHETTY, 2004), because they are responsible for the α-amilase and α-glucosidase inhibition (APOSTOLIDIS; KWON; SHETTY, 2007; ZHANG et al., 2016), reducing the postprandial increase of blood glucose, and this way can be an important strategy in the blood glucose control (CHEN; KANG, 2013). However, according to Kwon et al. (2006), natural inhibitors of these enzymes from plants show lower inhibitory effects in α-amylase activity when compared to α-glucosidase. These authors suggest that this potentiality could represent an effective therapy for postprandial hyperglycemia. Quercetin and its derivatives can decrease post-prandial hyperglycemia by limiting glucose absorption (WANG et al., 2016).

The doses evaluated in the following studies are shown in Table 3. Jaiswal et al. (2009) showed that leaf extracts from *Moringa oleifera* reduced hyperglycemia in severe diabetic rats after 21 days of treatment. Baroni et al. (2016) reported that leaf yacon extracts decreased hyperglycemia by improving insulin sensibility through its phenolic compounds in diabetic rats. Ntchapda et al. (2015) evaluated aqueous extracts from fig leaves, suggesting that they are effective in the treatment of diabetes. A study conducted by Al-Attar and Zari (2010) concluded that crude extracts of *Camellia sinensis* leaves reduced the levels of serum glucose in diabetic mices. Pomegranate leaves have been used in Ayurvedic medicines for the treatment of diabetes (PATEL, 2008). Persimmon leaves have been showed a significative decrease in insulin resistance index (DENG et al., 2011).

Table 3. Effects on the hyperglycemia and hypertension of extracts from vegetables leaves with their respective doses associated to phenolic compounds contents.

Leaves	Doses evaluated	Effects	Authors
Moringa oleífera (moringa)	200 mg·kg^{-1}	Hyperglycemia control	JAISWAL et al. (2009)
Smallantus sonchifolius (yacon)	400 mg·kg^{-1}, a day	Hyperglycemia decreasing	BARONI et al. (2016)
Ficus glumosa (fig)	225.40, 300.0 and 375.0 mg·kg^{-1}	Effective in the diabetes control	NTCHAPDA et al. (2015)
Camellia sinensis (indian tea)	0.5 ml, a day	Serum glucose decreasing	AL-ATTAR; ZARI (2010)
Stevia rebaudiana (stevia)	50 µg·ml^{-1}	Antihypertensive activity	YILDIZ-OZTURK et al. (2015)
Punica granatum L. (pomegranate)	200 and 400 mg·kg^{-1}	Antihypertensive activity	MESTRY et al. (2017)
Hancornias peciosa (mangaba)	0.03, 0.10 and 1.00 mg·kg^{-1}	Antihypertensive activity	SILVA et al. (2016)
Olea europaea (olives)	12.5 - 25.0 mg·kg^{-1}, twice a day	Antihypertensive activity	SUSALIT et al. (2011)
Hibiscus sabdariffa L. (hibiscus)	< 5 g. kg^{-1}	Antihypertensive activity	AJAY et al. (2007)
Morus alba (mulberry)	50 and 100 mg·kg^{-1}	Antihypertensive activity	ONYENEKWE et al. (1999)

*kg of body weight.

Several studies have been related phenolic compounds presenting antihypertensive effects, since it has been demonstrated that type 2 diabetes and hypertension are metabolic disorders strongly related to each other (BAKRIS et al., 2000). The mechanism by which these compounds act consists in their ability to inhibit the action of angiotensin I-converting enzyme (ACE) *in vitro* and *in vivo* (KWON; VATTEM; SHETTY, 2006). The ACE converts angiotensin I to angiotensin II, that is a vasoconstrictor, and its inhibition could contribute to the treatment of high blood pressure, and can represent an alternative to decrease the consumption of synthetic ACE inhibitors (APOSTOLIDIS; KWON; SHETTY, 2007).

Leaves from stevia (YILDIZ-OZTURK et al., 2015), pomegranate (MESTRY et al., 2017), mangaba (SILVA et al., 2016), olive (SUSALIT et al., 2011), hibiscus (AJAY et al., 2007; ONYENEKWE et al., 1999; ZHEN et al., 2016), mulberry (ZHANG et al., 2009), *Moringa oleifera* (GOYAL et al., 2007; INGALE; GANDHI, 2016; MISHRA et al., 2011) and sweet potatoes (TRAVALINI et al., 2014) have been also reported by the scientific literature as phenolics holders (Table 3), which antioxidant capacity have been related to their antihypertensive activity.

4. Potentialities of vegetables leaves in food technology

Byproducts of many vegetables can be introduced in the daily diet and /or used in the food industries due to their high content of phenolic compounds and other nutrients (LAGHA-BENAMROUCHE; MADANI, 2013). There are several ways of using vegetable crops leaves; dried and used as teas (AYBAR et al., 2001); dried and powdered as flours; (MISHRA et al., 2011) or *in natura*.

Recent studies have reported the use of dried leaves as ingredients in the preparation of several processed food products (Table 4). These applications include the production of ingredients for bakery products such as quinoa leaves (SWIECA, 2014), cassava leaves (FERRARI; LEONEL; MISCHAN, 2014) carrot leaves (CASTILHO JUNIOR; OLIVEIRA, 2013; PEREIRA et al., 2014) and beet leaves (MISHRA et al., 2011). These studies demonstrated that the use of by-products provided increments in the antioxidant capacity and the levels of several nutrients such as fibres, proteins, minerals, carotenoids and phenolic compounds of processed products. Moreover, the addition of flour from carrots and beets leaves (6%), as carrot and beet leaves in the formulation of cookies provided good acceptability and favorable technological properties, with potential to be used in extrusion and bakery (MISHRA et al., 2011). In all applications, the leaves were used in the form of powder (dried and powdered).

Table 4. Studies on leaves flours, obtained by drying and powdering, and their application in food technology.

Leaves	Study	Doses of the product used	Technological and/ or sensory effects	Authors
Chenopodium quinoa (quinoa)	Breads formulation	3%	Valuable supplements to the development of bread with improved functional properties; the supplementation increased antioxidant potential of the product without compromising sensory quality.	GAWLIK-DZIKI et al. (2015)
Chenopodium quinoa (quinoa)	Bread fortification	1%	Fortification affected positively the antioxidant and phenolic contents, the quality and biological effects; interactions were identified between phenolics, proteins and starch, wich affected the antioxidant capacity, starch digestibility and functional properties of breads.	SWIECA et al. (2014)
Moringa oleifera (moringa)	Ice cream formulation	7-13%	The product presented high nutritional value and sensory viability.	MADRONA et al. (2011)
Manihot esculent (cassava)	Flour preparation	-	Source of proteins, vitamins C, β-carotene and minerals. The processing reduced the cyanide levels.	TROMBINI; LEONEL (2014)
Manihot esculent (cassava)	Preparation of snacks	10%	High levels of proteins and fibres; can be used as materials for extrusion process.	FERRARI et al. (2014)
Daucus carota L. (carrot)	Preparation of cookies	10%	Excellent sources of fibres and minerals; good acceptability.	CASTILHO JUNIOR; OLIVEIRA (2013)
Daucus carota L. (carrot)	Flour preparation for food products	30%	Alternative method for producing products in general, such as pasta, cakes and breads.	PEREIRA et al. (2014)
Beta vulgaris L. (beet)	Cookies preparation	6%	Source of proteins and fibres; satisfactory sensory properties.	TRAVALINI et al. (2014)

The addition of flour (1, 2, 3, 4 and 5%) from quinoa leaves to bread can decrease loaf volume and increase properties as hardness, cohesiveness and gumminess of this food product (SWIECA, 2014). The incorporation of green tea extracts in breads (1.5 $g \cdot kg^{-1}$ and 5.0 $g \cdot kg^{-1}$) showed changes in crumb appearance, texture properties and taste profiles of them (WANG; ZHOU; ISABELLE, 2007).

Phenolics and proteins may raise two types of interactions, covalent or non-covalent, which might lead to precipitation of proteins through multisite interactions and multidentate interactions (OZDAL; CAPANOGLU; ALTAY, 2013). The non-covalent binding of phenolic compounds has not presented effects on the secondary structure of proteins, but in the tertiary structure (RAWELL; MEIDTNER; KROLL, 2005), while covalent binding affects both secondary and tertiary structures (KROLL; RAWELL; ROHN, 2003). These characteristics can be responsible for changes in the solubility, thermal stability and digestibility of food proteins (HAN; KOH, 2011; RAWELL; MEIDTNER; KROLL, 2005).

The use of phenolics sources as natural additives in food has also been studied. Researches on polyphenols have shown that they can positively affect lipid oxidation, colour stability, and antioxidant activity in meat products (BASTIDA et al., 2009). Nowak et al. (2016) found that the addition of leaf extracts of cherry and blackurrant leaves (5 $mg \cdot kg^{-1}$ and 10 $mg \cdot kg^{-1}$, respectively) increased the shelf life of vacum-packed sausages.

5. Main technologies in the reuse of leaves by the food industry

The crude extracts of polyphenols from leaves contain chlorophyll, proteins, polysaccharides, and other impurities, which limits the application of leaf polyphenols. For this reason, an efficient purification method is required to obtain high purity polyphenols from leaves (XI; MU; SUN, 2015).

The extraction of polyphenolic compounds depends on the polarity of the solvent, method and time of extraction, which determine both the qualitative and the quantitative composition of these compounds. The polarities of phenolics vary significantly, and it is difficult to develop a specific method for optimal extraction of all phenolic compounds (RODRÍGUEZ-PÉREZ et al., 2015).

The methods of purification of plant polyphenols are made by extraction with organic solvents, separation membranes, and by supercritical extraction (DAI; MUMPER, 2010; FARÍAS-CAMPOMANES; ROSTAGNO; MEIRELES, 2013). However, these methods present some drawbacks, such as long extraction cycles and high costs, which make them unviable for industrial scale use. It may also be performed using solid-liquid or liquid-liquid extraction, followed by chromatography. These methods is not fully effective due to the extensive use of reagents, energy consumption and because it is laborious (FU et al., 2006).

Recently, macroporous adsorption resins have been used in the purification of bioactive constituents of natural extracts due to their high efficiency (LIU et al., 2010a; LIU et al., 2010b; LIU et al., 2010c). They have been used in the separation and purification of biologically active substances due to their physicochemical stability, high adsorption, and easy utilization (WAN et al., 2014). In addition, adsorption is an environmentally friendly technique, allowing the separation of selected compounds from diluted solutions, and it can be used to recover phenolic compounds from plants (SUARÉZ-QUIROZ et al., 2014; SUN et al., 2015).

Macroporous resins exhibit high adsorption capacity not only due to polarity similar to the target compounds, but also because of their large contact surface and average pore diameter (LIU et al., 2010a; LIU et al., 2010b; LIU et al., 2010c), and can selectively adsorb aqueous and non-aqueous system constituents through electrostatic force, hydrogen bonding and complexation interactions (GAO; HUANG; LIU, 2007).

On the other hand, acccording to Nantitanon et al. (2010), the use of ultrasound was the best method to extract phenolics from guava leaves, followed by Soxhlet extraction and

maceration, based on extraction efficiency. These authors also concluded that the maturation stage of the leaves are important factors, and should be considered.

A study conducted by Xi et al. 2015) concluded that the purification of polyphenols with AB-8 macroporous resin from sweet potato leaves was highly economically and environmentally efficient, with great potential for industrial production. In addition, the purified products had high antioxidant capacity (62 $\mu g \cdot ml^{-1}$ of ascorbic acid equivalent).

6. Challenges for the use of the leaves by the food industry

The use of leaves in human food means a viable alternative to valorize wastes (CANINI et al., 2007). However, there are relevant aspects that which have to be considered in the study of these food matrices.

Some leaves may contain potentially toxic compounds. The toxicity of cassava leaves, for example, restricts their use. This toxicity is related to the presence of cyanide and the technique of kneading and tearing the cassava leaves before drying them can decrease the hydrocyanic acid contents. Effectively, it has been observed that the drying process of cassava leaves is efficient to promote the reduction of hydrocyanic acid (TROMBINI; LEONEL, 2014). Antia et al. (2006) have found low levels of toxic compounds in sweet potato leaves, except for oxalate, whose content can be reduced through cooking.

The extraction and purification of the phytochemicals present in the leaves are also challenges for the food industry, since several extraction techniques can lead to decreasing of these compounds or even generate negative impacts to the environment and/or the consumers' health.

7. Conclusion

Several studies have reported that phenolic compounds found in vegetables leaves can be related to health properties, such as anti-inflammatory, antioxidant, anti-bacterial, antihypertensive and antihyperglicemic activities. These materials of high nutritional value can be incorporated in the human diet or used as ingredients for food products. Furthermore, the practice of full use of food is a strategy to promote sustainability, reducing wastes and improving the economy.

The obtainment of phenolic compounds from leaves is an alternative to add value to these by-products, with the purpose to provide new opportunities for industries to develop functional foods, cosmetics and pharmaceuticals products.

8. References

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CAPÍTULO II: Angiotensin I-converting enzyme (ACE), inhibitory potential, antioxidant capacity and phytochemicals contents from organic oleraceous leaves

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Angiotensin I-Converting Enzyme (ACE) inhibitory potential, antioxidant capacity and phytochemicals contents from organic oleraceous leaves

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Abstract
Antioxidant activity is used to study the potential health benefits of natural antioxidants found in several plants. The aim of this study was to evaluate the phenolic and chlorophyll contents, the antioxidant capacity (measured by DPPH and FRAP assays) and the anti-hypertensive activity (measured by Angiotensin I-Converting Enzyme- ACE inhibitory potential) of leaf extracts obtained from 9 organic oleraceous. Leaf extracts of sweet potatoes presented higher phenolic contents. Higher free radical scavenging (FRS) potentials were expressed by leaf extracts from *Rosinha* and *Capivara* sweet potato cultivars (62 and 63.3%, respectively). We demonstrated that sweet potatoes leaves presented higher total chlorophylls contents (*Beterraba, Cenoura, Capivara, Rosinha* and *Nusay* cultivars), and the amounts of chlorophyll "a" presented no differences ($p>0.05$) between cassava, sweet potatoes and arrowroot leaf extracts. In general, leaves of sweet potatoes presented the highest phenolics and chlorophylls contents, and provided higher antioxidant capacity. Leaf extracts of arrowroot and sweet potatoes *beterraba* and *cenoura* cultivars presented higher percentages of Angiotensin-Converting enzyme inhibition. The use of these materials in the human diet or as ingredients for food industry can be encouraged because they present high levels of antioxidants such as phenolics and can lead to the waste decreasing and promotion of sustainability.
Keywords: byproducts, antioxidants, phytochemicals, anti-hypertensive activity.

1. Introduction

Several oleraceous (for example: tubers or roots) are considered important sources of bioactive substances, including phenolic compounds (TIERNO et al., 2015). Oleraceous leaves have been also studied, and arouse interest because they present higher contents of flavonoids and phenolic acids, with high bioactivity, compared with those of major commercial leafy vegetables (CARVALHO et al., 2010).

Phenolic compounds are closely associated with strong antioxidant activity (MOUSSI et al., 2015). According to several studies, these compounds have present a significant contribution to the antioxidant activity of roots and tubers (GRACE et al., 2014; KITA et al., 2015; LIAO et al., 2013; SOUSA et al., 2015; TIERNO et al., 2015). Furthermore, many vegetables leaves have shown their functional properties related to the high anti-hyperglicemic and anti-hypertensive activities due to phenolic contents (GOGNA et al., 2015) which have been reported as responsible for the α-amilase and α-glucosidase inhibition, and can promote postprandial decrease of blood glucose (WANG et al., 2016). Moreover, these compounds can contribute to the inhibiting action of angiotensin I-converting enzyme (ACE), acting as a vasoconstrictor and, thus, represents an alternative to the treatment of high blood pressure (KWON et al., 2006).

Moreover, leaves contain many nutrients including protein, dietary fiber, carotenoids, vitamins and minerals. In addition, leaves could be harvested several times a year and their annual yield is much higher than vegetables (HUANG et al., 2013).

Numerous neglected and underutilized species offer the potential to diversify not only the human diet, but also the increase of food production levels, and, thus, enable more sustainable and resilient agro- and horti-food systems (THOMPSON et al., 2007). Moreover, their use in fortified products could avoid the waste of these materials, which commonly are used for animal feed or discarded (HUE; BOYCE, 2012; XI; MU; SUN, 2015).

However, studies that report the phytochemical composition and antioxidant capacity of leaves obtained from organic vegetables are scarce and focused on traditional species from conventional farming, and there is not any study available on leaves of sweet potato (*Cenoura, Beterraba, Capivara, Nusay* and *Rosinha*), cassava (*IAC, Cachoeira* and *Saracura*) or arrowroot. Furthermore, there are no studies on the ACE inhibitory activity of organic oleraceous leaves. Thus, the aim of this study is at evaluating the phenolic and chlorophyll contents, the antioxidant capacity and the Angiotensin I-Converting Enzyme (ACE) inhibitory potential of leaves obtained from different cultivars of organic oleraceous (sweet potato, cassava and arrowroot).

2. Materials and methods

2.1. Chemical reagents

The compounds 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox), 2,4,6-tri(2-pyridyl)-S-triazine (TPTZ), 1,1-diphenyl-2-picrylhydrazyl (DPPH), Folin–Ciocalteu's phenol reagent and Angiotensin-Converting Enzyme (ACE) were purchased from Sigma–Aldrich (St. Louis, MO, USA).

