UNIVERSIDADE ESTADUAL DE CAMPINAS

FACULDADE DE ENGENHARIA DE ALIMENTOS

ANGELA MARIA FARIAS CAMPOMANES DUARTE

PRESSURIZED FLUID EXTRACTION OF BIOACTIVE COMPOUNDS: POLYPHENOLS AND THIOSULFINATES

EXTRAÇÃO COM FLUIDOS PRESSURIZADOS DE COMPOSTOS BIOATIVOS: POLIFENÓIS E TIOSULFINATOS

Campinas

2016

ANGELA MARIA FARIAS CAMPOMANES DUARTE

PRESSURIZED FLUID EXTRACTION OF BIOACTIVE COMPOUNDS: POLYPHENOLS AND THIOSULFINATES

EXTRAÇÃO COM FLUIDOS PRESSURIZADOS DE COMPOSTOS BIOATIVOS: POLIFENÓIS E TIOSULFINATOS

Thesis presented to the Faculty of Food Engineering of the University of Campinas in partial fulfillment of the requirements for the degree of Doctor in Food Engineering

Tese de doutorado apresentada à Faculdade de Engenharia de Alimentos da Universidade Estadual de Campinas como parte dos requisitos exigidos para a obtenção do titulo de Doutora em Engenharia de Alimentos

Supervisor/Orientador: Prof[a]. Dr[a]. Maria Angela de Almeida Meireles Petenate

ESTE EXEMPLAR CORRESPONDE À VERSÃO FINAL DE TESE DEFENDIDA PELA ALUNA ANGELA MARIA FARIAS CAMPOMANES DUARTE E ORIENTADA PELA PROF[A]. DR[A]. MARIA ANGELA DE ALMEIDA MEIRELES PETENATE

Campinas

2016

Agência(s) de fomento e nº(s) de processo(s): CAPES, 5817-11-0

Ficha catalográfica
Universidade Estadual de Campinas
Biblioteca da Faculdade de Engenharia de Alimentos
Claudia Aparecida Romano - CRB 8/5816

D85e

Duarte, Angela Maria Farias Campomanes, 1985-
Extração com fluidos pressurizados de compostos bioativos : polifenóis e tiosulfinatos / Angela Maria Farias Campomanes Duarte. – Campinas, SP : [s.n.], 2016.

Orientador: Maria Angela de Almeida Meireles Petenate.
Tese (doutorado) – Universidade Estadual de Campinas, Faculdade de Engenharia de Alimentos.

1. Extração com líquido pressurizado. 2. Extração com fluido supercrítico. 3. Compostos bioativos. 4. Polifenóis. 5. Tiosulfinatos. I. Petenate, Maria Angela de Almeida Meireles. II. Universidade Estadual de Campinas. Faculdade de Engenharia de Alimentos. III. Título.

Informações para Biblioteca Digital

Título em outro idioma: Pressurized fluid extraction of bioactive compounds : polyphenols and thiosulfinates
Palavras-chave em inglês:
Pressurized liquid extraction
Supercritical fluid extraction
Bioactive compounds
Polyphenols
Thiosulfinates
Área de concentração: Engenharia de Alimentos
Titulação: Doutora em Engenharia de Alimentos
Banca examinadora:
Maria Angela de Almeida Meireles Petenate [Orientador]
Christianne Elisabete da Costa Rodrigues
Guilherme José Maximo
Losiane Cristina Paviani
Priscilla Carvalho Vegui
Data de defesa: 26-04-2016
Programa de Pós-Graduação: Engenharia de Alimentos

COMISSÃO EXAMINADORA

Profª. Drª. Maria Angela de Almeida Meireles Petenate
(ORIENTADORA) – DEA/FEA/UNICAMP

Profª. Drª. Christianne Elisabete da Costa Rodrigues
(MEMBRO) – FZEA/USP

Prof. Dr. Guilherme José Maximo
(MEMBRO)) – DEA/FEA/UNICAMP

Drª. Losiane Cristina Paviani
(MEMBRO) – AGROPALMA S/A

Profª. Drª. Priscilla Carvalho Veggi
(MEMBRO) – DCET/UNIFESP

Profª. Drª. Miriam Verginia Lourenço
(SUPLENTE) – UNAERP

Dr. Rodrigo Nunes Cavalcanti
(SUPLENTE) – DEA/FEA/UNICAMP

Drª. Vanessa Martins da Silva
(SUPLENTE) – DEA/FEA/UNICAMP

A Ata da defesa com as respectivas assinaturas dos membros encontra-se no processo de vida acadêmica do aluno.

Dedico especialmente esta tese a Mario Enrique,
meu esposo, amigo e companheiro,
pela paciência, compreensão, amor e por ser como ele é;
pela confiança depositada em mim
e por fazer parte desta etapa maravilhosa.

Aos meus pais, Angel e Carola,
tudo isto é graças a vocês.

Aos meus irmãos, Ernesto, Ana Paula e Gonzalo
pelo carinho e energia à distância.

AGRADECIMENTOS

A Deus, a força espiritual.

À minha orientadora, Maria Angela de Almeida Meireles, pela oportunidade, suas orientações, sugestões e paciência.

Ao programa de Pós-Graduação em Engenharia de Alimentos da Faculdade de Engenharia de Alimentos da Universidade Estadual de Campinas (UNICAMP).

À CAPES/ PEC-PG pelo financiamento deste trabalho através da concessão da bolsa de estudos de doutorado.

À FAPESP pelo apoio financeiro para a realização deste trabalho através dos projetos 2012/10685-8 e 2013/04304-4.

A todos que compõem o Grupo LASEFI, pelos treinamentos, discussões e apoio técnico-científico.

Ao Ari, técnico do LASEFI, pela presteza, paciência e amizade e aos secretários da Pós-Graduação e do departamento de Engenharia de Alimentos, sempre muito solícitos.

Aos meus amigos e colegas de laboratório Carol, Rodrigo, Renata, Sylvia, Juan Felipe, Pedro, Abel, Tahmasb, Adina, Júlio, Gislaine, Eric e todos aqueles que contribuíram de alguma maneira com meu trabalho.

RESUMO

Nos últimos anos, resíduos do processamento de alimentos vêm sendo considerados e utilizados como fontes de baixo custo para a obtenção de compostos bioativos. Os compostos bioativos, entre eles os polifenóis e tiosulfinatos, são encontrados em pequenas concentrações nas plantas e são utilizados na indústria de alimentos, cosmética e farmacêutica já que acrescentam propriedades sensoriais e farmacológicas interessantes aos produtos onde são aplicados. A grande incidência de doenças degenerativas e a crescente exigência de qualidade dos extratos estão promovendo o desenvolvimento de tecnologias que visem a obtenção mais eficiente e sustentável de extratos vegetais. Os processos de extração que utilizam fluidos pressurizados como solvente de extração (Extração com Líquido Pressurizado - PLE e Extração com Fluido Supercrítico - SFE) têm se mostrado mais eficientes, respeitosos ao meio ambiente e economicamente viáveis quando comparados aos métodos convencionais. Nesse contexto, este tese visou estudar a obtenção de polifenóis e tiosulfinatos a partir de sabugo e grãos do milho roxo (*Zea mays* L.), semente de piquiá (*Caryocar villosum*), semente de açai (*Euterpe precatória*) e alho (*Allium sativum* L.) via PLE, e casca de abiú (*Pouteria caimito*) via SFE, utilizando como solventes de extração etanol pressurizado e dióxido de carbono supercrítico nos processos PLE e SFE, respectivamente. Inicialmente foi feita uma revisão bibliográfica sobre os fundamentos da PLE e SFE e sua aplicação na obtenção de polifenóis e tiosulfinatos. Na sequencia, a etapa de extração foi realizada e os efeitos das variáveis de extração sobre o rendimento e composição dos extratos foram avaliados. Em seguida, a viabilidade econômica do processo PLE na obtenção de compostos bioativos foi avaliada utilizando o simulador SuperPro Designer 8.5 ®. O processo PLE mostrou-se um processo eficiente na obtenção de polifenóis e tiosulfinatos uma vez que extratos altamente concentrados foram obtidos utilizando temperaturas e pressões moderadas, curtos períodos de extração e baixo consumo de etanol. Por outro lado, SFE de casca de abiu produz extratos com baixo potencial bioativo e com presença de polímeros. Assim, a SFE pode ser usada como pré-tratamento para melhorar a extração de compostos bioativos a partir de cascas de abiu através do uso de outras técnicas como PLE. Através da análise econômica, o processo PLE mostrou-se um processo economicamente viável para a obtenção de compostos bioativos a partir de sabugo e grãos de milho roxo.

Palavras-chave: *extração com líquido pressurizado, extração com fluido supercrítico, compostos bioativos, polifenóis, tiosulfinatos.*

ABSTRACT

In recent years, food processing wastes have been considered and used as low–cost sources for obtaining bioactive compounds. The bioactive compounds, including polyphenols and thiosulfinates, are found in low concentrations in plants and are used in the food, cosmetics and pharmaceutical industries due to add interesting sensorial and pharmacological properties to products which are applied. The high incidence of degenerative diseases and the growing demand for quality of extracts are promoting the development of more efficient technologies that aim the production of plant extracts in a sustainable manner. Extraction processes which use pressurized fluids as extraction solvent (Pressurized Liquid Extraction – PLE and Supercritical Fluid Extraction - SFE) have shown to be more efficient, environmentally friendly and economically viable when compared to conventional extraction methods. In this context, this thesis aimed to study the obtaining of polyphenols and thiosulfinates from purple corn waste (*Zea mays* L.), piquia seeds (*Caryocar villosum*), assai seeds (*Euterpe precatória*) and garlic (*Allium sativum* L.) via PLE, and abiu peels (*Pouteria caimito*) via SFE, using as extraction solvents pressurized ethanol and supercritical carbon dioxide in the PLE and SFE processes, respectively. Initially, a review about fundamentals of PLE and SFE and its applications for obtaining polyphenols and thiosulfinates was performed. Afterwards, the extractions were performed and the effects of extraction parameters on the global yield and the extracts composition were evaluated. Then, the economic feasibility of a PLE process for obtaining bioactive compounds was evaluated using the software SuperPro Designer 8.5®. PLE proved to be an efficient extraction process for obtaining polyphenols and thiosulfinates because highly concentrated extracts were obtained by using moderate pressures and temperatures, short extraction times and low ethanol consumption. SFE of abiu peel produced extracts with low bioactive potential and with presence of polymers. Thus, SFE can be used as a pretreatment to improve the extraction of bioactive compounds from abiu peels through the use of other extraction techniques such as PLE. From economic analysis, PLE proved to be an economically feasible process for obtaining bioactive compounds from purple corn waste.

Keywords: *pressurized liquid extraction, supercritical fluid extraction, bioactive compounds, polyphenols, thiosulfinates.*

LISTA DE FIGURAS

LISTA DE TABELAS

LISTA DE ABREVIATURAS E SIGLAS

AA	Atividade antioxidante
$\boldsymbol{Area_{Allicin}}$	Área do pico da alicina (*Area of the allicin peak*)
$\boldsymbol{Area_{IS}}$	Área do pico do padrão interno (*Area of the internal standard peak)*
$\boldsymbol{C_{Allicin}}$	Concentração de alicina (*Allicin concentration*)
$\boldsymbol{C_{IS}}$	Concentração de padrão interno (*Concentration of the internal standard*)
CE	Equivalentes de catequina
COL	Custo de mão de obra (*Cost of operating labor*)
COM	Custo de manufatura (*Cost of manufacturing*)
CRM	Custo de matéria prima (*Cost of raw materials*)
CUT	Custo de utilidades (*Cost of utilities*)
CWT	Custo de tratamento de resíduo (*Cost of waste treatment*)
DET	Tempo de extração dinâmica (*Dynamic extraction time*)
FCI	Custo fixo de investimento (*Fixed capital of investment*)
GAE	Equivalentes de ácido gálico
GRAS	Geralmente reconhecido como seguro (*Generally recognized as safe*)
HPLC	Cromatografia líquida de alta eficiência (High-performance liquid chromatography)
$\boldsymbol{m_{Extract}}$	Massa de extrato (*Mass of extract*)
$\boldsymbol{m_{RM}}$	Massa de matéria prima (Mass of raw material)
$\boldsymbol{m_{sample}}$	Massa de amostra (*Sample mass*)
N/A	Não aplicável (*Not applicable*)
OEC	Curva global de extração (*Overall extraction curve*)
PCC	Sabugo de milho roxo (*Purple corn cobs*)
PCW	Resíduo de milho roxo (*Purple corn waste)*
PLE	Extração com líquido pressurizado (*Pressurized liquid extraction)*

QE	Quantidade de extratores (*Quantity of extractors*)
S/F	Razão de massa de solvente e massa de matéria prima (*solvent to feed mass ratio*)
SC	Modo semi contínuo (*Semi-continuous mode*)
SET	Tempo de extração estática (*Static extraction time*)
SFE	Extração com fluido supercrítico (*Supercritical fluid extraction*)
SFE+C	Extração com fluido supercrítico e cossolvente (*Supercritical fluid extraction with cosolvent*)
SSC	Modo contínuo em regime permanente (*Steady-state continuous mode*).
SSC-E	Modo contínuo em regime permanente eficiente (*Steady-state continuous efficient mode*)
SSC-IE	Modo contínuo em regime permanente não eficiente (*Steady-state continuous inefficient mode*)
$\mathbf{T_g}$	Temperatura de transição vítrea (*Glass-transition temperature*)
USC	Modo contínuo em regime transiente (*Unsteady-state continuous mode*)
$\boldsymbol{X_{0,S/F}}$	Rendimento global de extração (*Global yield of extraction*)
$\boldsymbol{V_{IS}}$	Volume da mistura de padrão interno e amostra (*Volume of the mixture of the internal standard and the sample*)

SUMÁRIO

Capítulo 1

Introdução e objetivos

1. Introdução

Neste trabalho foi investigado o processo de extração de polifenóis e tiosulfinatos a partir de diversas matrizes vegetais: milho roxo (*Zea mays* L.), piquiá (*Caryocar villosum*), açaí (*Euterpe precatória*), alho (*Allium sativum* L.) e abiu (*Pouteria caimito*) utilizando técnicas de extração que utilizam um fluido pressurizado como solvente: extração com líquido pressurizado (PLE) e extração com fluido supercrítico (SFE). O estudo foi realizado no LASEFI (Laboratório de Tecnologia Supercrítica: Extração, Fracionamento e Identificação de Extratos Vegetais).

A primeira etapa deste trabalho apresenta uma revisão de literatura sobre os fundamentos das técnicas de PLE e SFE, os parâmetros que influenciam na eficiência dos processos e sua aplicação na obtenção de compostos bioativos: polifenóis e tiosulfinatos. Posteriormente, na etapa de extração, são apresentados os resultados da obtenção de polifenóis e tiosulfinatos a partir de resíduo de milho roxo, semente de piquiá, semente de açaí, alho e casca de abiú através das técnicas de PLE e SFE, usando como solventes de extração etanol e dióxido de carbono supercrítico, respectivamente. Adicionalmente, uma análise econômica do processo de PLE de polifenóis foi realizada a fim de avaliar sua viabilidade econômica.

Trabalhos anteriores realizados pelo nosso grupo de pesquisa demonstraram que em relação às técnicas convencionais de extração, a PLE é a técnica mais eficiente para a obtenção de compostos bioativos de caráter polar, sensíveis à temperatura, tais como os polifenóis, devido ao o uso da alta pressão e temperatura moderada que permitem uma extração rápida sem promover sua degradação (Cardenas-Toro et al., 2015; Osorio-Tobón et al., 2014, Veggi et al., 2014). Assim também, a partir de diversos trabalhos de pesquisa realizados pelo nosso grupo de pesquisa concluiu-se que dentre as técnicas de extração utilizadas para a obtenção de compostos bioativos de polaridade baixa ou intermediaria, tais como alguns polifenóis, a SFE se destaca devido ao uso de temperatura moderada e pressão alta que permitem uma rápida extração sem promover a degradação dos compostos de interesse. Adicionalmente, a SFE permite a obtenção de um extrato livre de solvente.

Por outro lado, a literatura indica que a SFE é a técnica mais recente e eficiente já aplicada para a obtenção de tiosulfinatos (Valle et al., 2012; Raybak et al., 2004). Porém, os tiosulfinatos são moléculas bastante instáveis não só às altas temperaturas e presença de oxigênio, entre outros fatores, mas também em solventes orgânicos não polares (Ilić et al.,

2011). Assim, aceites vegetais e hexano são solventes que oferecem baixo rendimento de extração devido principalmente ao alto nível de instabilidade dos tiosulfinatos (Fujisawa et al., 2008). Portanto, o desenvolvimento de processos de extração que visem o aumento do rendimento de tiosulfinatos sem promover sua degradação é necessário.

Devido ao etanol se mostrar como um solvente bastante favorável para a extração dos polifenóis (alta solubilidade e alta estabilidade) (Moldovan et al., 2012), pela sua classificação GRAS (*Generally recognized as safe*) e já que seu uso é permitido na indústria de alimentos, este foi selecionado como solvente na PLE. Enquanto, na SFE foi utilizado dióxido de carbono como solvente de extração uma vez que é inerte, possui classificação GRAS, não é inflamável nem tóxico, tem baixo custo, etc. Os tiosulfinatos foram extraídos via PLE utilizando etanol como solvente de extração uma vez que os tiosulfinatos são mais estáveis em solventes orgânicos polares (Ilić et al., 2011). Adicionalmente, a diferencia do metanol o qual já foi usado na obtenção de tiosulfinatos (González et al 2007), o etanol permitiria o uso dos extratos na industria de alimentos.

Resíduos do processamento do milho roxo, açaí, piquiá e abiú foram selecionados como matérias primas para a obtenção de polifenóis devido ao seu baixo custo, uma vez que são resíduos da indústria, e porque seu potencial bioativo ainda não foi amplamente explorado. Por outro lado, alho *in natura* foi selecionado como matéria prima para a obtenção de tiosulfinatos uma vez que representa uma das maiores fontes deste grupo de compostos.

A fim de determinar a viabilidade econômica da PLE na obtenção de compostos bioativos, o resíduo de milho roxo foi escolhido como matéria prima já que é obtido em grandes quantidades a partir do processamento de suco e porque possui, dentre as matéria primas estudadas, maior informação quanto à composição química e aplicação de processos de extração; o que permite fazer aumento de escala do processo e comparar a qualidade dos extratos produzidos via PLE com os encontrados na literatura, determinando a viabilidade econômica do processo.

Os polifenóis foram selecionados como compostos alvo de obtenção devido ao seu efeito antioxidante, antiviral, anticancerígeno, antibacteriano e anti-inflamatório, os quais são amplamente reportados na literatura (An-Na et al., 2014), e que permitem seu uso na indústria de alimentos, cosmética e farmacêutica. Assim também, os tiosulfinatos foram selecionados devido à sua propriedade antibacteriana, antiparasitária, antioxidante, antifúngica e anticancerígena (Mane et al., 2011) e o crescente interesse da indústria de

alimentos para uso como conservante natural e substituto de sódio na formulação de alimentos.

Em função da funcionalidade e importância econômica desses dois grupos de compostos bioativos, este trabalho teve como objetivo estudar a obtenção de polifenóis e tiosulfinatos a partir de diversas matrizes vegetais via PLE e SFE utilizando como solventes de extração etanol pressurizado e dióxido de carbono supercrítico, respectivamente, a fim de produzir extratos de alto valor econômico, ricos em polifenóis e tiosulfinatos e que possam ser amplamente utilizados na indústria de alimentos.

2. Objetivos

2.1. Objetivo geral

Avaliar o processo de extração de polifenóis e tiosulfinatos via PLE, através do rendimento global, composição química e custo de manufatura dos extratos.

2.2. Objetivos específicos

- Analisar o estado da arte da extração de polifenóis e tiosulfinatos a partir do uso de fluidos pressurizados;
- Determinar a condição ideal do processo PLE de resíduo de milho roxo para maior rendimento, considerando os seguintes parâmetros: temperatura (313, 323 e 333 K), pressão (2, 4 e 6 MPa) e tempo de extração estática (5, 10 e 15 min);
- Determinar a condição ideal do processo PLE de semente de açaí para maior rendimento, considerando os seguintes parâmetros: temperatura (313, 323 e 333 K) e pressão (2, 4 e 6 MPa);
- Determinar a condição ideal do processo PLE de semente de piquiá para maior rendimento, considerando os seguintes parâmetros: temperatura (313, 323 e 333 K), pressão (2, 4, 6, 8 e 10 MPa) e razão entre a massa de solvente e a massa de matéria prima (5, 10, 15, 20 e 25);
- Selecionar uma condição de processo PLE de alho para maior rendimento de alicina considerando as informações obtidas a partir do estado da arte;
- Analisar o teor de polifenóis totais, flavonóides totais, antocianinas, alicina e atividade antioxidante dos extratos;
- Realizar a análise econômica do processo PLE de milho roxo utilizando o simulador comercial SuperPro Designer 8.5®.

3. Estrutura do trabalho

A tese está dividida em 7 capítulos, os quais apresentam o desenvolvimento da pesquisa. Estes capítulos estão compostos pela revisão bibliográfica, artigos publicados, artigos submetidos para publicação e os que ainda serão submetidos. No **Capítulo 1 – Introdução e objetivos** é apresentada de maneira geral o tema principal deste trabalho, o objetivo geral da pesquisa e os objetivos específicos que permitirão atingir o desenvolvimento pleno do trabalho. Na Figura 1.1 são apresentadas as etapas que foram realizadas durante o doutorado.

Com o objetivo de compreender os fundamentos dos processos de extração com fluido pressurizado, foi escrito o **Capítulo 2 – Revisão Bibliográfica.** Este capítulo apresentará o estado da arte dos fundamentos dos processos PLE e SFE e suas aplicações na extração de compostos bioativos, especificamente na extração de polifenóis e tiosulfinatos. Neste capítulo foram estabelecidos os principais parâmetros que influenciam os processos de PLE e SFE. Esta primeira etapa teve como objetivo discutir os fundamentos das técnicas que emprega fluidos pressurizados na extração de compostos bioativos, focando naqueles processos cujo objetivo é obter extratos ricos em polifenóis e tiosulfinatos.

O **Capítulo 3 - Pressurized liquid extraction of anthocyanins from purple corn (*Zea mays* L.) waste: technical and economic viability** apresenta um estudo do processo de extração de antocianinas via PLE usando como matéria prima resíduo de milho roxo e etanol como solvente de extração. O efeito da temperatura, pressão e tempo de extração estática foi avaliado utilizando um planejamento fatorial completamente aleatorizado. Os extratos foram analisados em relação ao rendimento global de extração e atividade antioxidante. Após a análise estatística foram estabelecidas as condições ótimas de extração. Na condição ótima, foram realizadas curvas globais de extração e a concentração de antocianinas nos extratos foi determinada. Na simulação do processo PLE, foi avaliado o efeito das características químicas dos extratos, o aumento de escala do processo e o efeito do uso de extratores de diferentes volumes no aumento de escala.

O **Capítulo 4 – Recovery of bioactive compounds from Amazonian fruit wastes via Green techniques** apresenta um estudo do processo de extração de polifenóis e flavonóides com atividade antioxidante via PLE e SFE (extração com fluido supercrítico) usando como matérias primas: sementes de açaí, sementes de piquiá e casca de abiu; e como

solventes de extração etanol e dióxido de carbono, respectivamente. O efeito da temperatura, pressão e razão entre a massa de solvente e massa de matéria prima foi avaliado. Os extratos foram analisados em relação ao rendimento global de extração, teor de polifenóis totais, teor de flavonóides totais e atividade antioxidante. Este capítulo é o resultado da parceria realizada com a Universidade Federal do Amazonas – UFAM e a Universidade de Vigo, Espanha.

No **Capítulo 5 – Allicin-rich extract obtained from garlic by pressurized liquid extraction: quantitative determination of allicin in garlic samples** é apresentado o resultado da parceria realizada com o Departamento de Tecnologia de Alimentos – DTA/FEA/UNICAMP. Este capítulo apresenta um estudo da obtenção de extrato rico em alicina, um poderoso antioxidante e antifúngico, obtido do alho via PLE. A extração foi realizada a 313 K, 6 MPa e razão entre massa de solvente e massa de matéria prima (S/F) de 1.2 usando etanol como solvente. Foi determinada a concentração de alicina no extrato PLE de alho, e em amostras comerciais de alho fresco, alho em pó e óleo de alho usando o método do padrão interno.

O **Capítulo 6 – Discussão geral** apresenta uma discussão geral dos capítulos da tese. Finalmente, o **Capítulo 7 – Conclusões gerais** apresenta os principais resultados obtidos em cada um dos capítulos apresentados neste trabalho. Adicionalmente, é apresentada a memória do período do doutorado com todos os trabalhos acadêmicos realizados.

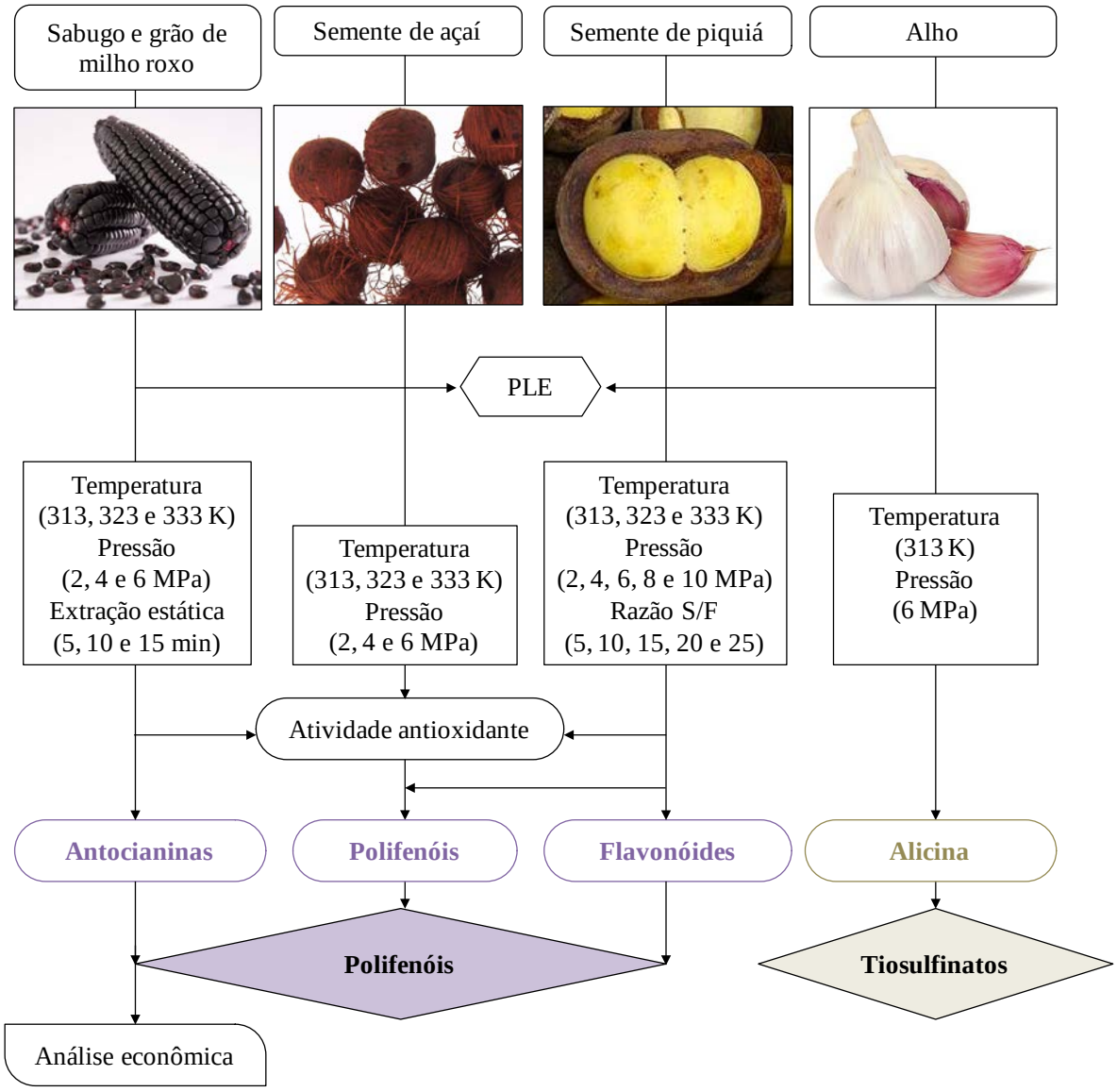


Figura 1.1. Esquema das etapas do desenvolvimento do projeto e as atividades realizadas.

Referencias

AN-NA, L., SHA, L., YU-JIE, Z., XIANG-RONG, X., YU-MING, C. & HUA-BIN, L. 2014. Resources and biological activities of natural polyphenols. *Nutrients*, 6, 6020-6047.

CARDENAS-TORO, F. P., ALCÁZAR-ALAY, S. C., COUTINHO, J. P., GODOY, H. T., FORSTER-CARNEIRO, T. & MEIRELES, M. A. A. 2015. Pressurized liquid extraction and low-pressure solvent extraction of carotenoids from pressed palm fiber: Experimental and economical evaluation. *Food and Bioproducts. Processing*, 94, 90-100.

FUJISAWA, H., SUMA, K., ORIGUCHI, K., KUMAGAI, H., SEKI, T. & ARIGA, T. 2008. Biological and chemical stability of garlic–derived allicin. *Journal of Agricultural and Food Chemistry*, 56, 4229-4235.

GONZÁLEZ, R. E., CAMARGO, A. B. & BURBA, J. L. 2007. Obtention of a quantitative secondary standard for allicin synthesis and purification. *Revista de la Facultad de Ciencias Agrarias UNCuyo*, 2, 61-70.

ILIĆ, D. P., NIKOLIĆ, V. D., NIKOLIĆ, L. B., STANKOVIĆ, M. Z., STANOJEVIĆ, L. P. & CAKIĆ, M. D. 2011. Allicin and related compounds: Biosynthesis, synthesis and pharmacological activity. Physics, Chemistry and Technology, 9, 9-20.

MANE, P., MAYEE, R. & ATRE, K. 2011. Medicinal properties of allium sativum (garlic): A review. *International Journal of Pharma Research & Development-Online*, 3, 145-150.

MOLDOVAN, B., DAVID, L., CHISBORA, C. & CIMPOIU, C. 2012. Cranberrybush (*Viburnun opulus* L.) fruit extracts. Effect of temperature, pH and storage solvent. *Molecules*, 17, 11655-11666.

OSORIO-TOBÓN, J. F., CARVALHO, P. I. N., ROSTAGNO, M. A., PETENATE, A. J. & MEIRELES, M. A. A. 2014. Extraction of curcuminoids from deflavored turmeric (*Curcuma longa* L.) using pressurized liquids: Process integration and economic evaluation. *Journal of Supercritical Fluids*, 95, 167-174.

RAYBAK, M. E., CALVEY E. M. & HARNLY, J. M. 2004. Quantative determination of allicin in garlic: Supercritical fluid extraction and standard addition of alliin", *Journal of Agricultural and Food Chemistry*, 52, 682-687.

VALLE, M., GLATZEL, V. & MARTÍNEZ, J. L. 2012. Supercritical CO_2 extraction of allicin from garlic flakes: Screening and kinetic studies. *Food Reviews International*, 45, 216-224.

VEGGI, P. C., CAVALCANTI, R. N. & MEIRELES, M. A. A. 2014. Production of phenolic-rich extracts from Brazilian plants using supercritical and subcritical fluid extraction: Experimental data and economic evaluation. *Journal of Food Engineering*, 131, 96-109.

Capítulo 2

Revisão Bibliográfica

1. Introdução

Os compostos bioativos antioxidantes vêm sendo alvo de estudo das empresas de alimentos, farmacêuticas e cosméticas devido à sua atividade biológica comprovada no combate ao envelhecimento celular e as doenças degenerativas tais como o câncer, doenças cardiovasculares, diabetes, Alzheimer, entre outras. Numerosos estudos científicos tentam explicar a origem das doenças degenerativas convergindo todas na ação dos radicais livres. Os radicais livres são substâncias químicas altamente reativas que a fim de atingir sua estabilidade, modificam outras moléculas através da subtração ou adição de um elétron, causando a desestabilização deles e resultando em novos radicais livres os quais produzirão uma reação em cadeia. Os radicais livres podem atacar proteínas e lipídios na membrana celular impedindo a célula desempenhar suas funções vitais (transporte e eliminação de resíduos, a divisão celular, etc.) ou podem atacar o DNA impedindo a replicação celular e favorecendo o envelhecimento celular (Lobo et al., 2010).

Naturalmente, o organismo humano produz radicais livres a partir dos processos de respiração, o metabolismo de alimentos e exercício físico. Da mesma maneira, a fim de combater o efeito dos radicais livres, antioxidantes são produzidos pelo organismo humano. Porém, quando no organismo se apresenta uma perda de equilíbrio entre os radicais livres e os antioxidantes, as doenças degenerativas são desenvolvidas. Adicionalmente, dos radicais livres produzidos naturalmente, estes também produzidos pela poluição ambiental, tabaco, radiação, aditivos químicos, entre outros. Desta forma, o consumo de antioxidantes se faz necessária a fim de combater o efeito dos radicais livres (Pham-Huy et al., 2008).

Os antioxidantes são substancias que impedem ou retardam a oxidação da molécula e podem ser classificados como antioxidantes endógenos, aqueles que são produzidos pelo próprio organismo e os antioxidantes exógenos, aqueles que são encontrados em pequenas concentrações em plantas e frutos e podem ser classificados pela sua estrutura química em carotenoides, polifenóis, glucosinolatos, tiosulfinatos entre outros. Além de possuir atividade antioxidante, estes compostos apresentam outras funções de proteção, por tal motivo são conhecidos como compostos bioativos (Irshad e Chaudhuri, 2002).

2. Polifenóis

Os polifenóis, também conhecidos como compostos fenólicos são os compostos bioativos mais abundantes na dieta. Estima-se que mais de 8000 compostos têm sido isolados

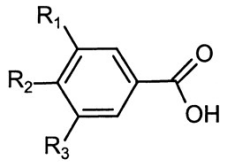
e descritos (Martinez-Valverde et al., 2000). Os polifenóis são metabólitos secundários produzidos pelas plantas em resposta à radiação UV, ao ataque de patógenos e insetos e para atrair polinizadores (Manach et al., 2004), e por esse motivo são encontrados em pequenas concentrações em frutas, hortaliças e sementes.

A concentração dos polifenóis nas plantas depende da variedade, clima, solo e condições de cultivo, enquanto a disponibilidade e concentração de polifenóis nos alimentos processados e seus subprodutos tem relação com a tecnologia aplicada durante seu processamento. Parâmetros de processo tais como temperatura, tipo de solvente, pH, presença de luz e oxigênio, presença de outros compostos fenólicos, enzimas e açucares devem ser controlados a fim de evitar a degradação dos polifenóis (Ozela et al., 2007, Shahidi e Nazck, 2004)

Os polifenóis caracterizam-se por possuir duas ou mais hidroxilas ligadas a um anel aromático, porém podem apresentar mais anéis aromáticos e hidroxilas. De acordo com a estrutura química, os polifenóis podem ser divididos em várias classes, dentre as quais se destacam a dos ácidos fenólicos e a dos flavonóides (Figura 2.1.).

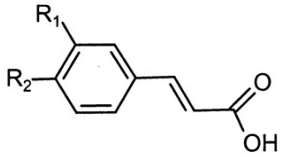
Os flavonóides possuem em sua estrutura química dois ou mais anéis aromáticos, cada um com um ou mais grupos hidroxilas ligados por uma ponte carbono e podem ser classificados em flavonas, flavanonas, flavonóis, diidroflavonóis, isoflavonas, antocianinas e chalconas (Han et al., 2007). Os ácidos fenólicos são divididos em dois grandes grupos: os derivados do ácido cinâmico e os derivados do ácido benzoico; sendo os mais representativos os ácidos gálico, clorogênico, cafeico, ferúlico, p-cumárico e gentísico. A subclasse menor é formada pelos estilbenos sendo o mais representativo o resveratrol (Rosa et al., 2010).

Ácidos hidroxibenzóicos



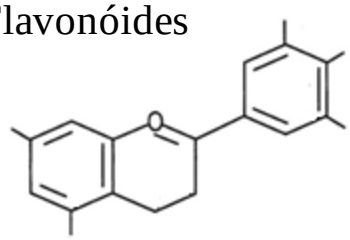
$R_1 = R_2 = OH, R_3 = H$: *ácido protocatecuico*
$R_1 = R_2 = R_3 = OH$: *ácido gálico*

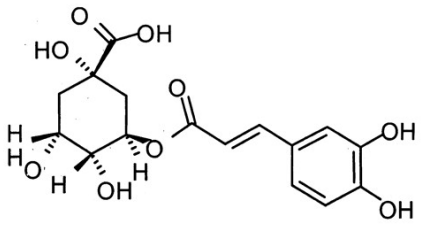
Ácidos hidroxicinâmicos



$R_1 = OH$: *ácido cumárico*
$R_1 = R_2 = OH$: *ácido cafeico*
$R_1 = OCH_3, R_2 = OH$: *ácido ferúlico*

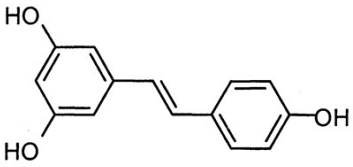
Flavonóides





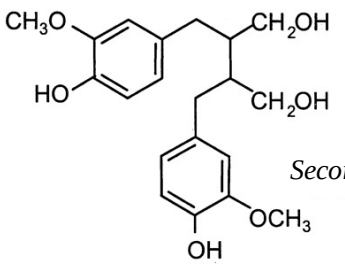
Ácido clorogênico

Estilbenos



Resveratrol

Lignanas



Secoisolariciresinol

Figura 2.1. Estrutura química dos polifenóis, adaptado de Manach et al. (2004).

Os polifenóis são os antioxidantes mais abundantes na dieta e o benefício à saúde pelo consumo de alimentos ricos em polifenóis é bastante conhecido. Estudos epidemiológicos têm demonstrado associação entre o consumo de polifenóis e uma diminuição da incidência de doenças cardiovasculares, câncer, doenças neurológicas e gastrointestinais, doenças do fígado, aterosclerose, osteoporose, obesidade, alergias e diabetes mellitus (Scalbert et al., 2005). A Figura 2.2. apresenta algumas das propriedades associadas aos polifenóis. A intensidade da atividade antioxidante dos polifenóis depende principalmente do número e posição dos grupos hidroxila presentes na molécula (Rice-Evans et al., 1997).

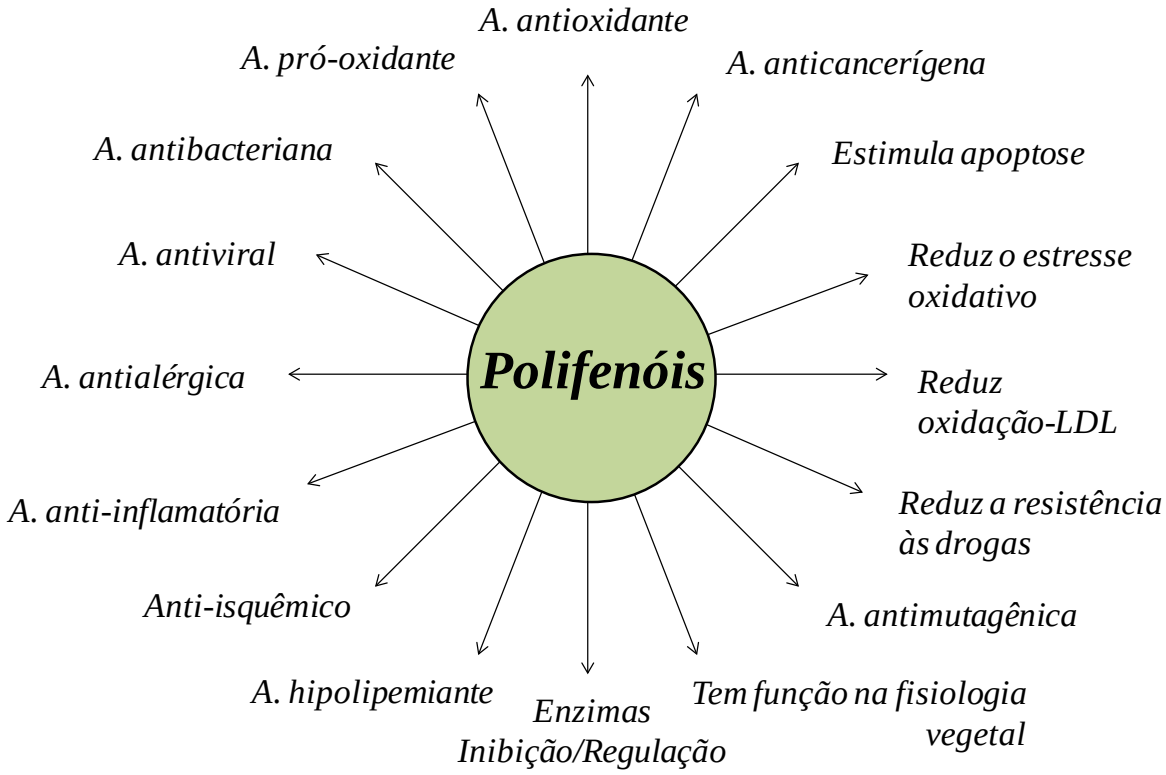


Figura 2.2. Propriedades atribuídas aos polifenóis

3. Tiosulfinatos

Os tiosulfinatos são compostos organosulfurados e consistem em dois átomos de enxofre ligados a uma molécula de oxigênio. Os tiosulfinatos são altamente reativos, isto é, transformam-se relativamente em outras substâncias de degradação (Benkeblia e Lanzotti, 2007). O alho, a cebola e outros vegetais da espécie *Allium* são fontes ricas de tiosulfinatos. Os compostos organosulfurados presentes nos vegetais da espécie *Allium* têm sido indicados como os responsáveis pelos aspectos benéficos à saúde da espécie. Entre estes tiosulfinatos presentes no alho se encontra a alicina. Estudos desenvolvidos com os vegetais da espécie *Allium*, seus extratos ou compostos organosulfurados específicos tem demonstrado seus diversos efeitos farmacológicos e biológicos tais como antimicrobiano, antibacteriano, antifúngico, antioxidante, antitumoral, antiviral e antiasmático (Lanzotti, 2006).

Os tiosulfinatos não estão presentes nos bulbos intactos, estes são formados por ação da enzima alinasa e na presença de água depois do tecido vegetal ter sido danificado (Cavallito e Bailey, 1944). A inativação da alinasa como resultado do aquecimento prolongado causa a perda da atividade antimicrobiana (Lawson, 1996).

Estudos demonstraram que os tiosulfinatos de forma individual exibem baixa atividade antimicrobiana quando comparados com o extrato cru da éspecie *Allium* (Lawson, 1998). Assim, acredita-se que algumas proteínas, saponinas e polifenóis presentes nos vegetais podem contribuir com a atividade antimicrobiana (Griffiths et al., 2002).

Na década dos anos 90, as substâncias que mostravam atividade antibacteriana, presentes na espécie *Allium*, foram identificadas. Alicina foi o primeiro composto organosulfurado que foi isolado e definido como agente antibacteriano no alho. A alicina é um notável bacteriostático e bactericida e tem demonstrado efeito contra uma grande variedade de organismos gram-negativo e gram-positivo (Borlinghaus et al., 2014). Estudos realizados sobre atividade antibacteriana dos tiosulfinatos tiveram como foco o alho e cebola, e mostraram que o alho foi mais efetivo em inibição bacteriana que a cebola uma vez que o teor de compostos sulfurados do alho é quatro vezes maior comparado com o nível encontrado nas cebolas (Lawson, 1996).

Os tiosulfinatos também possuem atividade antiparasitária. Tem sido reportado que a alicina numa concentração de 30 ug.mL^{-1} pode inibir eficientemente o crescimento de algumas parasitas (Ankri e Mirelman, 1999). A Figura 2.3. apresenta as propriedades atribuídas aos tiosulfinatos.

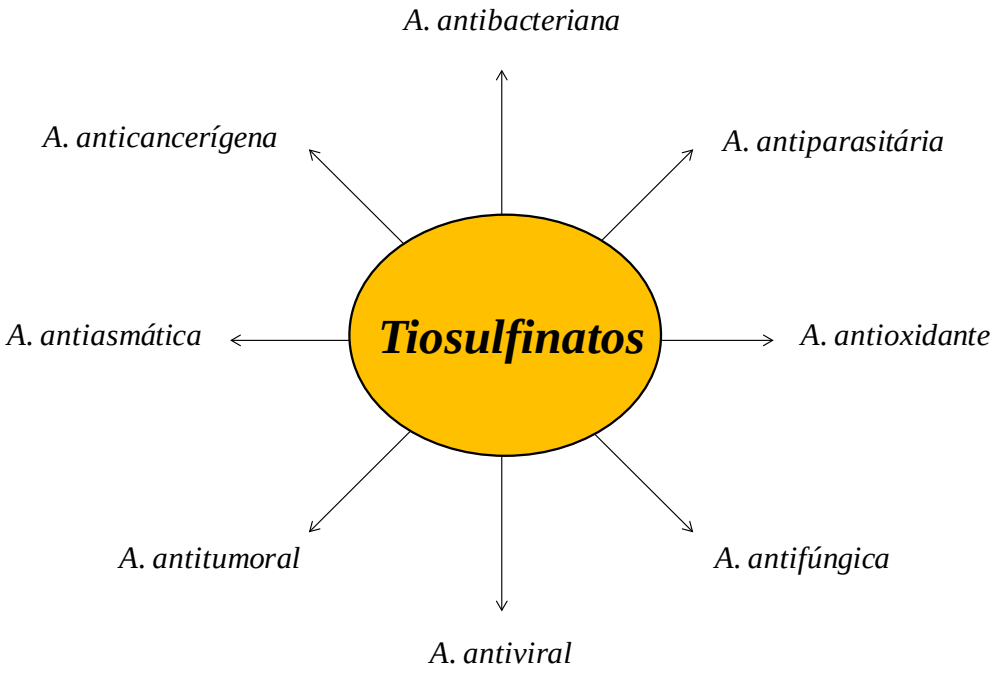


Figura 2.3. Propriedades atribuídas aos tiosulfinatos.

4. Extração com fluidos pressurizados

O processo de extração consiste em operações unitárias que visam a separação de determinados compostos a partir de uma dada matriz, através de processos químicos, físicos ou mecânicos (Cavalcanti, 2013). Existem várias técnicas de extração que podem ser aplicados para a obtenção de compostos bioativos, porém o custo, rendimento, produtividade e seletividade devem ser considerados na escolha da técnica mais adequada.

As técnicas convencionais de extração empregadas na obtenção de compostos bioativos apresentam vários inconvenientes, entre eles o alto tempo de residência e o consumo de grandes quantidades de solvente os quais são muitas vezes tóxicos, a baixa seletividade da extração e a difícil separação do solvente e extrato (Bernardo-Gil et al., 2002). Atualmente, técnicas de extração que superam os inconvenientes das técnicas convencionais e são menos nocivas ao meio ambiente estão sendo desenvolvidas e aplicadas mostrando resultados satisfatórios. A extração com líquido pressurizado (PLE) e extração com fluido supercrítico (SFE) são técnicas que utilizam como solvente de extração um fluido pressurizado que permite o aumento da eficiência de extração.

De forma geral, os processos de extração à alta pressão podem ser realizados em modo estático e/ou em modo dinâmico. A extração em modo estático é um processo em batelada que consiste em um ou mais ciclos com adição de solvente fresco entre cada ciclo. O extrator que contem a matriz vegetal é pressurizado com o solvente enquanto a válvula de saída é mantida fechada. Após a extração, a válvula é aberta liberando a mistura de extrato e solvente ao frasco coletor. No modo dinâmico, o solvente é continuamente enviado para o extrator enquanto a válvula de saída é mantida aberta (Carabias-Martinez et al., 2005, Osorio-Tobón et al., 2013).

Diferentemente das técnicas convencionais, os processos de extração realizados à alta pressão minimizam as reações de degradação por exposição à luz e oxigênio porque a extração é realizada em um extrator fechado.

4.1. Extração com líquido pressurizado

O processo de extração com líquido pressurizado (PLE - *Pressurized Liquid Extraction*), também conhecido como extração com solvente pressurizado (PSE - *Pressurized Solvent Extraction*) e extração acelerada com solvente (ASE - *Accelerated Solvent*

Extraction), tem sido usado satisfatoriamente na extração de compostos termolábeis a partir de diversas matrizes vegetais, mostrando maior eficiência de extração comparado aos métodos convencionais tais como soxhlet e maceração (Kaufmann e Christen, 2002).

PLE é um processo de extração ambientalmente amigável já que os solventes mais utilizados têm sido solventes GRAS (*Generally recognized as safe*), dentre eles etanol e água. Por tal motivo, a PLE tem sido principalmente aplicada na a obtenção de compostos bioativos de média e alta polaridade. Os polifenóis (Machado et al., 2015; Veggi et al., 2011; Paes et al., 2014; Osorio-Tobón et al., 2014; Garcia-Mendoza et al., 2015) e carotenóides (Cardenas-Toro et al., 2015; Garcia-Mendoza et al., 2015; Zaghdoudi et al., 2015) são algumas das classes de compostos bioativos que têm sido extraídos satisfatoriamente via PLE.

A Figura 2.4 apresenta o esquema básico do processo PLE. O procedimento de PLE envolve o empacotamento do leito de extração formado pela matriz vegetal. O leito pode ser formado no interior do extrator ou fora dele com auxilio de uma célula de extração feita de nylon que depois é depositada no extrator. Em seguida, pelo sistema de aquecimento, o extrator é aquecido até a temperatura de operação. A válvula de saída (*backpressure*) é mantida fechada enquanto a bomba de HPLC envia solvente para o interior do extrator, a uma vazão conhecida, até atingir a pressão de operação que é controlada pelo manômetro. No caso da extração em modo estático, o envio de solvente para o extrator é detido e a válvula de saída é aberta. No caso da extração em modo dinâmico, o solvente é enviado continuamente e a válvula de saída é aberta o suficiente para manter constante a pressão de operação. O solvente passa então pelo leito de extração solubilizando os compostos. Por último, os extratos são coletados em um frasco coletor. O processo de extração finaliza quando o tempo de extração ou a razão entre a massa de solvente e massa de matéria prima, os quais foram previamente estabelecidos, são atingidos. O solvente de extração é recuperado por evaporação.

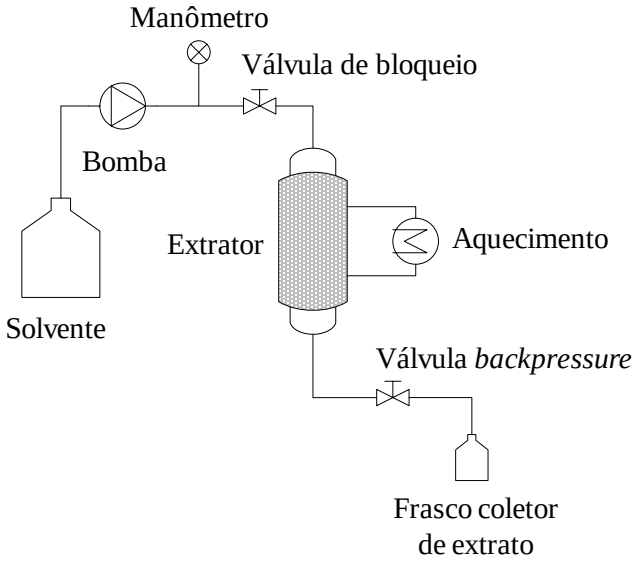


Figura 2.4. Esquema básico do processo de PLE.

4.2. Extração com fluido supercrítico

Assim como a PLE, o processo de extração com fluido supercrítico (SFE – *Supercritical Fluid Extraction*) é considerada uma técnica de extração ambientalmente amigável. A SFE utiliza um fluido supercrítico como solvente de extração, i.e. à temperatura e pressão acima do ponto crítico, T_C (temperatura crítica) e P_C (pressão crítica). Na região supercrítica, as propriedades do solvente são semelhantes às propriedades dos líquidos e gases: a densidade do solvente é próxima à dos líquidos, a viscosidade do solvente é próxima à dos gases e a difusividade do solvente é cerca de duas ordens de grandeza maior do que os líquidos (Brunner, 2005), e essas propriedades podem ser alteradas por pequenas variações de pressão e temperatura (Shi et al., 2012). Assim, os fluidos supercríticos podem dissolver uma maior gama de compostos que os gases e podem difundir-se através da matriz sólida de modo mais eficiente do que os líquidos.

O dióxido de carbono (CO_2) é o solvente mais utilizado na SFE devido à sua natureza não tóxica, baixo custo, alta disponibilidade e classificação GRAS (*geralmente reconhecido como seguro*) más principalmente pelo seu baixo ponto crítico (31.1 ºC e 7.4 MPa) que permite a extração de compostos termo sensíveis (Martínez e Vance, 2007).

A SFE e tem sido utilizada principalmente na obtenção de compostos de baixa polaridade tais como terpenoides (Uquiche et al., 2015; Albuquerque and Meireles, 2012), capsaicinoides (Aguiar et al., 2014), polímeros (Daneshvar et al., 2012) e alcalóides (Pereira

et al., 2007) porém compostos mais polares tais como carotenoides (Moraes et al., 2015; Przygoda e Wejnerowska, 2015) e polifenóis (Farías-Campomanes et al., 2013a; Farías-Campomanes et al., 2015; Cavalcanti et al., 2011) também têm sido extraídos via SFE. Para isso, o poder de solvatação do fluido supercrítico é aumentado a través da adição de pequenos volumes de um solvente orgânico polar (cossolvente).

A Figura 2.5 apresenta o esquema básico do processo SFE. O procedimento de SFE envolve o empacotamento do leito de extração formado pela matriz vegetal. Assim como a PLE, o leito pode ser formado no interior do extrator ou fora dele com auxilio de uma célula de extração feita de nylon que depois é depositada no extrator. Em seguida, o extrator é aquecido até a temperatura de operação e o CO_2 é resfriado a -10 ºC para garantir que o mesmo alcance a bomba de CO_2 na fase líquida. As válvulas de saída (bloqueio e micrométrica) são mantidas fechadas enquanto a bomba de CO_2 envia solvente para o interior do extrator até atingir a pressão de operação que é controlada pelo manômetro. Após o período de extração estática, inicia a extração dinâmica onde o solvente é enviado continuamente e as válvulas de saída são abertas o suficiente para manter constante a pressão de operação. Quando há necessidade do uso de cossolvente, o CO_2 e o cossolvente são misturados antes de entrar no extrator. O solvente passa então pelo leito de extração solubilizando os compostos. Após expansão na válvula micrométrica, o extrato é coletado em frascos de vidro imersos em banho de gelo a pressão atmosférica. O CO_2 na fase gasosa passa pelo totalizador de vazão e é então liberado para o ambiente ou recirculado. O processo de extração finaliza quando são atingidos o tempo de extração ou a razão entre a massa de solvente e massa de matéria prima que foram previamente estabelecidos.

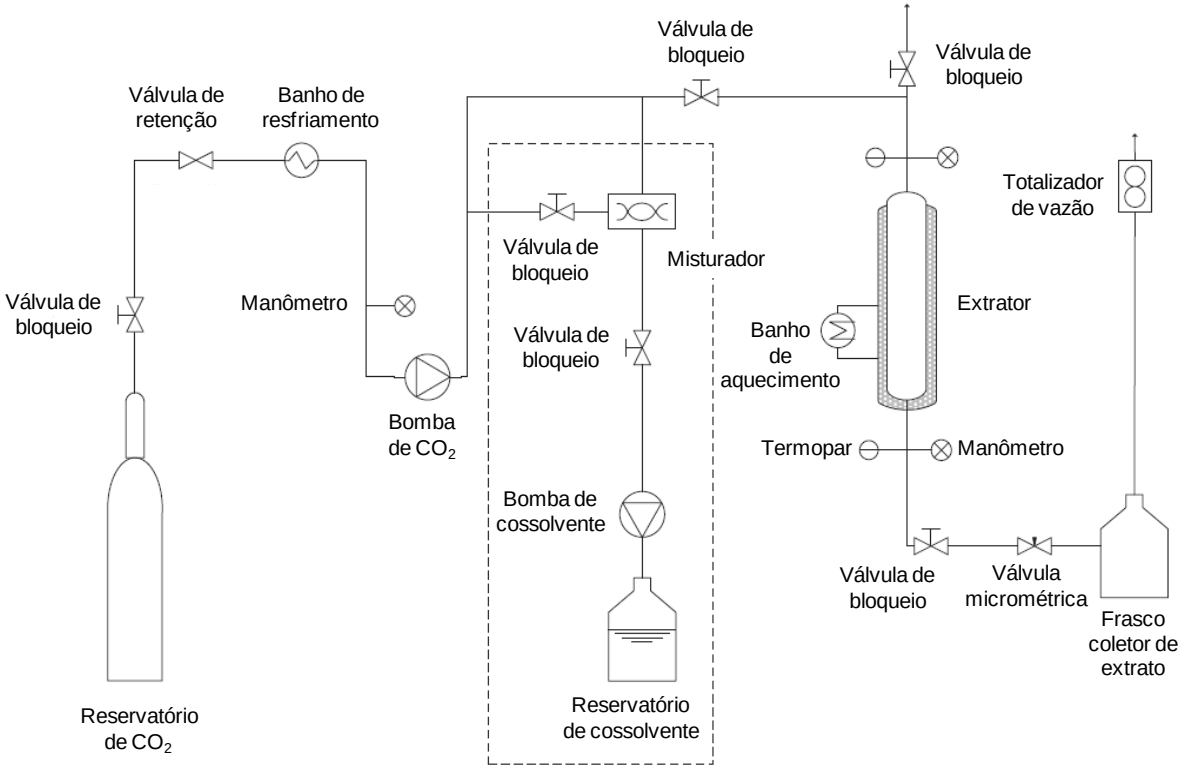


Figura 2.5. Esquema básico do processo de SFE (Farías-Campomanes et al., 2013b).

5. Parâmetros de extração

O desempenho dos processos de extração com fluido pressurizado é regido pelo solvente, temperatura, pressão, tempo de extração, a natureza da matriz vegetal e as características específicas dos compostos de interesse e sua localização no interior da matriz (Mustafa e Turner, 2011); portanto, conhecer e estabelecer a influencia destes fatores é necessário a fim de obter altos rendimentos de extração.

Na Tabela 2.1 são apresentados os fatores principais que regem os processos de extração com fluido pressurizado e seu efeito.

Tabela 2.1. Fatores que influenciam os processos de extração com fluido pressurizado.

Extração com líquido pressurizado (PLE)	***Extração com fluido supercrítico (SFE)***
Solvente	
Um aspecto fundamental na escolha do solvente é a característica de solubilidade dos compostos de interesse. Além disso, a seletividade do solvente é fundamental uma vez que	

deve minimizar a coextração de outros componentes da matriz (Mustafa e Turner, 2011). O solvente escolhido deve auxiliar na liberação de compostos a partir da matriz e ajudar na quebra das interações entre a matriz e os compostos (Runnqvist et al., 2010).

Os solventes mais utilizados na PLE são etanol, metanol e água, porém, solventes com baixo custo, seguros, sustentáveis, com baixa toxicidade e fáceis de remover ou recuperar devem ser escolhidos (Cárdenas-Toro et al., 2015; Osorio-Tobón et al., 2013; Machado et al., 2015).

O solvente mais utilizado na SFE é o dióxido de carbono supercrítico uma vez que é inerte, não inflamável, estável, atóxico, não corrosivo, apresenta-se disponível em grande quantidade e a um baixo custo, possui alto grau de pureza e ponto crítico relativamente baixo: 31,1 ºC e 7,4 MPa (Brunner et al., 2005).

Temperatura

O uso de altas temperaturas permite romper as interações entre os analitos e a matriz sólida (van der Walls, pontes de hidrogênio e atração dipolar), aumentado a solubilidade dos compostos de interesse no solvente (Richter et al., 1996).

A temperatura é um dos fatores mais críticos na PLE uma vez que afeta a eficiência e seletividade da extração. O uso de altas temperaturas de extração na PLE diminui a tensão superficial e a viscosidade do solvente aprimorando a transferência de massa.

A diminuição da tensão superficial do solvente líquido favorece o umedecimento da amostra e a formação de cavidades de solvente permitindo os analitos se dissolver mais rapidamente Enquanto, a diminuição da viscosidade do solvente líquido melhora sua penetração na partícula da matriz que resulta na melhora do processo de extração (Mustafa e Turner, 2011). Alta temperatura melhora a

A medida que a temperatura aumenta, ocorre uma diminuição relativa da densidade aumentando a difusividade do solvente e a pressão de vapor dos analitos, diminuindo seu poder de solvatação (Reverchon et al., 1995).

A baixa temperatura crítica do CO_2 permite a extração de compostos voláteis e termolábeis uma vez que os compostos de interesse não são expostos a altas temperaturas, as quais poderiam induzir a degradação (Cavalcanti, 2013).

taxa de difusão do solvente, i.e. a transferência de massa da molécula no solvente, que permite extrações mais rápidas com reduzido consumo de solvente (Osorio-Tobón et al., 2013).

Entre os principais inconvenientes do uso de alta temperatura esta a coextração de analitos que diminui a seletividade da extração e a degradação de compostos termolábeis.

Pressão

O uso de altas pressões facilita a extração de componentes localizados dentro dos poros da matriz, devido a que o aumento da pressão força o solvente penetrar na matriz vegetal em lugares onde normalmente não penetra o solvente a pressão atmosférica. Também, melhora o poder de solvatação do solvente aumentando os rendimentos de extração (Osorio-Tobón et al., 2013).

A pressão é um parâmetro que no apresenta grande influencia na PLE devido à que os fluidos não são compressíveis, portanto, mesmo sob grandes mudanças de pressão, o poder de solvatação não é significativamente afetado. Porém quando a pressão de vapor dos componentes é um fator importante para sua solubilização no solvente, a pressão pode ter um papel importante no processo de PLE (Rizvi, 2010).

O aumento da pressão permite usar o solvente a temperatura acima do seu ponto de ebulição enquanto é mantido em estado líquido melhorando seu poder de solvatação (Xynos et al., 2012).

Na região supercrítica, os gases apresentam densidade próxima à dos líquidos possuindo poder de solvatação dependente da pressão, onde pequenas variações de pressão acarretam grandes variações de solubilização (McHugh e Krukonis, 1994).