2.2. Materials

Leaf extracts from sweet potatoes (*Ipomoea batatas)* cultivar *Rosinha de Verdan* (LESR), *Cenoura* (LESCe), *Beterraba* (LESB), *Capivara* (LESCa) and *Nusay* (LESN), cassava (*Manihot esculenta*) cultivar *IAC* (LECI), *Cachoeira* (LECC) and *Saracura* (LECS) and arrowroot (*Maranta arundinacea*) (LEA) were analyzed. The 9 different cultivars of oleraceous were obtained from organic production systems at the Integrated Agroecological Production System (IAPS), located at Seropédica, Rio de Janeiro, Brazil . The samples were grown in 2015 (from March to June) under the same agricultural conditions and the leaves were collected at the mature stage. The leaves were washed in tap water, sanitized into a 200 ppm solution of sodium hypochlorite for 15 minutes and blotted with absorbent paper, freeze-dried (48 h) and kept at -4 °C until the analysis were realized.

2.3. Methods

2.3.1. Extraction for antioxidants and ACE inhibition assays

The extractions were performed according to Brand-Williams and Berset (1995). Due to the complex nature of phytochemicals, two or more methods should always be used in order to evaluate the total antioxidant capacity of plant extracts (16). For this reason, two complementary assays were performed, including free radical scavenging activity (FRS) and ferric reducing-antioxidant power (FRAP).

2.3.2. DPPH assay

DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging activity was determined by the method proposed by Brand-Williams and Berset (1995) with modifications as follows: 150 µL of extract reacted with 2.85 mL of the DPPH* solution (0.06 mM) for 1 h in the dark. Then

the absorbance was taken at 517 nm and the antioxidant capacity was expressed as μM of Trolox equivalent per gram of dry weight (μM TE g^{-1}) and as free radical scavenging (%FRS).

2.3.3. Ferric-reducing antioxidant power (FRAP) assay

The FRAP assay was performed based on the procedure described by Thaipong et al. (2006) with changes, as follows: 90 μL of extract was diluted in distilled water (270 μL) and allowed to react with 2.7 mL of FRAP, wich were mixture by vortex and held in a water bath at 37ºC for 30 minutes in absence of light The absorbance was taken at 595 nm. The standard curve was linear between 100 and 1000 μM of Trolox equivalent. Results were expressed in μM of Trolox equivalent per gram of dry weight (μM TE g^{-1}).

2.3.4. Determination of total phenolic content (TPC)

The Folin-Ciocalteu method, by means of which a crude estimate of the amount of phenolic compounds present in an extract can be obtained (18) followed a methodology proposed by Swain and Hillis (1959) with slightly modifications, as follows: 1 mL of extract was mixed with 10 mL of distilled water and 1 mL of aqueous solution of Folin–Ciocalteu reagent (0.25 N), and after 3 min, the mixture was added to 1.5 mL of an aqueous solution (10% w/w Na_2CO_3). After that, the reaction was taken at room temperature (25±1ºC) for 2 hours in the dark. The absorbance was recorded at 725 nm, and the results were expressed as mg of gallic acid equivalents (GAE) per gram of dry weight (mg GAE g^{-1}) using a gallic acid (0.01 to 0.05 mg mL^{-1}) standard curve.

2.3.5. Chlorophylls determination

Chlorophylls contents were determined using Lichtenthaler (1987) methodology, with changes, as follows: 1 g of a sample was added to 10 mL of a solution of 80% acetone (v / v). The mixture was centrifuged at 4000 g for 10 min and the supernatant transferred to a volumetric flask of 25 mL, completing the volume with the acetone solution at 80% (v / v). Then, readings were taking in a spectrophotometer at the wavelengths of 647 nm and 663 nm using 80% acetone (v/v) as a blank. The results were expressed in mg 100 g^{-1}.

2.3.6. ACE inhibition assay

The Angiotensin-Converting Enzyme (ACE) inhibitory property was evaluated to measure the anti-hypertensive activity and was performed according to Cushman and Cheung (1971) using hippuryl-histidyl-leucine (HHL) as substrate. The percentage of inhibitions of different leaf extracts were compared with the percentage of inhibition value of the standard cardioprotective drug lisinopril.

2.3.7. Statistical analysis

Statistically differences were performed by analysis of variance (ANOVA) and Tukey test where differences were detected. The significance of 5% was used when necessary.

Principal Component Analyses were used to associate variables among then and with the samples. PCA was performed using a correlation matrix after standardization, the PCA result is a map, it becomes easier the exploratory and visual relations among variables-variables and variables-samples.

Hierarchical cluster analysis (HCA) was made with the aim of classify samples according their similarities or dissimilarities, the HCA was done after standardization and using Euclidean distances to group the variables and the Ward method as the join method.

The statistical analyses were performed using R 3.2.4 (2016) Software developed by Core Team (2011) R, and FactoMineR package 1.32.

3. Results and discussion

3.1. Antioxidant capacity (AC)

Table 1 shows DPPH radicals scavenging capacity and ferric-reducing antioxidant power (FRAP). LESR and LESCa presented the highest %FRS, and were able to scavenge 62 and 63.3% of the DPPH free radicals, respectively.

Table 1. Total polyphenol contents, free radical scavenging and antioxidant capacity in 9 cultivars of oleraceous leaves (n=3).

Leaves	**Cultivars**	**TPC (mg GAE 100g^{-1})**	**Antioxidant Capacity**		
			FRS (%)	DPPH (μM TE g^{-1})	FRAP (μM TE g^{-1})
Sweet potatoes	LESCe	54.50 ± 0.71^{b}	48.95 ± 0.68^{b}	39.10 ± 1.50^{e}	505.90 ± 1.27^{a}
	LESB	76.34 ± 1.33^{a}	30.20 ± 0.77^{e}	427.83 ± 1.29^{a}	$110.30 \pm 1.82^{b,c}$
	LESR	51.90 ± 0.66^{b}	62.02 ± 0.19^{a}	$45.31 \pm 1.76^{d,e}$	55.28 ± 0.55^{c}
	LESCa	53.92 ± 0.61^{b}	63.35 ± 0.61^{a}	6.61 ± 1.15^{f}	443.70 ± 1.90^{a}
	LESN	45.60 ± 0.58^{c}	2.20 ± 0.58^{f}	134.60 ± 1.21^{c}	184.60 ± 0.61^{b}
Cassava	LECS	4.71 ± 0.50^{f}	$45.22 \pm 0.64^{b,c}$	224.88 ± 4.40^{b}	$96.51 \pm 2.80^{b,c}$
	LECC	5.08 ± 0.31^{f}	$39.8 \pm 4.45^{b,c,d}$	237.10 ± 2.40^{b}	$143.65 \pm 0.56^{b,c}$
	LECI	6.09 ± 1.16^{f}	$41.87 \pm 2.43^{b,c,d}$	$49.90 \pm 3.60^{d,e}$	$89.78 \pm 2.54^{b,c}$
Arrowroot	LEA	25.53 ± 1.18^{d}	$39.62 \pm 1.18^{c,d}$	$58.20 \pm 2.54^{d,e}$	$117.30 \pm 3.21^{b,c}$

LESCe-leaf extracts from sweet potatoes cultivar *Cenoura*; LESB- leaf extracts from sweet potatoes cultivar *Beterraba*; LESR- leaf extracts from sweet potatoes cultivar *Rosinha de Verdan*; LESCa- leaf extracts from sweet potatoes cultivar *Capivara*; LESN- leaf extracts from sweet potatoes cultivar *Nusay*; LECS- leaf extracts from cassava cultivar *Saracura*; LECC- leaf extracts from cassava cultivar *Capivara*; LECI- leaf extracts from cassava cultivar *IAC*; LEA- leaf extracts from arrowroot. Values are determined by at least triplicate individual experiments. Means with diferente small letter superscripts indicate significantly differences ($p \leq 0.05$) for rows means.

The antioxidant capacity measured by DPPH assay and expressed in Trolox equivalent (TE) revealed that LESB showed the highest value (427.83 μM TE.g^{-1}), followed by LECS and LECC (224.8 and 237.1 μM TE.g^{-1}, respectively). On the other hand, LESCe and LESCa presented the lower antioxidant capacities (Table 1). These values are in agreement with Jeng et al. (2015), who reported antioxidant capacity in a range of 9.2 to 237.9 mg TE g^{-1} for sweet potatoes leaf extracts, except for LESB, which was higher than the other samples studied. Cassava leaves presented high antioxidant capacity according to Simão et al. (2013), who found values in a range of 155.9 to 680.2 μM TE g^{-1} (measured by ABTS assay). LESCa and LESCe showed higher antioxidant capacity determined by FRAP method, ranging from 443.7 and 505.9 μM TE g^{-1}, respectively. These results for FRAP are in agreement with Jeng et al. (2015) who reported values between 117.5 and 885.64 μM TE g^{-1} for sweet potatoes leaves.

The leaf extracts presented higher AC measured by FRAP, when compared to the values reported by Peñarrieta et al. (2011) who found AC values ranged from 1.9 to 8 μM TE g^{-1} for processed potatoes and Bontempo et al. (2013) who detected 42.5 μM TE g^{-1} for extracts from different potato varieties, also determined by FRAP assays.

3.2. Total phenolics contents (TPC)

Leaf extracts of sweet potatoes presented the highest amount of phenolics (45.59 to 76.34 mg GAE g^{-1}), followed by arrowroot leaves (25.5 mg GAE g^{-1}). Carvalho et al. (2010) evaluated different photoperiod exposures of sweet potatoes leaves and reported TPC in a range of 4.9 to 25 mg GAE g^{-1}. Zhang et al. (2015) found TPC between 48.6 and 569.4 mg GAE g^{-1} for leaf extracts from different sweet potatoes cultivars. Fidrianny et al. (2013) found TPC of 0-196.4 mg GAE g^{-1} for leaves extracts from 5 colored varieties of sweet potatoes tubers.

It is important to note that several leaves studied in the present study showed higher TPC compared to some oleraceous reported in the literature. Shekhar et al. (2015) found TPC of 1.49 and 1.58 mg GAE g^{-1} for orange and white sweet potatoes, respectively. Uarrota et al. (2014) reported that four cultivars of cassava presented TPC in a range from 44.9 to 70 μg GAE g^{-1}.

3.3. Chlorophylls contents

LEA, LESCe, LESCa and LESN presented the highest total chlorophylls contents, and cassava leaves showed the lower values. The results obtained for chlorophyll "a" were statistically similar for different cultivars of sweet potatoes, arrowroot and cassava leaves (Table 2). Lins et al. (2015) found 4.52, 4.17 and 0.18 mg per 100 g of fresh sample of total chlorophylls and chlorophyll "a" and "b", respectively, for lemongrass. Machado et al. (2014a) evaluated olive leaves, and reported values in a range of 12.5 to 46.07, 8.15 to 33.1 and 4.35 to 12.97 mg 100 g^{-1} for total chlorophylls and chlorophylls "a" and "b", respectively. Aquino et al. (2011) reported total chlorophylls contents between 0.61 and 1.19 mg 100 g^{-1} for broccoli. Eucalyptus leaves was evaluated by Machado et al. (2014b) the authors found values for total chlorophylls, chlorophyll "a" and "b" ranging from 28.26 to 35.5, 21.1 to 25.9, and 7.12 to 9.57 mg 100 g^{-1}, respectively.

Table 2. Total chlorophylls, chlorophylls "a" and "b" of 9 cultivars of oleraceous leaves (n=3).

Leaves	Cultivars	Chlorophylls (mg 100 g^{-1})		
		Chlorophyll "a"	Chlorophyll "b"	Total chlorophylls
Sweet potatoes	LESCe	91.97 ± 0.16^{a}	$115.15 \pm 3.53^{b,c}$	$207.12 \pm 3.37^{d,e}$
	LESB	84.14 ± 1.07^{a}	110.80 ± 1.50^{b}	$164.35 \pm 1.60^{b,c}$
	LESR	76.40 ± 3.40^{a}	52.67 ± 2.43^{a}	121.30 ± 1.68^{a}
	LESCa	85.21 ± 0.25^{a}	$123.3 \pm 1.24^{b,c}$	$208.50 \pm 1.74^{d,e}$
	LESN	83.34 ± 0.25^{a}	$136.90 \pm 0.43^{c,d}$	220.30 ± 0.68^{e}
Cassava	LECS	95.01 ± 0.45^{a}	70.70 ± 3.10^{a}	$164.50 \pm 4.26^{b,c}$
	LECC	95.90 ± 2.90^{a}	62.13 ± 0.17^{a}	$135.40 \pm 4.61^{a,b}$
	LECI	84.00 ± 2.60^{a}	150.20 ± 2.40^{d}	$184.90 \pm 3.70^{c,d}$
Arrowroot	LEA	95.71 ± 2.60^{a}	$141.90 \pm 0.36^{c,d}$	236.90 ± 0.68^{e}

LESCe-leaf extracts from sweet potatoes cultivar *Cenoura*; LESB- leaf extracts from sweet potatoes cultivar *Beterraba*; LESR- leaf extracts from sweet potatoes cultivar *Rosinha de Verdan*; LESCa- leaf extracts from sweet potatoes cultivar *Capivara*; LESN- leaf extracts from sweet potatoes cultivar *Nusay*; LECS- leaf extracts from cassava cultivar *Saracura*; LECC- leaf extracts from cassava cultivar *Capivara*; LECI- leaf extracts from cassava cultivar *IAC*; LEA- leaf extracts from arrowroot. . Values are determined by at least triplicate individual experiments. Means with diferente small letter superscripts indicate significantly differences ($p \leq 0.05$) for rows means.

Carvalho et al. (2010) reported averages of 0.09 to 0.24 mg 100 g^{-1} for chlorophyll "a" and 0.09 to 0.23 mg 100 g^{-1} for chlorophyll "b" contents for sweet potatoes leaves. These results are lower than the values found in the present study. This disagreement may be due to the fact that the studied samples were from organic cultivation (different production system), and also showed different cultivars.

3.4. ACE inhibition assay

There are no studies evaluating the antihypertensive activity of organic oleraceous leaves. According to Table 3, LESB and LEA presented higher percentages of inhibition (78.30 and 82.05%, respectively). Comparing the extracts of cassava leaves, LECI presented a higher percentage of inhibition (71.22%).

Table 3. Angiotensin I-Converting Enzyme (ACE) inhibitory activity (%) of 9 leaves from organic oleraceous assayed *in vitro.*

Leaves	Cultivars	Inhibitory activity (%)
Sweet potatoes	LESCe	75.12 ± 0.78^{b}
	LESB	$78.30 \pm 0.84^{a,b}$
	LESR	57.25 ± 0.77^{e}
	LESCa	38.85 ± 1.48^{g}
	LESN	63.45 ± 3.13^{d}
Cassava	LECS	44.85 ± 1.06^{f}
	LECC	57.38 ± 1.47^{e}
	LECI	71.22 ± 1.58^{c}
Arrowroot	LEA	82.05 ± 0.35^{a}

LESCe-leaf extracts from sweet potatoes cultivar *Cenoura*; LESB- leaf extracts from sweet potatoes cultivar *Beterraba*; LESR- leaf extracts from sweet potatoes cultivar *Rosinha de Verdan*; LESCa- leaf extracts from sweet potatoes cultivar *Capivara*; LESN- leaf extracts from sweet potatoes cultivar *Nusay*; LECS- leaf extracts from cassava cultivar *Saracura*; LECC- leaf extracts from cassava cultivar *Capivara*; LECI- leaf extracts from cassava cultivar *IAC*; LEA- leaf extracts from arrowroot. Values are determined by at least triplicate individual experiments. Means with diferente small letter superscripts indicate significantly differences ($p \leq 0.05$) for rows means.

Kwon et al. (2006) found ACE inhibitory activity of 90.5%, 81.9% and 37.4% for aqueous extracts of rosemary, lemon balm and oregano. Mamilla and Mishra (2017) reported % ACE inhibition of 29.41 to 67.21% for chickpea, 33.28 to 83.53% for soybean, 48.80 to 79.13% for red lentil, 25.33 to 82.34% for mung bean and 31.68 to 71.73% for kidney bean.

Studies on the pathology of hypertension have been demonstrated that Angiotensin-Converting Enzyme (ACE) plays an important role in the blood pressure control (WIJESEKARA; KIM, 2010). ACE is an important enzyme which converts angiotensin I into angiotensin II (an important vasoconstrictor) (SKEGGS; KAHN; SHUMWAY, 1956) and indirectly increases the blood pressure (ATLAS, 2007). The inhibition of ACE consists in an important therapeutic alternative in the treatment of high blood pressure (KWON et al., 2006). Thus, the control of hypertension via modulation of ACE by food matrices can represent an important strategy to manage this risk fator (APOSTOLIDIS; KWON; SHETTY, 2007). Previous studies have been related phenolic compounds to antihypertensive effects, and this

activity is associated to their ability to inhibit the action of ACE in vitro and in vivo (KWON et al., 2006).

3.5. Multivariate statistical analysis (PCA)

3.5.1. Principal component analysis (PCA)

PCA (Figure 1) allows associate similar variables and a simple map, thus became easy to interpret big data tables summarizing the information. Three principal components were necessary to explain 75.60% of the total variance of experiment (CP1=31.96%, CP2=24.07% and CP3=19.57%), this amount is consider reasonable to explain the experiment variance variable. PCA could confirm the samples similarities, e.g. LESCa and LESCe were characterized due both have the greatest values of FRAP (443.7 and 505.9 μM TE. g^{-1} respectively), this relation may be done because LESCa and LESCe are in the right top side of Figure 1b and FRAP is in the same side in Figure 1a. Other group could be formed by LEA and LESN (right side of Figure 1b), they were the samples with biggest amount of the total clhorophylls (right side of Figure 1c). On the other hand, LESB, LECC and LECS the highest values of DPPH (427, 237 and 224), the order coincide with the distances with DPPH when both Figure 1a and 1b are analyzing overlapping.

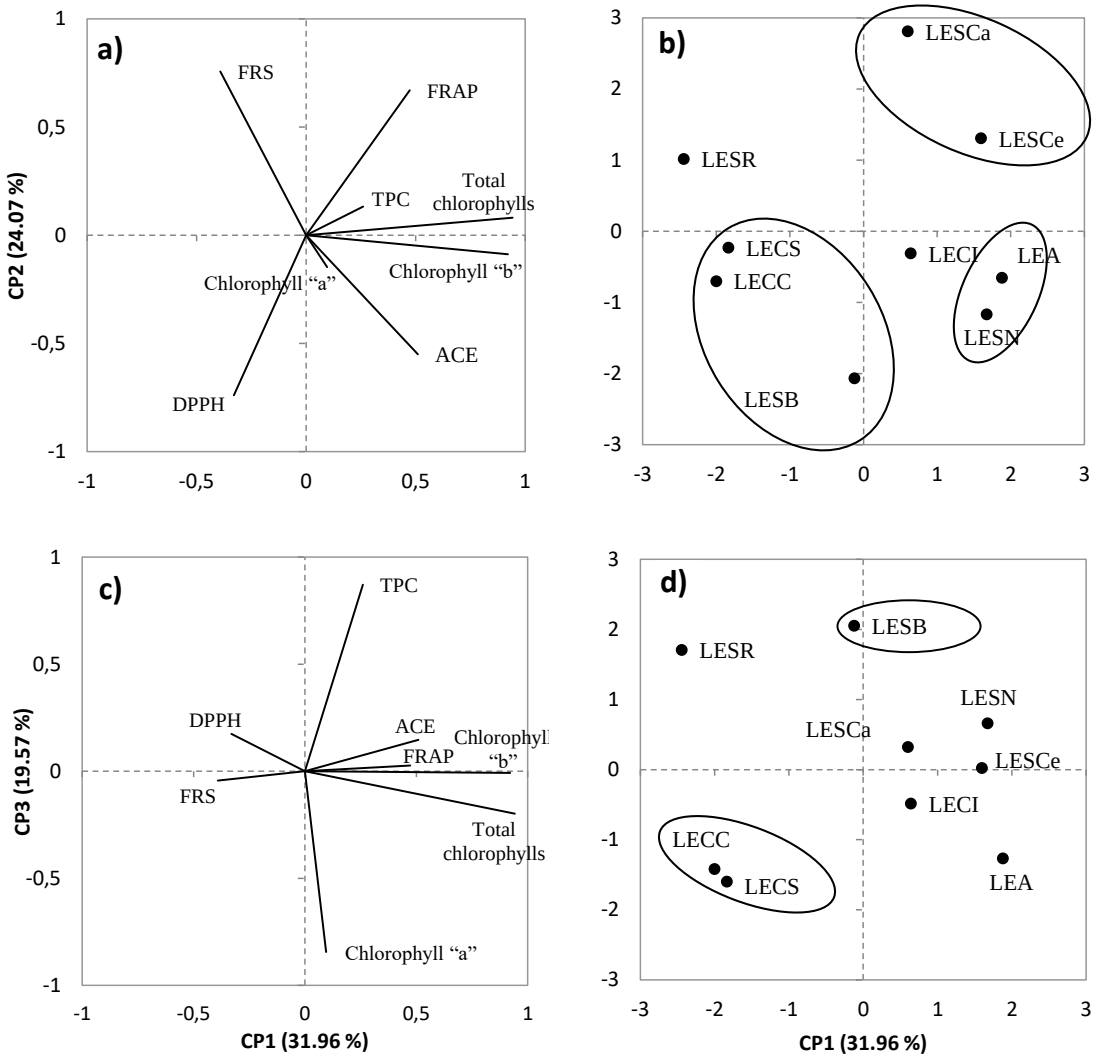

Figure 1. Principal component analysis making from 8 variables (antioxidant properties, chlorophyls and ACE) in the left side and 9 samples of leaves from different vegetables in the

right side. LESCe-leaf extracts from sweet potatoes cultivar *Cenoura*; LESB- leaf extracts from sweet potatoes cultivar *Beterraba*; LESR- leaf extracts from sweet potatoes cultivar *Rosinha de Verdan*; LESCa- leaf extracts from sweet potatoes cultivar *Capivara*; LESN- leaf extracts from sweet potatoes cultivar *Nusay*; LECS- leaf extracts from cassava cultivar *Saracura*; LECC- leaf extracts from cassava cultivar *Capivara*; LECI- leaf extracts from cassava cultivar *IAC*; LEA- leaf extracts from arrowroot.

The first three PC explain 75.60% of the total variability of the experiment. In the top side are the PC1 and PC2, whereas in the down side are the PC1 and PC3. In general, the antioxidant capacities evaluated by different methods demonstrated a similar trend. Sweet potatoes leaves presented the highest amounts of total phenolics and expressive antioxidant capacities. This suggest that is important to choose two or more methods to determine quantitative values for AC and to relate then to TPC.

The variables TPC and Chlrophyll "a" were not well represented by CP1 and CP2 due both vectors as small, additionally the sum of the variance from these two first components was 56.03%, thus a third component was necessary to a better global understanding of the experiment. In this way, LESB (Figure 1d) is nearly to TPC (Figure 1c) when both are observed overlapping, finally LECC and LECS had the lowers values of TPC because they are in diametrically opposite when Figures 1c and 1d are overlap observed.

3.5.2. Hierarchical cluster analysis (HCA)

The HCA (Figure 2) was made to form groups from the nine samples evaluating the multidimensional analysis of the eight evaluated variables. The HCA used the Euclidian distances and the ward method. The result allow join three group of leaves vegetables using in the present study. Three groups were formed as follows: LESCe and LESCa belong to the firsth goup, the second group was form by LESR, LESN, LECI and LEA, finally LESB, LECS and LECC were located in the third group.

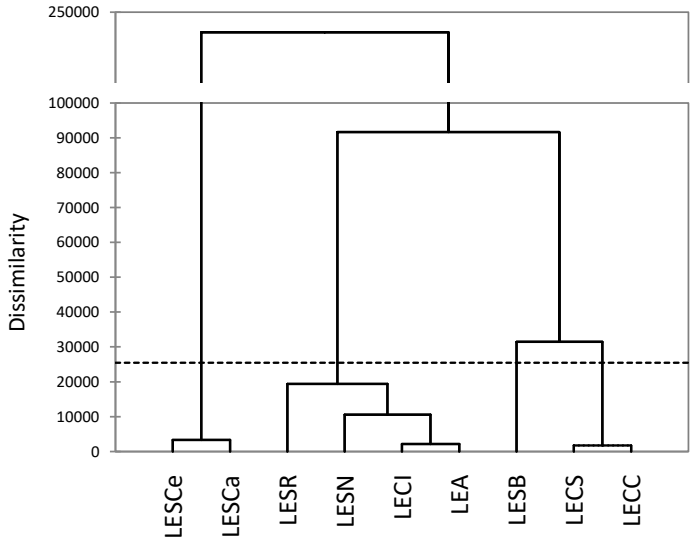

Figure 2. Hierarchical cluster analysis from the 9 samples and 8 variables using Euclidean distances and the Ward method. LESCe-leaf extracts from sweet potatoes cultivar *Cenoura*; LESB- leaf extracts from sweet potatoes cultivar *Beterraba*; LESR- leaf extracts from sweet potatoes cultivar *Rosinha de Verdan*; LESCa- leaf extracts from sweet potatoes cultivar *Capivara*; LESN- leaf extracts from sweet potatoes cultivar *Nusay*; LECS- leaf extracts from

cassava cultivar *Saracura*; LECC- leaf extracts from cassava cultivar *Capivara*; LECI- leaf extracts from cassava cultivar *IAC*; LEA- leaf extracts from arrowroot.

4. Conclusions

In general, sweet potatoes leaves presented the highest phenolic contents and antioxidant capacity, emphasizing LESCe and LESB, in contrast to cassava leaves that had lower TPC and AC, respectively. Nonetheless, the leaf extracts evaluated present higher TPC and AC when compared to their respective vegetables reported in literature. The chlorophylls contents were not related to antioxidant capacity of leaf extracts. However, sweet potatoes leaves presented higher amounts of these phytochemical.

The high phenolic and chlorophylls contents found in oleraceous leaves are considered an indicator that the use of these materials should be evaluated more carefully by researches, once they are sources of phenolics and present high antioxidant capacity. Moreover, there has been considerable interest in the potential for using natural food components as functional foods, which potential for ACE inhibition and consequent control of hypertension may be an important strategy for the use of these materials that are mostly discarded, even in substitution or to minimize the use of drugs.

For this reason, these leaves could be used to enrich food products or as ingredients in human diet.

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CAPÍTULO III: Adsorption/desorption characteristics and separation of phenolic compounds from organic purple-fleshed sweet potatoes (*Ipomoea batatas*) leaves usong microporous resins

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Adsorption and desorption characteristics and separation of phenolic compounds from organic purple-fleshed sweet potatoes (*Ipomoea batatas* L.) leaves using macroporous resins

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Abstract

In recent years, current trends have been noticed to study unedible parts of food as sources of phytochemicals or as ingredients in order to develop new uses for discarded materials and to promote sustainability. This study aimed to evaluate the performance of six macroporous resins in purification of phenolic compounds from organic purple-fleshed sweet potatoes. Static adsorption and desorption showed that XAD 16 and XAD 7HP had the highest adsorption and desorption ratio and recovery yield. Adsorption process was well fitted by pseudo second order kinetics. Furthermore, the isotherm adsorption study on XAD 16 and XAD 7HP showed that Freundlich model presented the best fit to experimental adsorption data. The process temperature did not affect the adsorption capacity of XAD 16. However, XAD 7HP showed higher adsorption capacity when performed at 30 °C, compared to 40 °C and 50 °C. Dynamic adsorption process on XAD 7HP resin in a glass column showed that phenolic compounds in the water extracts of purple-fleshed sweet potatoes leaves started to break through after 4.5 bed volumes of extract was loaded. A complete desorption was reached using 5 bed volumes of 95% ethanol. The purification of phytochemicals using adsorption is an alternative to replace the use of organic solvents and provide a safety process that presents a potential industrial application.

Keywords: phenolics, leaves, adsorption, desorption, resins.

1. Introduction

The interest on natural antioxidants for potential application as food antioxidants and dietary supplements keeps increasing in late years (LIN et al., 2012). Food ingredients from by-products of fruit and cereal processing might have great market potential opportunities when the consumption of 'ready-to-eat' products with health promoting properties is increasing (FAVA et al., 2013).

Recently, Mirabella et al. (2014) reported that 39% of food waste is produced by the food manufacturing industries in developed countries. They also reported that these wastes could be used as raw material for new products and applications.

Sweet potatoes leaves have been considered sources of flavonoids and phenolic acids, compared with those of major commercial leafy vegetables (CARVALHO et al., 2010). Owing to their high antioxidant capacity, polyphenols may have possible beneficial applications on human health, such as treatment and prevention of cancer, cardiovascular disease and other pathologies (IGNAT; VOLF; POPA, 2011; QUIDEAU et al., 2011).

At present, high purity of phenolics is greatly needed in the field of pharmacy and functional food. Conventional extraction methods such as maceration and Soxhlet have shown low efficiency and potential environmental pollution due to large volumes of organic solvents

and long extraction time required (DAI;MUMPER, 2010). The main organic solvents used are methanol, ethanol, acetone, ethyl acetate, and their combinations (XU; CHANG, 2007). Several methods have been developed such as microwave, ultrasound-assisted extractions, subcritical water extraction (SWE), supercritical fluid extraction (SFE), pressurized fluid extraction (PFE) or accelerated solvent extraction (ASE) (DAI; MUMPER, 2010). These methods have some disadvantages, such as long production cycles or high cost (FARÍAS-CAMPOMANES; ROSTAGNO; MEIRELES, 2013; FU et al., 2006).

Therefore, it is necessary to study simple and efficient environmentally friendly methods to extract polyphenols. With the trends of green chemistry, a new class of promising solvents to replace volatile organic solvents in samples preparation has emerged (WEI et al., 2015). Alternatively, adsorption/desorption is an environmental friendly technique allowing the separation of selected compounds from sample extract. It has been widely used for recovery of plant phenolic compounds (SUAREZ-QUIROZ et al., 2014). Activated carbon and macroporous resins (MARs) are the most widely used adsorbent of this technology (SUAREZ-QUIROZ et al., 2014). MARs can effectively separate active compounds (WU et al., 2015) and enable recovery of phytochemicals from different natural sources such as plant peels, seeds and leaves (D'ALESSANDRO et al., 2013). MARs are porous cross-linked polymer beads that have been developed as useful adsorbents. They are considered more applicable than common adsorbents due to their favorable physical and chemical stabilities, large surface areas, easy regeneration, long service life (CHEN et al., 2010), fast adsorption rate, strong adsorption capacity, and easy elution (DU et al., 2012; LIU et al., 2011), physicochemical stability, high adsorption selectivity and easy recycling (WAN et al., 2014).

The use of byproducts of plants for obtaining purified extracts of bioactive compounds using macroporous resins has been an alternative way in order to obtain high-valued food ingredients. Although there are some studies regarding the adsorption of phenolics compounds from oleraceous leaves, studies on the use of MARs for adsorption/desorption of polyphenols from organic sweet potato leaves are lacking. The aim of this study was to evaluate the adsorption/ desorption charateristics of resins to separate phenolic compounds from organic purple-fleshed sweet potatoes and to determine the optimal separation conditions of a selected resin. The effects of adsorption/ desorption processes on the phenolics contents were also examined.

2. Materials and methods

2.1. Chemicals

6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox), 2,4,6-tri(2-pyridyl)-S-triazine (TPTZ), 1,1-diphenyl-2-picrylhydrazyl (DPPH) and Folin–Ciocalteu's phenol reagent were purchased from Sigma–Aldrich (St. Louis, MO, USA).

2.2. Materials

Sweet potatoes were grown in 2015 (from March to June) under the same agricultural conditions in open fields in Integrated Research System in Agroecological Production-Agroecological Farm, Embrapa (Seropédica- Rio de Janeiro, Brazil). The organic purple-fleshed sweet potatoes leaves (PFSPL) were collected at mature stage of the commodities. After sampling, the leaves were washed, drained, frozen overnight (-18° C), dried in a freeze-dryer (Liotop L101, São Carlos, SP, Brazil) and grounded for subsequent extractions.

Amberlite resins (XAD 16, XAD 7HP, XAD 2, XAD 4, DAX 8 and XAD 1180N) were purchased from Sigma-Aldrich (St. Louis, Missouri, EUA).The chemical and physical properties are summarized in Table 1.

Table 1. Chemical and physical properties of resins.

Amberlite resins	Chemical matrix	Polarity	Surface área (m^2/ g)	Pore envelope (Ã)
XAD 16	Styrene-divinylbenzene	nonpolar	800	200
XAD 7HP	Acrylic ester	polar	500	450
XAD 2	Styrene-divinylbenzene	nonpolar	300	90
XAD 4	Styrene-divinylbenzene	nonpolar	750	100
XAD 1180N	Styrene-divinylbenzene	nonpolar	500	400
DAX 8	Acrylic ester	moderalety polar	160	225

2.3. Methods

2.3.1. Preparation of organic PFSPL extracts

Dried PFSPL (10 g) was weighted and mixed with 1 L of water. The mixture was put in a shaker (Nova Tecnica NT 715, Piracicaba, SP, Brazil) at room temperature for one hour. The mixture was decanted and filtered under vacuum through Whatmann No.5 filter paper. The filtered extracts were stored in refrigerator at 4 °C.

2.3.2. Pre-treatment of resins

The pre-treatment of resins were performed according to the procedures written by Buran et al. (2014). Five grams of resins were firstly soaked in water, and treated in ethanol (140 mL, 95%). Resins were then washed with water until the eluent was clear, and eluted with 140 mL of 4% HCl. After that, resins were washed with distilled water until neutral pH and then washed with 140 mL of 5% NaOH, followed by distilled water until a pH of 7.0. Lastly, all the resins were dried at 60 ˚C in an oven for 24 h to reach a constant weight for moisture determination [19].

2.3.3. Static adsorption and desorption tests

Pre-treated resins (1 g) and 25 mL of leaf extracts were added to a 125 mL Erlenmeyer flask. The adsorption process of each resin was tested at different pHs (3.0, 5.0, 7.0, 9.0 and 11.0). The pHs were adjusted using 0.01 N HCl and 0.01 N NaOH. After adjusting pH, the flasks were taken to a shaker at 100 rpm for 48 h at 30 °C. For static desorption test, the resins were filtered, washed with distilled water and added to 50 mL of ethanol (95%). The pH was adjusted to 3.0. The flasks were kept in a shaker at 100 rpm for 24 h at 50 °C. Adsorption and desorption ratios and capacities were determined according to the following equations:

Adsorption ratio: $A\ (\%) = \frac{(C_o - C_e)}{C_o}$ Equation 1

Adsorption capacity: $q_e = (C_o - Ce) \times \frac{(V_i)}{m}$ Equation 2

Where: A is the adsorption ratio (%). q_e is the adsorption capacity (mg/g dry resin) at equilibrium; C_o is the initial concentration of phenolics in the extracts (mg/L); C_e is the equilibrium concentration of phenolics in the extracts (mg/L); m is the initial weight of resin (g); V_0 is the volume of extract used (mL).