Assim, um aumento da pressão acarreta o aumento do poder de extração do solvente diminuindo sua seletividade (Reverchon et al., 1995) enquanto a alta difusividade e a baixa viscosidade, própria dos gases, permitem alto poder de penetração na matriz vegetal favorecendo a transferência de massa (Rizvi et al., 1986).

Ademais, devido à relativa baixa pressão

Dependendo da estrutura da matriz o uso de pressão alta pode ter uma influencia negativa no processo de extração, por exemplo, a pressão alta, a matriz pode ser compactada afetando o fluxo de solvente (Kronholm et a., 2007).

crítica do dióxido de carbono, as pressões de operação não são necessariamente altas, reduzindo os custos de investimento e, consequentemente, os de manufatura. (Brunner, 2005; Valle e Aguilera, 1999).

6. PLE aplicada à obtenção de polifenóis e tiosulfinatos

A eficiência da PLE frente às outras técnicas de extração, na obtenção de polifenóis, tem sido demonstrada em vários estúdios. Machado et al. (2015) estudaram a extração de polifenóis a partir de resíduos de amora-preta via PLE e as técnicas convencionais de extração soxhlet (etanol e metanol, S/F de 40, 80ºC, 5 h) e maceração (metanol acidificado, temperatura ambiente, 24 h). Os autores avaliaram a influência do tipo de solvente (água, água acidificada, etanol e etanol+água 50% v:v) e temperatura (60, 80 e 100ºC) no rendimento global, fenóis totais, antocianinas monoméricas totais e atividade antioxidante dos extratos. A pressão de operação, vazão e S/F usada nos ensaios de PLE foi 7,5 MPa, 3,0 – 3,8 $mL.min^{-1}$ e 18, respectivamente. Os resultados mostraram que o tipo de solvente e a temperatura influenciaram significativamente na obtenção dos polifenóis. PLE usando a mistura de água e etanol (50% v:v) a 100ºC forneceu os melhores resultados de teor fenóis e antocianinas no extrato e atividade antioxidante. A atividade antioxidante dos extratos foi fortemente relacionada ao teor de fenóis nos extratos e em menor intensidade ao teor de antocianinas.

Paes et al. (2014) estudaram a obtenção de extratos ricos em polifenóis, antioxidantes e antocianinas a partir dos resíduos de mirtilo (*Vaccinium myrtillus L*) via PLE usando água, etanol, acetona em diferentes proporções a 40ºC, 20 MPa, vazão de 10 mL/min e 15 min de extração dinâmica. Soxhlet com metanol, etanol e acetona (S/F de 30) foi realizado como técnica de extração convencional para comparação dos resultados de rendimento e composição de extrato. Os resultados mostraram que PLE com água e/ou etanol foi eficiente na extração de polifenóis, antioxidantes e antocianinas a partir de mirtilo. Os extratos que foram obtidos com água acidificada mostraram a menor atividade antioxidante enquanto etanol puro e etanol+água (50% v:v) produziram os extratos com maior teor de

polifenóis e atividade antioxidante. Os extratos com maior teor de antocianinas foram obtidos com 100% de água acidificada.

Osorio-Tobón et al. (2014) estudaram o processo de obtenção de um extrato etanólico rico em curcuminóides a partir de cúrcuma desaromatizada via PLE. O efeito dos parâmetros operacionais temperatura (60, 70 e 80ºC) e pressão (10, 15, 20, 30 e 35 MPa) no rendimento global e extração de curcuminóides foram avaliados. A vazão (8,4 mL/min) o tempo de extração estática (20 min), tempo de extração dinâmica (60 min) e razão S/F (9.5) foram mantidos constantes. Extração soxhlet (S/F de 9,5, 78 ºC, 6 h) e leito agitado (S/F de 9,5, 40ºC, 3 h) foram realizados para comparação. Os resultados mostraram que apenas a temperatura influenciou significativamente o rendimento de extração, enquanto que a extração de curcuminóides foi influenciada pela temperatura e a pressão Assim, o aumento da temperatura favoreceu o rendimento de extração, enquanto o aumento da pressão afetou negativamente a extração de curcuminóides. A condição ótima de extração foi determinada em 60ºC e 10 MPa. A PLE utilizou três e seis vezes menos tempo de extração que leito agitado e soxhlet, respectivamente, para produzir rendimentos similares. Por último, o custo de manufatura dos extratos produzidos via PLE foi de até 2,5 vezes menor que o custo dos extratos produzidos via soxhlet e leito agitado mostrando a viabilidade econômica do processo de PLE.

Veggi et al. (2011) compararam economicamente os processos de PLE (5 MPa, 80ºC, 9 min de extração estática, 1,67 mL de etanol.min^{-1} e 12 min de extração dinâmica), extração assistida com ultrassom (S/F de 10, frequência de 81 W, temperatura ambiente, 2 h), leito agitado (30ºC, S/F de 10, agitação a 150 rpm, 2 h) e soxhlet (S/F de 10, 8 h) para a produção de extratos de cascas de jabuticaba ricos em antocianinas. Etanol foi utilizado como único solvente de extração. Os resultados indicaram maior eficiência de extração para PLE seguido de UAE, soxhlet e leito agitado. Também, PLE mostrou-se como a técnica mais viável economicamente para a obtenção de extratos ricos em antocianinas.

Diferentemente dos polifenóis, os tiosulfinatos são moléculas bastante instáveis frente a vários fatores ambientais, por esse motivo, a literatura referente a processos que visem sua obtenção ainda é escassa. A obtenção de tiosulfinatos tem sido convencionalmente realizada a baixa pressão com uso de metanol como único solvente de extração (González et al., 2007) inviabilizando seu uso na indústria de alimentos. Nesse sentido, técnicas de extração mais respeitosas ao meio ambiente e que visem a obtenção de extratos ricos em

tiosulfinatos com potencial para ser utilizados na formulação de alimentos e outros produtos, já tem sido estudadas, entre elas a extração com dióxido de carbono supercrítico (Valle et al., 2012; Raybak et al., 2004). No entanto, até a elaboração dos artigos derivados desta tese, o uso da técnica de extração com líquido pressurizado (PLE) para a obtenção de tiosulfinatos não tinha sido aplicada; portanto, o artigo que é apresentado no capítulo 5 da presente tese e o artigo de Horita et al. (2016) apresentam resultados inovadores e não foram considerados na revisão bibliográfica.

7. SFE aplicada na obtenção de polifenóis e tiosulfinatos

A obtenção de polifenóis através da extração com dióxido de carbono supercrítico tem sido bastante explorada durante os últimos anos. As principais vantagens da SFE frente às técnicas convencionais de extração são o uso de solvente GRAS, menor tempo de extração e reduzido ou nenhum consumo de solvente orgânico. O solvente orgânico mais utilizado para modificar o poder de solvatação do dióxido de carbono é o etanol devido principalmente à sua classificação GRAS. Santos et al. (2012) estudaram o processo de extração supercrítica de polifenóis a partir de casca de eucalipto. Dióxido de carbono supercrítico puro e modificado com etanol, acetato de etila e água foram usados como solvente de extração. Os melhores resultados em termos de seletividade, teor de polifenóis, rendimento e atividade antioxidante foram obtidos com a mistura CO_2+etanol. Ademais os extratos produzidos com CO_2+etanol apresentaram maiores quantidades dos compostos de interesse que os produzidos através das técnicas convencionais de extração usando água+metanol e água+etanol como solventes. A quantidade de etanol na mistura CO_2+etanol teve influencia significativa no rendimento, teor de polifenóis no extrato e a atividade antioxidante dos mesmos, enquanto o aumento da temperatura afetou negativamente os polifenóis quantificados por HPLC, indicando a provável degradação dos mesmos. A condição ótima de extração foi determinada em 30 MPa, 70ºC, 20% de etanol e vazão de 10 g de CO_2.min^{-1}.

Pereira et al. (2016) estudaram o processo de extração supercrítica e convencional de antioxidantes e polifenóis a partir de folhas e bagas de murta-comum (*Myrtus communis* L.). Os ensaios de SFE foram realizados a 23 MPa, 45ºC e vazão de 0.3 kg de CO_2/h usando etanol como cossolvente a uma vazão de 0.09 kg/h. Hidrodestilação foi realizada como técnica convencional de extração. Os resultados mostraram que os extratos produzidos via SFE apresentaram rendimento de extração e atividade antioxidante significativamente maior. A análise de composição dos extratos mostrou que a principal diferença entre os extratos

produzidos via SFE e hidrodestilação esta relacionada como a forma conjugada do glicosídeo dos polifenóis. Assim, os extratos SFE mostraram principalmente as formas de glicosídeo (flavonóides e antocianinas), enquanto os extratos produzidos via hidrodestilação mostraram as formas de agliconas (ácidos fenólicos).

Os estudos relacionados com a extração de tiosulfinatos têm como objetivo principal a obtenção de alicina uma vez que é o tiosulfinato que confere o maior poder antioxidante, antibacteriano e antifúngico aos extratos. (Lawson, 1996). Alicina é uma molécula de baixa polaridade que com frequência é extraída com solventes polares tais como etanol e água devido à sua instabilidade em solventes orgânicos não polares (Ilić et al., 2011). Assim, hexano e aceite vegetal são solventes que proporcionam baixos rendimentos de extração de alicina (Fujisawa et al., 2008). Adicionalmente, a temperatura é um dos fatores que devem ser considerados a fim de prevenir sua degradação (Fujisawa et al., 2008; Raybak et al., 2004). Processos de secagem que envolva o uso de alta temperatura podem resultar na perda da atividade ou destruição da enzima alinase prejudicando a formação de alicina (Shi et al., 2002).

Nesse contexto, Valle et al. (2012) estudaram a extração com dióxido de carbono supercrítico de alicina a partir de flocos de alho. O processo foi avaliado em termos de rendimento de oleorresina e seletividade de extração de alicina. O fenômeno de aglomeração característico do alho foi diminuído usando alho com umidade reduzida e temperatura abaixo de 65ºC. Os resultados mostraram que o rendimento de oleorresina aumento levemente com a pressão de extração (15-45 MPa) e fortemente com a temperatura (35-65ºC) porém a concentração de alicina no extrato diminuiu conforme aumentou a temperatura. Assim, uma condição de extração intermediária de pressão e temperatura foi selecionada como a condição ótima de extração. 30 MPa, 55ºC, 4 h e S/F de 55 proporcionou rendimentos de 19 g de oleorresina per kg de matéria prima e extratos com concentração de 75 mg de alicina per kg de oleorresina.

Referencias

ALBUQUERQUE, C. L. C. & MEIRELES, M. A. A. 2012. Defatting of annatto seeds using supercritical carbon dioxide as a pretreatment for the production of bixin: Experimental, modeling and economic evaluation of the process. *Journal of Supercritical Fluids*, 66, 86-95.

AGUIAR, A. C., SANTOS, P., COUTINHO, J. P., BARBERO, G. F., GODOY, H. T. & MARTÍNEZ, J. 2014. Supercritical fluid extraction and low pressure extraction of Biquinho pepper (*Capsicum chinense*). *LWT-Food Science and Technology*, 59, 1239-1246.

ANKRI, S. & MIRELMAN, D. 1999. Antimicrobial properties of allicin from garlic. *Microbes and Infection*, 2,125-129.

BENKEBLIA N. & LANZOTTI V. 2007. Allium thiosulfinates: chemistry, biological properties and their potential utilization in food preservation. Food, 1, 193-201.

BERNARDO-GIL, M. G., RIBEIRO, M. A. & ESQUÍVEL, M. M. 2002. Produção de extratos para a indústria alimentar: uso de fluidos supercríticos. *Boletim da Sociedade Portuguesa de Biotecnologia: Métodos em Biotecnologia*, 73, 14-21.

BORLINGHAUS, J., ALBRECHT, F., GRUHLKE, M. C. H., NWACHUKWU, I. D., & SLUSARENKO, A. J. 2014. Allicin: Chemistry and Biological Properties. *Molecules*, 19, 12591-12618.

BRUNNER, G. 2005. Supercritical fluids: technology and application to food processing. *Journal of Food Engineering*, 67, 21-33.

CARABIAS-MARTINEZ, R., RODRIGUEZ-GONZALO, E., REVILLA-RUIZ, P. & HERNANDEZ-MENDEZ, J. 2005. Pressurized liquid extraction in the analysis of food and biological samples. *Journal of Chromatography A*, 1089, 1-17.

CARDENAS-TORO, F. P., ALCÁZAR-ALAY, S. C., COUTINHO, J. P., GODOY, H. T., FORSTER-CARNEIRO, T. & MEIRELES, M. A. A. 2015. Pressurized liquid extraction and low-pressure solvent extraction of carotenoids from pressed palm fiber: Experimental and economical evaluation. *Food and Bioproducts Processing*, 94, 90-100.

CAVALCANTI, R. N. 2013. Extração de antocianinas de resíduo de Jabuticaba (Myrciaria cauliflora) utilizando líquido pressurizado e fluido supercrítico: caracterização química,

avaliação econômica e modelagem matemática. Tese de Doutorado, Universidade Estadual de Campinas (UNICAMP).

CAVALCANTI, R. N., VEGGI, P. V. & MEIRELES, M. A. A. 2011. Supercritical fluid extraction with a modifier of antioxidant compounds from jabuticaba (*Myrciaria cauliflora*) byproducts: economic viability. *Procedia Food Science*, 1, 1672-1678

CAVALLITO, C. J. & BAILEY, J. H. 1944. Allicin, the antibacterial principle of *Allium sativum*. Isolation, physical properties and antibacterial action. *Journal of the American Chemical Society*, 66, 950-1951.

DANESHVAR, M., KAMALI, H., MASOOMI, M. & GHAZIASKAR, H. S. 2012. Supercritical carbon dioxide grafting of glycidyl methacrylate onto medium density polyethylene and purification of residual monomer and initiator. *Journal of Supercritical Fluids*, 70, 119-125.

FARÍAS-CAMPOMANES, A. M., ROSTAGNO, M. A. & MEIRELES, M. A. A. 2013a. Production of polyphenol extracts from grape bagasse using supercritical fluids: Yield, extract composition and economic evaluation. *Journal of Supercritical Fluids*, 77, 70-78.

FARÍAS-CAMPOMANES, A. M. & MEIRELES, M. A. A. 2013b. Pisco Bagasse as a Potential Source of Bioactive Compounds – A Review. *Recent Patents on Engineering*, 7, 41-50.

FARÍAS-CAMPOMANES, A. M., ROSTAGNO, M. A., COAQUIRA-QUISPE, J. J. & MEIRELES, M. A. A. 2015. Supercritical fluid extraction of polyphenols from lees: overall extraction curve, kinetic data and composition of the extracts. *Bioresources and Bioprocessing*, 2, 45.

FUJISAWA, H., SUMA, K., ORIGUCHI, K., KUMAGAI, H., SEKI, T. & ARIGA, T. 2008. Biological and chemical stability of garlic–derived allicin. *Journal of Agricultural and Food Chemistry*, 56, 4229-4235.

GARCIA-MENDOZA, M. P., PAULA, J. T., PAVIANI, L. C., CABRAL, F. A. & MARTINEZ-CORREA, H. A. 2015. Extracts from mango peel by-product obtained by supercritical CO_2 and pressurized solvent processes. *LWT-Food Science and Technology*, 62, 131-137.

GONZÁLEZ, R. E., CAMARGO, A. B. & BURBA, J. L. 2007. Obtention of a quantitative secondary standard for allicin synthesis and purification. *Revista de la Facultad de Ciencias Agrarias UNCuyo*, 2, 61-70.

GRIFFITHS, G., TRUEMAN, L., CROWTHER, T., THOMAS, B. & SMITH, B. 2002. Onions – a global benefit to health. *Phytotherapy Research*, 16, 603-615.

HAN, X., SHEN, T. & LOU, H. 2007. Dietary polyphenols and their biological significance. *International Journal of Molecular Sciences*, 9, 950-988.

HORITA, C. N., FARÍAS-CAMPOMANES, A. M., BARBOSA, T. S. ESMERINO, E. A., GOMEZ DA CRUZ, A., BOLINI, H. M. A., MEIRELES, M. A. A. & POLLONIO, M. A. R. 2016. The antimicrobial, antioxidant and sensory properties of garlic and its derivatives in Brazilian low-sodium frankfurters along shelf-life. *Food Research International*, 84, 1-8.

ILIĆ, D. P., NIKOLIĆ, V. D., NIKOLIĆ, L. B., STANKOVIĆ, M. Z., STANOJEVIĆ, L. P. & CAKIĆ, M. D. 2011. Allicin and related compounds: Biosynthesis, synthesis and pharmacological activity. Physics, Chemistry and Technology, 9, 9-20.

IRSHAD, M. & CHAUDHURI, P. S. 2002. Oxidant-antioxidant system: role and significance in human body. *Indian Journal of Experimental Biology*, 40, 1233-1239.

KAUFMANN, B. & CHRISTEN, P. 2002. Recent extraction techniques for natural products: microwave-assisted extraction and pressurized solvent extraction. *Phytochemical Analysis*, 13, 105-113.

KRONHOLM, J., HARTONEN, K. & RIEKKOLA, M. 2007. Analytical extractions with water at elevated temperatures and pressures. *Trends in Analytical Chemistry*, 26, 396-412.

LANZOTTI, V. 2006. The analysis of onion and garlic. *Journal of Chromatography A*, 1112, 3-22.

LAWSON, L. D. 1996. The composition and chemistry of garlic cloves and processed garlic. *In:* KOCH, H. P. & LAWSON, L. D. (eds) *Garlic: the science and therapeutic application of Allium sativum L. and related species*. Baltimore: Williams and Wilkins Press.

LAWSON, L. D. 1998. Garlic: a review of its medicinal effects and indicated active compounds. *In:* LAWSON, L. D. & BAUER, R. (eds) *Phytomedicines of Europe: chemistry and biological activity*. Washington: ACS symposium series.

LOBO, V., PATIL, A., PHATAK, A. & CHANDRA, N. 2010. Free radicals, antioxidant and functional foods: Impact on human health. *Pharmacognosy Reviews*, 4, 118-126.

MACHADO, A. P. F., PASQUEL-REÁTEGUI, J. L., BARBERO, G. F. & MARTÍNEZ, J. 2015. Pressurized liquid extraction of bioactive compounds from blackberry (*Rubus fruticosus* L.) residues: a comparison with conventional methods. *Food Research International*, 77, 675-683.

MANACH, C., SCALBERT, A. & JIMENEZ, L. 2004. Polyphenols: Food sources and bioavailability. *The American Journal of Clinical Nutrition*, 79, 727-747.

MARTÍNEZ, J. L. & VANCE, S. W. 2007. Supercritical Extraction Plants. *In:* MARTÍNEZ, J. L. (ed.) *Supercritical Fluid Extraction of Nutraceuticals and Bioactive Compounds*. Boca Raton: CRC Press-Taylor and Francis Group.

MARTINEZ-VALVERDE, I., PERIAGO, M. J. & ROS, G. 2000. Significado nutricional de los compuestos fenólicos de la dieta. *Archivos Latinoamericanos de Nutrición*, 50, 5-18.

McHUGH, M. A. & KRUKONIS, V. J. 1994. Supercritical Fluid Extraction: Principles and Practice, 2nd Ed. Butterworth-Heinneman.

MORAES, M. N., ZABOT, G. L. & MEIRELES, M. A. A. 2015. Extraction of tocotrienols from annatto seeds by a pseudo continuously operated SFE process integrated with low-pressure solvent extraction for bixin production. *Journal of Supercritical Fluids*, 96, 262-271.

MUSTAFA, A. & TURNER, C. 2011. Pressurized liquid extraction as green approach in food and herbal plants extraction: A review. *Analytica Chimica Acta*, 703, 8-18.

OSORIO-TOBÓN, J. F. & MEIRELES, M. A. A. 2013. Recent Applications of Pressurized Fluid Extraction: Curcuminoids Extraction with Pressurized Liquids. *Food and Public Health*, 3, 289-303.

OSORIO-TOBÓN, J. F., CARVALHO, P. I. N., ROSTAGNO, M. A., PETENATE, A. J. & MEIRELES, M. A. A. 2014. Extraction of curcuminoids from deflavored turmeric (*Curcuma*

longa L.) using pressurized liquids: Process integration and economic evaluation. *Journal of Supercritical Fluids,* 95, 167-174.

OZELA, E. F., STRINGHETA, P. C. & CHAUCA, M. C. 2007. Stability of anthocyanins in spinach vine (*Basella rubra*) fruits. *Ciencia e Investigación Agraria,* 34, 115-120.

PAES, J., DOTTA, R., BARBERO, G. F. & MARTÍNEZ, J. 2014. Extraction of phenolic compounds and anthocyanins from blueberry (Vaccinium myrtillus L.) residues using supercritical CO_2 and pressurized liquids. *Journal of Supercritical Fluids,* 95, 8-16.

PEREIRA, C. G., ROSA, P. T. V. & MEIRELES, M. A. A. 2007. Extraction and isolation of indole alkaloids from *Tabernaemontana catharinensis* A.DC: Technical and economical analysis. *Journal of Supercritical Fluids,* 40, 232-238.

PEREIRA, P., CEBOLA, M. J., OLIVEIRA, M. C. & BERNARDO-GIL, M. G. 2016. Supercritical fluid extraction vs conventional extraction of myrtle leaves and berries: Comparison of antioxidant activity and identification of bioactive compounds. *Journal of Supercritical Fluids,* 113, 1-9.

PHAM-HUY, L. A., HE, H. & PHAM-HUY, C. 2008. Free radicals, Antioxidants in Disease and Health. *Journal of Biomedical Science,* 4, 89-96.

PRZYGODA, K. & WEJNEROWSKA, G. 2015. Extraction of tocopherol-enriched oils from Quinoa seeds by supercritical fluid extraction. *Industrial Crops and Products,* 63, 41-47.

RAYBAK, M. E., CALVEY E. M. & HARNLY, J. M. 2004. Quantative determination of allicin in garlic: Supercritical fluid extraction and standard addition of alliin", *Journal of Agricultural and Food Chemistry*, 52, 682-687.

REVERCHON, T., TADDEO, R. & DELLA PORTA, G. 1995. Extraction of sage oil by supercritical CO_2: influence of some process parameters. *Journal of Supercritical Fluids,* 8, 302-309.

RICE-EVANS, C. A., MILLER, N. J. & PAGANGA, G. 1997. Antioxidant properties of phenolic compounds. *Trends in Plant Science,* 4, 304-309.

RICHTER, B. E., JONES, B. A., EZZELL, J. L., PORTER, N. L., AVDALOVIC, N. & POHL, C. 1996. Accelerated solvent extraction: a technique for sample preparation, *Analytical Chemistry*, 68, 1033–1039.

RIZVI, S. S. H., BENADO, A. L., ZOLLWEG, J. A. & DANIELS, J. A. 1986. Supercritical fluid extraction fundamental principles and modeling methods. *Food Technology,* 40, 55-65.

RIZVI, S. 2010. Separation, extraction and concentration processes in the food, beverage and nutraceutical industries. Philadelphia, PA: Woodhead Pub Ltd, Cambridge, U, K.

ROSA, L. A., ALVAREZ-PARRILLA, E. & GONZÁLEZ-AGUILAR, G. A. 2010. Fruit and vegetable phytochemicals: chemistry, nutritional value, and stability. First Edition. Wiley J. & Sons, Inc.

RUNNQVIST, H., BAK, S. A., HANSEN, M., STYRISHAVE, B., HALLING-SØRENSEN, B. & BJÖRKLUND, E. 2010. Determination of pharmaceuticals in environmental and biological matrices using pressurized liquid extraction—Are we developing sound extraction methods?. *Journal of Chromatography A*, 1217, 2447-2470.

SANTOS, S. A. O., VILLAVERDE, J. J., SILVA, C. M., NETO, C. P. & SILVESTRE, A. J. D. 2012. Supercritical fluid extraction of phenolic compounds from Eucaliptus globulus Labill bark. *Journal of Supercritical Fluid*, 71, 71-79.

SCALBERT, A., JOHNSON, I. T. & SALTMARSH, M. 2005. Polyphenols: antioxidants and beyond. *The American Journal of Clinical Nutrition*, 81, 2155-2175.

SHAHIDI, F. & NAZCK, M. 2004. Phenolics in Food and Nutraceuticals. Boca Raton, FL, USA, CRC Press-Taylor and Francis Group.

SHI, J., MAZZA, G. & MAGUER, M. L. 2002. Functional Foods: Biochemical and Processing Aspects. Boca Raton: CRC Press.

SHI, J., XUE, S. J., Ma, Y., JIANG, Y., YE, X. & YU, D. 2012. Green separation technologies in food processing: supercritical-CO_2 fluid and subcritical water extraction. *In:* Boyce, J. I. & Arcand, Y. (eds.) *Green Technologies in Food Production and Processing*. New York, NY, USA: Springer-Verlag New York Inc.

UQUICHE, E., CIRANO, N., & MILLAO, S. 2015. Supercritical fluid extraction of essential oil from *Leptocarpha rivularis* using CO_2. *Industrial Crops and Products*, 77, 307-314.

VALLE, J. M. D & AGUILERA, J. M. 1999. Extracción con CO_2 a alta presión: fundamentos y aplicaciones en la industria de alimentos (review). *Food Science and Technology International*, 5, 1-24.

VALLE, M., GLATZEL, V. & MARTÍNEZ, J. L. 2012. Supercritical CO_2 extraction of allicin from garlic flakes: Screening and kinetic studies. *Food Reviews International*, 45, 216-224.

VEGGI, P. C., SANTOS, D. T. & MEIRELES, M. A. A. 2011. Anthocyanin extraction from jabuticaba (*Myrciaria cauliflora*) by different techniques: economic evaluation. *Procedia Food Science*, 1, 1725-1731.

XYNOS, N., PAPAEFSTATHIOU, G., PSYCHIS, M., ARGYROPOULOU, A., ALIGIANNIS, N. & SKALTSOUNIS, A. 2012. Development of a green extraction procedure with super/subcritical fluids to produce extracts enriched in oleuropein from olive leaves. *Journal of Supercritical Fluids*, 67, 89-93.

ZAGHDOUDI, K., PONTVIANNE, S., FRAMBOISIER, X., ACHARD, M., KUDAIBERGENOVA, R., AYADI-TRABELSI, M., KALTHOUM-CHERIF, J., VANDERESSE, R., FROCHOT, C. & GUIAVARC'H, Y. 2015. Accelerated solvent extraction of carotenoids from: Tunisian Kaki (*Diospyros kaki* L.), peach (*Prunus persica* L.) and pricot (*Prunus armeniaca* L.). *Food Chemistry*, 184, 131-139.

Capítulo 3

Pressurized liquid extraction of anthocyanins from purple corn (*Zea mays* L.) waste: technical and economic viability

Angela M. Farías-Campomanes, Maurício A. Rostagno, M. Angela A. Meireles

Artigo que será submetido no periódico Industrial Crops and Products

O material suplementar desde artigo encontra-se com Apêndice A

Pressurized liquid extraction of anthocyanins from purple corn (Zea mays L.) waste: technical and economic viability

Angela M. Farías-Campomanes[1], Mauricio A. Rostagno[2], M. Angela A. Meireles[1]*

[1]LASEFI/DEA/ School of Food Engineering (FEA), University of Campinas (UNICAMP), Rua Monteiro Lobato, 80, Campinas 13083-862, SP, Brazil.

[2]LAPFAL/ School of Applied Science (FCA), University of Campinas (UNICAMP), Rua Pedro Zaccaria, 1300, Limeira 13484-350, SP, Brazil

Corresponding author: Profa. Dra. M. Angela A. Meireles
E-mail: maameireles@gmail.com
Telephone/Phone: +55 19 35214033
Fax: +55 19 35214027

Pressurized Liquid Extraction of Anthocyanins from Purple Corn (*Zea mays* L.) Waste: Extraction Parameters, Extracts Composition and Technical and Economic Evaluation

Angela M. Farías-Campomanes[1], Mauricio A. Rostagno[2], M. Angela A. Meireles[1]*

[1]LASEFI/DEA/ School of Food Engineering (FEA), University of Campinas (UNICAMP), R. Monteiro Lobato, 80, Campinas 13083-862, SP, Brazil.

[2]LAPFAL/ School of Applied Science (FCA), University of Campinas (UNICAMP), R. Pedro Zaccaria, 1300, Limeira 13484-350, SP, Brazil

Emails: maameireles@gmail.com; meireles@fea.unicamp.br

Abstract

This work aimed to study the pressurized liquid extraction of anthocyanins from purple corn waste, using ethanol as extraction solvent, and to determine the technical and economic feasibility of the process. The effects of pressure (2, 4 and 6 MPa), temperature (313, 323 and 333 K), and static extraction time (5, 10 and 15 min) on the yield and antioxidant activity of the extracts were evaluated. At the optimal condition, overall extraction curves were performed and through high-performance liquid chromatography, the profile and content of anthocyanins in the extracts were determined. The manufacturing costs of the extracts were estimated by the SuperPro Designer v.8.5 software. High global yield and extracts with high antioxidant activity were obtained by PLE at 333 K, 6 MPa and 5 min of static extraction. The most concentrated extracts in anthocyanins were obtained when purple corn cobs were used and more than 92 % of the total extracted anthocyanins were recovered with 15 min of dynamic extraction. The use of purple corn cobs as raw material turned feasible the process from extraction units with 2 extractors of 0.025 m^3. The use of a large number of small-volume extractors instead of large-volume extractors can be used for scaling-up process without affecting the manufacturing cost and keeping the feasibility of the process.

Keywords: Pressurized liquid extraction, Antioxidant, Anthocyanins, Purple corn; manufacturing cost.

Highlights

- Pressurized liquid extraction of anthocyanins and antioxidants from purple corn waste was conducted.
- Maximum antioxidant activity of the extracts was observed at 5 min of SET, 6 MPa and 333 K.
- 90% of the total yield was obtained after 30 min of extraction.
- Extracts with higher concentration of anthocyanins were obtained from purple corn cobs.
- Obtaining anthocyanins from purple corn waste by PLE is economically feasible.

Graphical Abstract

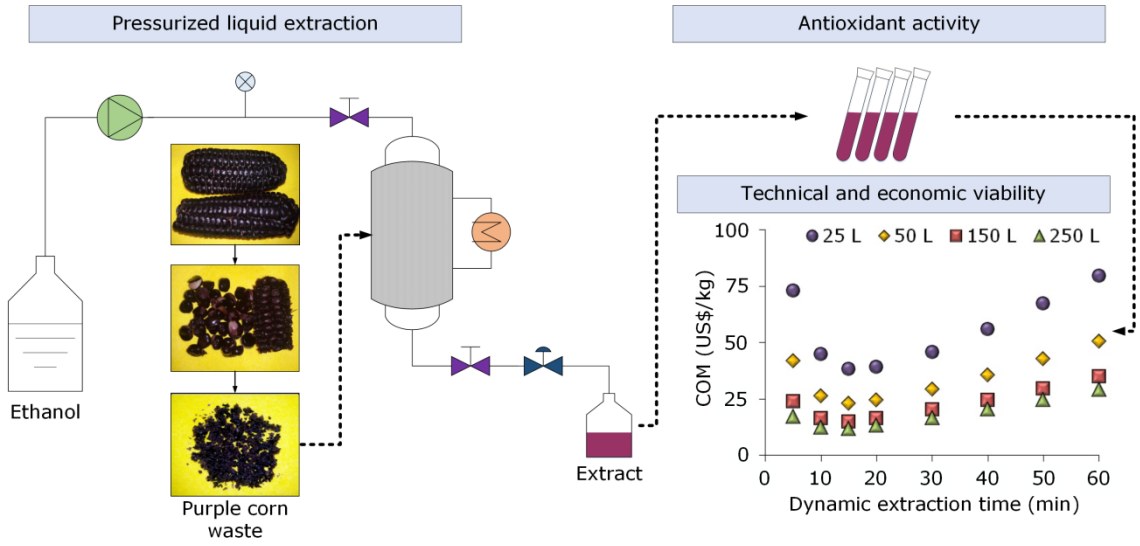


Figure 1. Graphical abstract.