Desorption ratio: $D\% = C_d \frac{V_d}{(C_d - C_e)V_0} \times 100$ Equation 3

Desorption capacity: $q_d = C_d \times \frac{V_d}{m}$ Equation 4

% Recovery: $R = \frac{C_d V_d}{C_o V_o} \times 100\%$ Equation 5

Where: *D* is the desorption ratio (%); q_d is the desorption capacity (mg/ g dry resin); *R* is the recovery after complete desorption; C_d is the concentration of phenolics in the desorption solution (mg/L); V_d is the volume of the desorption solution (mL); C_o, C_e, *m* and V_0 are the same as those in Equations 1 and 2.

2.3.4. Adsorption kinetics test

XAD16 and XAD 7HP were selected for adsorption kinetics and adsorption isotherms. Pre-treated resins (1 g) and 25 mL of leaf extracts were added to a 125 mL erlenmeyer flask. The pH was adjusted as described in item 2.3.3. Flasks were taken to a shaker (100 rpm) at 30 °C for 48 h. An aliquot of 200 µL was taken out every 30 min for the first 6 hours, every 60 min from 6 to 12 hours, and then at 24 and 48 hours to measure total phenolics contents. Two kinetic models, pseudo first order and pseudo second order models were applied to fit the adsorption process.

Pseudo-first-order model: $\ln(q_e - q_t) = \ln q_e - K_f t$ Equation 6

Pseudo-second-order model: $\frac{t}{q_t} = \frac{1}{K_s q_e^2} + \frac{1}{q_e} t$ Equation 7

Where: K_f is the rate constant of the pseudo-first-order-model and *Ks* is the rate constant of the pseudo-second-order-model, q_t (µg.g^{-1}) is the amount of total phenolics adsorbed at time *t* and q_e is the adsorption capacity at equilibrium.

2.3.5. Adsorption isotherm tests

1 gram of pre-treated resin was added to 25 mL of leaf extracts of different concentrations of phenolic compounds (10, 40, 60, 80 and 100 µg.mL^{-1}) to a 125 mL Erlenmeyer flask and the pH was adjusted to 5.0 and 3.0 (XAD 16 and XAD 7HP, respectively). The adsorption took place at three temperatures (30, 40 and 50 ˚C) in a shaker at 100 rpm. Total phenolics in the solutions were quantified after 24 hours of adsorption. The equilibrium adsorption isotherms for phenolics were calculated using Langmuir, Freundlich and Henry equations:

Langmuir equation: $q_e = \frac{q_m K_L C_e}{1 + K_L C_e}$ Equation 8

Freundlich equation: $q_e = K_F C_e^{1/n}$ Equation 9

Henry equation: $q_e = K C_e$ Equation 10

Where: q_m is the maximum adsorption capacity (µg.g^{-1}); K_L is constant of Langmuir model; K_F is the Freundlich constant that indicates the adsorption capacity; 1/n is an empirical constant related to the adsorption affinity of the adsorbent for the absorbate; K is the constant of Henry model.

2.3.6. Dynamic adsorption and desorption tests

Resin XAD 7HP was loaded into a glass column (I.D. x L, 22 x 350 mm) in a bed volume of 20 mL. PFSPL water extract was loaded into this column at a flow rate of 1, 3 or 5 mL/ min by a peristaltic pump. The flow rates were choosen according to preliminary test results The adsorbate-laden column was washed with 5 bed volumes (BV) of distilled water. Phenolic compounds were desorbed using 95% ethanol at a flow rate of 5, 6 or 7 mL/ min. The eluent was collected and analyzed for total phenolic contents.

2.3.7. Determination of total phenolic content

Total phenolics content was measured according to Swain and Hillis (1959) with modifications, as follows: 1 mL of extract was mixed with 10 mL of distilled water and 1 mL of aqueous solution of Folin–Ciocalteu reagent (0.25 N). After 3 min, 1.5 mL of an aqueous solution (10% w/w Na_2CO_3) were added. The reaction occurred at room temperature (25±1ºC) for 2 hours in the dark. The absorbance was measured at 725 nm. A standard curve was performed using gallic acid. The results were expressed as milligram of gallic acid equivalents (GAE) per gram of dry weight (mg GAE. g^{-1}).

2.3.8. Statistical analysis

All analysis were performed in triplicate. Data were presented as means ± standard deviations. Significant differences between means ($p \leq 0.05$) were estimated by Student's t-tests using XLSTAT 7.5. Data analysis was performed using SAS® 9.2 and Origin® 8.0 data analysis software.

3. Results and discussion

3.1. Static adsorption and desorption

Adsorption of phenolic compounds on macroporous resins is a complicated process (WANG et al., 2017). Resins present different adsorption characteristics, which may be due to different adsorption and desorption mechanism (SUN et al., 2015).

Adsorption and desorption of phenolic compounds on resins in different pHs are presented in Figure 1. Amberlite resins XAD 16, XAD 1180N and XAD 2 had the highest adsorption capacity and adsorption ratio at pH 5.0, while the resins XAD 7HP, XAD 4 and DAX 8 showed better adsorption performance at pH 3.0. Comparing the six resins, XAD 7HP had the highest adsorption capacity (1705.6 µg. g^{-1}). XAD 2 presented the lowest adsorption capacity (357.3 µg. g^{-1}), even in best pH.

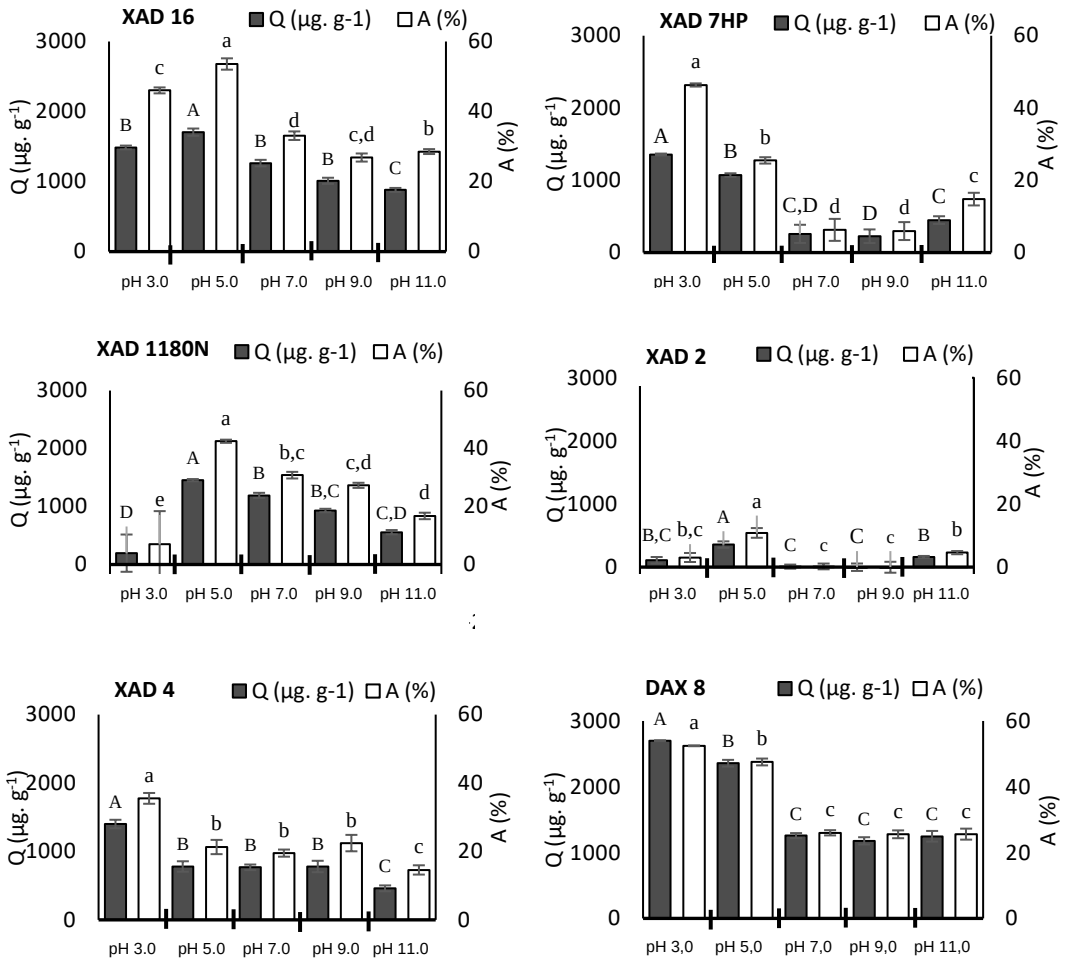


Figure 1. Static adsorption capacity (µg.g^{-1}) and adsorption ratio (%) of phenolic compounds from organic PFSPL using different macroporous resins. Results are mean of three determinations. Different upper-case letters indicate significant differences of black bars. Different lower-case letters indicate significant differences of white bars ($p \leq 0.05$).

Results showed that the resins presented higher adsorption capacity at lower pHs. This behaviour is also reported in other studies (XI; MU; SUN, 2015; WANG et al., 2013), who found that the adsorption capacity of resins decreased as the pH value of the extract was enhanced.

The removal of phenols by resins from aqueous solutions under different pHs are related to the species distribution of phenols in solutions (LIN; JUANG; 2009). Zhu et al. (2017) indicated that hydrogen bonding might play an important role in the adsorption process of resins. According to these studies, at a high pH values, the hydrogen groups of the analyte are dissociated to H^+ and their corresponding anions, which will result in lower adsorption capacity. Wang et al. (2013) reported that in high pH solutions, the polyphenols may be in a form of ions, which are more difficult to be adsorbed. Moreover, polyphenols are relatively stable when the pH value ranges from 3.0 to 5.0 (ZHANG et al., 2008).

Desorption percentages of resins are showed in Figure 2. Based on the results of Figure 2A, XAD 16, XAD 7HP and XAD 4 showed the highest adsorption percentages (%A) among the six resins. However, XAD 4 presented low (45.2%) desorption percentage (%D), in comparison with XAD 16 and XAD 7HP. These results are corroborated in the Figure 2B, which showed that the highest percentage of recovery (%R) is observed in XAD 16 (83.2%)

and XAD 7HP (82.6%). These results are consistent to Silva et al. (2007) which reported that XAD 7 showed the bests results regarding the adsorption process of phenolics, reaching very high polyphenolic contents from *Inga edulis* leaves. Static tests indicated that XAD 16 and XAD 7HP were more efficient at adsorption and desorption than other resins. Therefore, they were chosen for further kinetic tests.

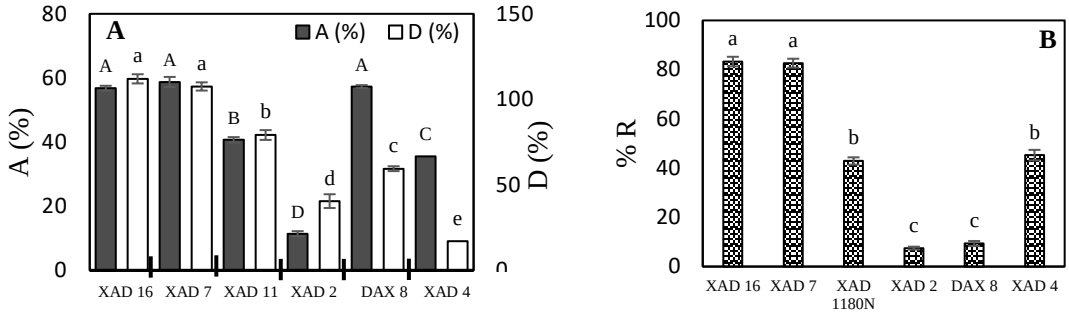


Figure 2. Static adsorption and desorption (A) and recovery ratio (B) of phenolic compounds from organic PFSPL using different macroporous resins according to the best pH of adsorption from each one. Different upper-case letters indicate significant differences of black bars. Different lower-case letters indicate significant differences of white bars ($p \leq 0.05$).

3.2. Adsorption kinetics test

Adsorption kinetics of total phenolics on XAD 16 and XAD 7HP are represented in Figure 3. The adsorption of total phenolics on XAD 16 at 30 °C, 40 °C and 50 °C reach equilibrium after 6 hours. The adsorption of total phenolics on XAD 7HP at 30 °C, 40 °C and 50 °C reached equilibrium more quickly, after 1 hour. Although XAD 7HP needed less time to reach the adsorption equilibrium, it presented lower adsorption capacity at equilibrium (1894.7 μg. g^{-1}) when compared to that of XAD 16 (2537.4 μg. g^{-1}). The ideal resin should provide higher adsorption capacity and shorter processing time.

The temperature at which the adsorption process occurs can affect both the adsorption rate and the adsorption capacity. Adsorption rates vary directly with temperature. However, since the adsorption is an exothermic process, the adsorption capacity will vary inversely with the temperature (BENEFIELD; JUDKINS; WEAND, 1982). As illustrated in Figure 3, the adsorption capacity decreases with the temperature increasing from 30 to 50 °C at the same initial concentration, which corroborates that the adsorption process is an exothermic process. Similar results were obtained for the recovery of phenolics using macroporous resin (ZHU et al., 2017). For this reason, 30 °C was selected in the following experiments.

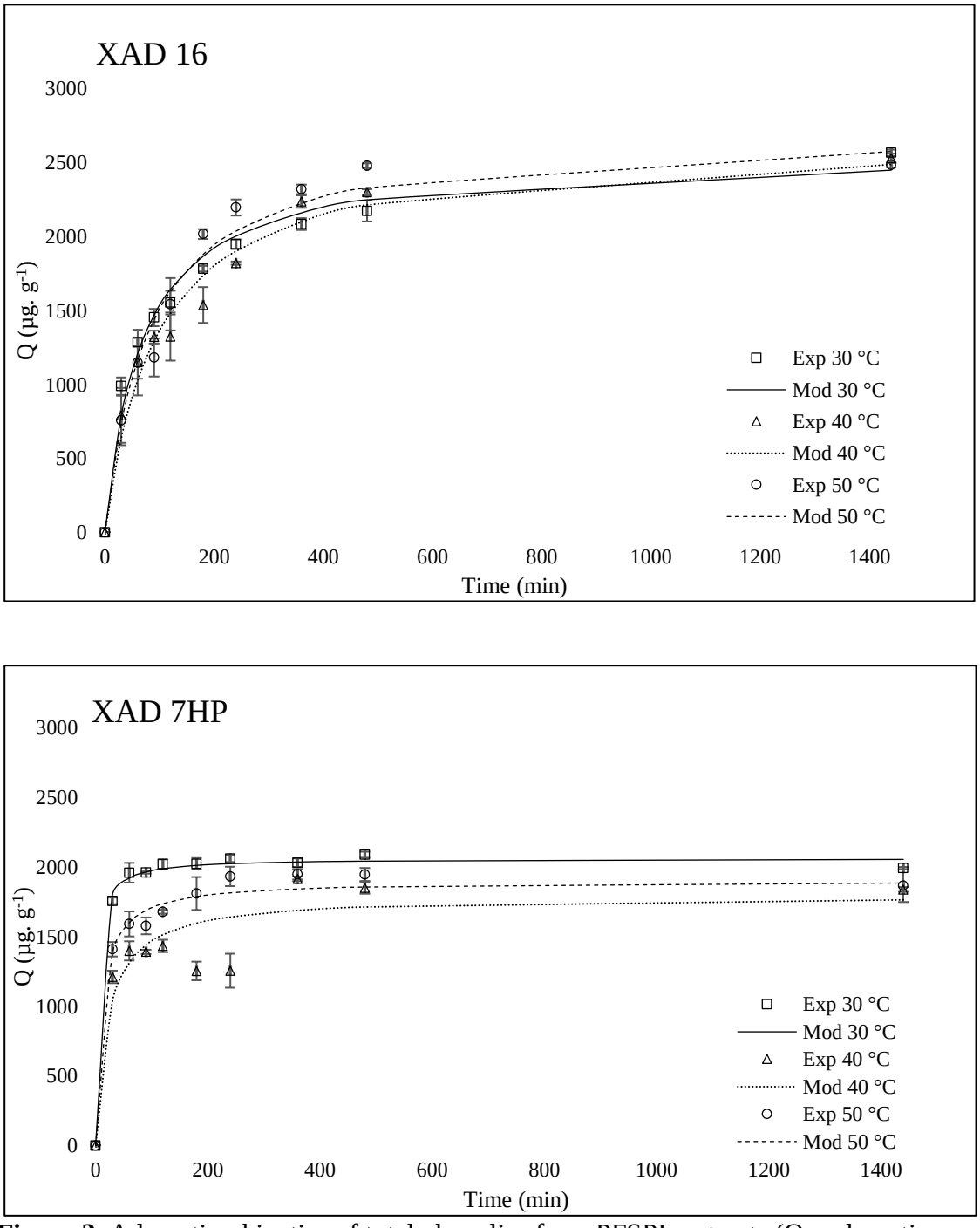


Figure 3. Adsorption kinetics of total phenolics from PFSPL extracts (Q= adsorption capacity; Exp= experimental values; Mod= pseudo-second-order model values). Results are mean of three determinations.

It was seen that regression of kinetic data using pseudo first-order model rendered straight lines between log (q_e - q_t) and time. The correlation coefficients (R^2) ranged from 0.7865 to 0.9969 (Table 2). For the pseudo second-order model, it was observed that the correlation coefficient (R^2) model was in a range of 0.8596 to 0.9960, higher than those of the pseudo first-order model. These findings indicated that the kinetics data for both resins XAD

16 and XAD 7HP under three studied temperatures were described better by the pseudo second-order model. Chabaane et al. (2011) reported that in general, the pseudo-second order kinetic model provides a very good fit to adsorption data over the whole adsorption curve. Similar results were observed in previous studies using macroporous resins on phenolic compounds (FIRDAOUS et al., 2017).

Table 2. Pseudo first and second order rate constants of resins calculated on the basis of total phenolics.

	Temperature (°C)	**K_f**	**q_e**	**Correlation coefficient (R^2)**
Pseudo First order				
XAD 16	30	0.011	2315.06	0.9219
	40	0.007	2417.80	0.9476
	50	0.009	2479.44	0.9866
XAD 7HP	30	0.067	2022.11	0.9969
	40	0.036	1629.97	0.7865
	50	0.042	1818.43	0.9572

	Temperature (°C)	K_s	q_e	Correlation coefficient (R^2)
Pseudo second order				
XAD 16	30	5.80×10^{-6}	2563.76	0.9829
	40	3.97×10^{-6}	2652.26	0.9775
	50	4.57×10^{-6}	2719.72	0.9740
XAD 7HP	30	1.10×10^{-4}	2062.08	0.9960
	40	2.53×10^{-5}	1791.79	0.8596
	50	4.64×10^{-5}	1900.90	0.9742

K_f- rate constant of pseudo first order; K_s- rate constant of pseudo second order ; q_e- adsorption capacity at equilibrium.

3.3. Adsorption isotherm tests

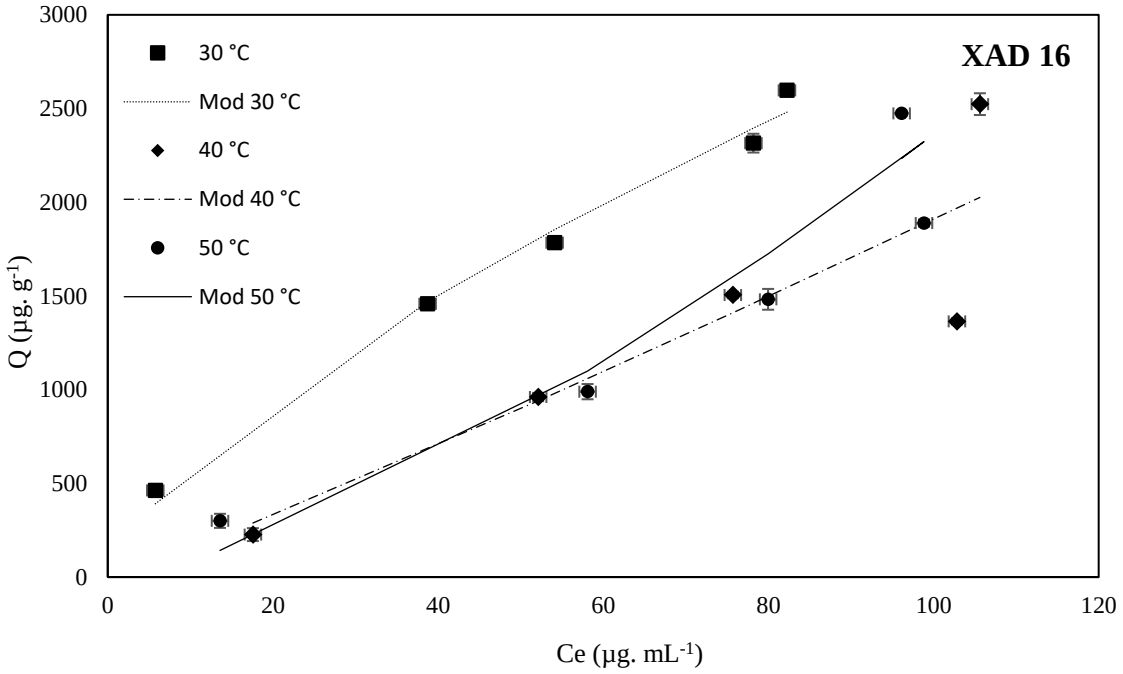
Adsorption equilibrium measurements are used to determine the maximum or the final adsorption capacity, and are formulated into an isotherm model (LIN; JUANG, 2009). According to static adsorption/ desorption and kinetics tests, XAD 16 and XAD 7HP showed the best results for adsorption. Therefore, they were selected for studies of isotherms adsorption of total phenolics. The total phenolics data (Table 3) were regressed according to the Langmuir, Freundlich and Henry isotherms equations. The experiments were performed at 30 °C, 40 °C and 50 °C. Results are presented in Figure 4. The adsorption capacity on both resins increased

when the phenolics concentration was higher (100 µg.mL^{-1}). Similar results were reported by Liu et al. (2015), which found that adsorption capacity increased with the increment of capsaicinoids concentration.

Table 3. Langmuir, Freundlich and Henry equation constants of total phenolics on Amberlite XAD 16 and XAD 7HP.

	Temperature (° C)	**K_l/ K_F/ K**	**p value**	**q_e**	**p value**	**Correlation coefficient (R^2)**
Langmir						
XAD 16	30	133.81	0.20	6483.55	0.09	0.9758
	40	7.68 x 10^6	0.99	1.44 x 10^8	0.99	0.7754
	50	4.67 x 10^7	0.00	9.75 x 10^8	0.00	0.8795
XAD 7HP	30	84.36	0.20	4.11 x 10^3	0.07	0.9441
	40	82.34	0.47	2.84 x 10^3	0.23	0.8561
	50	53.57	0.16	2.76 x 10^3	0.01	0.9599
Freundlich						
XAD 16	30	116.04	0.04	1.44	0.00	0.9891
	40	12.79	0.71	0.92	0.13	0.7774
	50	3.60	0.68	0.71	0.06	0.9010
XAD 7HP	30	96.57	0.22	1.43	0.02	0.9175
	40	66.74	0.46	1.43	0.08	0.8340
	50	150.80	0.21	1.84	0.03	0.9253
Henry						
XAD 16	30	31.75	1.53			0.9414
	40	18.85	0.00			0.7754
	50	20.87	0.00			0.8795
XAD 7HP	30	27.61	0.00			0.8253
	40	18.27	0.00			0.7767
	50	19.20	0.00			0.7310

K_l- rate constant of Langmuir model; K_F- rate constant of Freundlich model; K- rate constant of Henry model; q_e- adsorption capacity at equilibrium.



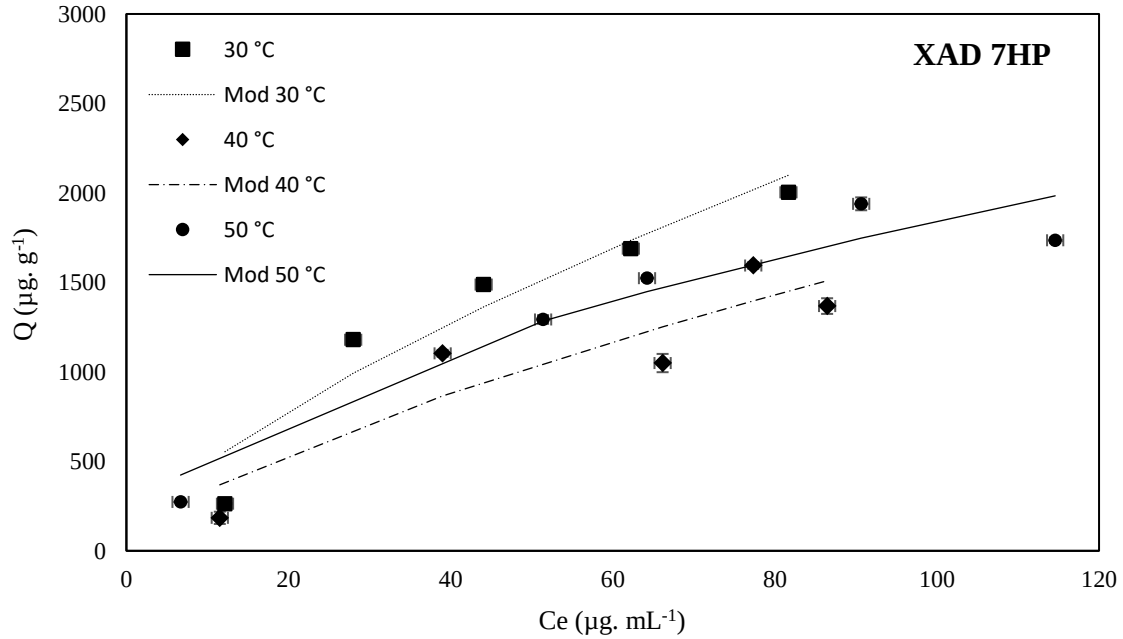


Figure 4. Adsorption isotherms of total phenolics based on Freundlich equation on XAD 16 and XAD 7HP resins from PFSPL extracts (Mod= model values).

According to the correlative coefficients (R^2) showed in the Table 3, data were well fitted by the Freundlich model for both resins. The Langmuir and Freundlich isotherms are the most common models to express adsorption mechanism (BURAN et al., 2014). The Langmuir model describes a monolayer adsorption with energetically identical sorption sites and without mutual interactions between the adsorbed molecules. The Freundlich model assumes adsorption to heterogeneous surfaces, which is characterized by sorption sites at different energies (DURAN et al., 2011). The Freundlich expression is an exponential equation. It assumes that as the adsorbate concentration increases, the concentration of adsorbate on the adsorbent surface (ALLEN; McKAY; PORTER, 2004) will also increase.

Based on the Figure 4, for the resin XAD 16, the adsorption capacity was not significantly affected by the elevation of temperature. However, for XAD 7HP, the adsorption capacity at 50 °C was lower.

3.4. Dynamic adsorption and desorption

The Figure 5A shows that as the SPL extracts were pumped into the column, no phenolic compounds were detected in the eluent. However, in the course of time, a higher volume of extract was pumped into the column. The resin slowly reached the adsorption saturation and phenolic compounds breakthrough and were detected in the eluent. According to Zhu et al. (2017), the adsorption capacity decreases as soon as the resin is saturated. and it is important to find the breakthrough curve in order to determine the volume of sample solution. Breakthrough volume is the volume of extract pumped into the column when the concentration of phenolic compounds in the eluent is 5% of that in the original extract (BURAN et al., 2014).

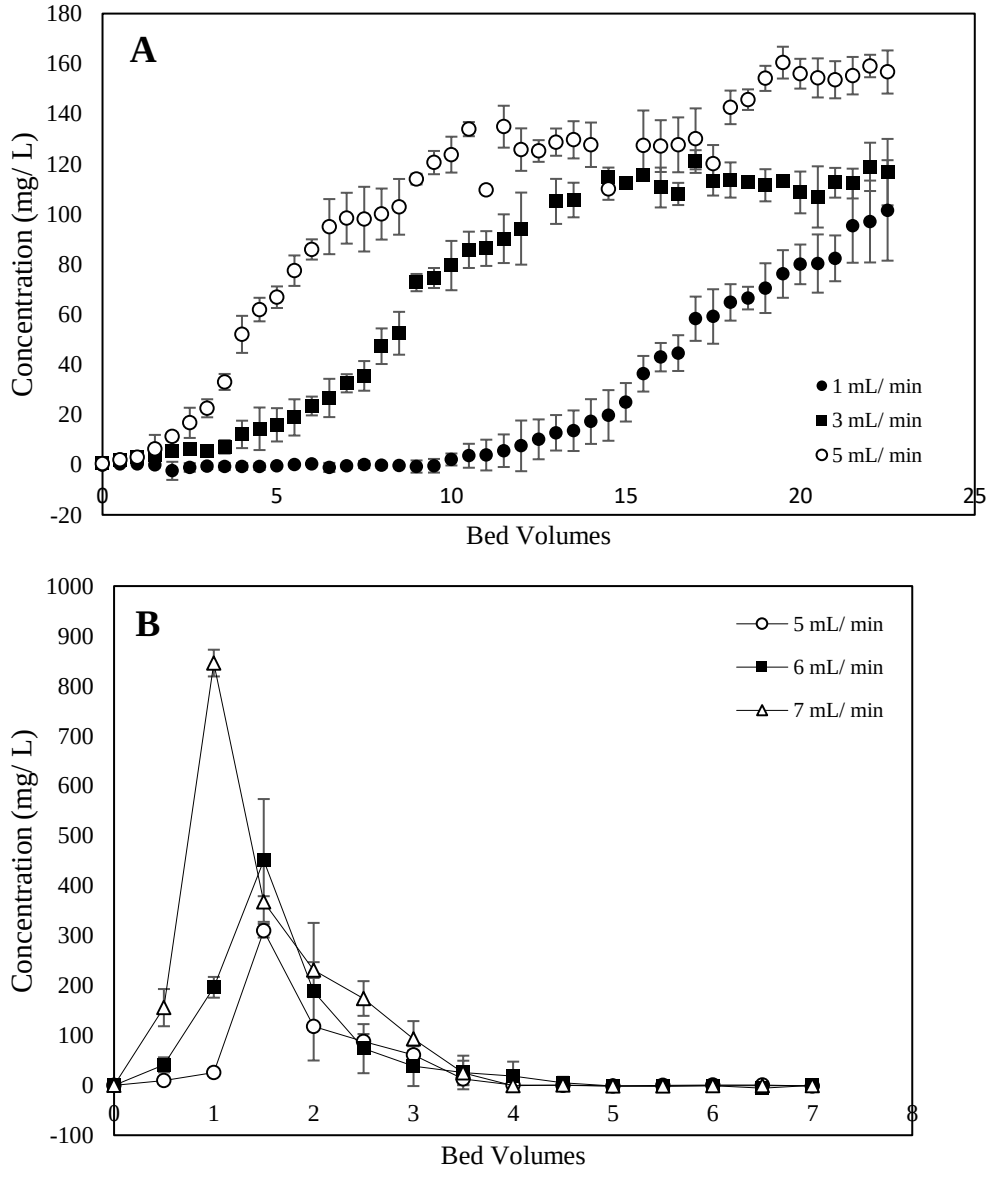


Figure 5. Dynamic adsorption (A) and desorption (B) curves of total phenolics on Amberlite XAD7HP resin at different flow rates. Results are mean of three determinations.

Dynamic adsorption was performed evaluating three flow rates (1 mL/ min, 3 mL/ min and 5 mL/ min). At a flow rate of 3 mL/ min and 5 mL/ min, the breakthrough point was reached at 4.5 and 2.3 bed volumes. Although, at a flow rate of 1 mL/ min, the breakthrough point took a longer time to be reached (13.5 bed volumes).

The use of higher flow rate may lead to incomplete adsorption of phenolic compounds on XAD 7HP resin, because there is a shorter contact time with the resin (MA et al., 2009).

The resin was eluted by 95% (v/v) ethanol solution at 5 mL/min, 6 mL/min and 7mL/ min. According to Liu et al. (2004), for industrial applications, higher % of ethanol is preferred because it is considered easier to concentrate compared to one with a higher amount of water.

In the Figure 5B can be observed that a complete desorption of phenolic compounds was performed with 4 and 5 bed volumes. The higher flow rate (7mL/ min) was choose because the desorption of phytochemicals from resin required a shorter time.

4. Conclusions

Resins XAD 16 and XAD 7HP showed better recovery of phenolics from purple-fleshed sweet potatoes leaves (*Ipomoea batatas* L.), which is related to higher adsorption and desorption capacity. The adsorption of phenolics on XAD 16 and XAD 7HP followed a pseudo second order kinetics and were better fitted in a Freundlich isotherm model. The method of purification of phenolic compounds from leaves could represent an alternative of reuse of these materials, for their use in food and pharmaceutical industries, contributing to sustainable and to economic benefits.

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CAPÍTULO IV: Study of oxidative stability of soybean oil incorporated with phenolic extracts from sweet potatoes (Ipomoea batatas) leaves obtained by microporous resins purification

Manuscrito submetido para a revista Food Chemistry
(Qualis Capes A1/ Ciência de Alimentos)

Study of oxidative stability of soybean oil incorporated with phenolic extracts from sweet potatoes leaves obtained by macroporous resins purification

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Abstract

The most important oils to the human diet have high contents of polyunsaturated fatty acids. For this reason, they are susceptible to changes due to oxidation of these compounds, which may compromise the composition of these fatty acids and the quality of these oils during storage and heating. To minimize these reactions, alternative natural sources which have antioxidant action have been studied to replace synthetic antioxidants used by industry. Phenolic compounds are phytochemicals found in sweet potatoes leaves (SPL) and these compounds have been demonstrated antioxidant potential when applied to food products. The aim of this study was to improve the oxidative stability of soybean oils by using purified extracts of SPL obtained by adsorption with macroporous resins. Soybean oil added from SPL extracts at two concentrations (2000 and 3000 mg. kg^{-1}) and synthetic antioxidant TBHQ (500 mg. kg^{-1}) were subjected to heating for 20 days at 60 °C and evaluated by fatty acids profile, volatile compounds, acidity and peroxide indexes, conjugated dienes, p-anisidine value, color, total phenolic compounds and frying stability. The control samples presented higher oxidation levels by evaluating all the parameters. TBHQ antioxidant was the most efficient during the accelerated oxidation period induced through heating of soybean oil. However, a higher concentration of SPLE was more effective in the inhibition of peroxide, acidity and p-anisidine values. The phenolic contents of all samples did not decrease during the accelerated oxidation. The color parameters of samples added to natural and synthetic antioxidants did not change over time, in contrast to the color of control samples, which acquired a darker color.

Keywords: oxidative stability, leaves extracts, soybean oil, adsorption.

1. Introduction

The oxidative processes, which are responsible for changes in the color, taste, texture and nutritional value of food, can be decreased by modifying environmental conditions or by using antioxidants, which prevent or reduce the oxidative reactions (AMAROWICZ et al., 2004; TAGHVAEI; JAFARI, 2015).