Chemical compounds studied in this article

Cyanidin-3-glucoside (PubChem CID: 197081); Peonidin-3-glucoside (PubChem CID: 443654); Pelargonidin-3-glucoside (PubChem CID: 443648).

Abbreviations

AA, antioxidant activity; COM, cost of manufacturing; COL, cost of operating labor; CRM, cost of raw materials; CUT, cost of utilities; CWT, cost of waste treatment; DET, dynamic extraction time; FCI, fixed capital of investment; GRAS, generally recognized as safe; N/A; not applicable; PCW, purple corn waste; PLE, pressurized liquid extraction; QE, quantity of extractors; S/F, solvent to feed mass ratio (g of solvent/g of raw material); SC, semi-continuous mode; SET, static extraction time; SSC, steady-state continuous mode; SSC-E, steady-state continuous efficient mode; SSC-IE, steady-state continuous inefficient mode; USC, unsteady-state continuous mode.

1 Introduction

In recent years, the interest in consuming food with health benefits has increased. Thus, the search for new sources of bioactive compounds with antioxidant activity has diversified, especially because the use of some synthetic antioxidants is being restricted due to their carcinogenicity (Kahl, 1984). Antioxidants are compounds able to scavenge free radicals, which are produced by chemical reactions in the human body, environmental pollution, UV radiation, alcohol consumption, consumption of food with chemical residues, preservatives, hormones, saturated fats, etc. Free radicals are related to be responsible for several diseases such as coronary and respiratory diseases, cancer, and diabetes (Collins, 2005; Sharan et al., 2011). Antioxidants are found in plants and some foods, even in their processing waste (Baydar et al., 2007; Melo et al., 2011; Farías-Campomanes and Meireles, 2013; Santos et al., 2010; Farías-Campomanes et al., 2013). However, many forms of vegetable wastes are currently being discarded due to lack of studies on their health benefits.

As well as some food wastes such as grape seed, potato skin and apple pomace, purple corn waste can be an important source of antioxidant compounds (Farías-Campomanes and Meireles, 2013; Albishi et al., 2013; García et al., 2009); however, the variety, the farming and the processing conditions must be considered because they determine the antioxidant concentration in the products and wastes (Lafka et al., 2007). Purple corn (*Zea mays* L.) is a native plant of Peru and is found between 1,000 to 2,400 meters above sea level (m.a.s.l.) in the Andean valleys. Purple corn is composed of kernels (65-85 %) and cobs (15-35 %), whose content of anthocyanin pigment is higher than that found in grains skin (Quispe-Jacobo et al., 2011). Studies have shown that anthocyanins from purple corn fight cervical cancer (Hagiwara et al., 2001) and its extracts prevent heart diseases, obesity and diabetes (Tsuda et al., 2003; Tsuda et al., 2004).

Purple corn is harvested throughout the year but its availability increases in April, November and December (Ministry of Production). In household, the whole purple corn is used for the obtaining of pigment for use in food preparation; while industrially, only purple corn cobs are used. Industrially, pigment from purple corn cobs is used as additive for coloring food products, dyeing textile fibers and manufacturing cosmetics, while grains are used as a source of starch or animal feed (Risco-Mendoza, 2007). The most consumed byproduct from purple corn is a beverage produced at the industrial and household level named "*chicha morada*". Due to the solubility of anthocyanins in water, chicha morada is

prepared by adding purple corn in boiling water. Cobs and kernels obtained after chicha morada preparation, show intense purple color suggesting that processing conditions were not extreme enough for depletion of anthocyanins from purple corn. Hence, purple corn waste from chicha morada production may be a potential source of recoverable anthocyanins. Since, purple corn wastes are discarded without any treatment; then, the development of sustainable and environmentally friendly techniques for recovering anthocyanins from purple corn wastes is a very important issue in the food industry.

Pressurized liquid extraction (PLE) is a green technique used for the extraction of bioactives from vegetal matrices. PLE is also known as accelerated solvent extraction (ASE), enhanced solvent extraction (ESE) and pressurized solvent extraction (PSE) and is referred as pressurized hot water extraction (PHWE) when water is used as extraction solvent. PLE uses a liquid solvent at elevated temperature and pressure, improving the solvation of target compounds and increasing the diffusion rates (Mendiola et al., 2011). Hence, the extraction time and solvent consumption are reduced because the solvent penetrates the solid matrix more efficiently (Mustafa and Turner, 2011). Due to PLE is performed in a closed extractor, avoids the degradation of bioactives sensitive to light and oxygen. In that sense, PLE has been successfully applied for extracting temperature sensitive compounds such as polyphenols (Santos et al., 2010; Veggi et al., 2014; Osorio-Tobón et al., 2014) and carotenoids (Cardenas-Toro et al., 22; Zaghdoudi et al., 2015).

Since, PLE involves extraction using polar solvents such as methanol and ethanol, PLE has been mainly employed for extracting more polar bioactive compounds.

Considering that stability of anthocyanins depends on the temperature, light, oxygen and solvents, among others; PLE is an appropriated technique for the anthocyanins extraction because their operating principles aim to minimize degradation reactions. Hence, PLE of purple corn waste from chicha morada processing could be an alternative process for obtaining low-cost anthocyanin-rich extracts. However, a study of the technical and economic feasibility of anthocyanins extraction from purple corn waste by PLE has not been yet performed. The aim of this work was to investigate the PLE of anthocyanins from purple corn waste. This evaluation was based on the effects of the extraction parameters (temperature, pressure and static extraction time), on the global yield, anthocyanins yield, antioxidant activity of the extracts and its manufacturing cost.

2 Materials and methods

2.1 Chemicals

Ethanol (99.5%) and formic acid were purchased from Dinamica (Campinas, Brazil). The color reagent 2,2-diphenyl-1-picrylhydrazyl and anthocyanin standards HPLC-grade with purity higher than 90%: cyaniding-3-glucoside, peonidin-3-glucoside and pelargonidin-3-glucoside; were supplied by Sigma Aldrich (Saint Louis, USA). HPLC grade methanol was purchased from Merk (Darmstadt, Germany). Ultra-pure water was obtained with a purification system (Milli-Q, Millipore, Bedford, MA, USA).

2.2 Plant material

Purple corn (*Zea mays* L.) cultivated in Cuzco city (Kculli variety) was obtained from the Central Market of Cuzco (Cuzco, Cuzco, Peru). Purple corn samples were cleaned and inedible parts were removed. Purple corn beverage “chicha morada” was prepared according to the traditional recipe. 1 kg of purple corn, manually separated in kernels and cobs, was added in 4 liters of water. After boiling for 30 min, kernels and cobs were filtered; this process was performed two times. The aqueous phases from the filtering stages were used for preparing the beverage. Wet kernels and cobs from preparing chicha morada were divided into two fractions: (1) cobs and kernels, labeled as purple corn waste (PCW) and (2) only cobs labeled as purple corn cobs (PCC). PCW and PCC were packed in plastic bags and stored in a domestic freezer (Metalfrio, DA420, Sao Paulo, Brazil) at 255 K until the experiments were performed. PCW and PCC were subjected to size reduction (particle diameter of 3.5 mm) using a knife grinder (Marconi, model MA340, Piracicaba, Brazil). The moisture of samples was determined by placing approximately 3 g of sample in dried containers inside an oven at 373 K (model TE 395-1, Tecnal, Sao Paulo, Brazil). The weight of the samples was monitored until no changes were observed. The moisture of PCW and PCC was calculated in 67.8 ± 0.1% and 79 ± 1%, respectively.

2.3 Pressurized liquid extraction

In order to minimize subsequent stages to the obtaining purple corn waste, which might increase the manufacturing cost of the PLE extracts; the whole wet purple corn waste (PCW) was used in the PLE experiments.

2.3.1 Extraction unit

PLE assays were performed using a home-made PLE unit which consisted of: a HPLC pump (Thermo Separation Products, model 3200 ConstaMetric P/F, Fremont, USA), a manometer, an extraction vessel (Thar Designs, CL 1373, Pittsburg, USA) of 6.3 cm^3 (2.0 cm diameter and 2.0 cm height; internal dimensions), an electric heating jacket, a blocking valve and a backpressure valve.

2.3.2 Extraction procedure

The PLE procedure was the following: (1) the bed extraction, formed by approximately 6 g of PCW (wet basis), was placed between two glass beds, inside the extraction vessel; (2) the extraction vessel was heated until the extraction temperature was reached; (3) the blocking and backpressure valves were closed; (4) ethanol, used as the extraction solvent, was pumped into the extraction vessel at flow rate of 2.6×10^{-5} kg/s until to extraction pressure was reached; (5) the extraction vessel containing the PCW and ethanol, was kept at the extraction temperature and pressure for a period of time, named static extraction time; (6) after static extraction, ethanol was pumped into the extraction vessel at flow rate of 2.6×10^{-5} kg/s; (7) the blocking valve was opened; (8) the backpressure valve was open enough to keep the extraction pressure and (9) the extracts were collected in glass flasks until the solvent mass to feed mass ratio (S/F) of 8 g ethanol/g PCW was achieved; this extraction period is named as dynamic extraction due to fresh solvent is continuously fed to the extraction vessel. S/F of 8 was determined from preliminary essays. Ethanol from the extracts was removed by vacuum evaporation (Laborota, model 4001, Viertrieb, Germany) at 313 K and the samples were weighed (Sartorius, model A200S, Göttingen, Germany).

2.3.3 Global yield

The global yield of extraction ($X_{0,S/F}$) was calculated as the percentage of the mass of extract ($m_{extract}$) obtained from the total initial mass of the raw material used to form the extraction bed in dry basis ($M_{raw\ material}$) as shown in Equation 1.

$$X_{0,S/F}\ (\%) = \frac{m_{extract}}{m_{raw\ material}} \times 100 \qquad (1)$$

2.3.4 Overall extraction curves

Overall extraction curves (OEC) provide information of the kinetic behavior of the extraction processes and are important in the technical and economic assessment (Jesus et al., 2013). After determining the best extraction condition in terms of global yield and antioxidant activity of extracts, a first OEC, at 333 K, 6 MPa and 5 min of static extraction, with PCW was performed. Moreover, based on the literature and to increase the content of antioxidants and anthocyanins in the extracts; a second OEC at the same extraction condition but using purple corn cobs (PCC) as the raw material, was performed. OECs were conducted for 1 h and the extracts were collected in glass flasks every 10 min. OECs were performed in duplicate. Extract yield was calculated throughout the OECs.

2.4 Chemical characterization of the extracts

2.4.1 Antioxidant activity

The radical scavenging activity of DPPH (2,2-diphenyl-1-picrylhydrazyl) was used to determine the antioxidant activity of the PCW extracts according to the method of Kordali et al. (2005). The DPPH solution was prepared by dissolving DPPH radical in ethanol at a concentration of 60 µM. From DPPH solution, a standard calibration curve at different concentrations of DPPH (0, 10, 20, 30 40, 50 and 60 µM) was prepared. Then, extract solutions in ethanol with concentration of 5 mg of extract per cm^3 of ethanol were prepared. An aliquot of 0.1 cm^3 of the extract solutions was transferred to tubes with 3.9 cm^3 of DPPH solution (60 µM) and mixed in a shaker tube in the dark. A 0.1 cm^3 aliquot of ethanol was used as the control solution. Pure ethanol was used as a blank to calibrate the spectrophotometer. Readings were taken immediately at the beginning and after 30 min of reaction time at 517 nm in a UV-vis spectrophotometer (Femto, model 800XI, Sao Paulo, Brazil). Antioxidant activity of the extracts was calculated as the percentage of inhibition (PI) according to Equation 2.

$$PI = \frac{(Abs^{Control} - Abs^{Sample})}{Abs^{Control}} \times 100 \quad (2)$$

2.4.2 Separation, identification and quantification of anthocyanins

Anthocyanins from PCW and PCC extracts collected from the OECs were determined by high performance liquid chromatography on a Waters Alliance separation

module (model 2695D, Milford, MA, USA) using a diode array detector (2998). Samples were filtered through 0.45 µm nylon syringe filters directly into HPLC vials. The injection volume was 10 µL. Anthocyanins were separated on a Poroshell column (C18, 2.6 µm, 100A, 100 mm × 4.6 mm, Agilent Technologies, Sunnyvale, USA) at 300 K with a flow rate of 0.6 cm^3/min. A gradient mobile phase consisted of water containing 5 % of formic acid (A) and methanol (B). The gradient solvent system was as follows: 0 min – 0% B, 1.25 min – 15% B, 4 min – 20% B, 8 min – 30% B; 12 min – 40% B, 15 min – 50% B, 19 min – 55% B, 21 min – 60% B, 22 min – 70% B, 23 min – 80% B; 25 min – 90% B, 27.5 min – 95% B, 29.5 min – 95% B, 31.5 min – 15% B, 33.5 min – 0% B. Delay time of 7 min was considered. Anthocyanins were detected at 520 nm and quantified using Empower 2 software (Water, Milford, MA, USA). Anthocyanins: cyaniding-3-glycoside (cy-3-glu), pelargonidin-3-glucoside (pg-3-glu) and peonidin-3-glucoside (pn-3-glu) were identified by comparing their UV-vis spectra and the retention time to those of standards. The anthocyanins were quantified using calibration curves. Regression equations (cy-3-glu, y = 35041x-670; pg-3-glu, y = 39187x-1291; pn-3-glu, y = 20016x-709, were "x" is the standard concentration in $mg.L^{-1}$ and "y" is area) and correlation coefficients (r2 = 1) were calculated using Minitab v.16 software. The limits of detection (cy-3-glu, 0.12 mg/L; pg-3-glu; 0.11 mg/L; pn-3-glu, 0.18 mg/L) and quantification (cy-3-glu, 0.34 mg/L; pg-3-glu; 0.29 mg/L; pn-3-glu, 0.53 mg/L) were also calculated by using Minitab v.16 software. Anthocyanins that did not correspond to the standards were quantified using the regression equation of the respective anthocyanins from which derives.

2.5 Statistical analysis

Full factorial design 3^k where $k = 3$ (27 runs) was used to evaluate the effect of the extraction parameters: temperature, pressure and static extraction time, coded as X_1, X_2 and X_3; on the global yield and antioxidant activity of the extracts. Minitab v.16 software was used for the statistical analysis. The global yield and antioxidant activity of the extracts for different selected levels of variables are shown in Table 1. The main effects of extraction parameters were analyzed.

2.6 Process simulation: Technical and economic evaluation

2.6.1 Process simulation

SuperPro Designer v8.5 (Intelligent Inc., Scotch Plains, NJ, USA) software was used to perform the technical and economic evaluation of the PLE of anthocyanins from

purple corn wastes. High-pressure processes are often operated in batch mode, i.e. only one extractor is used.

Table 1. Global yield and antioxidant activity of the PCW extracts for the different selected levels of variables.

Run	T (K) *X*1	P (MPa) *X*2	SET (min) *X*3	GY (%, d.b.)	DPPH (%, PI)
1	313	2	5	7.3	21 ± 1
2	313	2	10	8.9	21 ± 1
3	313	2	15	7.6	33.0 ± 0.3
4	313	4	5	10.8	26.1 ± 0.3
5	313	4	10	7.0	6.7 ± 0.1
6	313	4	15	7.3	11.4 ± 0.2
7	313	6	5	6.8	29.2 ± 0.1
8	313	6	10	7.5	14.6 ± 0.1
9	313	6	15	8.7	10.3 ± 0.2
10	323	2	5	8.5	31 ± 1
11	323	2	10	7.4	33 ± 1
12	323	2	15	7.8	19 ± 1
13	323	4	5	7.4	13.7 ± 0.1
14	323	4	10	7.5	12.2 ± 0.1
15	323	4	15	8.6	23.6 ± 0.1
16	323	6	5	7.3	27.9 ± 0.4
17	323	6	10	6.7	17 ± 1
18	323	6	15	7.2	25 ± 1
19	333	2	5	7.0	27.8 ± 0.3
20	333	2	10	8.0	14.4 ± 0.2
21	333	2	15	7.3	16.0 ± 0.1
22	333	4	5	10.2	13.4 ± 0.4
23	333	4	10	8.4	26.28 ± 0.01
24	333	4	15	8.2	21.5 ± 0.1
25	333	6	5	9.5	31.8 ± 0.2
26	333	6	10	7.8	20 ± 1
27	333	6	15	7.9	22.5 ± 0.4

Temperature (T), pressure (P), static extraction time (SET), global yield (GY), antioxidant activity of the extracts determined by DPPH method (DPPH).

Batch mode allows mimicking laboratory experiments, reducing the process development and design complexity; however, several disadvantages including low production rates caused by “dead-times”; i.e. stages where there is not production, are observed. The stages of charging, pressurization, static extraction, depressurization, discharging and cleaning are considered dead-time stages.

Their effects can be reduced by operating several extractors in parallel; thus, plant can produce extract constantly (continuous mode) or in time intervals (semi-continuous mode). Continuous mode allows increasing production rates with smaller investment capital because more than one extractor can be operated using a low-capacity pump, reducing the manufacturing cost (COM) of extracts. Figure 2 shows a classification of extraction processes according to the operating mode.

A continuous process can operate in two different modes: steady state (SSC) and unsteady state (USC). In this work, the SSC mode identifies processes in which the number of operating extractors is constant over time. Moreover, an SSC process may be classified according its efficiency.

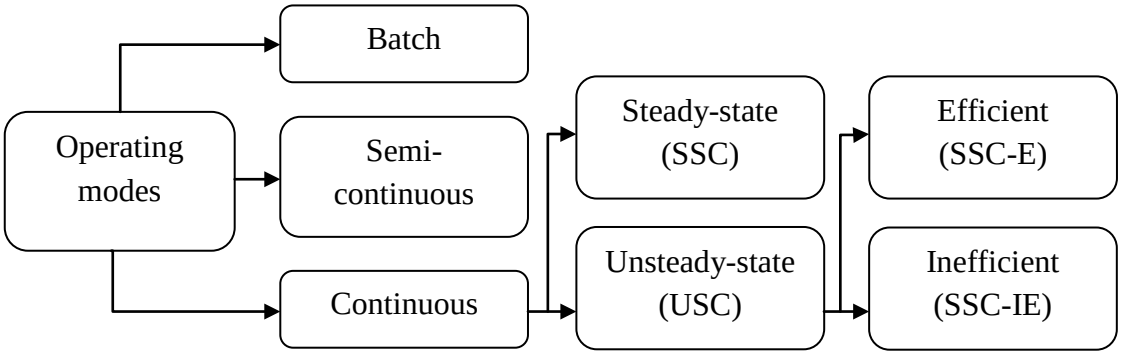


Figure 2. Operating mode classification

Thus, an SSC process is defined as efficient (SSC-E), if n-1 extractors (n≥2) are always operated in the dynamic extraction stage, meaning that only one extractor at a time is in a dead-time stage. An SSC-E process must satisfy the relationship given in Equation 3.

$$t_D = \frac{t_{DE}}{n-1} \tag{3}$$

where t_D is the dead-time of the process, t_{DE} is the dynamic extraction time and n is the number of extractors in the system. For example, t_D must be equal to t_{DE} for a SSC-E process with two extractors (n=2). In this process, while one of the extractors is under operation (dynamic extraction), the second extractor is discharged, cleaned, charged again and set on

operating condition (temperature and pressure) to start the extraction process when the first extractor stops (Figure 3a) (Moraes et al., 2015).

A system with n≥3 extractors operates in SSC inefficient (SSC-IE) mode when at most n-2 extractors are in the dynamic extraction stage; i.e. at least two extractors are in dead-time stages. For example, Figure 3b shows a system with n=3 extractors operating in SSC-IE mode, where n-2=1 extractor is always under operation. In this work, the COMs of purple corn waste extracts obtained by PLE in batch and continuous mode (USC, SSC-E and SCC-IE) were estimated.

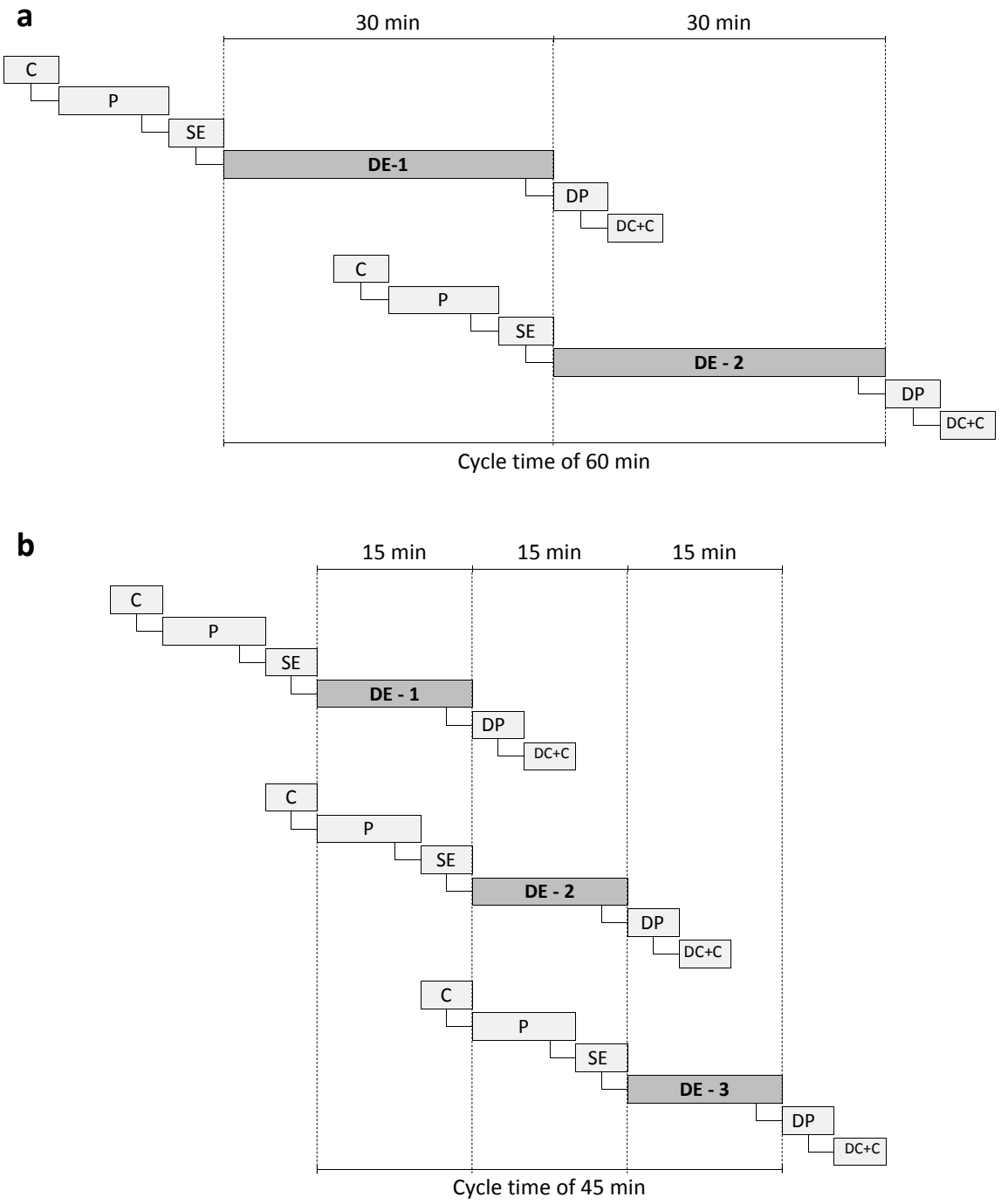


Figure 3. Schematic diagram of a PLE process with *n*=2 extractors operating in SSC-E mode (a) and *n*=3 extractors operating in SSC-IE mode (b): charging (C), pressurization (P), static extraction (SE), dynamic extraction (DE), depressurization (DP), discharging and cleaning (DC+C).

2.6.2 Economic evaluation

SuperPro Design v8.5 software estimates the mass and energy balances for the whole process, equipment costs, operating cost and cost of manufacturing, among other parameters. The costs of manufacturing (COM) for the purple corn waste extracts produced by PLE were estimated by the simulator whose calculation is based on the methodology proposed by Turton et al. (Turton et al., 1998). COM is mainly comprised by the fixed capital of investment (FCI), cost of raw materials (CRM), cost of utilities (CUT), cost of waste treatment (CWT) and cost of operating labor (COL). COM is expressed as unit of production cost (US$/kg extract) by calculating the ratio between the annual operating cost (US$/year) and the production rate per year (kg of extract/year). The economic data entered to the simulator are presented in Table 2.

Table 2. Economic parameters used for COM estimation.

	Industrial units					
	2×0.025 m^3	2×0.05 m^3	2×0.15 m^3	2×0.25 m^3	3×0.1 m^3	1 × 0.3 m^3
Fixed capital of investment (FCI)						
PLE unit extraction	US$ 77,191 [a]	US$ 117,000 [b]	US$ 226,182 [a]	US$ 307,304 [a]	US$ 260,000 [a]	US$ 287,000 [a]
Distiller	US$ 13,000 [a]	US$ 20,000[b]	US$ 39,000 [a]	US$ 53,000 [a]	US 45,000[a]	US$ 59,000 [a]
Depreciation rate[b]	10%/year	10%/year	10%/year	10%/year	10%/year	10%/year
Operational labor cost (COL)						
Basic rate[b]	US$ 6.9/h	US$ 6.9/h	US$ 6.9/h	US$ 6.9/h	US$ 6.9/h	US$ 6.9/h
Number of workers [c]	1	1	2	2	2	3
Cost of the raw material (CRM)						
Purple corn waste	US$ 0	US$ 0	US$ 0	US$ 0	US$ 0	US$ 0
Purple corn cobs	US$ 0	US$ 0	US$ 0	US$ 0	US$ 0	US$ 0
Ethanol [b]	US$ 0.85/kg	US$ 0.85/kg	US$ 0.85/kg	US$ 0.85/kg	US$ 0.85/kg	US$ 0.85/kg
Water [b]	US$ 0.04/kg	US$ 0.04/kg	US$ 0.04/kg	US$ 0.04/kg	US$ 0.04/kg	US$ 0.04/kg
Cost of utilities (CUT)						
Electricity [b]	US$ 0.092/ kWh	US$ 0.092/ kWh	US$ 0.092/ kWh	US$ 0.092/ kWh	US$ 0.092/ kWh	US$ 0.092/ kWh
Water steam [b]	US$ 4.2/ton	US$ 4.2/ton	US$ 4.2/ton	US$ 4.2/ton	US$ 4.2/ton	US$ 4.2/ton

[a]Estimed values by six tenths rule, [b]Osorio-Tobón et al., 2014.

Once the purple corn is harvested throughout the year, the PLE process was designed to operate 7,920 h per year, which corresponds to 330 days per year of continuous operation (24 h per day). Equipment prices were estimated by six tenths rule using the data provided by Osorio-Tobón et al. (2014) and the simulator. The annual equipment depreciation rate was considered to be 10%. The plant had an estimated useful life of 15 years. To estimate the COL, the number of operators required per shift was estimated by Turton et al. (1998). The CUT considered heat exchange agents and the electricity used in the process which were estimated by the simulator through an energy balance. The CWT was neglected because the process residue is a nontoxic vegetal material and can be used in other processes or incorporated into the soil. The CRM is related to the plant material and solvent lost during the process. Since, purple corn waste is a byproduct from beverage preparation; then the raw material cost was considered as zero. The discharge and distillation stages have solvent loss which was taken to be 2% of the total ethanol. The sum of the dead-time stages was defined as 30 min for all the simulated processes.

2.6.3 Scale-up

Prado et al. (2012) concluded that the laboratory and industrial scales have the same performance when the ratio between the solvent mass to feed mass (S/F) is kept constant. Therefore, for the PLE process scale-up, the same assumption was considered. A scale-up study was performed using two extractors with individual volumes of 25, 50, 150 and 250 L. The COM was estimated throughout the OEC. The amount of purple corn waste required to fill the extractors was calculated by multiplying the apparent density and the extractor volume.

2.6.4 Comparison of operating modes using small and large volume extractors

PLE process scale-up has been permanently performed by the increase of extractor volumes (Santos et al., 2010; Veggi et al., 2014; Osorio-Tobón et al., 2014). However, besides the increasing of the production capacity associated with the use of large volume extractors, some drawbacks are also associated. The transporting, installation and start-up costs are high for large-volume extractors. For seasonal raw materials, the use of large-volume extractors may become the process economically not feasible because, might have no raw material available in sufficient quantity to completely fill the extractors, then the operating cost can overcome the winnings. Also, large-volume extractors often operated in

batch mode where dead-times are observed. To overcome the drawbacks, the PLE scale-up by the increase of the number of small-volume extractors instead of increase the extractors volumes, is proposed in this work.

The COMs of the extracts were estimated for a system with 2 and 3 small-volume extractors and compared to the COM provided for a system with one large-volume extractor. Production capacity of 300 L was considered for both systems. In addition, for the comparing of the operating modes (batch, SC, USC, SSC-E, SSC-IE), extractors of 100, 150 and 300 L were used in the simulations.

3. Results

3.1 Pressurized liquid extraction

The selection of the extraction solvent took into account the stability and solubility of anthocyanins. Anthocyanins are more stable in ethanol than water (Moldovan et al., 2012) and, under subcritical conditions, higher level of ethanol in the extraction solvent (hydroalcoholic solution) provides higher anthocyanin yield (Monrad et al., 2010). Moreover, the solvation and extraction of bioactives water-soluble from purple corn during chicha morada preparation, was assumed.

Based on the statistical results (ANOVA) with confidence level of 95%, the effects of temperature, pressure and static extraction time on the global yield and antioxidant activity of the extracts, were not significant (p-value > 0.05). Also, the first order interactions were not significant. However, despite the effects of extraction parameters were no significant; the main effects were plotted and analyzed. Figure 4 shows the main effects of temperature, pressure and static extraction time on the global yield (%, d.b) and the antioxidant activity (percentage of inhibition of DPPH radical, PI) of the extracts.

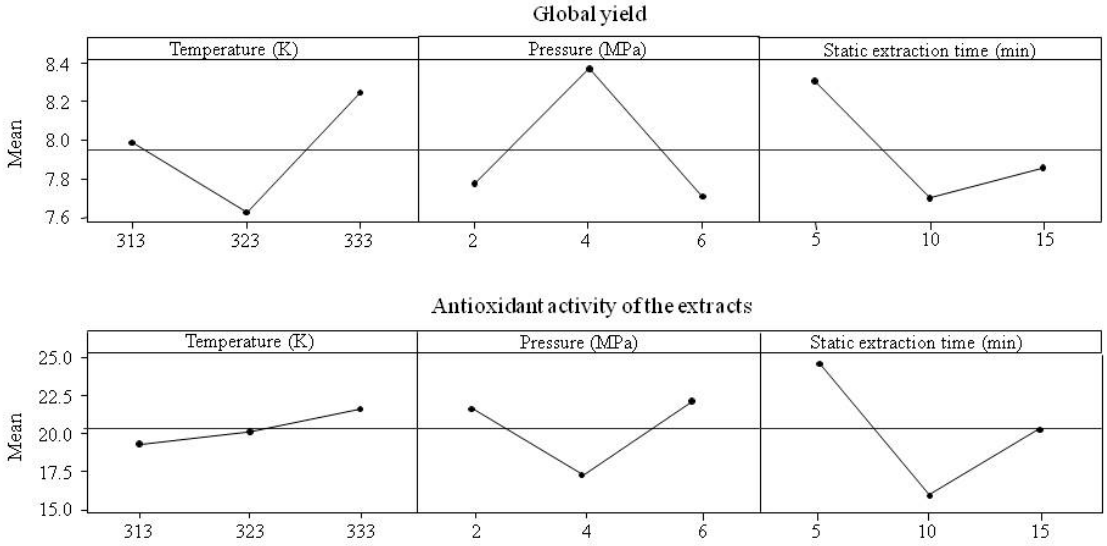


Figure 4. Main effects of the extraction parameters on the global yield and antioxidant activity of the extracts.

3.1.1 Effect of extraction temperature

High temperatures are associated with high extraction yields because the solvating power of the solvent is increased and the interactions between analytes and solid matrix are disrupted; then, a wide variety of compounds are dissolved (Mustafa and Turner, 2011). However, the limitless increase of temperature might promote the degradation of the major responsible for the antioxidant activity of the extracts, the anthocyanins, and thus, a decrease of global yield. In Figure 4, high temperature favored the global yield, however, 323 K promoted a decrease of the global yield, probably indicating two extraction stages. Also, the studied temperature range (313–333 K) did not affect the antioxidant activity of the extracts. The degradation of the compounds that are sensitive to that temperature range and responsible for the antioxidant activity of the extracts, during purple corn processing where the boiling temperature of water was reached, was assumed. The maximums inhibition percentages of DPPH radical varied from 31 to 33 % and were obtained in the range of 313 to 333 K.

3.1.2 Effect of extraction pressure

High pressures are also associated with high global yields and small extraction times; however, not always rising pressure enhances the efficiency of the extraction process. According to Kronholm et al. (2007), the pass of solvent throughout the extraction bed can be affected by a compaction promoted due to the use of elevated pressure. Thus, the increase of pressure from 2 to 4 increased the global while the increased from 4 to 6 MPa promoted a

decrease of the global yield. The effect of increasing pressure was also observed in the PLE of phenolics and antioxidants from *Scutellaria pinnatifida* (Golmakani et al., 2014); where pressures above 4 MPa promoted a reduction of the extract yield. The pressure effect on the antioxidant activity of the extracts was opposite to that observed for the global yield. Extracts with the lowest antioxidant activity were obtained at 4 MPa and it might be caused by the interaction with other parameters.

3.1.3 Effect of static extraction time

Static extraction is often performed to equilibrate the system at the extraction conditions (with respect to temperature and pressure) and is conducted before dynamic extraction where the vessel extractor is continuously fed of fresh solvent. During static extraction time, the equilibrium between target compounds and solvent could be achieved promoting the degradation of some sensitive compounds (Domínguez, 2013). Therefore, determining the static extraction time that allows enhance the extraction efficiency and avoid the degradation of the target compounds is a very important issue. Global yield and antioxidant activity of the extracts were affected by static extraction time and the effect was the same for both. The lowest level of static extraction time (5 min) provided the best results. Short period of static extraction may have minimized the thermal degradation of anthocyanins and others compounds which gave the antioxidant activity to the extracts (Pedreschi and Cisneros-Zevallos, 2007). Since, the best balance between yield and antioxidant activity, i.e. high global yield without decreasing the antioxidant activity of the extracts, was showed by 333 K, 6 MPa and 5 min of static extraction; these extraction conditions were considered as the optimum and were used to perform the OEC and the economic evaluation.

3.2 Overall extraction curve and anthocyanins content in the extracts

According to Yang et al. (2010), cobs from purple corn provide extracts with higher antioxidant activity than those produced by using kernels from purple corn; and there is a high correlation between antioxidant activity and anthocyanins content of the extracts. In that sense, aiming to produce more anthocyanin-rich extracts, purple corn cobs (PCC) were also used as raw material. Thus, overall extraction curves (OEC) were performed for purple corn waste (PCW) and purple corn cobs (PCC). The effects of temperature, pressure and static extraction time on the PLE-PCW performance were assumed as the same for PLE-PCC; therefore, overall extraction curves using PCC were also performed at 333 K, 6 MPa and 5

min of static extraction. The global yield and anthocyanin yield were determined along the OECs.

Figure 5a shows the extract yield throughout the OEC performed for PCW and PCC. For dynamic extraction times higher than 10 min, PLE with PCW showed higher global yield than PLE with PCC. In addition, the maximum global yield, achieved by using PCW, was 9 ± 1 % and almost 70 % of the total extractable mass was obtained at 20 min of dynamic extraction. In contrast, when PCC was used, the same fraction was reached in the first 10 min of dynamic extraction. The maximum global yield achieved for PLE of PCC was 6.4 ± 0.2 %. Figure 5b presents the anthocyanin yield obtained when PCW and PCC were used as raw materials. The anthocyanin yield from the waste of purple corn processing was increased roughly 5 times when PCC instead of PCW, was used. The highest anthocyanin yield was 46 ± 1 mg per 100 g PCC. Since, purple corn cobs *in nature* contain 92 to 94 mg of anthocyanins per 100 g cobs (Yang and Zhai, 2010; Jin and Giusti, 2007); then, approximately 50 % of the total anthocyanin content in cobs was recovered from the cobs of purple corn processing. In addition, from a simple mass balance and considering that percentage of cobs in PCW varied from 15 to 21 % (Quispe-Jacobo et al., 2011), therefore, it may be assumed that the contribution of anthocyanins derived from cobs represented among 69 to 97 % of the total anthocyanins extracted from PCW.

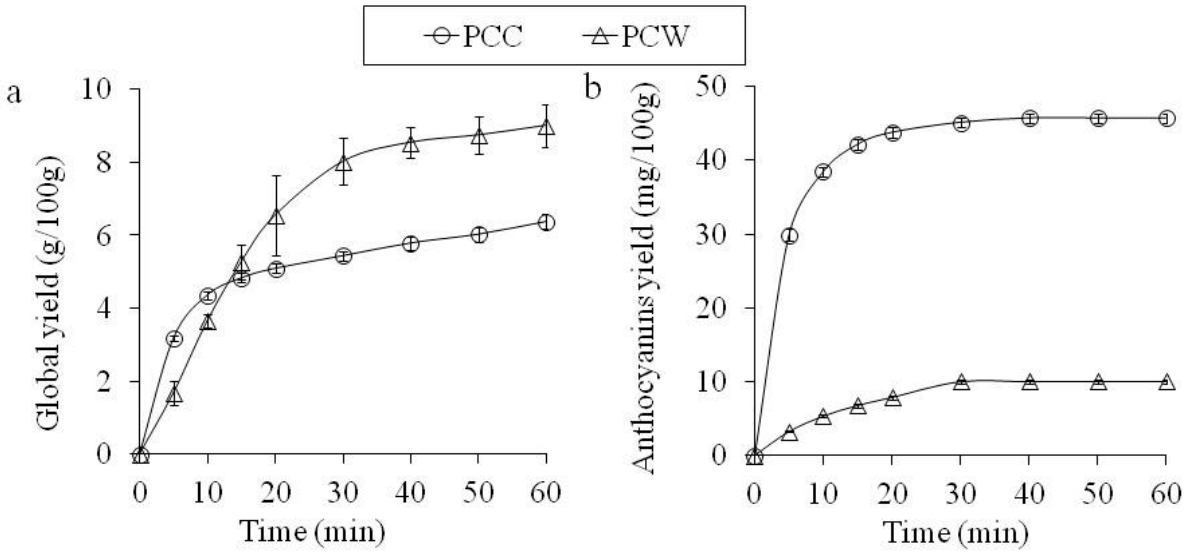


Figure 5. Global yield (a) and anthocyanins yield (b) of PLE at 333 K, 6 MPa and 5 min of static extraction time, using PCW and PCC as raw materials.

Also, this assumption may be supported by difference of intensity between PCW and PCC extracts, obtained under the same extraction conditions (Figure 6). These results agree with those who indicate the higher concentration of anthocyanins in cobs than in kernels

(Yang and Zhai, 2010). The following facts makes us assume that a hydrolysis reaction could have been carried out during PLE of PCW: high extract yield, low anthocyanin yield, turbidity of the extracts and the presence of a white thin layer at the bottom of the collector flasks after a resting period. Also, high temperature (333 K), high content of water (68 %) and starch (65-85 %) in samples of PCW (Quispe-Jacobo et al., 2011) are factors that could have contributed for producing a hydrolysis reaction and, therefore, an increase of the extract yield without the increasing anthocyanins yield.

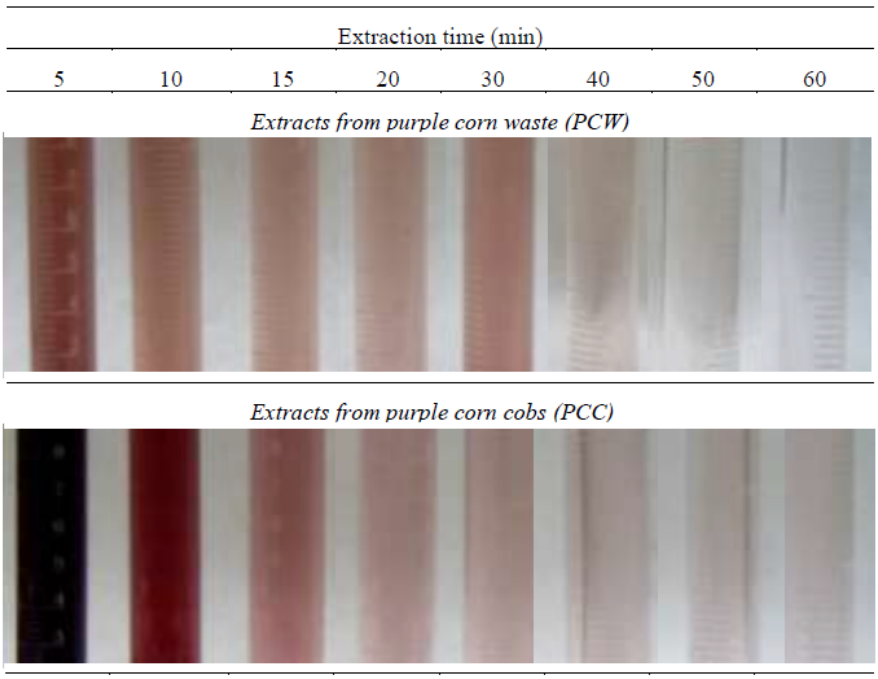
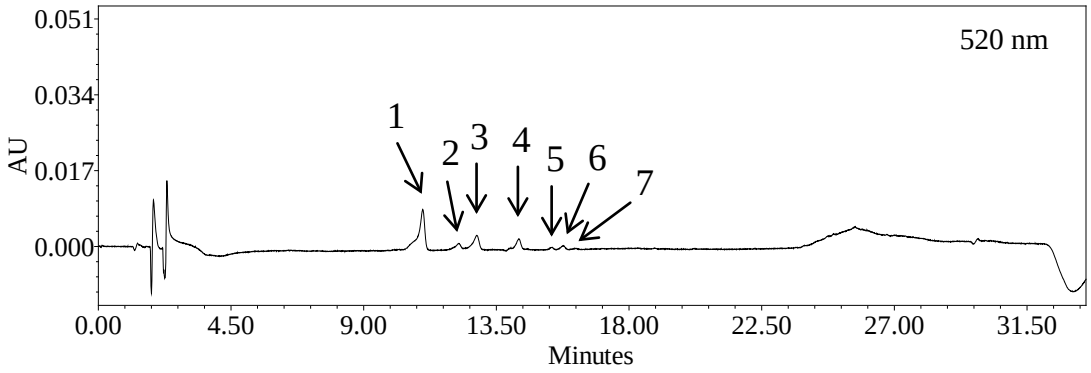


Figure 6. Extracts of PCW and PCC collected during the kinetic of extraction performed at 333 K, 6 MPa and 5 min of static extraction time.

Figure 7 shows the chromatogram of anthocyanins related to the extracts obtained from PCW and PCC by PLE. Seven anthocyanins were detected in the extracts and three were identified as cyanidin-3-glucoside (peak 1, R_t = 11.01 min), pelargonidin-3-glucoside (peak 2, R_t = 12.23 min) and peonidin-3-glucoside (peak 3, R_t = 12.84 min), as were previously identified in Andean purple corn (Pedreschi and Cisneros-Zevallos, 2007; Yang and Zhai, 2010; Aoki et al., 2002). In addition, according to the literature, other 6 anthocyanins including their respective malonyl and etylmalonyl derivatives have been found in Andean purple corn (Jin and Giusti et al., 2007; Pascual-Teresa et al., 2002). In this work, the wavelength of maximum absorbance and relative retention time of three of the unidentified anthocyanins matched with those for cyanidin-3-(6-malonyl)-glucoside (peak 4, R_t = 14.26 min), pelargonidin-3-(6-malonyl)-glucoside (peak 5, R_t = 15.38 min) and peonidin-3-(6-

malonyl)-glucoside (peak 6, R_t = 15.77 min) (Yang and Zhai, 2010; Pascual-Teresa et al., 2002). Anthocyanin of the peak 7 and R_t =16.20 min was not identified and quantified because its concentration in the extracts was lower than limits of quantification.

Purple corn waste (PCW)



Purple corn cobs (PCC)

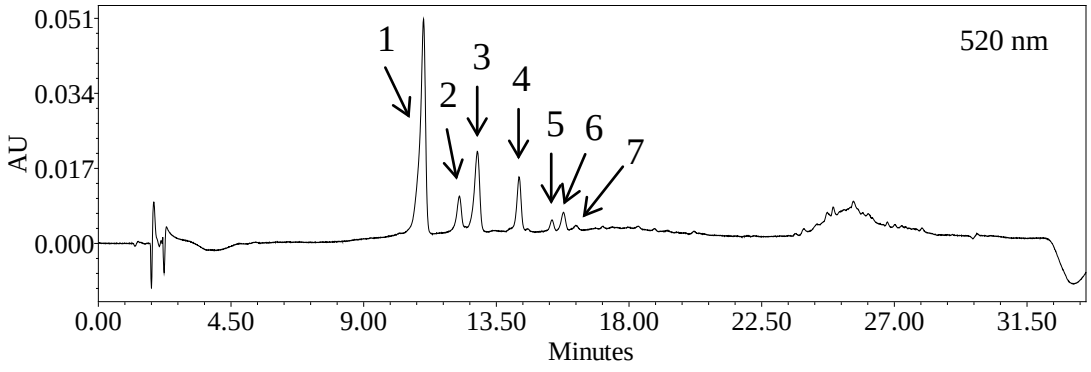


Figure 7. HPLC chromatogram of anthocyanins extracted from PCW and PCC by PLE using ethanol as solvent at 333 K, 6 MPa and 5 min of static extraction time. 1. cyanindin-3-glucoside; 2. pelargonidin-3-glucoside; 3. peonidin-3-glucoside; 4. cyanidin-3-(6-malonylglucoside); 5. pelargonidin-3-(6-malonylglucoside); 6. peonidin-3-(6-malonylglucoside); 7. not identified.

Anthocyanins extraction was completed at 30 and 40 min when PCW and PCC, respectively, were used. The relative percentages of extracted anthocyanins throughout the overall extraction curve are presented in Table 3. The distribution of the extracted anthocyanins was similar for PCW and PCC; and cyanidin-3-glucoside, pelargonidin-3-glucoside and peonidin-3-glucoside were the major anthocyanins in the extracts.

3.3 Scale-up using 2 extractors of 0.025, 0.05, 0.15 and 0.250 m^3

The manufacturing cost (COM) was estimated for the extracts obtained in dynamic extraction times in which anthocyanins extraction was observed. Table 4 and Table 5 show the manufacturing cost (COM) of the extracts produced using extraction units with two extractors (with the same volume) and different capacities (2×0.025, 2×0.05, 2×0.15 and 2×0.25 m^3). Despite the similar COMs of the extracts obtained from PCW and PCC, only the PLE of anthocyanins from PCC is a profitable inversion.

Table 3. Relative percentages of the anthocyanins extracted from PCW and PCC along the overall extraction curves.

			Cy-3-glu[a]	Pg-3-glu[a]	Pn-3-glu[a]	Others[a]
	DET	*Rt*	11.01	12.23	12.84	14.26/ 15.38/15.77
PCW	5		50 ± 2	17.6 ± 0.5	20.5 ± 0.2	12 ± 1
	10		49 ± 2	17.6 ± 0.5	20.8 ± 0.2	12 ± 1
	15		49 ± 1	18 ± 1	21 ± 1	12 ± 1
	20		56 ± 1	20 ± 1	23.4 ± 0.5	-
	30		55 ± 1	21 ± 1	23.43 ± 0.02	-
PCC	5		53 ± 1	14 ± 1	17.9 ± 0.4	15 ± 1
	10		54.9 ± 0.4	15.7 ± 0.2	19.64 ± 0.04	10 ± 1
	15		54.6 ± 0.4	15.9 ± 0.2	19.88 ± 0.01	10 ± 1
	20		60.2 ± 0.1	17.8 ± 0.4	22.08 ± 0.03	-
	30		72.9 ± 0.1	-	27.10 ± 0.04	-
	40		100.0 ± 0.2	-	-	-

[a]Quantified by using the respective anthocyanin standard. Retention time in min (*Rt*), dynamic extraction time in min (DET).

Return on investment (ROI) and payback time are key economic indicators of the profitability of an inversion. ROI is used to evaluate the feasibility of an investment and is calculated by dividing the annual profit by the total capital investment while payback time is a measure of the time needed for the total capital investment to be recovered by cumulative profits and is calculated by dividing the total capital investment charged to the project by the annual profit (Mexandre, 2003). The project is more economically feasible as the ROI increments, then in many situations, a ROI of 10% to 15% is a minimum value to determine whether to approve or deny a project (Fernández-Ronco et al., 2013). The shorter the payback time, the more attractive the project appears to be. Throughout the overall extraction curve for

PLE of anthocyanins from PCW, the ROI values were negative indicating that the project is not profitable (Table 4). PLE with PCW as raw material is not profitable mainly due to it produces extracts with low anthocyanins concentration which are non-competitive with those available in the market. In contrast, when PCC was used to produce extracts with higher content of anthocyanins, the ROI values were higher than 17% even using the lowest capacity extraction unit (2×0.025 m^3). Also, ROI increased when the dynamic extraction time of the process and the capacity of the extraction units were increased.

Table 4. Economic evaluation of PLE process using purple corn waste (PCW) as the raw material.

DET (min)	COM (US$/kg extract)	CRM (%)	COL (%)	FCI (%)	CUT (%)	Operating Cost (US$/year)	ROI (%)	Payback Time (years)
2 × 0.025 m^3								
5	38	10	62	21	7	146000	-79	N/A
10	26	14	57	19	11	161000	-83	N/A
15	25	16	53	18	12	170000	-85	N/A
20	26	18	51	17	13	177000	-90	N/A
30	27	20	49	17	15	187000	-92	N/A
2 × 0.025 m^3								
5	24	15	49	25	11	187000	-58	N/A
10	17	21	42	21	15	216000	-64	N/A
15	18	24	39	20	18	235000	-67	N/A
20	18	26	37	19	19	249000	-73	N/A
30	19	28	34	17	21	267000	-76	N/A
2 × 0.15 m^3								
5	18	20	43	21	15	419000	-59	N/A
10	14	27	36	18	20	507000	-68	N/A
15	14	30	32	16	22	565000	-73	N/A
20	15	32	30	15	23	606000	-82	N/A
30	16	34	27	14	25	662000	-86	N/A
2 × 0.25 m^3								
5	14	26	33	22	19	550000	-48	N/A
10	11	32	26	18	24	695000	-59	N/A
15	12	35	23	15	26	792000	-65	N/A
20	13	37	21	14	27	862000	-76	N/A
30	14	39	19	13	29	954000	-81	N/A

Dynamic extraction time in min (DET), cost of manufacturing (COM), cost of raw material (CRM), cost of labor (COL), fixed cost of investment (FCI), cost of utilities (CUT), return of investment (ROI).

Conversely to ROI, payback time decreased as the dynamic extraction time and the capacity of the extractor were increased. In that sense, the payback time varied from 5.6 to 0.7 years when using extraction units of 2×0.025 m^3 and 2×0.25 m^3 (Table 5). Therefore, PLE of anthocyanins from PCC is feasible when using 2 extractors of 0.025 m^3 and a minimum dynamic extraction time of 10 min.

Table 5. Economic evaluation of PLE process using purple corn cobs (PCC) as the raw material.

DET (min)	COM (US$/kg extract)	CRM (%)	COL (%)	FCI (%)	CUT (%)	Operating Cost (US$/year)	ROI (%)	Payback Time (years)
2 × 0.025 m^3								
5	38	10	62	21	8	146000	-3	N/A
10	38	14	57	19	11	161000	18	5.6
15	44	16	54	18	12	170000	29	3.5
20	51	18	51	18	14	177000	35	2.9
30	64	20	49	17	15	187000	41	2.5
40	76	21	47	16	16	192000	41	2.5
2 × 0.025 m^3								
5	24	15	49	25	11	187000	29	3.4
10	26	21	42	21	15	216000	51	2.0
15	30	24	39	20	17	235000	65	1.5
20	35	26	37	18	19	249000	73	1.4
30	46	28	34	17	21	267000	80	1.2
40	55	29	33	16	22	279000	81	1.2
2 × 0.15 m^3								
5	18	20	43	21	15	419000	61	1.7
10	20	27	36	18	20	507000	93	1.1
15	24	30	32	16	22	565000	114	0.9
20	29	32	30	15	23	606000	125	0.8
30	38	34	27	14	25	662000	136	0.7
40	46	35	26	13	26	697000	136	0.7
2 × 0.25 m^3								
5	14	25	33	22	19	550000	89	1.1
10	17	32	26	18	24	695000	128	0.8
15	20	35	23	15	26	792000	152	0.7
20	25	37	21	14	27	861000	166	0.6
30	33	39	19	13	29	954000	179	0.6
40	40	40	18	12	30	1013000	178	0.6

Dynamic extraction time in min (DET), cost of manufacturing (COM), cost of raw material (CRM), cost of labor (COL), fixed cost of investment (FCI), cost of utilities (CUT), return of investment (ROI).

The lowest COMs of the extracts were obtained at 5 min of dynamic extraction and it kept approximately constant when dynamic extraction time was increased to 10 min. The lowest COM was US$ 14 per kg of extract and was obtained when 2 extractors of 0.25 m^3 and 5 min of dynamic extraction were used; however, slightly higher COMs with similar ROI and payback time were obtained for low capacity extractors and higher dynamic extraction times. From the results, only the PLE of anthocyanins from purple corn is feasible when PCC is used as raw material due to the COMs and the anthocyanin concentrations (9.4 to 7.9 g/kg extract) of the extracts were comparable with those available in the market, which are obtained by conventional process using higher amounts of solvent and longer extraction times. The selling price of purple corn extracts in the international market depends on the anthocyanins content; thus, the selling price of purple corn extract powder containing 25% anthocyanins can cost up to US$ 170.00/kg (Hangzhou New Asia International Co. Ltd.). This selling price was used to calculate the ROI and payback time.

3.4 Comparison of operating modes using small and large-volume extractors

Table 6 shows the COMs obtained for systems comprised of one large-volume (0.3 m^3) extractor and 2-3 small-volume (0.15-0.1 m^3) extractors. Since, 30 min was considered as the sum of the dead-times; then dynamic extraction times of 15 and 30 min were used to evaluate the influence of all operating modes on the COM of the extracts. The COM of the PCC extracts obtained by the PLE process in batch, semi-continuous and continuous (USC, SSC-E and SSC-IE) increased with the dynamic extraction time, since more than 92% of the total anthocyanins were extracted at 15 min, then additional 15 min of extraction leads to a consumption of energy, electricity, solvent and labor, that are inversely proportional to the increase of productivity. Thus, the performing of more cycles resulted in extracts with the highest COM (US$ 32 to 38/kg).

The dynamic extraction of 15 min, batch, SC and SSC-IE modes were evaluated. The operating modes did not have great influence on the COM due to it varied from US$ 21 to 25 per kg of extract; however, the ROI and payback time were more profitable for extraction units with 2 and 3 extractors than extraction units with 1 extractor in batch mode. Large-volume extractors have some disadvantages; one of them is the high initial investment.

The difference on COM and annual operating cost between SC and SSC-IE mode was mainly caused by the extraction unit price. The purchase cost of a unit with 3 extractors of 0.1 m^3 is higher than that with two extractors of 0.15 m^3. However, due to the unit production rate of SSC-IE mode was higher than SC mode, because SSC-IE system used 3 extractors of 100 L, via the consecutive 15 min operation of each extractor; then, the lowest COM (US$ 21/kg) was obtained when 3 extractors of 0.1 m^3 operating in SSC-IE mode, were used.

Table 6. Economic evaluation of PLE of PCC using 1 to 3 extractors with a total capacity of 300 L.

DET (min)	NE	Extractor volume (L)	Operating mode	COM (US$/kg)	Unit production rate (kg/year)	Operating cost (US$/year)	ROI (%)	Payback time (years)
15	1	300	Batch	25	31,061	760,000	103	1.0
15	2	150	SC	24	23,295	565,000	115	0.9
15	3	100	SSC-IE	21	31,040	659,000	123	0.8
30	1	300	Batch	32	26,225	835,000	44	2.3
30	2	150	SSC-E	38	17,482	662,000	136	0.7
30	3	100	USC	32	26,130	846,000	46	2.2

Dynamic extraction time in min (DET), number of extractors (NE), cost of manufacturing (COM), return of investment (ROI), semi-continuous mode (SC), inefficient steady-state continuous mode (SSC-IE), efficient steady-state continuous mode (SSC-E), unsteady-state mode (USC).

For 30 min of dynamic extraction, the batch ad USC mode showed similar unit production rate and annual operating cost and, therefore, close ROIs and payback times. Both operating modes showed similar profitability due to differences on the COL of the extraction unit with one extractor of 0.3 m^3, caused by the number of workers, was equivalent to the difference of FCI, caused by the purchase price of the extraction unit with 3 extractors of 0.1 m^3. In contrast, the SSC-E mode showed the highest COM of the extracts. The difference of US$ 6.00/kg is mainly related to the increasing of pump purchase cost. The 2-extractor system in SSC-E mode needs one pump to supply one extractor of 150 L all the time; in contrast, the 3-extractors system in USC mode needs one pump to supply two extractors of 100 L in some periods (Figure 8). Therefore, since SSC-E is the most efficient mode, it also shows the lowest operating cost and the best values of ROI and payback time. Note that the

difference between the COMs obtained using small-volume and large-volume extractors is not large. Thus, several small-volume extractors can be used to scale-up a process. Scaling-up using small-volume extractors may result in several advantages over that using large-volume extractors, such as cheaper transportation of the extractors to the plant and greater versatility of processes using all or only some extractors in times of low raw material availability.

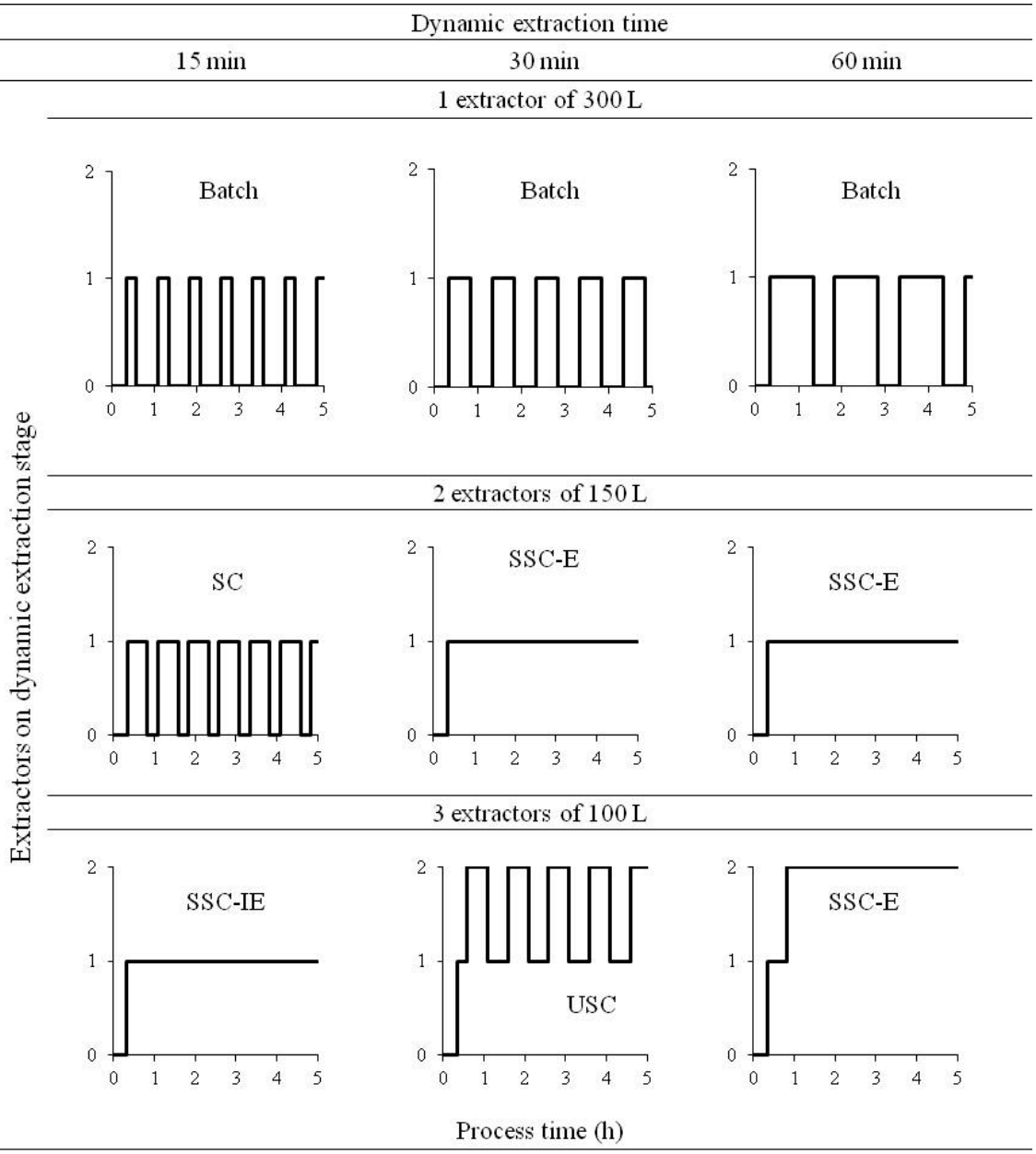


Figure 8. Number of the extractors in the dynamic extraction stage over time according to operating mode: semi-continuous mode (SC), unsteady-state mode (USC), efficient steady-state continuous mode (SSC-E), inefficient steady-state continuous mode (SSC-IE).