The interest in the identification and purification of new natural compounds with antioxidant activity as an alternative to prevent the oxidative deterioration of foods and to limit the use of synthetic antioxidants, has been increased, since the synthetic ones are related to negative health effects (KULISICA et al., 2004). Some studies have reported that the dosage of some synthetic antioxidants used in the industry, not only have no adverse effect on human but also have anticarcinogenic and antimutagenic properties (SLAMENOVA et al. 2003; VALENZUELA; SANHUEZA; NIETO, 2003), in contrast to natural antioxidants with similar characteristics.

Several natural compounds have antioxidant activity, and the most important natural antioxidants are phenolic compounds (flavonoids, phenolic acids and tannins), nitrogen compounds (alkaloids, amino acids, peptides, amines and chlorophyll derivatives), carotenoids,

tocopherols and ascorbic acid (AMAROWICZ et al., 2004). These compounds could be present in any part of plants including leaf, stem, root, seed, fruit, bark (TAGHVAEI; JAFARI, 2015). Leaves are sustainable sources of bioactive compounds, such as phenolics, when compared to the own vegetable (OLIVEIRA et al., 2013).

Sweet potatoes leaves are natural by-products considered of important nutritional and functional value (ISLAM, 2006), containing functional compounds with antioxidant activities such as carotenoids, flavonoids, chlorogenic acids, and several caffeoylquinic derivatives, all of which contribute to improve the human health (CHEN et al., 2017; ISHIGURO, 2002). These leaves are considered as a by-product of sweet potato products, and their extracts (SPLE) contain large amounts of phenolic compounds with biological activity (ISLAM, 2006).

The most abundant polyphenols identified in SPLE are chlorogenic acids and caffeoylquinic acid (CQA) derivatives (LUO et al., 2013; JENG et al., 2015), wich have health-promoting bioactive properties, such as antioxidant, antimicrobial, anticancer, antidiabetes, and antimutagenicity (LIAO et al., 2013).

Thus, it has been necessary to enrich and identify key antioxidants from these abundant substances present in sweet potatoes leaves (LI et al. 2018). In general, the conventional separation methods are expensive and only suitable for small-scale production (YANG; ZHAO; LIN, 2016) and demand much time, energy and high cost (FU et al., 2006). Macroporous resins have received significant attention in separation and purification of bioactive components from various natural products due to their favorable adsorption characteristics (LIU et al., 2010) as fast adsorption rate, low costs, convenient regeneration process and can be applied to a large-scale production (LIU et al., 2010; XI; MU; SUN, 2015). Furthermore, several studies have been showed that macroporous resin are effective in the separation of phenolics (DONG et al., 2015; ZHAO et al., 2015), which could be applied as bioactive ingredients in food industry (LI et al., 2017).

Soybean oil is a highly-consumed polyunsaturated vegetable oil worldwide (MALHEIRO et al., 2013; RODRIGUES et al., 2012). Tocopherols, naturally present in soybean oil are generally themselves first oxidized and are quickly decomposed by oxidation reactions or during the refining process of the oil (ROSSI; ALAMPRESE; RATTI, 2007). However, there is still doubt about safety and the approval, usage level, type and application of synthetic antioxidants (TAGHVAEI et al., 2014).

Therefore, this work aims the valorization of purified extracts of leaves from PFSP obtained by MARs as active sources of natural antioxidants added to provide oxidative stability to soybean oil.

2. Materials and methods

2.1. Total phenolics extraction by MARs

The studies on optimization of phenolics separation and purification from PFSPL with macroporous resins have been previously carried out in our laboratory. For phenolics extraction, pre-treated Amberlite XAD7 was used and the procedures were performed according to Buran et al. (2014). 25 mL of leaf extract (100 mg. mL^{-1}) were added to 1 g of resin in an Erlenmeyer flask and the pH was adjusted to 3.0. The adsorption process occurred at 30 °C in a shaker at 100 rpm. For desorption, the resins were filtered, washed with distilled water, added to 50 mL of ethanol (95%), and the pH was adjusted to 3.0. The process was performed in a shaker at 100 rpm for 24 h at 50 °C.

2.2. Sample preparation and oxidation test (Schaal oven test)

For the experiment, refined soybean oil was used, with no addition of synthetic antioxidants. Packages of 900 mL of soybean oil, processed by Cargill Agrícola S/A

(Uberlândia, MG- Brazil), were used. The synthetic antioxidant used was t-butyl-hidroxyquinone (BHT). Three treatments were carried out: soybean oil, without addition of antioxidants (control); soybean oil, with addition of 500 mg/ kg of TBHQ; soybean oil, with addition of 2000 and 3000 mg/ kg of PFSPL extracts.

The mixtures were stored at 4° C until the start of the experiments. The Schaal oven test was performed according to Taghvaei et al. (2014), as follows: the mixtures were strongly shaken and distributed (55 g) in glass bottles (100 mL, diam. 4 cm), which were stored for 15 days at 60° C (accelerated aging) in a ventilated oven (SOLAB SL 102, Piracicaba- São Paulo, Brazil). Periodically (every 3 days), samples were removed from oven, flushed with nitrogen, and stored at -20° C until analysis.

2.3 Acidity Index

The acidity index represents the quantity of KOH, in mg, necessary for the neutralization of free fatty acids in a gram of fat (CAUNII et al., 2015). The acidity was measured by AOAC methods (AOAC).

2.4. Peroxide value

The hydroperoxides were measured using the iodometric titration method (AOAC, 1995) as described as follows: 5 g of sample were diluted in 30 mL acetic acid/chloroform solution (3:2, v/v). Then, 0.5 mL of a saturated potassium iodide solution, 30 mL of water and 1 mL of starch solution were added and a titration was performed with a 0.1 N $Na_2S_2O_3$ solution. Results were expressed as meq O_2. kg^{-1} of sample.

2.5. p-anisidine value (PA) and Kreiss Index

The PA value was determined by dillution of 0.5 g of sample with 25 mL of isooctane and adding PA (0.25% in glacial acetic acid) (AOCS, 1998). PA value was measured at 350 nm in a spectrophotometer (NOVA 2000 UV, Piracicaba- São Paulo, Brazil). The Kreiss index is a qualitative analysis, which provides an indication of the occurrence of oxidation lipids at an early stage of rancid development, showing the presence of secondary oxidation products (Hamilton et al., 1983). In the presence of lipix oxidation, the bottom layer acquires a red color (Pregnolatto & Pregnolatto, 1985). The Kreiss index was measured by AOAC methods (1995).

2.6. Color

The color was evaluated using a HunterLab colorimeter (Miniscan EZ BrasEq, São Paulo, Brazil) in the L, a, b, mode of CIE (L, a, b, indicate lightness, redness/greenness, and yellowness/blueness, respectively).

2.7. Total Phenolic Compounds

Total phenolics content was measured according to Swain and Hillis (1959) with some modifications. One mL of extract, 10 mL of distilled water and 1 mL of aqueous solution of Folin–Ciocalteu reagent (0.25 N) were mixture, and after 3 min, 1.5 mL of an aqueous solution (10% w/w Na_2CO_3) was added. The reaction occurred for 2 hours in the absence of light, at room temperature (25±1ºC). The absorbance was measured at 725 nm in a spectrophotometer (NOVA 2000 UV, Piracicaba- São Paulo). The results were expressed as milligram of gallic acid equivalents (GAE) per gram of dry weight (mg GAE. g^{-1}), according to a standard curve performed using gallic acid.

2.8. Statistical analysis

All analysis were performed in triplicate. Data were presented as means ± standard deviations. Significant differences between means ($p \leq 0.05$) were estimated by Student's t-tests using XLSTAT 7.5. Data analysis was performed using SAS 9.2 and Origin 8.0 data analysis software.

3. Results and discussion

3.1. Acidity Index

The results for acidity index determination are presented in the Figure 1.

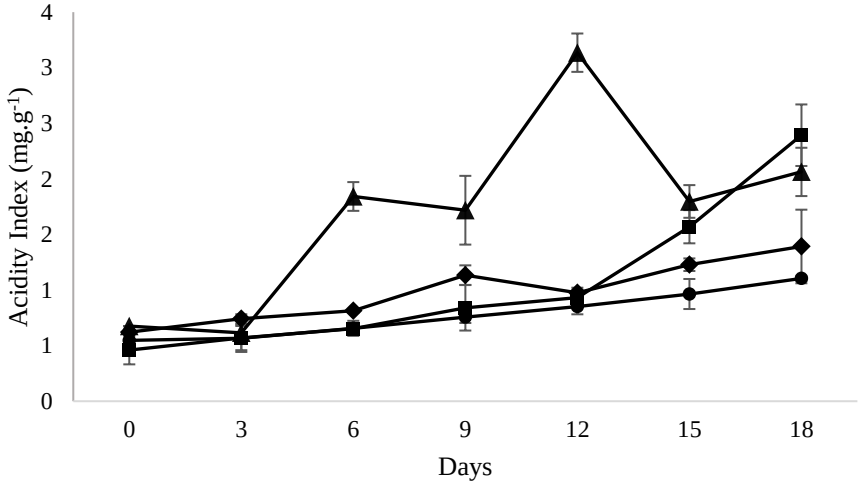

Figure 1: Acidity index of control (▲, sample A), SPLE at 2000 $mg.kg^{-1}$ (■, sample B), SPLE at 3000 $mg.kg^{-1}$ (♦, sample C) and TBHQ at 500 $mg.kg^{-1}$ (●, sample D) during the accelerated oxidation test. Values are expressed in mg KOH. g^{-1} of sample. Means (n = 3) ± SD.

According to the Figure 1, there was statistic differences between the acidity indexes of the control sample and the other samples. The samples containing 3000 $mg.kg^{-1}$ of extracts and TBHQ were equivalent in the acidity contents. The control and the sample containing 2000 $mg.kg^{-1}$ of extract showed an increase in the acidity index during 18 days of heating. However, the samples containing 3000 $mg.kg^{-1}$ and TBHQ preserved the acid content over time, showing that the presence of the synthetic antioxidant and the natural antioxidant in a higher concentration were able to minimize the effects of the lipid oxidation on the acidity of the samples.

3.2. p-anisidine and Kreiss Index

Thermo-oxidation of frying oils involves both primary and secondary oxidation (KIRAN et al., 2015). The p-anisidine value provides estimations of the secondary oxidation products (principally 2-alkenals and 2, 4-alkadienals) generated during decomposition of hydroperoxides (TAGHVAEI et al., 2014; KIRAN et al., 2015). The results for p-anisidine value are shown in Figure 2.

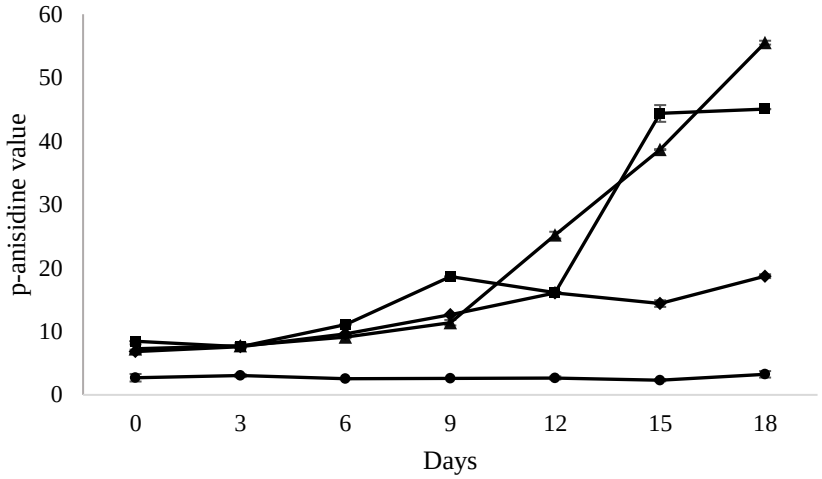

Figure 2: The p-anisidine value of control (▲, sample A), SPLE at 2000 mg.kg^{-1} (■, sample B), SPLE at 3000 mg.kg^{-1} (♦, sample C) and TBHQ at 500 mg.kg^{-1} (●, sample D) during the accelerated oxidation test. Means (n = 3) ± SD.

According to the results showed in the Figure 2, the p-anisidine value of the control sample and the sample containing SPLE at both concentrations increased during the 18 days.

The oils added to the highest concentration of extract presented a slight increase in the p-anisidine, but more discreet when compared to the lower concentration and to the control sample, being compatible with the results demonstrated by the other analysis. However, the results for the samples containing TBHQ at 500 mg.kg^{-1} did not increase over time suggesting that this treatment is more effective in the inhibition of p-anisidine value increasing.

The effectiveness of natural antioxidants from plants in decreasing of anisidine values was reported and discussed in several studies (CHE MAN; JASWIR, 2000; IDRIS et al., 2008; KIRAN et al., 2015).

The results for Kreiss Index are presented in the Table 1.

Table 1. Positive (+) and negative (-) results for Kreiss Index along the days of storage due to heating of samples.

	Day 0	**Day 3**	**Day 6**	**Day 9**	**Day 12**	**Day 15**	**Day 18**
Sample A	-	-	+	+	+	+	+
Sample B	-	-	-	-	+	+	+
Sample C	-	-	-	-	-	-	+
Sample D	-	-	-	-	-	-	+

Sample A (control); sample B (containing SPLE at 2000 mg.kg^{-1}); sample C (containing SPLE at 3000 mg.kg^{-1}); sample D (containing TBHQ at 500 mg.kg^{-1}).

The samples that presented positive results for Kreiss Index acquired red color at the end of the reaction, which means that there was a formation of secondary oxidation products from rancid formation at the mentioned times.

In the control sample, the formation of oxidation products occurs very early on day 6, in contrast to the other samples. Sample B showed formation of the oxidation products on day 12 and samples C and D on day 18. Evaluating the results for Kreiss reaction, it can be suggested that SPLE at 3000 mg.kg^{-1} and TBHQ at 500 mg.kg^{-1} are more efficient in lipid oxidation inhibition, because the formation of oxidation products in these samples occurred later.

3.3. Peroxide Value

The peroxide values of the samles are showed in the Figure 3.

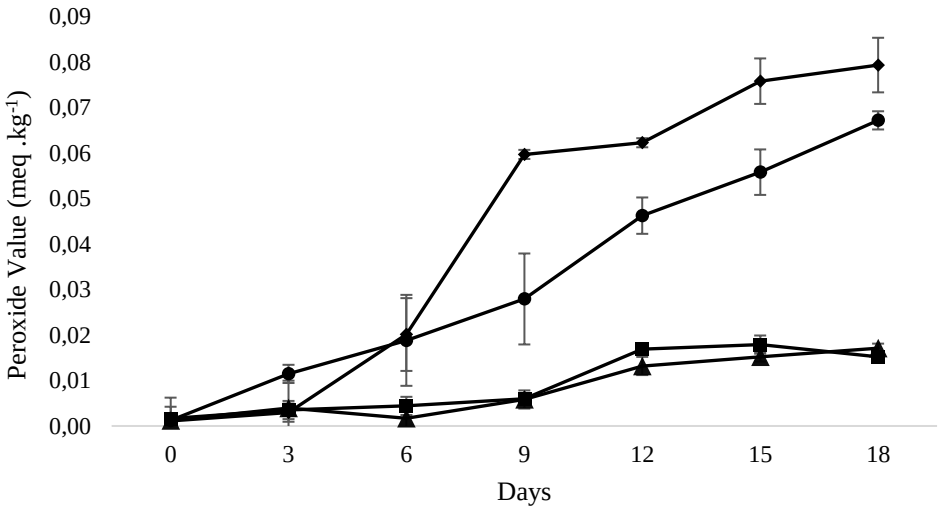

Figure 3: Peroxide index of control (▲, sample A), SPLE at 2000 mg.kg^{-1} (■, sample B), SPLE at 3000 mg.kg^{-1} (♦, sample C) and TBHQ at 500 mg.kg^{-1} (●, sample D) during the accelerated oxidation test. Values are expressed in meq. kg^{-1} of sample. Means (n = 3) ± SD.

Some authors suggest that determination of the peroxide value is an important quality control index for oils, because it measures the concentration of hydroperoxides which are unstable and can easily break down to oxygenated constituents such as alcohols, aldehydes, free fatty acids, and ketones, indicating the primary oxidation of the oils (MOHAMMADI et al., 2016; PIZARRO et al, 2013).

The peroxide value of the control increased to a maximum value of 171.08 meq KOH. 1000 g^{-1} after 18 days heating (Figure 3). The control sample (without any antioxidant) had the highest increase of peroxide value with a significant difference with the other samples, in accordance with Afshari and Sayyed-Alangi (2015) which reported that soybean oil added to leaf extracts from *Cressa cretica* were efficient in the inhibition of peroxide formation.

The peroxide value of soy oil containing 2000 mg/kg of extract, 3000 mg/kg of extract and 500 mg/kg of TBHQ were 151.81, 79.36 and 67.20 meq KOH. 1000 g^{-1} respectively. The inhibition rates also were 2.5, 11.26, 53.61 and 60.7 %, for samples A, B, C and D, respectively, after 18 days heating compared with blank sample.

The increasing in peroxide value during heating was slow at the first 6 days and then, a sharp increase was observed until the end of 18 days, for all samples.

The results for peroxide value demonstrated SPLE at 3000 mg. kg^{-1} is more effective than SPLE at 2000 mg. kg^{-1} in oxidation inhibition of soybean oil. These results are consistent with Mohammadi et al. (2016), which found that an increase in the concentration of phenolic extracts of olive leaf, significantly decreased the peroxide value of soybean oil samples.

On the other hand, SPLE was less effective in the oxidation prevention when compared to TBHQ after 18 days storage at 55 ˚C. The results for peroxide are in accordance with Almeida-Doria and Regitano (2000), which verified that TBHQ (200 mg. kg^{-1}) was more efficient decreasing the formation of peroxides in soybean oil on accelerated storage test at 63 ° C when compared to natural antioxidants. Ribeiro and Jorge (2017) reported that TBHQ antioxidant was the most efficient during the accelerated oxidation period induced through heating of soybean oil added to coffee husk extract, leading to lower peroxide values.

We observed that at a level of 3000 mg. kg^{-1} of SPLE is as an effective antioxidant for the stabilization of soybean oil, and primary and secondary oxidation inhibition was equivalent to the obtained with TBHQ. These results are consistent with Franco et al. (2017) which found peanut skin extracts as importants antioxidants in soybean oil, decreasing the peroxide index and the p-anisidine value when compared to the control sample. Similar results were found by Sayyad et al. (2017), which reported that rosemary extracts could increase the oxidative stability of soybean oil on storage.

3.4. Color

The color changes of oils samples are presented in the Table 2.

Table 2. Color changes of oil samples during 18 days storage at 60° C.

	Day 1			Day 3			Day 6			Day 9			Day 12			Day 15			Day 18		
	L	a	b	L	a	b	L	a	b	L	a	b	L	a	b	L	a	b	L	a	b
A	7.9	-0.5	-0.5	4.2	-0.7	-0.7	3.7	-0.5	-0.7	4.0	-0.7	-0.5	2.3	-0.4	-0.4	3.1	0.02	-0.9	1.5	0.1	-1.3
B	2.8	-0.8	-0.4	2.1	-0.7	-0.5	2.2	-0.5	-0.4	3.0	-0.5	-0.5	2.4	-0.5	-0.4	3.2	-0.6	-0.6	2.8	-0.5	-0.5
C	2.2	-0.6	-0.4	1.9	-0.5	-0.5	1.7	-0.5	-0.4	1.9	-0.5	-0.4	1.8	-0.6	-0.5	2.0	-0.7	-0.5	2.6	-0.5	-0.6
D	2.9	-0.5	-0.4	2.7	-0.7	-0.5	2.2	-0.6	-0.5	2.2	-0.5	-0.5	2.2	-0.6	-0.4	2.7	-0.6	-0.5	2.7	-0.6	-0.5

Sample A (control); sample B (containing SPLE at 2000 $mg.kg^{-1}$); sample C (containing SPLE at 3000 $mg.kg^{-1}$); sample D (containing TBHQ at 500 $mg.kg^{-1}$). Data are the average of three replicates.

Color is an important quality parameter of edible oils. “L” indicates lightness, “a” represents redness/greenness, and “b” shows yellowness/blueness of oil samples (TAGHVAEI et al., 2014).

We observed that lightness, redness/greenness and yellowness/blueness of the samples added to SPLE and TBHQ did not change significantly ($p > 0.05$) during storage. However, the lightness and the yellowness of the control sample decreased on the third and eighteenth days, respectively, showing that the lipid changes occurred in this sample with no antioxidants were able to change the color parameters leading to a darkening (lower lightness and yellowness).

It is important to emphasize that the soybean oils added to extracts and TBHQ maintained their color over time due to inhibition of lipid oxidation, in contrast to the control sample, which presented higher levels of oxidation and color changes.

3.5. Total phenolics

The changes of phenolic compounds from the samples 18 days storage at heating was evaluated by total phenolic content analysis. The results are illustrated in Figure 4.

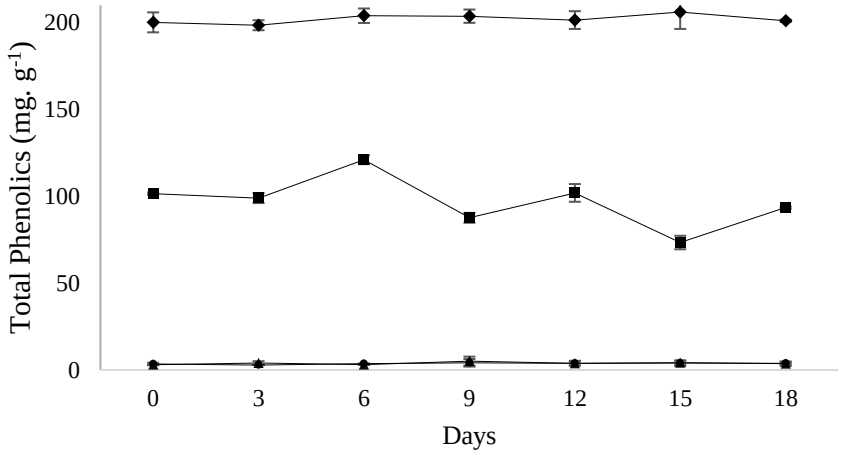

Figure 4: Total phenolics compounds of control (▲, sample A), SPLE at 2000 mg.kg^{-1} (■, sample B), SPLE at 3000 mg.kg^{-1} (♦, sample C) and TBHQ at 500 mg.kg^{-1} (●, sample D) during the accelerated oxidation test. Values are expressed in mg Galic Acid. g^{-1} of sample. Means (n = 3) ± SD.

According to Brewer (2011), the phenolic compounds are able to stopping progressive oxidative damages, by scavenging free radicals and chelate transition metals.

The results show that soybean oil with no antioxidants presented low phenolic content, which did not change over time. Samples B and C had a stable phenolic content during the 18 days, with no statistically significant decrease. This behavior suggests that at the temperature which the experiment was conducted, the phenolic compounds were not degraded, and for this reason, the capacity of lipid oxidation inhibition of soybean oil added with antioxidants was maintained. It was showed that phenolics of SPLE had thermal stability for 18 days at 65 °C.

4. Conclusions

SPLE obtained by adsorption with MARs, in the studied concentrations, were able to improve the polyphenols content of soybean oil and consequently resulted in a better oxidative stability comparing to the control oil. A higher concentration of SPLE was found to be more efficient in the oxidative stability of soybean oils. The formulated oils presented the lower acidity and peroxide indexes and anisidine value until 18 days of heating. The phenolic

compounds found in SPLE were not degraded during the 18 days of heating and contributed to maintain a longer stability and preservation of color parameters of the oils.

In addition, the strong antioxidant capacity of these extracts indicates that the purification with macroporous adsorption resins is an effective technique, allowing its application in the development of several food products.

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Conclusões Gerais

As folhas de olerícolas orgânicas estudadas apresentam um importante perfil de nutrientes. Realizando uma triagem em relação ao teor de compostos fenólicos e capacidade antioxidante, pôde-se constatar que as folhas de batatas-doce apresentaram resultados mais promissores, viabilizando o estudo de extração e purificação.

Foram demonstradas diferenças significativas nos teores de fenólicos totais e capacidade antixidante entre folhas de cultivares diferentes de uma mesma olerícola, o que pode ser explicado pelas diferenças de natureza de cada cultivar, e ainda pelo tipo de composto de interesse que foi alvo de biofortificação.

Foram investigados a capacidade inibidora de angiotensina com o objetivo de avaliar a bioatividade dos compostos fenólicos das folhas, uma vez que estes são os compostos de interesse do presente estudo, e uma de suas propriedades consiste em inibir a atividade da enzima conversora de angiotensina por uma mecanismo que bloqueia a produção de aldosterona e a vasoconstrição e, consequentemente, permite o controle de pressão arterial.

Dentre as folhas de todos os cultivares de batatas-doce avaliadas, foram selecionadas as folhas de batata-doce de polpa roxa, devido ao maior teor de compostos fenólicos totais, maior atividade antioxidante e maior atividade da enzima conversora de angiotensina, permitindo que os estudos de extração e purificação fossem mais concretos e eficazes devido à alta atividade destes compostos.

Dentre os 6 diferentes tipos de resinas estudadas, as resinas XAD16 e XAD 7HP demonstraram-se mais eficientes na obtenção de extratos purificados de fenólicos provenientes das folhas de batata-doce de polpa roxa, o que foi definido pelos parâmetros de maior capacidade de adsorção e dessorção e maior percentual de recuperação durante os testes estáticos. As diferenças de comportamento entre as resinas podem ser explicadas devido às distintas propriedades físicas e afinidade que as mesmas apresentam em relação ao composto de interesse estudado.

Cada resina foi estudada em 5 diferentes faixas de pH, dentre os quais foram observados que os maiores percentuais de adsorção foram atingidos em menores pHs (3.0 e 5.0). Este comportamento é justificado pelo fato de que em elevado pH, os compostos fenólicos podem estar presentes em forma de íons, sendo dessa maneira mais dificíceis de serem adsorvidos pelas resinas.

Os testes de adsorção cinética demonstraram que a resina XAD 16 permite maior capacidade de adsorção dos fenólicos em relação à resina XAD 7HP, embora apresente um maior tempo para atingir o equilíbrio de adsorção, o que justifica a escolha feita pela resina XAD 7HP quando se trata de processo a nível industrial, uma vez que demandaria menor custo com enenrgia. Na adsorção e dessorção dinâmica foram escolhidas as taxas de fluxo de 3 mL/ min para adsorção, pois permite que o extrato tenha um tempo intermediário de contato com a resina, suficiente para permitir a interação necessária para o processo de adsorção. Já para a dessorção dinâmica, foram escolhidas as taxas de fluxo de 5 e 6 mL/min, pois foram suficientes para promover a dessorção completa dos compostos.

Após definir todos os parâmetros ótimos para o processo de purificação dos compostos fenólicos de folhas de batata-doce roxa utilizando resinas macroporosas de adsorção, os extratos purificados foram aplicados em sistemas modelo de óleo de soja com o objetivo de avaliar a capacidade de inibição da oxidação lipídica.

Os resultados demonstraram que óleos de soja adicionados de maior concentração de extrato foram capazes de inibir a oxidação de óleo de soja submetido à oxidação forçada, sendo comparados até mesmo com a eficiência do uso do antioxidante sintético TBHQ. As amostras controle (sem antioxidantes) apresentaram altos índices de oxidação primária e secundária durante os testes.

Os resultados deste estudo demonstraram que a temática do aproveitamento integral dos alimentos vem ganhando cada vez mais destaque, comprovando que a partir de materiais que são comumente descartados e destinados ao lixo, pode-se agregar valor através da extração e purificação de compostos de interesse, os quais podem ser aplicados em matrizes alimentícias como antioxidantes naturais, ou até mesmo serem utilizados na indústria cosmética. Além disso, o aproveitamento destes materiais pode culminar com vantagens econômicas para produtores agrícolas, sustentabilidade e redução do volume de lixo produzido após a colheita de vegetais.

ANEXOS

ANEXO A

1. Metodologias detalhadas utilizadas no Capítulo II

1.1. Extraction procedure for antioxidant assays

Samples (1 g) were macerated with mortar and pestle and placed into erlenmeyer with 25 mL of solvent (acetone:ethanol:water, 40:40:20 v/v/v) and stirred at room temperature and 3500 rpm for 1 hour, in absence of light. After extraction, the sample was filtered under vacuum through sinter funnel. Subsequently, the filtration residue was re-extracted in 15 mL of the same solvent at the early conditions. These filtrates were taken to a volumetric flask to 100 mL with distilled water and used to determine their antioxidant capacity using the free radical scavenging activity (DPPH), ferric reducing-antioxidant power (FRAP) and total phenolics content (TPC) assays.

1.2. DPPH assay

Extracts (150 µL) reacted with 2.85 mL of the DPPH* solution (0.06 mM) for 1 h in the dark. Then the absorbance was taken at 517 nm, in spectrophotometer (*Spectrophotometer Model NOVA 2000 UV*).

Trolox standard solutions were analyzed for the calibration curve construction. Free radical scavenging was expressed in percentual (%FRS) according to Equation 1.

$$\%\ FRS = \frac{(Abs_B - Abs_A)}{Abs_B} \times 10 \qquad \text{Equação (1)}$$

Where:
Abs_B = spectrophotometer absorbance of control read at 517 nm;
Abs_A = spectrophotometer absorbance of sample extract read at 517 nm;

The antioxidant capacity was expressed by µM of Trolox equivalent per gram of dry weight (µM TE. g^{-1}) from the regression coefficient obtained of calibration curve.

1.3. FRAP assay

90 µL of extract was diluted in distilled water (270 µL) and allowed to react with 2.7 mL of FRAP, wich were mixture by vortex and held in a water bath at 37ºC for 30 minutes in absence of light. Readings of the colored product were taken at 595 nm. The standard curve was linear between 100 and 1000 µM of Trolox equivalent. Results were expressed in µM of Trolox equivalent per gram of dry weight (µM TE. g^{-1})

1.4. Total phenolic contents assay

1 mL of extract, 10 mL of distilled water and 1 mL of 0.25 N Folin–Ciocalteu reagent was mixtured in a tube and then vortexed thoroughly. The mixture was allowed to react for 3 min and then 1.5 mL of an aqueous solution (10% w/w Na_2CO_3) was added and mixed well. The solution was incubated at room temperature (25±1ºC) in the dark for 2 hours and then the absorbance was taken at 725 nm using a spectrophotometer. The results were expressed in mg of gallic acid equivalents per gram of dry weight (mg GAE. g^{-1}) using a gallic acid (0.01 to 0.05 mg/mL) standard curve.

1.5. Chlorophylls determination

1 g of a sample was added to 10 mL of a solution of 80% acetone (v / v). The mixture was centrifuged at 4000 g for 10 min and the supernatant transferred to a volumetric flask of 25 mL, completing the volume with the acetone solution at 80% (v / v). Then, readings were taking in a spectrophotometer at the wavelengths of 647 nm and 663 nm using 80% acetone (v/v) as a blank. The results were expressed in mg $100g^{-1}$, using Equations 2 and 3 to calculate the contents of chlorophylls "a" and "b", and Equation 4 to determine total chlorophyll.

$$Chlorophyll\ "a" = (12.25\ x\ A_{663}) - (2.79\ x\ A_{647}) \qquad \text{Equation (2)}$$

$$Chlorophyll\ "b" = (21.5\ x\ A_{647}) - (5.1\ x\ A_{663}) \qquad \text{Equation (3)}$$

$$Total\ chlorophylls = (7.15\ x\ A_{663}) + (18.71\ x\ A_{647}) \qquad \text{Equation (4)}$$

Where: A_{663} and A_{647} are the absorbances.

1.6. ACE inhibition assay

100 mU. mL^{-1} of ACE from rabbit lung was prepared in 200 mM borate buffer (pH 8.3). A reaction mixture (0.25 mL of HHL(7 mM) in pH 8.3 borate buffer (200 mM), 0.2 mL of NaCl (2 M), 0.02 mL water, 0.015 mL aqueous leaf extract and 0.015 mL of ACE (100 mU. mL^{-1})) was incubated at 37 °C for 30 min. 0.25 mL of HCl (1 N) was used to stop the reaction. 1.5 mL of ethyl acetate was used to extract the hippuric acid liberated from the HHL by ACE. An aliquot (1.3 mL) of the extract was evaporated and the residue was dissolved in 0.4 mL of water. Distilled water (0.015 mL) was used in control set, instead of leaf extract. The hippuric acid concentration was determined by measuring the absorbance in spectrophotometer, at 228 nm. A blank solution was prepared by addition of buffer instead of ACE. Assays were performed in triplicate. The percentage inhibition of ACE activity by leaf extracts was calculated according to the Equation 5. The percentage of inhibitions of different leaf extracts were compared with the percentage of inhibition value of the standard cardioprotective drug lisinopril.