The main reason to the existence of few high-pressure extraction industries is the high initial investment cost needed to bring a plant into operation. Thus, it is possible to bring

a high-pressure extraction plant into operation with less initial investment when using small-volume extractors and equipments (pump, distiller, etc) of enough capacity to supply the plant's future production. This allows a long-term increase of the production capacity by increasing the number of extractors.

4 Conclusions

High global yield and extracts with high antioxidant activity were obtained by PLE at 333 K, 6 MPa and 5 min of static extraction, using ethanol as extraction solvent. Despite the low extract yield showed by PCC with respect to PCW, extracts more concentrated in anthocyanins were obtained when PCC was used. More than 92 % of the total extracted anthocyanins from purple cobs were recovered with 15 min of dynamic extraction. From the economic evaluation, the use of PCC instead of PCW as raw material turns feasible the PLE of anthocyanins from purple corn wastes even when using extraction units with low capacity (2×0.025 m^3). Similar COMs were obtained using small and large-volume extractors for equal volume production; therefore, the increase of the number of low-volume extractors can be used as a feasible alternative for scaling-up an extraction process.

Conflict of interest

The authors confirm that there are no conflicts of interest regarding this paper.

Acknowledgments

Angela Farias-Campomanes thanks CAPES (5817-11-0) for Ph.D. assistantships. M. A. A. Meireles is thankful to CNPq for a productivity grant (301301/2010-7). The authors acknowledge the financial support from CAPES, CNPq and FAPESP.

References

ALBISHI, T., JOHN, J. A., AL-KHALIFA, A. S. & SHAHIDI, F. 2013. Phenolic content and antioxidant activities of selected potato varieties and their processing by-products. *Journal of Functional Foods*, 5, 590-600.

AOKI, H., KUZE, V. & KATO, Y. 2002. Anthocyanins isolated from purple corn (*Zea mays* L.) Available in: http://www.ffcr.or.jp/zaidan/ffcrhome.nsf/7bd44c20b0dc562649256502001b65e9/c6698773361b42b249256ba60018e581/$file/anthocyanin-ffij199.pdf.

BAYDAR, N. G., OZKAN, G. & CETIN, E.S. 2007. Characterization of grape seed and pomace oil extracts. *Revista Argentina de Grasas Aceites*, 58, 29-33.

CARDENAS-TORO, F. P., ALCÁZAR-ALAY, S. C., COUTINHO, J. P., GODOY, H. T., FORSTER-CARNEIRO, T. & MEIRELES, M. A. A. 2015. Pressurized liquid extraction and low-pressure solvent extraction of carotenoids from pressed palm fiber: Experimental and economical evaluation. *Food and Bioproducts Processing*, 94, 90-100.

COLLINS, A. R. 2005. Antioxidant intervention as a route to cancer prevention. *European Journal of Cancer*, 41, 1923-1930.

DOMÍNGUEZ, H. 2013. Functional ingredients from algae for foods and nutraceutical, Woodhead Publishing Series in Food Science, *Technology and Nutrition*, 1-19.

FARIAS-CAMPOMANES, A. M. & MEIRELES, M. A. A. 2013. Pisco Bagasse as a Potential Source of Bioactive Compounds – A Review. *Recent Patents on Engineering*, 7, 41-50.

FARIAS-CAMPOMANES, A. M., ROSTAGNO, M. A. & MEIRELES, M. A. A. 2013. Production of polyphenol extracts from grape bagasse using supercritical fluids: Yield, extract composition and economic evaluation. *Journal of Supercritical Fluids*, 77, 70-78.

FERNÁNDEZ-RONCO, M. P., DE LUCAS, A., RODRÍGUEZ, J. F., GARCÍAS, M. T. & GRACIAS, I. 2013. New considerations in the economic evaluation of supercricitical processes: separation of bioactive compounds from multicomponent mixture. *Journal of Supercritical Fluids*, 79, 345-355.

GARCÍA, Y. D., VALLES, B. S. & LOBO, A. P. 2009. Phenolic and antioxidant composition of by-products from the cider industry: Apple pomace. *Food Chemistry*, 117, 731-738.

GOLMAKANI, E., MOHAMMADI, A., AHMADZADEH-SANI, T. & KAMALI, H. 2014. Phenolic and flavonoid content and antioxidant capacity of pressurized liquid extraction and perculation method from roots of *Scutellaria pinnatifida* A. Hamilt. subsp *alpina* (Bornm) Rech. F. *Journal of Supercritical Fluids*, 95, 318-324.

HAGIWARA, A., MIYASHITA, K., NAKANISHI, T., SANO, M., TAMANO, S., KADOTA, T., KODA, T., NAKAMURA, M., IMAIDA, K., ITO, N. & SHIRAI, T. 2001. Pronounced inhibition by a natural anthocyanin, purple corn color, of 2-amino-1-methyl-6-phenylimidazo 4,5-b pyridine (PhIP)associated colorectal carcinogenesis in male F344 rats pretreated with 1,2-dimethylhydrazine. *Cancer Letters*, 171, 17-25.

JESUS, S. P., CALHEIROS, M. N., HENSE, H. & MEIRELES, M. A. A. 2013. Simplified Model to Describe the Kinetic Behavior of Supercritical Fluid Extraction from a Rice Bran Oil Byproduct. *Food and Public Health*, 3, 215-222.

JIN, P. & GIUSTI, M. M. 2007. Effect of Extraction Conditions on Improving the Yield and Quality of an Anthocyanin-Rich Purple Corn (*Zea mays* L.) Color Extract. *Food and Chemical Toxicology*, 72, 363-368.

KAHL, R. 1984. Synthetic antioxidants: biochemical actions and interference with radiation, toxic compounds, chemical mutagens and chemical carcinogens. *Toxicology*, 33, 185-228.

KORDALI, S., KOTAN, R., MAVI, A., CAKIR, A., ALA, A. & YILDIRIM, A. 2005. Determination of the Chemical Composition and Antioxidant Activity of the Essential Oil of Artemisia dracunculus and of the Antifungal and Antibacterial Activities of Turkish Artemisia absinthium, A. dracunculus, Artemisia santonicum, and Artemisia spicigera Essential Oils. *Journal of Agricultural and Food Chemistry*, 53, 9452-9458.

KRONHOLM, J., HARTONEN, K. & RIEKKOLA, M. 2007. Analytical extractions with water at elevated temperatures and pressures. *Trends in Analytical Chemistry*, 26, 396-412.

LAFKA, T. I., SINANOGLOU, V. & LAZOS, E. S. 2007. On the extraction and antioxidant activity of phenolic compounds from winery wastes. *Food Chemistry*, 104, 1206-1214.

MELO, P. S., BERGAMASCHI, K. B., TIVERON, A. P., MASSARIOLI, A. P., OLDONI, T. L. C., ZANUS, M. C., PEREIRA, G. E. & DE ALENCAR, S. M. 2011. Phenolic composition and antioxidant activity of agroindustrial residues. *Ciencia Rural*, 41, 1088-1093.

MENDIOLA, J. A., HERRERO, M., CIFUENTES, A. & IBANEZ, E. 2007. Use of compressed fluids for sample preparation: Food applications. *Journal of Chromatography A*, 1152, 234-246.

MEXANDRE, C. D. 2003. Economic evaluation of projects, in: C.D. Mexandre (Ed.) Computer Aided Chemical Engineering, Elsevier, 571-604.

MINISTRY OF PRODUCTION. Perfil Comercial: Antocianina de maiz morado. Available in: http://www.sierraexportadora.gob.pe/perfil_comercial/ANTOCIANINA%20DE%20MAIZ%20MORADO.pdf.

MOLDOVAN, B., LUMINITA, D., CHISBORA, C. & CIMPOIU, C. 2012. Degradation Kinetics of Anthocyanins from European Cranberrybush (*Viburnun opulus* L.) Fruit Extracts. Effects of temperature, pH and Storage Solvent. *Molecules*, 17, 11655-11666.

MONRAD, J. K., HOWARD, L. R., KING, J. W., SRINIVAS, K. & MAUROMOUSTAKOS, A. 2010. Subcritical Solvent Extraction of Anthocyanins from Dried Red Grape Pomace. *Journal of Agricultural and Food Chemistry*, 58, 2862-2868.

MORAES, M. N., ZABOT, G. L. & MEIRELES, M. A. A. 2015. Extraction of tocotrienols from annatto seeds by a pseudo continuously operated SFE process integrated with low-pressure solvent extraction for bixon production. *Journal of Supercritical Fluids*, 96, 262-271.

MUSTAFA, A. & TURNER, C. 2011. Pressurized liquid extraction as green approach in food and herbal plants extraction: A review. *Analytica Chimica Acta*, 703, 8-18.

OSORIO-TOBÓN, J. F., CARVALHO, P. I. N., ROSTAGNO, M. A., PETENATE, A. J. & MEIRELES, M. A. A. 2014. Extraction of curcuminoids from deflavored turmeric (*Curcuma longa* L.) using pressurized liquids: Process integration and economic evaluation. *Journal of Supercritical Fluids*, 95, 167-174.

PASCUAL-TERESA, S., SANTOS-BUELGA, C. & RIVAS-GONZALO, J. 2002. LC-MS analysis of anthocyanins from purple corn cob. *Journal of the Science of Food and Agriculture*, 82, 1003-1006.

PEDRESCHI, R. & CISNEROS-ZEVALLOS, L. 2007. Phenolic profiles of Andean purple corn (Zea mays L.). *Food Chemistry*, 100, 956-963.

PRADO, J. M., DALMOLIN, I., CARARETO, N. D. D., BASSO, R. C., MEIRELLES, A. J. A., OLIVEIRA, V., BATISTA, E. A. C. & MEIRELES, M. A. A. 2012. Supercritical fluid extraction of grape seed: Process scale-up, extract chemical composition and economic evaluation. *Journal of Food Engineering*, 109, 249-257.

QUISPE-JACOBO, V., ARROYO-CONDORENA, K. & GUTIERREZ, A. 2011. Morphological and chemistry characteristics from three cultivars of purple corn (*Zea mays* L.) in Arequipa – Perú. *Revista de la Sociedad Químic del Perú*, 77, 205-217.

RISCO-MENDOZA, M. 2007. Conociendo la cadena productiva del maiz morado en Ayacucho. Available in: http://www.solidinternational.ch/wp-content/themes/solid/sources/img/Conociendo-la-cadena-productiva-del-maiz-morado-en-Ayacucho11.pdf.

SANTOS, D. T., VEGGI, P. C. & MEIRELES, M. A. A. 2010. Extraction of antioxidant compounds from Jabuticaba (*Myrciaria cauliflora*) skins: Yield, composition and economical evaluation. *Journal of Food Engineering*, 101, 23-31.

SHARAN, R. N., ODYUO, M. M. & PURKAYASTHA, S. 2011. Oxygen free radicals and their biomedical implications: A mini review. *Mini-Reviews in Organic Chemistry*, 8, 372-376.

TSUDA, T., HORIO, F., UCHIDA, K., AOKI, H. & OSAWA, T. 2003. Dietary cyanidin 3-O-beta-D-glucoside-rich purple corn color prevents obesity and ameliorates hyperglycemia in mice. *Journal of Nutrition*, 133, 2125-2130.

TSUDA, T., UENO, Y., AOKI, V., KODA, T., HORIO, F., TAKAHASHI, N., KAWADA, T. & OSAWA, T. 2004. Anthocyanin enhances adipocytokine secretion and adipocyte-specific gene expression in isolated rat adipocytes. *Biochemical and Biophysical Research Communications*, 316, 149-157.

TURTON, R., BAILIE, R. C., WHITING, W. B. & SHAEIWITZ, J. A. 1998. Analysis, synthesis, and design of chemical process, 1 ed., Prentice Hall, Upper Saddle River, NJ.

VEGGI, P. C., CAVALCANTI, R. N. & MEIRELES, M. A. A. 2014. Production of phenolic-rich extracts from Brazilian plants using supercritical and subcritical fluid extraction: Experimental data and economic evaluation. *Journal of Food Engineering*, 131, 96-109.

YANG, Z. & ZHAI, W. 2010. Identification and antioxidant activity of anthocyanins extracted from the seed and cob of purple corn (*Zea mays* L.). *Innovative Food Science and Emerging*, 11, 169-176.

ZAGHDOUDI, K., PONTVIANNE, S., FRAMBOISIER, X., ACHARD, M., KUDAIBERGENOVA, R., AYADI-TRABELSI, M., KALTHOUM-CHERIF, J., VANDERESSE, R., FROCHOT, C. & GUIAVARC'H, Y. 2015. Accelerated solvent extraction of carotenoids from: Tunisian Kaki (*Diospyros kaki* L.), peach (*Prunus persica* L.) and pricot (*Prunus armeniaca* L.). *Food Chemistry*, 184, 131-139.

Capítulo 4

Recovery of bioactive compounds from Amazonian fruit wastes via green techniques

Angela M. Farías-Campomanes, Carlos V. Lamarão, Elena M. Balboa, Herminia Domínguez, Valdir F. Veiga, M. Angela A. Meireles

Artigo que foi submetido ao periódico Food and Bioproducts Processing em outubro de 2015

O material suplementar desde artigo encontra-se com Apêndice B

Recovery of bioactive compounds from Amazonian fruit wastes via green techniques

Angela M. Farías-Campomanes[1], Carlos V. Lamarão[2], Elena M. Balboa[3], Herminia Domínguez[3], Valdir F. Veiga[2], M. Angela A. Meireles[1]*

[1]LASEFI/DEA/FEA (School of Food Engineering), UNICAMP (University of Campinas), Rua Monteiro Lobato 80, 13083-862 Campinas, São Paulo, Brazil

[2]Department of Chemistry, ICE, UFAM (Federal University of Amazonas), Av. Gal. Rodrigo Octávio, 3.000, Coroado II, Manaus, Brazil

[3]Department of Chemical Engineering, University of Vigo (Campus Ourense) As Lagoas s/n, 32004, Ourense, Spain

Corresponding author: Profa. Dra. M. Angela A. Meireles

E-mail: maameireles@gmail.com

Telephone/Phone: +55 19 35214033

Fax: +55 19 35214027

Recovery of bioactive compounds from Amazonian fruit wastes via green techniques

Angela M. Farías-Campomanes[1], Carlos V. Lamarão[2], Elena M. Balboa[3], Herminia Domínguez[3], Valdir F. Veiga Jr.[2], M. Angela A. Meireles[1*]

[1]LASEFI/DEA/FEA (School of Food Engineering), UNICAMP (University of Campinas), Rua Monteiro Lobato 80, 13083-862 Campinas, Sao Paulo, Brazil

[2]Department of Chemistry, ICE, UFAM (Federal University of Amazonas), Av. Gal. Rodrigo Octávio, 3.000, Coroado II, Manaus, Brazil

[3]Department of Chemical Engineering, University of Vigo (Campus Ourense) As Lagoas s/n, 32004, Ourense, Spain

*Corresponding author: maameireles@gmail.com Phone: ±55 19 35214033, fax: +55 19 35214027

Abstract

In this paper, the obtaining of bioactive compounds via pressurized liquid extraction (PLE) and supercritical fluid extraction (SFE) was studied. Ethanol and carbon dioxide were used as extraction solvents, respectively. The effects of temperature, pressure and solvent mass to feed mass ratio (S/F) on the extract yield and composition were evaluated. The PLE of assai seeds (313 to 333 K, 2 to 6 MPa), PLE of piquia seeds (313 to 333 K, 2 to 10 MPa, S/F of 5 to 25) and SFE of abiu peels (313 to 333 K, 15 to 35 MPa) were studied. PLE is an efficient technique for extracting phenolics and antioxidants from assai seeds and even flavonoids from piquia seeds. Extracts with high content of bioactive compounds were produced at low pressures and low temperatures using only small amounts of ethanol. However, the increase of pressure, temperature and S/F increased the extract yield. Assai seed extracts with 33 % phenolics and high antioxidant activities were obtained at 313 K/ 4 MPa. The best extraction condition for PLE of piquia seeds was 313 K/ 8 MPa and S/F of 10 due to piquia seed extracts with 10 % phenolics, 5.3 % flavonoids and high antioxidant activity (EC_{50} of 12-16 µg/mL) were produced. SFE of abiu peels showed high extraction selectivity and produced extracts with antioxidant activity lower than those showed by assai and piquia seed extracts. SFE could be used to obtain polymers from abiu peels and/or as a pre-treatment to improve the antioxidants extraction.

Keywords: Pressurized liquid extraction, supercritical fluid extraction, phenolic, flavonoid, antioxidant activity.

Highlights

- Green extraction of bioactive compounds from Amazonian fruit wastes was conducted.
- Extracts rich in antioxidant and phenolics were obtained from assai seeds by PLE.
- PLE provided extracts rich in phenolic and flavonoid compounds from piquia seeds.
- Abiu peels extracts obtained by SFE showed low antioxidant activity.
- PLE and SFE showed high efficiency on the extraction of bioactive compounds.

Graphical abstract

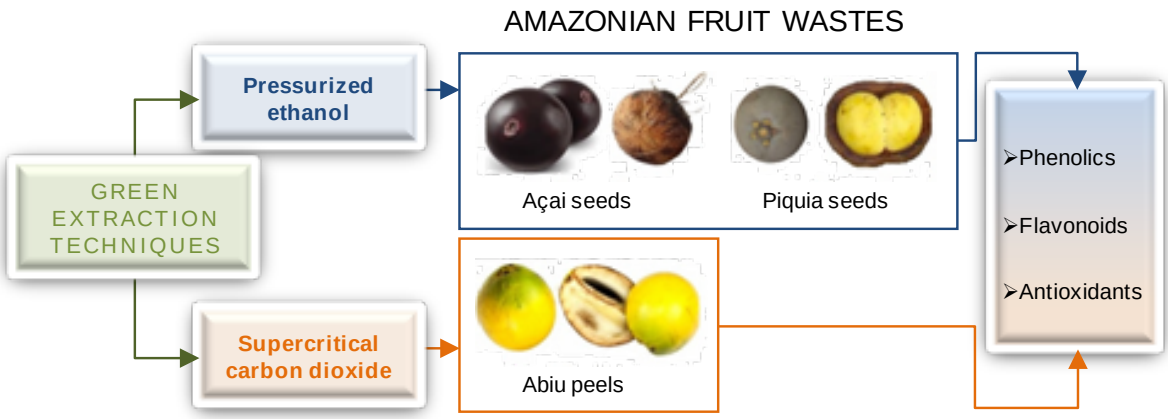


Figure 1. Graphical abstract

1. Introduction

The food, pharmaceutical and cosmetic industries have shown recent interest in Amazonian native plants, which are widely recognized for their nutritional value and potential bioactivity. Bioactive compounds have specific biological activities in humans and can be found in small quantities in plants and fruits (Sasidharan et al., 2010). Several properties have been attributed to bioactive compounds, including antioxidant, anticancer, and anti-inflammatory properties (Liu, 2013).

Assai (*Euterpe precatoria*) is a fruit indigenous to the Amazonian region of Brazil and is well known as an energetic fruit and an important source of phenolics and flavonoids. These phenolics and flavonoids consist mainly of anthocyanins, which are responsible for the fruit's antioxidant effects (Rosso et al., 2008; Bataglion et al., 2015; Rufino et al., 2011). Most of the published studies on assai only consider the pulp of the fruit. Few studies consider the assai seeds which represent up to 90 % of the fruit's weight. Assai seeds contain saturated and unsaturated fats. However, in contrast to pulp, assai seeds do not contain cyaniding-3-glucoside and cyanidin-3-rutinoside (Wycoff et al., 2015). On the other hand, assai seed extracts have antioxidant activity that can be attributed to the presence of procyanidins and other unidentified compounds (Rodrigues et al., 2006).

Piquia (*Caryocar villosum*) is an Amazonian fruit and is much appreciated after it is cooked. A few studies on piquia pulp have been conducted recently. Almeida et al. (2012) evaluated the genotoxic effect of piquia pulp on DNA, concluding that piquia pulp extracts protect against DNA damage. Carotenoids and phenolics were identified in piquia pulp extracts, but only phenolics were found to affect the *in vitro* antioxidant efficiency of the extracts (Chisté et al., 2012). However, despite growing researcher interest in piquia and its effects on human health, few studies have been performed on the fruit to date. To the author's knowledge, currently, there are no published studies of piquia seeds, even though the seeds could be a potential source of phenolics and carotenoids with antioxidant properties, as well as the pulp.

Abiu (*Pouteria caimito*) is widely known for its sweet taste and its sticky white liquid, popularly known as "latex", which sticks to the lips when eaten. Latex from abiu is traditionally removed with oil, applied on abscesses and given as a vermifuge and purge (Morton, 1987). Abiu has also been used by Amazonian groups to treat malaria and to relieve

coughs and bronchitis (Ruiz et al., 2011). However, to the author's knowledge, studies to elucidate the compounds responsible for these beneficial health effects have not been performed.

Despite the potential biological activity of these Amazonian fruits, the wastes from fruit consumption and processing are currently discarded and are not considered to be sources of bioactive compounds that are feasible for recovery. Moreover, efficient and environmentally friendly extraction techniques which aim to efficiently obtain bioactive compounds by producing highly concentrated extracts in short periods of time by using small amounts of non-toxic solvents, have not been applied to Amazonian fruit wastes.

Pressurized liquid extraction (PLE) has been used to generate value-added compounds from several raw materials and has demonstrated higher efficiencies than conventional extraction techniques such as soxhlet and maceration (Kaufmann and Christen, 2002). PLE uses a liquid solvent at high temperature and high pressure. High temperatures enable the interactions between the solid matrix and target compounds to be disrupted, thus increasing the solubilities of the target compounds in the solvent (Mustafa and Turner, 2011). Because increasing temperature decreases the surface tension and viscosity of the solvent, mass transfer is improved. In addition to increased temperature, pressure is applied to keep the solvent in the liquid state, thus improving the solvation power of the solvent (Xynos et al., 2012). Therefore, PLE can be performed in short periods of time with small amounts of solvent (Osorio-Tobón et al., 2014). Because PLE is conducted in a closed extractor, the degradation of oxygen- and light-sensitive compounds is avoided. PLE has been mainly applied for extracting polar compounds and is preferentially performed with water and ethanol (green solvents) as extraction solvents.

Similar to PLE, supercritical fluid extraction (SFE) is a green extraction technique. SFE uses a supercritical fluid as extraction solvent and has been mainly used for extracting non polar compounds. However, more polar compounds also have been extracted via SFE. For that, the solvation power of the supercritical fluid is increased through the addition of a small volume of a polar solvent (co-solvent). Moreover, SFE have been applied for removing undesirable non polar compounds such as fat (Albuquerque and Meireles, 2012), polymers (Daneshvar et al., 2012) and alkaloids (Pereira et al., 2007).

Supercritical fluid extraction (SFE) is performed at temperatures and pressures above the critical point of the solvent. In the supercritical region, the properties of the solvent

are similar to the properties of gases and liquids: the solvent's density is approached to that of liquids, the solvent's viscosity is close to that of normal gases, while solvent's diffusivity is about two orders of magnitude higher than liquids (Brunner, 2005). These properties can be modified with small variations in pressure and temperature (Shi et al., 2012). Thus, supercritical fluids can dissolve a greater range of compounds than gasses and can diffuse through the solid matrix more efficiently than liquids. Carbon dioxide is the most frequently used solvent for supercritical fluid extraction due to its nontoxic nature, low cost, generally recognized as safe (GRAS) status and high availability. Most importantly, carbon dioxide has a low critical point (304.3 K and 7.4 MPa) that allows the extraction of highly temperature-sensitive compounds (Martínez and Vance, 2007). Similar to PLE, SFE minimizes degradation from exposure to oxygen and light because the extraction is performed in a closed extractor.

PLE has been successfully used to obtain polyphenols (Machado et al., 2015; Veggi et al., 2014; Paes et al., 2014; Osorio-Tobón et al., 2014; Garcia-Mendoza et al., 2015), carotenoids (Cardenas-Toro et al., 2015; Garcia-Mendoza et al., 2015; Zaghdoudi et al., 2015) and thiosulfinates (Farías-Campomanes et al., 2014), all of which are polar bioactive compounds. Moreover, SFE has been successfully used to obtain non polar and slightly polar bioactive compounds such as terpenoids (Uquiche et al., 2015), carotenoids (Moraes et al., 2015; Przygoda and Wejnerowska 2015), and capsinoids (Aguiar et al., 2014).

The objective of this work was to evaluate the abilities of PLE to extract polar bioactive compounds from assai and piquia seeds, and SFE to extract non polar bioactive compounds from abiu peels, using pressurized ethanol and supercritical carbon dioxide as extraction solvents, respectively.

2. Material and methods

2.1. Raw materials and characterization

Assai (*Euterpe precatoria*), piquia (*Caryocar villosum*) and abiu (*Pouteria caimito*) were collected in the Amazonas state (Brazil) in March 2013. The fruits were cleaned in the Laboratory of Plant Products Technology at the Federal University of Amazonas (UFAM). Assai seeds, piquia seeds and abiu peels were manually separated from the fruits. The seeds and peels were dried in an oven at 308 K (model SP-100/27-A, Novatecnica, Sao Paulo, Brazil) for 30 h, then the raw materials were milled using a knife

grinder (model SP31, SP Labor, Sao Paulo, Brazil), packed in plastic bags, labeled and stored in a domestic freezer at 255 K until their transport to Campinas (Sao Paulo, Brazil). At the Laboratory of Supercritical Technology: Extraction, Fractionation and Identification of Extracts (LASEFI) at the University of Campinas (UNICAMP), the geometric mean diameter of the particles was determined according to ASAE (ASAE S319.4, 2008). The particle size distribution was determined using a vibratory system (Bertel, model 1868, Sao Paulo, Brazil) equipped with 16- to 80- mesh sieves (Tyler series, Wheeling, USA). The moisture contents of the raw materials were determined according to a gravimetric method based on water removal upon heating in an oven (Tecnal, model TE 395-1, Sao Paulo, Brazil) at 378 K. The bed's apparent density was calculated by dividing the mass of the raw material used in the experiments by the volume occupied by the material in the extractor vessel.

2.2. Extractions

PLE was conducted in a homemade PLE system previously described by Rodrigues et al. (2014). This system was slightly modified by replacing the back pressure valve with blocking and micrometric valves. The PLE system was equipped with a 5.7 cm^3 extraction vessel (Thar Designs, CL 1373, Pittsburg, USA) that was completely filled with raw material. Ethanol (99.5 % purity, Dinamica, Campinas, Brazil) was used as the extraction solvent. PLE experiments were performed in static mode followed by dynamic mode. Static extraction was performed to equilibrate the system with respect to temperature and pressure. After filling with raw material, the extraction vessel was brought to the operating temperature and then filled with solvent until the operating pressure was reached. Static extraction began when the operating conditions (temperature and pressure) were reached. During static mode, solvent was not fed into or removed from the extraction vessel. For PLE of assai and piquia seeds, static extraction times of 10 min were used. Dynamic extraction involved continuously flowing the solvent at the operating flow rate through the extraction bed within the vessel at the operating temperature and pressure. Dynamic extraction was performed until the required solvent mass-to-feed mass ratio (S/F) was reached. The PLE parameters used for assai and piquia seeds are shown in Table 1. After the PLE experiments, the ethanol in the assai seed extracts was immediately removed by vacuum evaporation at 0.01 MPa (Laborota, model 4001, Viertrieb, Germany) using a thermostatic bath at 313 K. Piquia seed extracts were first filtered through filter paper and then subjected to vacuum evaporation. Filtration stage before evaporation was performed because piquia seed extracts showed precipitation of fine white particles. Finally, the extract-containing collector flasks were weighed in a semi-analytical

balance (Sartorius, model A200S, Gottingen, Germany) to determine the extract masses. PLE experiments were performed in duplicate.

SFE was performed in the commercial SFE system described by Albuquerque and Meireles (2012). The extraction vessel volume was the same as for PLE (5.7 cm^3) and was completely filled with abiu peels. Carbon dioxide (99.9 % purity, Gama Gases, Sao Bernardo do Campo, Brazil) was used as the extraction solvent. The static extraction time was 10 min, and dynamic extraction was performed until the required solvent mass-to-feed mass ratio (S/F) was achieved. SFE parameters for abiu peels are shown in Table 1.

Table 1. Extraction parameters of PLE of assai seeds, PLE of piquia seeds and SFE of abiu peels.

Process	Temperature (K)	Pressure (MPa)	S/F ratio	Flow rate (kg/s)
PLE of assai seeds	313, 323, 333	2, 4, 6	2.3 ± 0.2	2.0×10^{-5}
PLE of piquia seeds	313, 323, 333	2, 4, 6, 8, 10	5, 10, 15, 20, 25	2.0×10^{-5}
SFE of abiu peels	313, 323, 333	15, 20, 25, 30, 35	50	1.11×10^{-4}

Pressurized liquid extraction (PLE), supercritical fluid extraction (SFE), solvent mass to feed mass ratio (S/F).

After extractions, the collector flasks were placed in desiccators for 10 minutes to completely remove the carbon dioxide from the extracts. Finally, the collector flasks were weighted using a semi-analytical balance (Sartorius, model A200S, Gottingen, Germany) to determine the extract masses. The SFE experiments were performed in duplicate.

Extract yield (EY) was calculated as the ratio of extract mass (M_E) to the total dry mass of the raw material (M_R) used to form the extraction bed, as shown in Eq. (1).

$$EY(\%) = \frac{M_E}{M_R} \times 100 \quad (1)$$

2.3. Extract characterization

2.3.1. Total phenolic content

The total phenolic content was determined according to the method proposed by Singleton et al. (1999). The Folin-Ciocalteau method is based on the reduction of

phosphomolybdic-phosphotungstic acid to a blue complex in an alkaline solution, a reaction that occurs in the presence of phenolic compounds. Quantification was done on the basis of a standard curve of gallic acid. Analyses were performed in triplicate, and the results are expressed in g of gallic acid equivalent (GAE) per 100 g of extract (%) and g of GAE per 100 g of raw material in dry basis (%).

2.3.2. Total flavonoid content

The total flavonoid content was determined according to the method described by Delcour and Devarebeke (1985). This method is a colorimetric assay based on the reaction of A-rings with the chromogen p-dimethylaminocinnamaldehyde (DMACA). Quantification was performed on the basis of a standard curve of catechin. The results are expressed in g of catechin equivalent (CE) per 100 g of extract (%) and g of CE per 100 g of raw material in dry basis (%). Analyses were performed in triplicate.

2.3.3. DPPH radical scavenging activity

The antioxidant activity of the extracts was determined using the method proposed by Kordali et al. (2005). This method involves measuring the radical scavenging ability of samples using the radical 2,2-diphenyl-1-picrylhydrazyl (DPPH). Analyses were performed in triplicate, and the results are expressed as percentages of inhibition (PI) according to Eq. (2)

$$PI(\%) = \frac{A_c - A_s}{A_c} \times 100 \quad (2)$$

where A_c is the absorbance of the control and A_s is the absorbance of the sample. The antioxidant activity of an extract was expressed as a PI when the extract concentration used in the analysis did not scavenge 50 % of the DPPH free radicals. The antioxidant activity was expressed as EC_{50} when the extract concentration resulted in the scavenging of 50% of the DPPH free radicals.

2.3.4. ABTS radical scavenging activity

This assay was performed according to the method described by Re et al. (1999) and is based on the ability of different substances to scavenge the 2,2-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) ABTS+ radical. Analyses were performed in triplicate, and the results were expressed as EC_{50}.

3. Results and discussion

The characterization data of Amazonian fruit wastes are presented in Table 2. Approximately the same mass of raw material from each fruit was required to form the extraction beds. Because extractor vessels with the same volume were used in all experiments, the apparent densities of the extraction beds must be similar.

Table 2. Physical and chemical characteristics of Amazonian fruit wastes.

		Assai seeds	Piquia seeds	Abiu peels
Moisture (%)		7.1 ± 0.1	21.5 ± 0.3	3.7 ± 0.2
Extraction bed (g, w.b.)		5.1 ± 0.3	5.06 ± 0.03	5.4 ± 0.1
Apparent density (g/cm^3)		0.90 ± 0.03	0.89 ± 0.01	0.96 ± 0.01
Geometric mean diameter of particle (mm)		0.99 ± 0.01	1.04 ± 0.03	0.73 ± 0.01
Mass retained (%)	16 mesh	68 ± 1	73 ± 12	18 ± 1
	24 mesh	15.5 ± 0.3	23 ± 9	10.5 ± 0.4
	32 mesh	5.3 ± 0.3	4 ± 3	12 ± 1
	48 mesh	3.9 ± 0.2	1 ± 1	19.8 ± 0.1
	80 mesh	5 ± 1	-	28 ± 1
	Plate	3 ± 1	-	12 ± 2

Values are reported as the means ± standard deviation

3.1. Pressurized liquid extraction of assai seeds

The performance of the PLE using assai seeds was evaluated in terms of extract yield, and extracts were evaluated according to phenolic content and antioxidant activity. The results are shown in Table 3. As expected, an increase in temperature increased the extract yield, since an increased temperature increases the solubility. Elevated temperatures decrease the solvent surface tension and therefore enable the formation of solvent cavities; thereby allowing a faster dissolution of analytes in the solvent, which results in the increasing of the extract yield (Mustafa and Turner, 2011). However, increased temperature is also associated with decreasing extraction selectivity, i.e. non-target compounds are also dissolved. Extract characteristics must, therefore, be considered to determine the optimal extraction temperature.

In addition to keeping the extraction solvent in the liquid state, pressure helps the solvent to efficiently penetrate the solid matrix, thus maximizing the contact between target compounds and solvent (Mustafa and Turner, 2011). In this study, the extract yield increased

when the pressure was raised from 2 to 6 MPa; however, this behavior was only observed in extractions performed at 323 and 333 K. At 313 K, an interaction between temperature and pressure may have occurred. Because viscosity increases as temperature decreases and density increases as pressure increases, an increase in the density of an already viscous solvent (at 313 K) may have hindered its passage through the extraction bed, resulting in a decreased extract yield.

Table 3. Extract yields, phenolic yields, and phenolic content and antioxidant activities of assai seed extracts obtained by PLE.

Temperature/ Pressure	Extract yield (g extract.100 g^{-1} assai seeds)	Phenolic yield (g GAE.100 g^{-1} assai seeds)	Phenolic content (g GAE.100 g^{-1} extract)	Antioxidant activity (PI)
313 K/2 MPa	0.67 ± 0.04	0.17 ± 0.01	26 ± 1	43 ± 1
313 K/4 MPa	0.64 ± 0.03	0.21 ± 0.03	33 ± 5	42 ± 1
313 K/6 MPa	0.64 ± 0.02	0.18 ± 0.01	29 ± 1	46 ± 1
323 K/2 MPa	0.7 ± 0.1	0.19 ± 0.01	27 ± 1	47 ± 1
323 K/4 MPa	0.71 ± 0.04	0.20 ± 0.02	29 ± 3	46 ± 7
323 K/6 MPa	0.72 ± 0.02	0.17 ± 0.04	23 ± 5	27 ± 1
333 K/2 MPa	0.7 ± 0.1	0.14 ± 0.02	20 ± 2	37 ± 1
333 K/4 MPa	0.8 ± 0.1	0.19 ± 0.01	25.6 ± 0.1	38 ± 2
333 K/6 MPa	0.82 ± 0.03	0.17 ± 0.05	21 ± 6	26 ± 10

Values are reported as the means ± standard deviation. Pressurized liquid extraction (PLE), percentage of inhibition (PI), gallic acid equivalents (GAE).

The highest extract yield obtained in this study from *E. precatoria* assai seeds (0.82 ± 0.03 %, achieved at 333 K/ 6 MPa) was lower than the yield reported by Wycoff et al. (2015) for white and purple assai seeds from *E. oleracea* Mart. Specie. Wycoff et al. (2015) reported extract yields of 3.4% for white assai seeds and 4.7% for purple assai seeds, using an accelerated solvent extractor at 373 K/ 10.3 MPa with methanol as the extraction solvent. The lower extract yields obtained in this study can be attributed to the operating conditions, the extraction solvent and the raw material source. However, it should be noted that unlike ethanol, methanol is a toxic solvent; therefore the use of methanol as extraction solvent represents a no green extraction process, besides reducing the field of application of the

extracts. Also, the use of elevated temperature may promote the bioactive compounds degradation.

Recently, the food, cosmetic and pharmaceutical industries have shown a special interest in assai because of its antioxidant capacity. This antioxidant capacity has been partially attributed to the fruit's phenolic content (Pacheco–Palencia et al., 2009). Thus, the phenolic yield of assai seeds using PLE was determined (Table 3). A maximum phenolic yield of 0.21 ± 0.03% was obtained from assai seeds at 313 K/ 4 MPa. This yield was higher than the yields reported for *E. oleracea* pulp (0.02 and 0.03%) whose extracts were obtained with methanol and water, respectively, after maceration, agitation, sparging, filtration and freeze drying stages (Pacheco-Palencia et al., 2009; Bataglion et al., 2015). However, the results obtained in this work are comparable with the phenolic yield reported for defatted *Euterpe edulis* pulp (0.22 %) whose extracts were obtained in a ultrasonic bath with methanol 0.1 M HCl (338 K for 15 min), centrifugation and filtration (Borges et al., 2011). Due to the lack of studies on the phenolic content of assai seeds, assai pulp was considered in this discussion of results. According to Table 3, the phenolic content in extracts decreased as the temperature increased, a trend likely caused by a decrease in extraction selectivity. High temperatures reduce the dielectric constant and polarity of a solvent (Mohsen-Nia et al., 2010), potentially favoring the extraction of less polar compounds such as fat. Wycoff et al. (2015) reported a saturated and unsaturated fat content of 0.22-0.33% in assai seeds. However, the degradation of phenolics in assai seed extracts by the using of increased temperature also could have occurred.

Assai seed extracts with a minimum phenolic content of 20% (dry basis) were produced by PLE with ethanol. The extract with the highest phenolic content (33 ± 5%) was obtained at 313 K/ 4 MPa. The phenolic concentration in the ethanolic extracts prior to solvent removal was 0.7 ± 0.1 g GAE. L^{-1} of extract. This result agrees with the results reported by Rodrigues et al. (2006), who produced assai seed extracts with polyphenol concentration of 0.68 g/L by repeated digestion with 1 L of methanol at room temperature during 3 days.

High DPPH radical inhibition percentages (PIs) were obtained despite the low concentrations of the extract solutions (0.25 mg. mL^{-1} of ethanol). Extracts produced at 2 and 4 MPa showed similar antioxidant activities, although the phenolic content in the extracts differed. This finding suggests that a pressure of 2 MPa may have promoted the co-extraction

of non-phenolic and antioxidant compounds. Better polyphenol recovery from assai seeds has been obtained using methanol as the extraction solvent rather than ethanol. Cold digestion with methanol at room temperature for 3 days produced extracts with higher polyphenol concentrations and higher antioxidant activities than those produced with ethanol by soxhlet extraction (Rodrigues et al., 2006). However, besides the methanol toxicity, the time is an important processing parameter due to longer extraction times not only involves an expenditure of energy but also implies the degradation of light and oxygen sensitive compounds.

As seen by the obtained results, PLE is a promising and environmentally friendly technique for producing assai seed extracts with high phenolic content and antioxidant activity. Passing a small amount of ethanol (S/F of ratio of 2.3 ± 0.2) at low temperature and pressure (313 K/ 4 MPa) through the extraction bed for a short time (10 min) was required to obtain the highest extract yields and to produce extracts with the highest phenolic content. Due to the features of assai seed extracts generated by PLE, these extracts may be used in the food industry to replace synthetic antioxidants or as ingredients in cosmetic and drug formulation.

3.2. Pressurized liquid extraction of piquia seed

In extraction processes with highly porous extraction beds, an increase in pressure is associated with compaction of the extraction bed, resulting in a decreased extract yield (Farías-Campomanes et al., 2013). This effect can be mitigated by using solvents with reduced viscosities, and reduced viscosities can be achieved by increasing the temperature. Therefore, to increase the extract yield at high pressures, elevated temperatures are desired. Conversely, at low operating temperatures, the extract yield can be increased by using low pressures. This hypothesis was verified by the isotherms measured at 313 and 323 K (Figure 2).

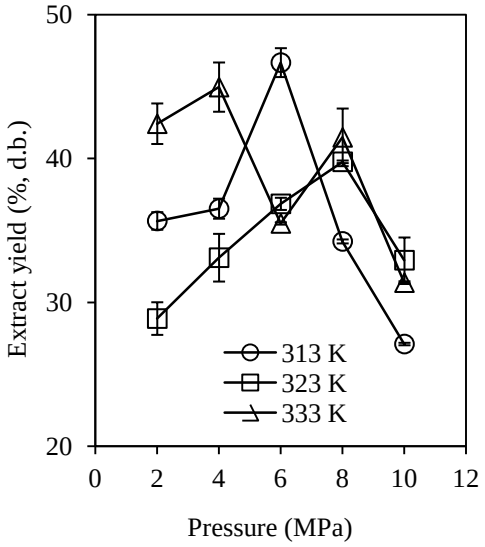


Figure 2. Extract yield isotherms obtained by PLE with S/F ratio of 25 from piquia seeds.

At the lowest operating temperature (313 K), a pressure of 6 MPa resulted in the maximum extract yield, while higher pressures decreased the extract yields. When the temperature was increased to 323 K, the pressure at which the maximum extract yield was obtained increased to 8 MPa. Once again, pressures above 8 MPa decreased the extract yields. For the isotherm performed at 333 K, the hypothesis was not confirmed, as the extract yield increased from 2 to 4 MPa and from 6 to 8. This behavior suggests the existence of two extraction periods and may be associated with increased compound solubility due to increased temperature (Mustafa and Turner, 2011). The highest extract yields of 47 ± 1 and 45 ± 2% were obtained at 313 K/ 6 MPa and 333 K/ 4 MPa, respectively, with an S/F ratio of 25.

In Figure 3, the extract yield obtained at 333 K/ 4 MPa is plotted as a function of S/F ratio. For the temperatures and pressures studied, the extraction degree (ED) of the extract (the ratio between the extract yield for a given S/F and the maximum extract yield that was achieved at the same extraction condition) varied from 50 to 86% at an S/F of 10. Moreover, high EDs of phenolics (92 to 99 %) and flavonoids (92 to 100 %) from piquia seeds were achieved at the same S/F ratio (Figure 3). Because nearly all of the phenolics and flavonoids were extracted at an S/F ratio of 10, S/F ratios higher than 10 likely promoted the co-extraction of non-target compounds, decreasing the extract purity.

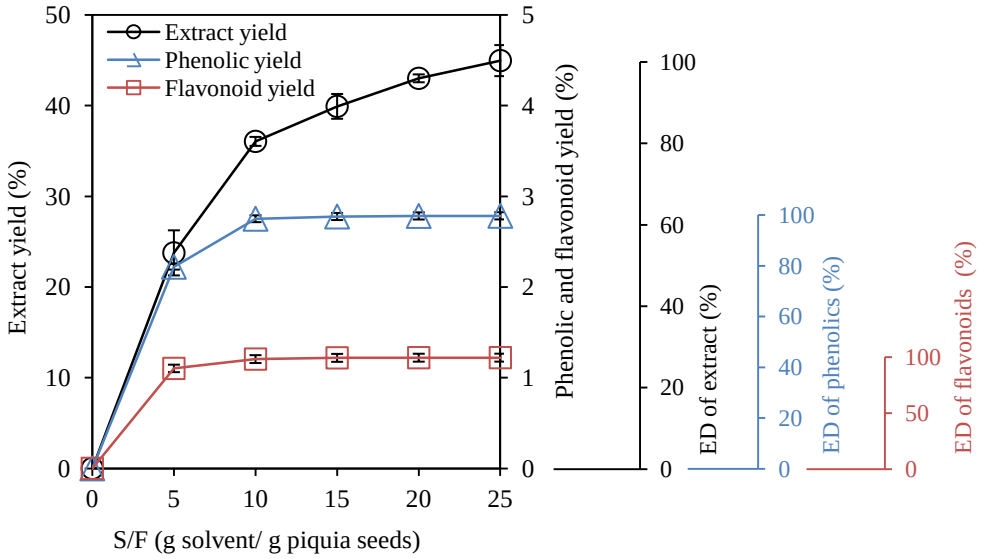


Figure 3. Yield (with respect to piquia seeds in dry basis) and extraction degree (ED) of extract, phenolics and flavonoids from piquia seeds by PLE at 333 K/ 4 MPa.

Figure 4 shows the extraction yields of phenolics and flavonoids from piquia seeds by PLE with an S/F ratio of 10. The 333 K/ 8 MPa condition yielded the highest extraction efficiency of phenolics and flavonoids, with a phenolic yield of 2.91 ± 0.02% and a flavonoid yield of 1.51 ± 0.02%. At 313 and 323 K, the extraction of phenolics and flavonoids was increased when the pressure was increased. Pressures above 8 MPa resulted in decreased extraction yields.

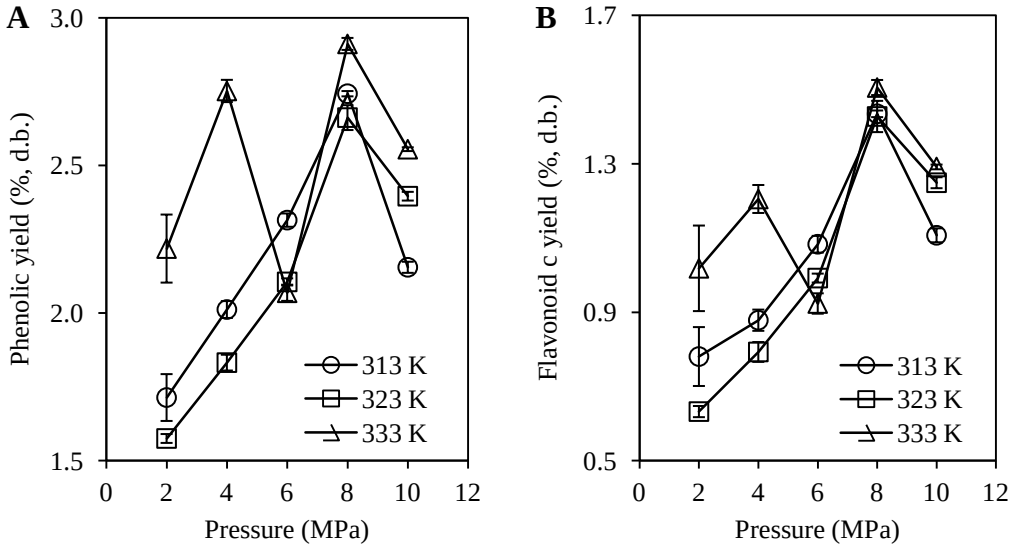


Figure 4. Phenolic yield (g GAE.100 g^{-1} of piquia seeds) (A) and flavonoid yield (g CE. 100 g^{-1} of piquia seeds) (B) obtained by PLE using S/F of 10.

Figure 5 shows the content of phenolics and flavonoids in piquia seed extracts obtained with an S/F of 10. Temperature did not influence the content of phenolics and flavonoids in the extracts. A pressure of 8 MPa yielded the highest contents. Piquia seed extracts with phenolic contents of 9.9 to 10.5% and flavonoid contents of 5.1 to 5.6% were obtained for temperatures between 313 and 333 K, a pressure of 8 MPa and an S/F ratio of 10. Due to piquia is a relatively novel fruit, few studies have been performed with piquia pulp. Furthermore, to the author's knowledge, piquia seeds have not yet been explored. Chisté et al. (2012) produced extracts from piquia pulp with a mixture of ethanol and water (1:1, v/v) at atmospheric pressure and room temperature. A maximum phenolic content of 5,163 ± 161 µg per g of extract or 0.52 ± 0.02%, determined by the sum of individual phenolic compounds, was reported. Piquia seed extracts also showed antioxidant activity (Figure 6). At constant temperature, extracts with the highest antioxidant activity (i.e., the lowest half maximal effective concentration, EC_{50}) were obtained at 8 MPa. At constant pressure, the antioxidant activities of the extracts were similar for all studied temperatures. These results suggest that there may be a strong correlation between antioxidant activity and phenolic and/or flavonoid content in the piquia seed extracts. Furthermore, the increase in EC_{50} when the S/F ratio was increased also support this hypothesis because the content of phenolics and flavonoids in the extracts decreased with increasing S/F ratio, higher concentrations of extract were required to reach the EC_{50}.

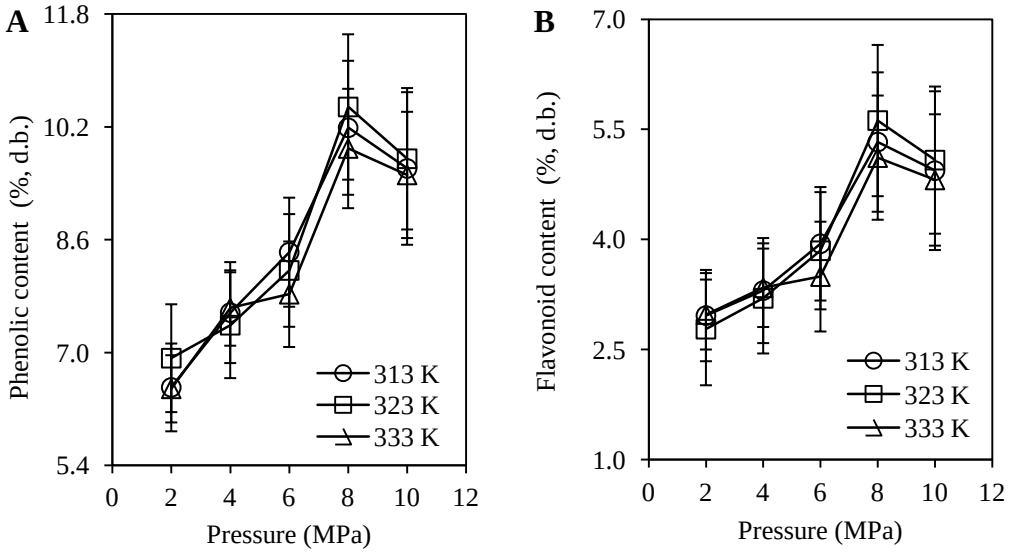


Figure 5. Phenolic content (g GAE.100 g^{-1} of extract) (A) and flavonoid content (g CE.100 g^{-1} of extract) (B) in the extracts of piquia seeds obtained by PLE using S/F of 10.

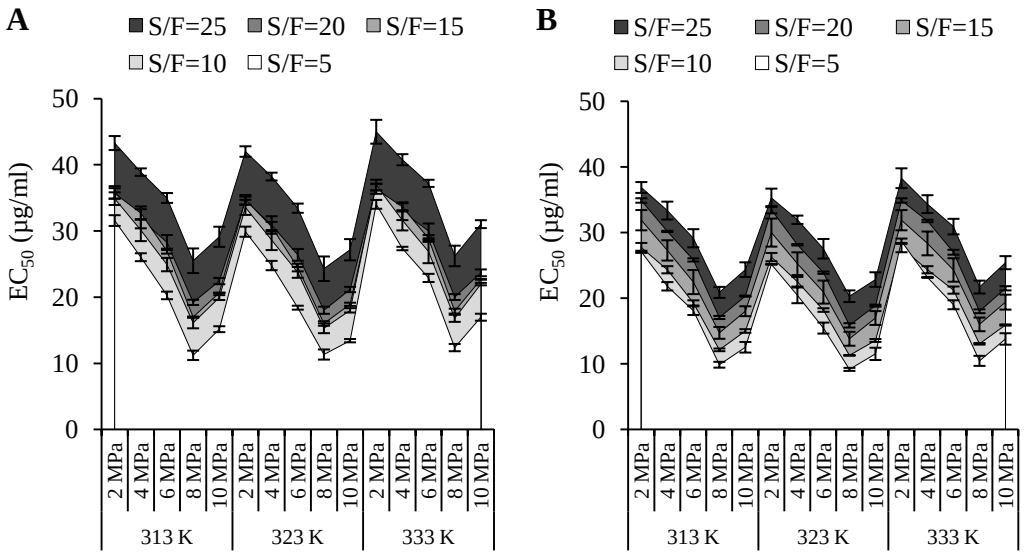


Figure 6. Antioxidant activity of the piquia seed extracts obtained by PLE determined by DPPH (A) and ABTS (B) methods. Solvent mass to feed mass ratio (S/F).

Due to higher temperatures increase energy consumption and operating costs and because temperature did not affect the extraction of bioactives from piquia seeds, the best conditions for PLE of piquia seeds are 313 K/ 8 MPa and an S/F ratio of 10.

Moreover, due to the scarcity of studies with piquia, the results obtained in this work are novel and may contribute to the development of industrial processes to obtain phenolics, flavonoids and antioxidants to manufacture food additives, drugs and cosmetics.

3.3. Supercritical fluid extraction of abiu peels

Figure 7 shows the extract yield isotherms obtained for SFE of abiu peels. Increasing pressure enhances the solvation power of carbon dioxide, dissolving more compounds and increasing the extract yield. For pressures below 20 MPa, isobaric increases in temperature increased the extract yield. An opposite trend was observed for pressures above 25 MPa.

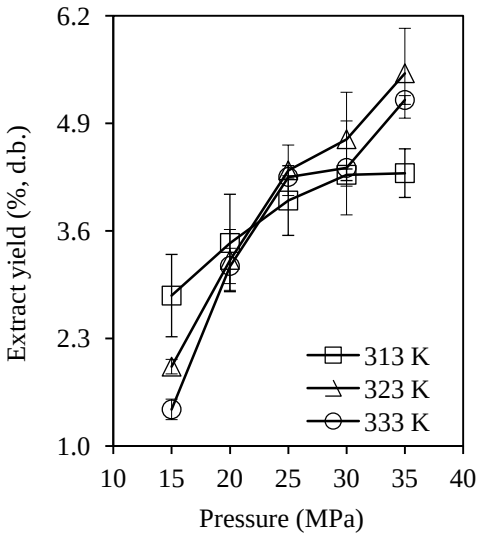


Figure 7. Extract yield isotherms obtained by SFE of abiu peels.

This behavior is known as a crossover phenomenon and is frequently observed in supercritical fluid extraction (Foster et al., 1991). In Figure 7, the crossover pressure for abiu peels was reached between 20 and 25 MPa. According to Mukhopadhyay (2000), density and volatility significantly contribute to the crossover effect. At pressures of 20 MPa and lower, high extract yields were obtained at low temperatures when the solvent density was high, suggesting that the effect of carbon dioxide density was more important than volatility. In contrast, at pressures of 25 MPa and higher, high extract yields were obtained at high temperatures when the compound volatility was high. Thus, for pressures higher than the crossover pressure, the effect of compound volatility was more important than density. Over the range of temperatures and pressures studied, the SFE extract yield of abiu peels varied from 2.3 to 5.8%.

Supercritical carbon dioxide has proven to be a good solvent for dissolving monomers and polymers with low molecular weights (Daneshvar et al., 2012) and has been successfully applied to polymer synthesis and processing (Cooper, 2000; Tomasko et al., 2003). Abiu fruit is widely known for adhering to the lips when eaten, and this characteristic has been attributed to a milky substance popularly named “latex” (Janick and Paull, 2008). Latex is present in abiu peels and has not yet been characterized.

The physical characteristics of abiu peel extracts change with temperature: at room temperature, the extracts were orange and liquid with sticky textures. When stored in a domestic freezer at a low temperature, the extracts were yellow, solids with thick textures.

Because latex is a polymer and polymers change their physical properties with temperature (Reis, 2012), supercritical carbon dioxide may have solvated the “latex” and removed it from the abiu peels. However, to prove this assumption, the abiu peel extracts must be characterized in terms of rheological properties to determine its molecule architecture.

Abiu peel extracts showed low antioxidant activity compared to PLE extracts of assai seeds and piquia seeds (Table 4) when analyzed by DPPH method. When using extract solutions at low concentrations (2 mg/mL ethanol), the highest PI values were obtained with extracts processed under the same conditions that gave the lowest extract yield (333 K/ 15 MPa). In contrast, extracts with the second lowest antioxidant activity were produced under conditions that provided the highest extract yield (323 K/ 35 MPa).

Due to the high selectivity of SFE, this process might be used as a green extraction technique to obtain polymers from abiu peels, instead of conventional techniques that use toxic solvents such as chloroform and methanol (Segallio, 2009). SFE can also be used as a pre-treatment technique to increase the availability of antioxidants in the solid matrix for later extraction by another environmentally friendly technique such as PLE.

Table 4. Antioxidant activities (PI) of the abiu peel extracts obtained by SFE.

Pressure (MPa)	313 K	323 K	333 K
15	4.7 ± 0.4	5.9 ± 1.0	8.8 ± 0.2
20	8.0 ± 2.4	4.1 ± 0.2	4.9 ± 0.3
25	4.1 ± 0.7	6.0 ± 0.1	5.2 ± 0.2
30	5.6 ± 0.8	4.70 ± 0.01	5.2 ± 1.0
35	4.7 ± 0.1	4.24 ± 0.01	5.2 ± 1.2

Values are reported as the means ± standard deviation.

4. Conclusion

Bioactive compounds from Amazonian fruit wastes were obtained using green techniques. PLE is an efficient technique for extracting phenolics and antioxidants from assai seeds and flavonoids from piquia seeds because extracts with high content of bioactive compounds were produced at moderate pressures (4 to 8 MPa) and low temperatures using only small amounts of a GRAS solvent. SFE of abiu peels showed high extraction selectivity

and produced extracts with low antioxidant activity. SFE could be used to extract polymers from abiu peels and/or as a pre-treatment prior to other processing techniques for antioxidant extraction.

Acknowledgments

Angela M. Farías-Campomanes is grateful to CAPES-PEC-PG (5817110) for the Ph.D. assistantship. C. Lamarão Pereira thanks FAPEAM. E. Balboa thanks the Spanish Ministry of Education and Science for her FPI grant (BES-2010-041807). M. Angela A. Meireles is thankful to CNPq for a productivity grant (301301/2010-7). The authors acknowledge the financial support from CAPES, CNPq and FAPESP.

References

AGUIAR, A. C., SANTOS, P., COUTINHO, J. P., BARBERO, G. F., GODOY, H. T. & MARTÍNEZ, J. 2014. Supercritical fluid extraction and low pressure extraction of Biquinho pepper (Capsicum chinense). *LWT-Food Science and Technology*, 59, 1239-1246.

ALBUQUERQUE, C. L. C. & MEIRELES, M. A. A. 2012. Defatting of annatto seeds using supercritical carbon dioxide as a pretreatment for the production of bixin: Experimental, modeling and economic evaluation of the process. *Journal of Supercritical Fluids*, 66, 86-95.

ALMEIDA, M. R., AISSA, A. F., DARIM, J. D. C., GOMEZ, T. D. U. H., CHISTÉ, R. C., MERCADANTE, A. Z., ANTUNES, L. M. G. & BIANCHI, M. L. P. 2012. Effect of Piquiá (Caryocar Villosum) Pulp Fruit Oxidative Stress, Ephx2 and Tp53 Gene Expressions in Liver of Rats. *Free Radical Biology and Medicine*, 53, S82.

ASAE, 2008. ASAE 319.4. Method of Determining and Expressing Fineness of Feed Materials by Sieving S319.4. pp. 602-605. American Society of Agricultural Engineers Standards, Michigan.

BATAGLION, G. A., DA SILVA, F. M. A., EBERLIN, M. N. & KOOLEN, H. H. F. 2015. Determination of the phenolic composition from Brazilian tropical fruits by UHPLC–MS/MS. *Food Chemistry*, 180, 280-287.

BORGES, G. S. C., VIEIRA, F. G. K., COPETTI, C., GONZAGA, L. V., ZAMBIAZI, R. C., FILHO, J. M. & FETT, R. 2011. Chemical characterization, bioactive compounds, and antioxidant capacity of jussara (*Euterpe edulis*) fruit from the Atlantic Forest in southern Brazil. *Food Research International*, 44, 2128-2133.

BRUNNER, G., 2005. Supercritical fluids: technology and application to food processing. *Journal of Food Engineering*, 67, 21-33.

CARDENAS-TORO, F. P., ALCÁZAR-ALAY, S. C., COUTINHO, J. P., GODOY, H. T., FORSTER-CARNEIRO, T. & MEIRELES, M. A. A. 2015. Pressurized liquid extraction and low-pressure solvent extraction of carotenoids from pressed palm fiber: Experimental and economical evaluation. *Food and Bioproducts Processing*, 94, 90-100.

CARDOSO, L. C., SERRANO, C. M., QUINTERO, E. T., LOPEZ, C. P., ANTEZANA, R. M. & DE LA OSSA, E. J. M. 2013. High Pressure Extraction of Antioxidants from Solanum stenotomun Peel. *Molecules*, 18, 3137-3151.

CHISTÉ, R. C., FREITAS, M., MERCADANTE, A. Z. & FERNANDES, E. 2012. The potential of extracts of Caryocar villosum pulp to scavenge reactive oxygen and nitrogen species. *Food Chemistry*, 135, 1740-1749.

COOPER, A.I., 2000. Polymer synthesis and processing using supercritical carbon dioxide. *Journal of Materials Chemistry*, 10, 207-234.

DANESHVAR, M., KAMALI, H., MASOOMI, M. & GHAZIASKAR, H. S. 2012. Supercritical carbon dioxide grafting of glycidyl methacrylate onto medium density polyethylene and purification of residual monomer and initiator. *Journal of Supercritical Fluids*, 70, 119-125.

DELCOUR, J. & DEVAREBEKE, D. J. 1985. A new colometric assay for flavonoids in pilsner beer. *Journal of the Institute of Brewing*, 91, 37-40.

FARÍAS-CAMPOMANES, A. M., HORITA, C. N., POLLONIO, M. A. R. & MEIRELES, M. A. A. 2014. Allicin-Rich Extract Obtained from Garlic by Pressurized Liquid Extraction: Quantitative Determination of Allicin in Garlic Samples. *Food and Public Health*, 4, 272-278.

FARÍAS-CAMPOMANES, A. M., ROSTAGNO, M. A. & MEIRELES, M. A. A. 2013. Production of polyphenol extracts from grape bagasse using supercritical fluids: Yield, extract composition and economic evaluation. *Journal of Supercritical Fluids*, 77, 70-78.

FOSTER, N. R., GURDIAL, G. S., YUN, J. S. L., LIONG, K. K., TILLY, K. D., TING, S. S. T., SINGH, H. & LEE, J. H. 1991. Significance of the crossover pressure in solid-supercritical fluid phase equilibria. *Industrial and Engineering Chemistry Research*, 30, 1955-1964.