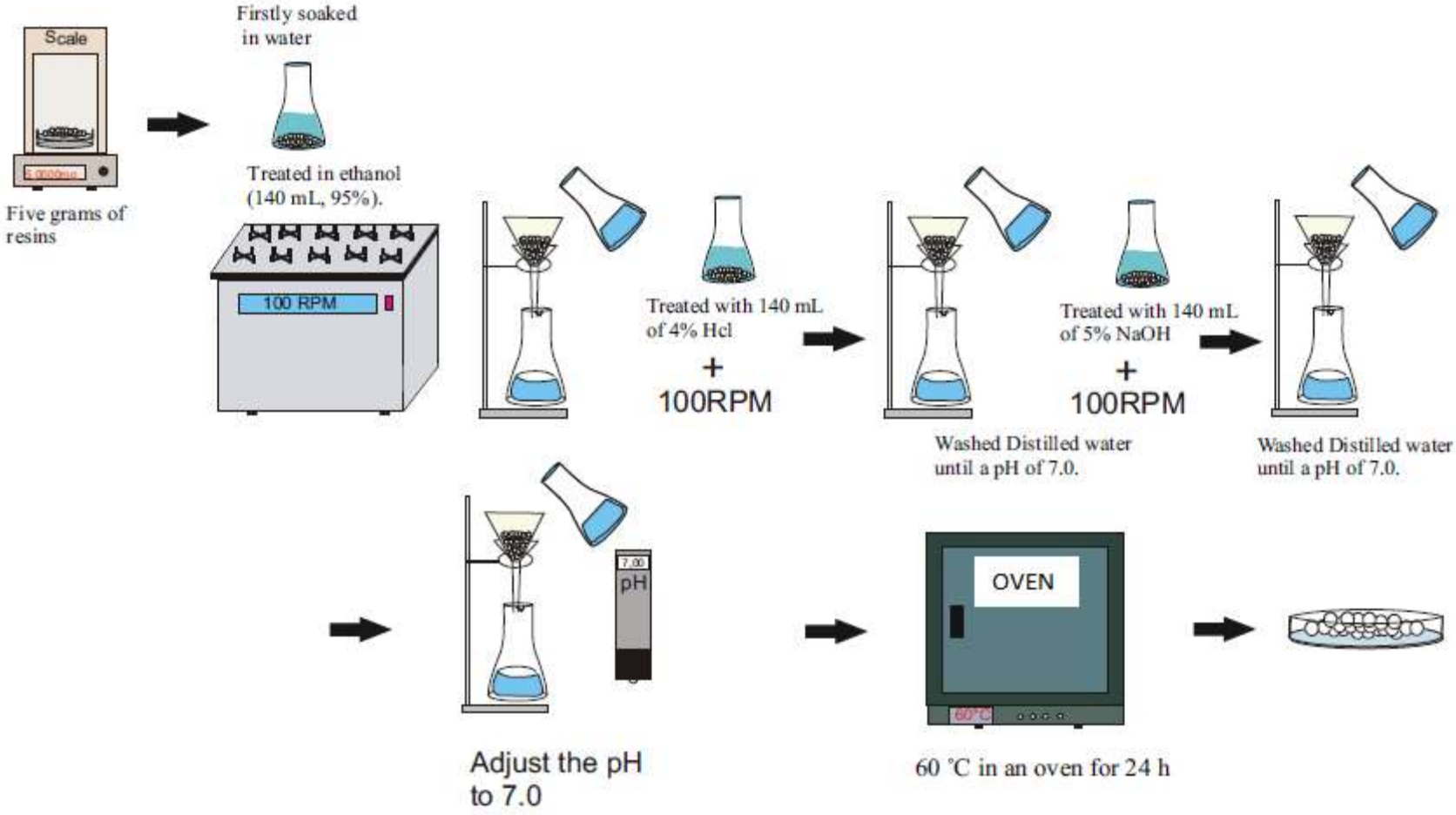
$$\%\ ACE\ inhibition = [(A_0 - A_e)/A_0] \times 100 \qquad \text{Equation (5)}$$

Where:
A_0= absorbance without extract
A_e= absorbance with extract

ANEXO B

2. Metodologias detalhadas utilizadas no Capítulo III

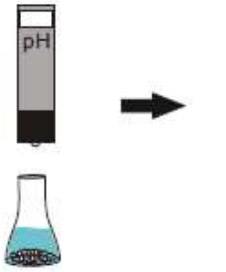
2.1. Pre-treatment of resins



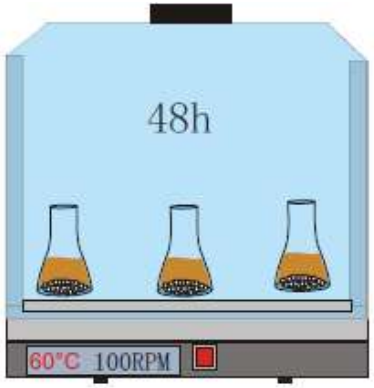
2.2. Static adsorption and desorption tests

Pre-treated resins (1 g) and 25 mL of leaf extracts were added to a 125 mL

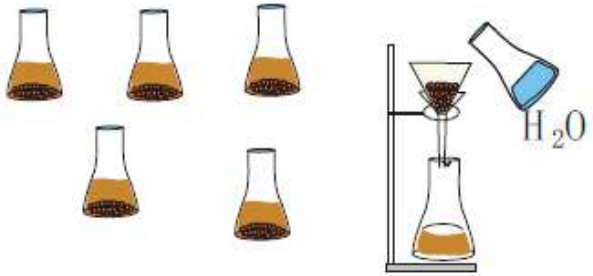
different pHs (3.0, 5.0, 7.0, 9.0 and 11.0).



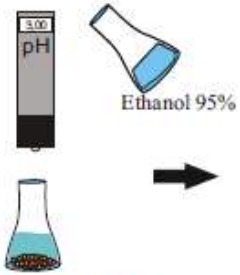
pHs were adjusted using 0.01 N HCl and 0.01 N NaOH



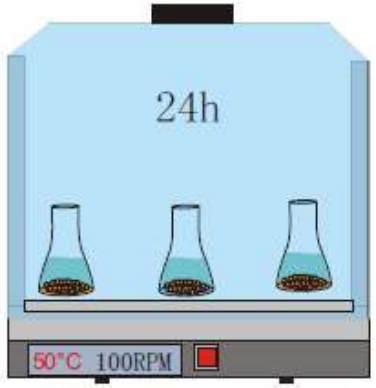
After adjusting pH, the flasks were taken to a shaker at 100 rpm for 48 h at 30 °C



washed with distilled water and added to 50 mL of ethanol (95%).

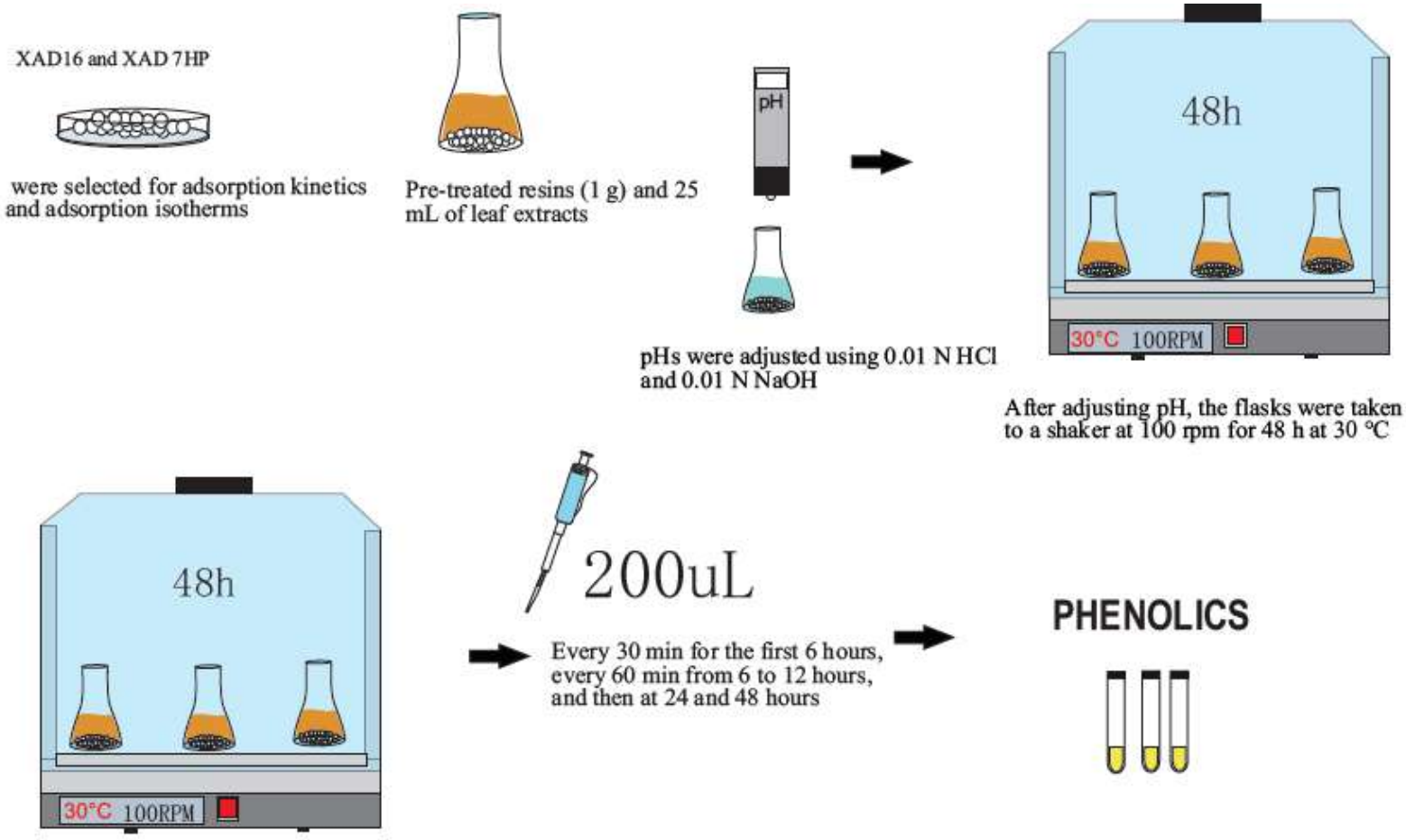


The pH was adjusted to 3.0

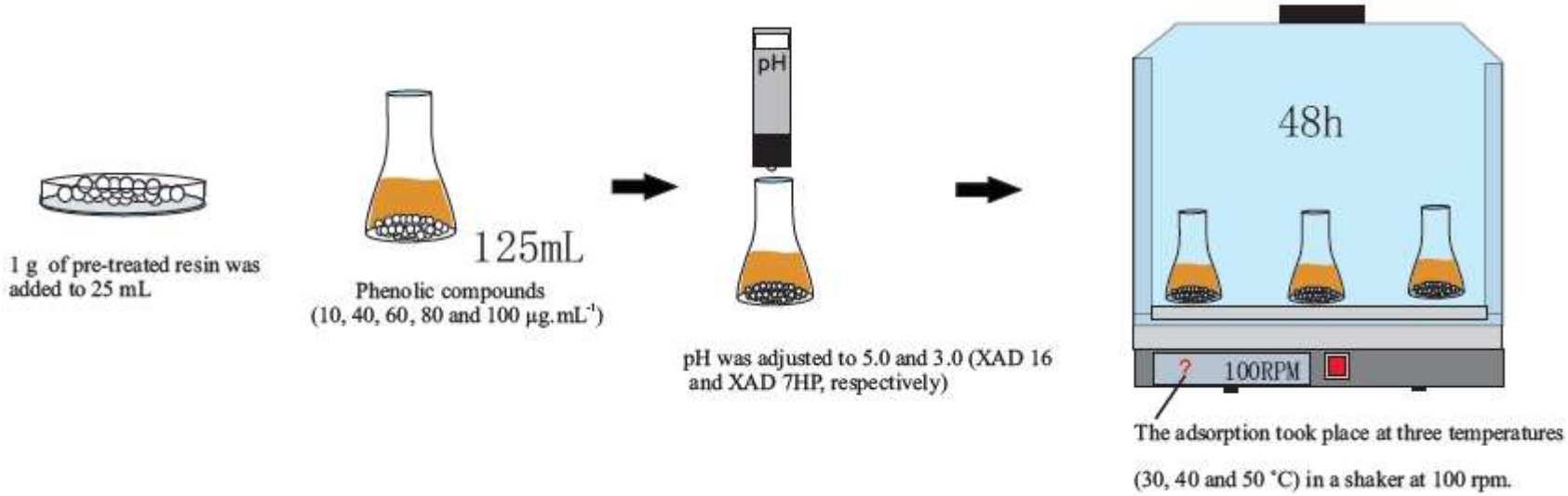


The Flasks were Kept in a shaker at 100 rpm for 24 h at 50 °C

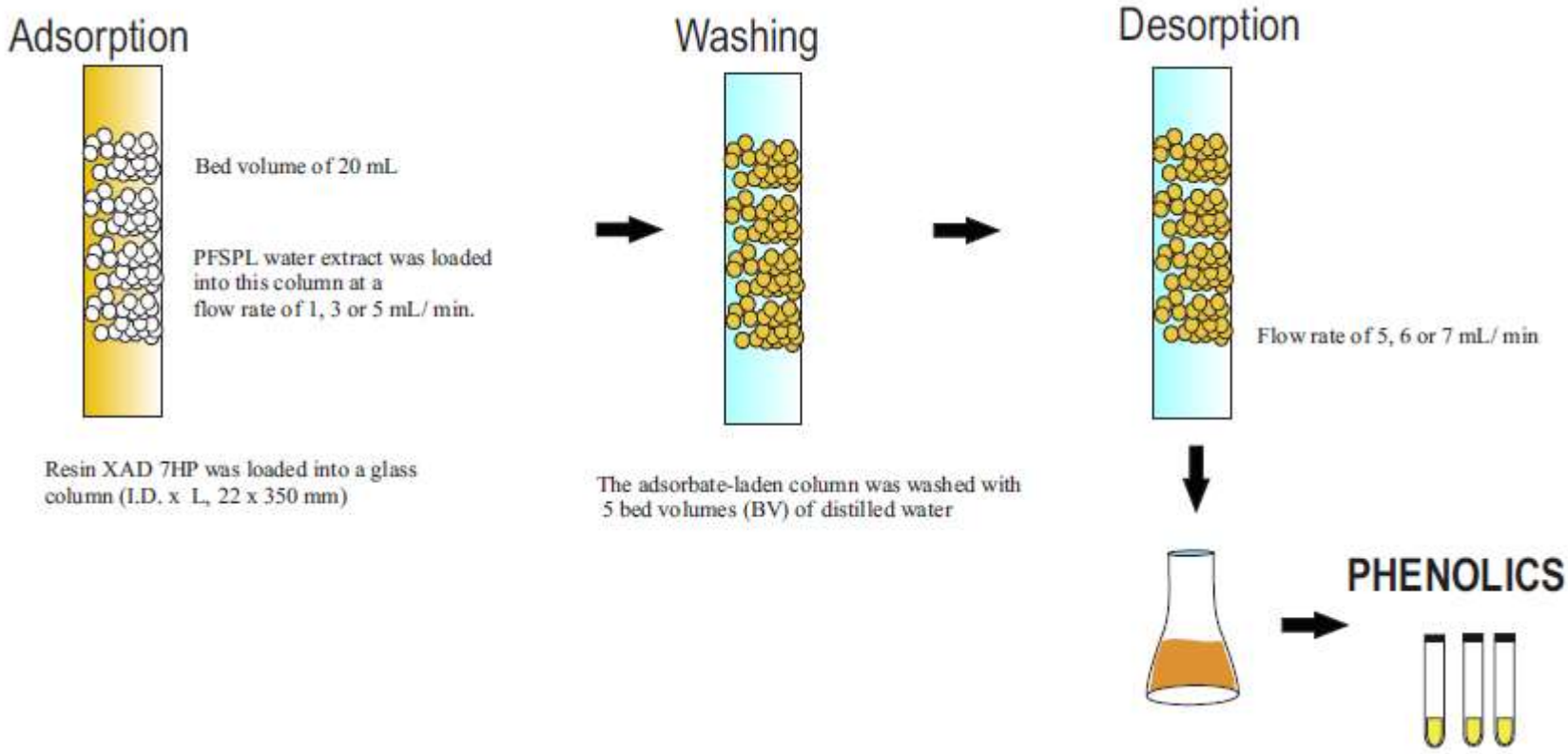
2.3. Adsorption kinetics tests



2.4. Adsorption isotherms



2.5. Dynamic adsorption and desorption tests



ANEXO C

3. Metodologias detalhadas utilizadas no Capítulo IV

3.1. Acidity index

Five grams of sample were weighted in a 250 ml Erlenmeyer flask and added to 50 ml of solvent and 0.5 ml of alcohol solution of phenolphthalein. The mixture was shaken until complete dissolution of the sample and titrated with 0.01 M NaOH solution by shaking the Erlenmeyer flask until a color appear for at least 30 seconds. Acidity index can be expressed as mg KOH per gram of sample and calculated according to the equation bellow:

$$AI = \frac{V*M*f*0.0561*10^3}{W} \qquad \text{Equation (6)}$$

Where:
V= volume (mL) of NaOH solution
M= molarity of NaOH solution
f = correction factor
w = weight of sample (g)

3.2. Peroxide Index

Five grams of sample were wheigted in a 250 mL erlenmeyer flask and added to 30 mL of acetic acid and chloroform mixture (3 + 2) and stirred. until the sample. After that, 0.5 mL of saturated solution of KI was added with shaking. 30 mL of distilled water and 1 mL of starch solution were added and the mixture was titrated with 0.01M of $Na_2S_2O_3$ solution until the disappearance of blue color. The results were expressed as mEq of peroxide per 1000 grams os sample, following the Equation 7:

$$PI = \frac{V*M*f*10^3}{W} \qquad \text{Equation (7)}$$

Where:
V= volume (mL) of $Na_2S_2O_3$ solution
M= molarity of $Na_2S_2O_3$ solution
f = correction factor
w = weight of sample (g)

3.3. Kreiss Index

Two mL os sample were transfered to a 25mL tube and added to 2 mL of concentrated HCl and stirred for 30 seconds. Two mL of 0.1% floroglucin solution in ether ethyl were added and shaken for 30 seconds more. The mixture was allow to stand for 10 seconds to separate the phases. In the presence of rancidity the inferior phase will present pink or red color.

3.4. p-anisidine Value

The PA value was determined by dillution of 0.5 g of sample with 25 mL of isooctane and adding PA (0.25% in glacial acetic acid) (AOCS, 1998). PA value was measured at 350 nm in a spectrophotometer.

$$IA = 10 * \frac{1.2*Abs}{w} \qquad \text{Equation (8)}$$

Where:
Abs= absorbance at 350 nm
w= weight of the sample

ANEXO D

4. Fotografias das análises referentes ao Capítulo IV

4.1. Figuras referentes ao processo de oxidação forçada das amostras analisadas

Na figura, pode-se observar a diferença na coloração dos óleos. A amostra A6 (óleo sem adição de antioxidantes) encontra-se com uma coloração mais escura que os demais, indicando que a ausência dos antioxidantes favoreceu a oxidação lipídica e consequentemente, alteração de cor. As amostras B6 e C6 são adicionadas dos extratos de folhas de batata-doce em diferentes concentrações.

4.2. Resultado da análise de determinação do índice de *Kreiss*

As figuras se referem às análises de Kreiss. A coloração marrom do conteúdo dos tubos de ensaio indica um resultado negativo, o que significa que não houve formação de compostos provenientes da oxidação lipídica. Em contraste, a coloração vermelha indica um resultado positivo para a reação, indicando a presença de produtos secundários de oxidação.