GARCIA-MENDOZA, M. P., PAULA, J. T., PAVIANI, L. C., CABRAL, F. A. & MARTINEZ-CORREA, H. A. 2015. Extracts from mango peel by-product obtained by supercritical CO_2 and pressurized solvent processes. *LWT-Food Science and Technology*, 62, 131-137.

KAUFMANN, B. & CHRISTEN, P. 2002. Recent extraction techniques for natural products: microwave-assisted extraction and pressurised solvent extraction. *Phytochemical Analysis*, 13, 105-113.

KORDALI, S., KOTAN, R., MAVI, A., CAKIR, A., ALA, A. & YILDIRIM, A. 2005. Determination of the Chemical Composition and Antioxidant Activity of the Essential Oil of Artemisia dracunculus and of the Antifungal and Antibacterial Activities of Turkish Artemisia absinthium, A. dracunculus, Artemisia santonicum, and Artemisia spicigera Essential Oils. *Journal of Agricultural and Food Chemistry*, 53, 9452-9458.

LIU, R.H., 2013. Dietary bioactive compounds and their health implications. *Journal of Food Science*, 78, 18-25.

JANICK, J. & PAULL, R.E., 2008. The Encyclopedia of Fruits and Nuts, first ed. London, UK.

MACHADO, A. P. F., PASQUEL-REÁTEGUI, J. L., BARBERO, G. F. & MARTÍNEZ, J. 2015. Pressurized liquid extraction of bioactive compounds from blackberry (*Rubus fruticosus L.*) residues: a comparison with conventional methods. *Food Research Internationa*l, 77, 675-683.

MARTÍNEZ, J. L. & VANCE, S. W. 2007. Supercritical Extraction Plants, in: Martínez, J.L. (Ed.), Supercritical Fluid Extraction of Nutraceuticals and Bioactive Compounbds CRC Press-Taylor and Francis Group, Boca Raton, pp. 25-49.

MOHSEN-NIA, M., AMIRI, H. & JAZI, B., 2010. Dielectric constants of water, methanol, ethanol, butanol and acetone: Measurement and computational study. J. Solution Chem. 39, 701-108.

MORAES, M. N., ZABOT, G. L. & MEIRELES, M. A. A. 2015. Extraction of tocotrienols from annatto seeds by a pseudo continuously operated SFE process integrated with low-pressure solvent extraction for bixin production. *Journal of Supercritical* Fluids, 96, 262-271.

MORTON, J.F., 1987. Abiu, in: Morton, J.F. (Ed.), Fruits of warm climates. Julia Morton, Miami, pp. 406-408.

MUKHOPADHYAY, M., 2000 Natural Extracts Using Supercritical Carbon Dioxide. CRC Press, New York.

MUSTAFA, A. & TURNER, C. 2011. Pressurized liquid extraction as green approach in food and herbal plants extraction: A review. *Analytical Chimica Acta*, 703, 8-18.

OSORIO-TOBÓN, J. F., CARVALHO, P. I. N., ROSTAGNO, M. A., PETENATE, A.J., MEIRELES, M. A. A. 2014. Extraction of curcuminoids from deflavored turmeric (Curcuma longa L.) using pressurized liquids: Process integration and economic evaluation. *Journal of Supercritical Fluids*, 95, 167-174.

PACHECO-PALENCIA, L. A. DUNCAN, C .E TALCOTT, S. T. 2009. Phytochemical composition and thermal stability of two commercial acai species, Euterpe oleracea and Euterpe precatoria. *Food Chemistry*, 115, 1199.

PAES, J., DOTTA, R., BARBERO, G. F., MARTÍNEZ, J. 2014. Extraction of phenolic compounds and anthocyanins from blueberry (*Vaccinium myrtillus* L.) residues using supercritical CO_2 and pressurized liquids. *Journal of Supercritical Fluids*, 95, 8-16.

PEREIRA, C. G., ROSA, P. T. V. & MEIRELES, M. A. A., 2007. Extraction and isolation of indole alkaloids from Tabernaemontana catharinensis A.DC: Technical and economical analysis. *Journal of Supercritical Fluids*, 40, 232-238.

PRZYGODA, K. & WEJNEROWSKA, G. 2015. Extraction of tocopherol-enriched oils from Quinoa seeds by supercritical fluid extraction. *Industrial Crop and Products*, 63, 41-47.

RE, R., PELLEGRINI, N., PROTEGGENTE, A., PANNALA, A., YANG, M. & RICE-EVANS, C. 1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine*, 59, 1231-1237.

REIS, J. M. L. 2012. Effect of Temperature on the Medicinal Properties of Polymer Mortars. *Materials Research Bulletin*, 15, 645-649.

RODRIGUES, L. M., ALCÁZAR-ALAY, S. C., PETENATE, A. J. & MEIRELES, M. A. A. 2014. Bixin extraction from defatted annatto seeds. Comptes Rendus Chimie, 17, 268-283.

RODRIGUES, R. B., LICHTENTHALER, R., ZIMMERMANN, B. F., PAPAGIANNOPOULOS, M., FABRICIUS, H. & MARX, F. 2006. Total oxidant scavenging capacity of Euterpe oleracea Mart. (Açaí) seeds and identification of their polyphenolic compounds. *Journal of Agricultural and Food Chemistry*, 54, 4162-4167.

ROSSO, V., HILLEBRAND, S., CUEVAS MONTILLA, E., BOBBIO, F. O., WINTERHALTER, P. & MERCADANTE, A. Z. 2008. Determination of anthocyanins from acerola (*Malpighia emarginata* DC.) and açai (*Euterpe oleracea Mar*t.) by HPLC–PDA–MS/MS. Journal of Food Composition and Analysis,. 21, 291-299.

RUFINO, M. S. M., PÉREZ-JIMÉNEZ, J., ARRANZ, S., ALVES, R. E., BRITO, E. S., OLIVEIRA M. S. P., SAURA-CALIXTO, F., 2011. Açaí (*Euterpe oleraceae*) 'BRS Pará': A tropical fruit source of antioxidant dietary fiber and high antioxidant capacity oil. Food of Research International, 44, 2100-2106.

RUIZ, L., RUIZ, L., MACO, M., COBOS, M., GUTIERREZ-CHOQUEVILCA, A.-L. & ROUMY, V. 2011. Plants used by native Amazonian groups from the Nanay River (Peru) for the treatment of malaria. *Journal of Ethnopharmacology*, 133, 917-921.

SASIDHARAN, S., CHEN, Y., SARAVANAN, D., SUNDRAM, K. M. & LATHA, L.Y. 2010. Extraction, Isolation and Characterization of Bioactive Compounds from Plants' Extracts. *African Journal of Traditional Complementary and Alternative Medicines,* 8, 1-10.

SEGALLIO, R.M., 2009. Estudo da Extração do Látex e do Óleo essencial do Abiu, in: UNIMEP (Ed.), 7a Mostra Acadêmica UNIMEP. Universidade Metodista de Piracicaba, Piracicaba, SP, pp. 475-481.

SHI, J., XUE, S. J., M, Y., JIANG, Y., YE, X. & YU, D. 2012. Green separation technologies in food processing: supercritical-CO2 fluid and subcritical water extraction, in: Boyce, J.I., Arcand, Y. (Eds.), Green Technologies in Food Production and Processing. Springer & Business Media, pp. 273-294.

SINGLETON, V. L., ORTHOFER, R. & LAMUELA-RAVENTOS, R. M. 1999. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteau reagent. *Methods in Enzymology*, 299, 152-178.

TOMASKO, D. L., LI, H., LIU, D., HAN, X., WINGERT, M. J., LEE, L. J. & KOELLING, K.W. 2003. A Review of CO2 Applications in the Processing of Polymers. *Industrial and Engineering Chemistry Research,* 42, 6431-6456.

UQUICHE, E., CIRANO, N. & MILLAO, S., 2015. Supercritical fluid extraction of essential oil from *Leptocarpha rivularis* using CO_2. *Industrial Crops and Products,* 77, 307-314.

VEGGI, P. C., CAVALCANTI, R. N., MEIRELES, M. A. A. 2014. Production of phenolic-rich extracts from Brazilian plants using supercritical and subcritical fluid extraction: Experimental data and economic evaluation. *Journal of Food Engineering*, 131, 96-109.

WYCOFF W., LUO, R., SCHAUSS, A. G., NEAL-KABABICK, J., SABAA-SRUR, A. U. O., MAIA, J. G. S., TRAN, K., RICHARDS, K. M. & SMITH. R. E. 2015. Chemical and nutritional analysis of seeds from purple and white açaí (*Euterpe oleracea* Mart.). *Journal of Food Composition and Analysis*, 41, 181-187.

XYNOS, N., PAPAEFSTATHIOU, G., PSYCHIS, M., ARGYROPOULOU, A., ALIGIANNIS, N. & SKALTSOUNIS, A.-L. 2012. Development of a green extraction procedure with super/subcritical fluids to produce extracts enriched in oleuropein from olive leaves. *Journal of. Supercritical. Fluids*, 67, 89-93.

ZAGHDOUDI, K., PONTVIANNE, S., FRAMBOISIER, X., ACHARD, M., KUDAIBERGENOVA, R., AYADI-TRABELSI, M., KALTHOUM-CHERIF, J., VANDERESSE, R., FROCHOT, C. & GUIAVARC'H, Y. 2015. Accelerated solvent extraction of carotenoids from: Tunisian Kaki (*Diospyros kaki* L.), peach (*Prunus persica* L.) and pricot (*Prunus armeniaca* L.). *Food Chemistry*, 184, 131-139.

Capítulo 5

Allicin-rich extract obtained from garlic by pressurized liquid extraction: quantitative determination of allicin in garlic samples

Angela M. Farías-Campomanes, Claudia N. Horita, Marise A. R. Pollonio, M. Angela A. Meireles

Artigo publicado no periódico Food and Public Health 2014, 4(6): 272-278

ISSN: 2162-8440 DOI: 10.5923/j.fph.20140406.03

Open Access: http://article.sapub.org/10.5923.j.fph.20140406.03.html

O material suplementar desde artigo encontra-se com Apêndice C

Food and Public Health 2014, 4(6): 272-278
DOI: 10.5923/j.fph.20140406.03

Allicin-Rich Extract Obtained from Garlic by Pressurized Liquid Extraction: Quantitative Determination of Allicin in Garlic Samples

Angela M. Farías-Campomanes[1], Claudia N. Horita[2], Marise A. R. Pollonio[2], M. Angela A. Meireles[1,*]

[1]LASEFI/DEA (Department of Food Engineering)/FEA (School of Food Engineering)/UNICAMP (University of Campinas), Rua Monteiro Lobato, Campinas, Brazil
[2]DTA (Department of Food Technology)/ FEA (School of Food Engineering)/ UNICAMP (University of Campinas), Rua Monteiro Lobato, Campinas, Brazil

Abstract Since ancient times, garlic has been used to prevent and treat various diseases. The health benefits of garlic are attributed to its content of thiosulfinates, of which allicin is the main bioactive compound. Allicin is not found in fresh garlic; it is derived from alliin through the enzymatic action of alliinase when the membranes of the garlic cloves are destroyed. Allicin is a very unstable compound, degrading within a few minutes of being produced, at high temperatures and in the presence of certain solvents. Pressurized liquid extraction (PLE) is a relatively new technique that has demonstrated high efficiency on the extraction of bioactive compounds. Thus, PLE of garlic at 313 K and 6 MPa, using ethanol as solvent, was performed. The concentration of allicin in the garlic PLE extract, garlic powder, garlic oil and fresh garlic was quantified using the Internal Standard method. The global yield of garlic PLE was 1.3 ± 0.3% on a wet basis. The garlic PLE extract had the highest allicin concentration (332 ± 5 μg of allicin.g^{-1} of sample), followed by fresh garlic and garlic powder. Allicin was not detected in garlic oil.

Keywords Pressurized Liquid Extraction, Ethanol, Allicin, Garlic, *Allium sativum L.*

1. Introduction

Garlic (Allium sativum L.) and its derived products have been widely used for culinary and medicinal purposes by many cultures. Research has demonstrated that garlic has a wide range of biological activities, including antihypertensive, lipid-lowering, antibacterial, antifungal, and antiviral activities, among others [1]. Studies have shown a relationship between a high intake of garlic and a low risk of certain cancers [2-4]. The health benefits of garlic have been attributed to its thiosulfinates content. Allicin is the most prevalent of these bioactive compounds, representing approximately 70% of the thiosulfinates in garlic [5].

Allicin was first reported in 1944 by Cavallito and Bailey [6], who described it as a colorless oil with low solubility in water that was relatively unstable. Allicin derives from the precursor alliin through the enzymatic activity of alliinase (Figure 1). According to Murray [7], 4 g of fresh garlic contains approximately 10 mg de alliin and can be converted into at least 4 mg of allicin. Alliin and alliinase are located in separate regions of garlic cloves, and the alliinase reaction is initiated only after the cells have been crushed [8, 9]. Allicin is completely formed in 0.3 min at 310 K [10] and, at room temperature, its half-life ranges from 10 days to few hours depending in the solvent in which it is dissolved [11]. Despite allicin being a low-polarity molecule, it is often extracted using polar solvents, such as water and ethanol, at room pressure (0.1 MPa) because it is very unstable in non-polar organic solvents [9]. Fujisawa et al. [12] concluded that vegetable oil and n-hexane are solvents that provide low extraction yields of allicin, most likely due to its high level of instability. Moreover, the temperature and concentration at which allicin is stored are factors that must be considered to prevent its degradation [12-14].

The composition of thiosulfinates compounds in garlic products depends on the processing conditions [15]. According to Shi [16], spray-drying, freeze-drying and oven-drying at high temperature, of fresh garlic; result in a loss of activity or destroying of the alliinase, thus preventing allicin production. In contrast, over drying garlic at low temperature (< 333 K) has little infect on the yield of the allicin and other thiosulfinates. Additionally, garlic products in the form of oils do not generate allicin, but rather, allicin-derived compounds [16].

* Corresponding author:
maameireles@gmail.com (M. Angela A. Meireles)
Published online at http://journal.sapub.org/fph

Food and Public Health 2014, 4(6): 272-278

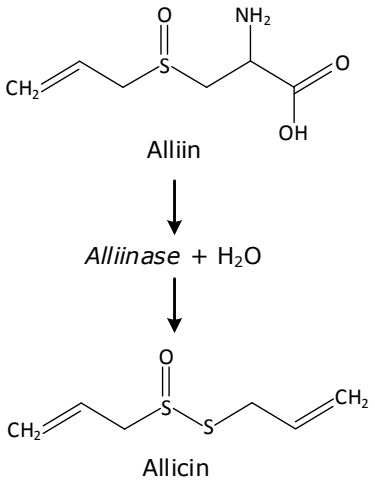


Figure 1. Formation of allicin in fresh garlic

Conventional extraction techniques have several drawbacks including the degradation of sensitive compounds due to the use of high temperatures, the consumption of large amounts of solvent, the toxicity of some solvents, the long processing times and the low selectivity, among others [17]. Thus, more environmentally friendly techniques that do not present health risks and provide high-quality extracts are being utilized.

Pressurized liquid extraction (PLE) is a promising technique that allows obtaining labile compounds through using a liquid solvent at high pressure [18, 19]. Moreover, PLE is simple, easy to operate, fast and it consumes low amounts of solvent compared with conventional techniques. Thus, an allicin-rich extract was obtained from garlic using PLE technique with ethanol as the solvent, was obtained. Additionally, allicin was identified and quantified in extract of fresh garlic, and of commercial samples of garlic oil and garlic powder.

2. Materials and Methods

2.1. Plant Material

Fresh garlic was obtained at local supermarkets (Campinas, SP, Brazil). The outer skin of the garlic cloves was peeled off. The garlic cloves were cut into small cubes (approximately 0.3 cm on all side) using a kitchen knife. The garlic samples were prepared immediately before PLE extraction was performed in order to avoid the degradation of allicin.

2.2. Pressurized Liquid Extraction

The PLE assays were performed using the home-made PLE system previously described by Rodrigues et al. [18], which consisted of an HPLC pump (Thermo Separations Products, model 3200 ConstaMetric P/F, Fremont, CA, USA), a manometer, an extraction vessel (Thar Designs, CL 1373, Pittsburg, PA, USA) that was heated using an electrical heating jacket and blocking and backpressure valves (Figure 2).

A 6.3 cm^3 (2.0 cm diameter and 2.0 cm height; internal dimensions) extraction vessel was completely filled with 6.6 ± 0.1 g of fresh garlic. The extraction assays were performed at 313 K and 6 MPa using a static extraction period of 5 min, in triplicate. Ethanol (99.5% purity, Dinamica, Campinas, Brazil) flowing at a rate of 2.6×10^{-5} $kg.s^{-1}$ was used as the solvent, and the solvent mass to feed mass ratio (S/F) was maintained at 1.2. Ethanol was removed from the extracts by vacuum evaporation (Laborota, model 4001, Viertrieb, Germany) at 313 K and 0.1 MPa. The global extraction yield ($X_{0,\,S/F}$) was calculated as the percentage (%) of the mass of the extract ($m_{Extract}$) relative to the total mass of the raw material on a wet basis (m_{RM}) that was used to perform the bed extraction, using Equation 1, as follows:

$$X_{0,S/F}(\%) = \frac{m_{Extract}}{m_{RM}} \times 100 \quad (1)$$

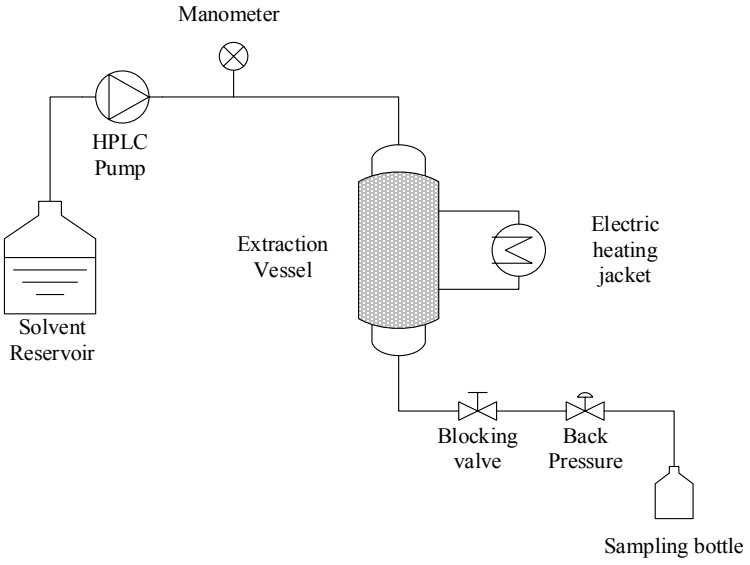


Figure 2. Flow diagram of the home-made PLE unit used in this study

2.3. Determination of the Allicin Concentration in the Garlic Samples

The concentration of allicin in the garlic samples was determined using the Internal Standard method [8]. The internal standard used in this study was ethyl p-hydroxybenzoate, which was mixed with the garlic samples prior to performing the HPLC analysis. The internal standard must have a known concentration, a structure similar to that of allicin and must not react with it.

2.3.1. Preparation of the Internal Standard Solution

Two internal standard solutions of different concentrations were prepared. First, an internal standard solution with a concentration of 0.5 $mg.cm^{-3}$ was prepared. For this, approximately 200 mg of ethyl p-hydroxybenzoate (99%, lot P500011, Fluka) was added to 8 cm^3 of methanol and the solution was shaken until it dissolved. Then, 360 cm^3 of Milli-Q water at 353 K was added. Finally, 32 cm^3 of Milli-Q water at room temperature was added and the solution was shaken. After performing the first HPLC analysis, it was observed that the concentration of this internal standard solution was much higher than the allicin concentration of the samples. Thus, to ensure the accuracy of the results, a second internal standard solution, with a concentration of 0.2 $mg.cm^{-3}$, was prepared.

2.3.2. Preparation of Garlic Samples

In addition to the samples of the garlic PLE extract, samples of fresh garlic (*Allium sativum L.*), garlic oil (International Flavors & Fragrance Inc., Sao Paulo, Brazil) and garlic powder (Fuchs, Gewürze, Brazil) were analyzed. The garlic sample preparation was performed according to Eagling and Sterling [8]. For this, garlic samples were dissolved in the internal standard solution with magnetic stirring for 5 min. The ratio between the volume of the internal standard solution and the mass of the garlic sample was set to 5 (Table 1).

Table 1. Masses of the garlic samples and volumes of internal standard solution used to prepare the samples that were analyzed using HPLC

Garlic sample	Volume of internal standard solution (cm^3)	Mass of garlic sample (g)	Ratio (internal standard solution/ garlic sample)
Fresh garlic	25	5	5
Garlic oil	25	5	5
Garlic powder	25	5	5
Garlic PLE extract	1.33	0.27	5

The samples were placed in an oven (Tecnal, model TE 395-1, Sao Paulo, Brazil) at 303 K for 20 min and then were centrifuged for 5 min at 4000 rpm. Afterward, an aliquot of the supernatant was mixed with the mobile-phase solution, and this mixture was then used in the chromatographic analysis. The ratio between the volume of the supernatant and the volume of the mobile-phase solution was set to 4 (Table 2).

Table 2. Volumes of the mobile-phase solution and supernatant used to prepare the samples that were analyzed using HPLC

Garlic sample	Volume of mobile-phase solution (cm^3)	Volume of supernatant (cm^3)	Ratio (mobile phase/ supernatant)
Fresh garlic	8	2	4
Garlic oil	8	2	4
Garlic powder	8	2	4
Garlic PLE extract	1.2	0.3	4

2.3.3. High-Performance Liquid Chromatography Analysis

For the high-performance liquid chromatography (HPLC) analyses, the mixtures of the mobile-phase solution and supernatant were filtered through 0.25-µM nylon syringe filters (VWR-International, Darmstadt, Germany) and were placed directly into HPLC vials. The chromatographic separation was performed using a Waters Alliance separation module (model 2695D, Milford, MA, USA) equipped with a diode array detector (2998). The separation was performed using a Poroshell C18 column (100 × 4.6 mm id, 2.5 µm, Agilent Technologies, Sunnyvale, CA, USA) at 323 K using a flow rate of 0.4 $cm^3.min^{-1}$. The initial mobile-phase consisted of water containing 0.1% acetic acid (solvent A) and methanol (solvent B). The mobile-phase composition of 50% A and 50% B was maintained for 12 min. The concentration of solvent A was decreased from 50% to 10% in 1 min, and this concentration was maintained for 5 min. Then, the concentration of solvent A was increased to its initial value (50% solvent A) in 2 min. The injection volume was 10 µL. UV detection was performed at 254 nm.

2.3.4. Identification of Allicin in the Garlic Samples

The allicin in the garlic samples was identified using the UV spectrum of pure allicin that was found in the literature [20] and by the order of allicin elution with respect to that of the internal standard [21].

2.3.5. Allicin Quantification

Allicin quantification in garlic extract samples was performed using ethyl p-hydroxybenzoate as the internal standard. The allicin concentration in the garlic samples was calculated using Equation 2 [21].

$$C_{Allicin} = \frac{C_{IS} \times Area_{Allicin} \times V_{IS}}{m_{Sample} \times Area_{IS}} \quad (2)$$

Where $C_{Allicin}$ is the allicin concentration ($mg.g^{-1}$ sample), C_{IS} is the concentration of the internal standard ($mg.cm^{-3}$), $Area_{Allicin}$ is the area of the allicin peak, $Area_{IS}$ is the area of the internal standard peak, V_{IS} is the volume of the mixture of the internal standard and the sample and m_{Sample} is the mass of the sample that was analyzed on a wet basis.

3. Results and Discussion

3.1. Preparation of the Plant Material

The agglomeration phenomenon, also called the caking phenomenon, consists of small particles aggregating to form dense lumps. This phenomenon occurs when the processing temperature rises above the glass-transition temperature (T_g) of a material [22]. Studies have related this phenomenon to the moisture content of a solid matrix. The caking phenomenon is commonly observed in vegetal matrixes that have high water content [23]. A study of a large variety of Brazilian garlics determined that the water content of fresh garlic ranges from 65 to 70% [24]. Additionally, small particles and high temperatures favor the occurrence of the caking phenomena [25]. Allicin is a very labile compound that readily decays, particularly at high temperatures, and drying allicin-containing materials (to reduce the water content) at a high temperature or even at a low temperature, such as when freeze-drying, can result in a significant reduction in the level of allicin [26]. Thus, in this study, to reduce the extent of agglomeration without promoting allicin degradation, fresh garlic cloves instead of dried garlic were used. Additionally, to prevent caking, the fresh garlic was cut into small cubes using a knife instead of crushing it until it formed sticky lumps.

3.2. Pressurized Liquid Extraction

PLE was performed at 313 K and 6 MPa. A low temperature was used to avoid allicin degradation. A pressure of 6 MPa was used because it is the maximal pressure at which the unit can operate in the dynamic mode.

The selection of an extraction solvent was based on the solubility and stability of allicin. Fujisawa et al. [12] investigated extracting allicin from garlic using several different solvents at low pressure. The authors concluded that ethanolic solutions, of various concentrations (20–100%) produced extracts with higher allicin yields than those obtained using water, n-hexane or vegetable oil. Moreover, they concluded that allicin is more stable in ethanolic solutions than in water and that it is very unstable in vegetable oil. Ilić et al. [9] reported that allicin and other thiosulfinates could be transformed into more stable compounds when they were in non-polar organic solvents, such as n-hexane and oil.

Table 3. Garlic pressurized liquid extraction data

	Exp. 1	Exp. 2	Exp. 3
Fresh garlic (g)	6.6	6.5	6.7
Ethanol (cm^3)	9.7	9.6	9.8
S/F	1.2	1.2	1.2
Extraction time (min)	4.9	4.8	4.9
Global yield (%)	1.0	1.6	1.5

Therefore, due to the instability of allicin in non-polar organic solvents and the high yield of allicin obtained using ethanolic solutions, ethanol was used as the solvent in this study. Because the garlic samples were wet, bed extraction was performed within the extraction vessel after placing the sample particles without compacting them.

The global yield ($X_{0,S/F}$) of PLE extraction for fresh garlic was 1.3 ± 0.3% on a wet basis. More information about the extraction assays is shown in Table 3.

3.3. Identification of Allicin in the Garlic Samples

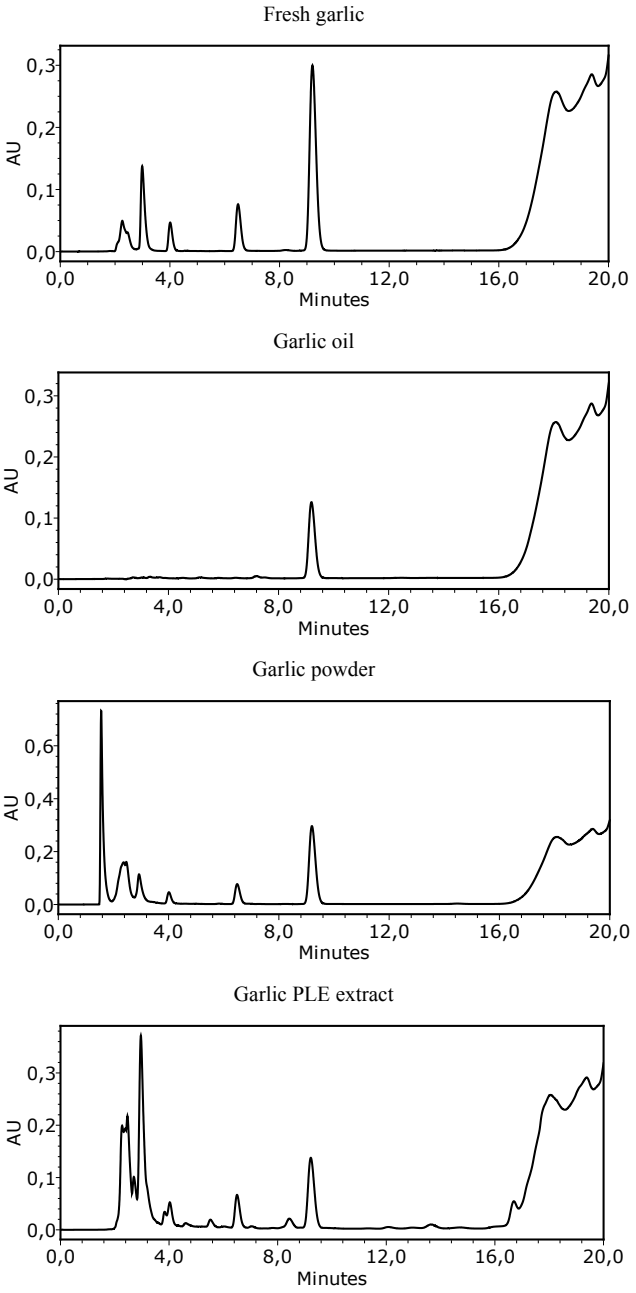


Figure 3. Chromatograms of the garlic samples obtained using 0.2 $mg.cm^{-3}$ of the internal standard; the allicin t_R = 6.5 min and the internal standard t_R = 9.2 min

The main peaks in the UV chromatograms of all of the garlic samples (Figure 3) were analyzed. The UV spectrum of allicin was identified and it is shown in Figure 4. The typical shoulder of the allicin peak at 240 nm was observed

[27]. The retention times (t_R) of allicin and ethyl p-hydroxybenzoate (used as the internal standard) were 6.5 and 9.2 min, respectively. The order of elution, with allicin eluted before the internal standard, was as expected [21].

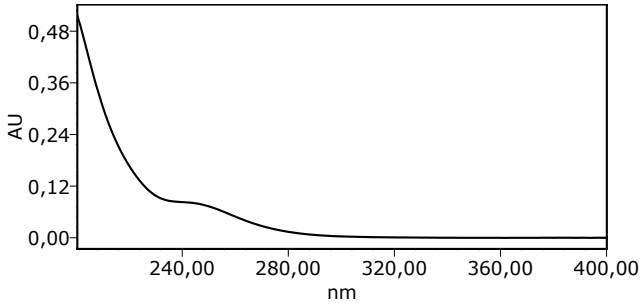


Figure 4. UV spectrum of allicin that was identified in the garlic samples

The average allicin concentrations (± amplitude) are reported in Table 4. In order to evaluate the method reproducibility, two concentrations of the internal standard (0.5 and 0.2 mg.cm^{-3}) were employed. The allicin concentration of garlic depends on the crop, the location of the plants and the processing, storage and handling conditions [28]. Because the fresh garlic samples were taken from different batches, a small variation in the allicin concentration was expected. In contrast, the garlic powder samples were taken from the same batch, so their allicin concentrations were similar. Therefore, it is possible to conclude that changing the concentration of internal standard did not affect the allicin quantification.

The garlic PLE extract obtained at 313 K and 6 MPa, using an S/F of 1.2, had the highest allicin concentration: 332 ± 5 μg of allicin.g^{-1} of sample, followed by the fresh garlic and garlic powder. Allicin was not detected in garlic oil samples, most likely because its instability in non-polar solvents as explained. There are two reasons for the high allicin concentration in the sample of garlic PLE extract. First, allicin is more soluble in ethanol than in water (the other garlic samples were obtained using water). Second, allicin is stable in ethanol because ethanol contains a hydroxyl group that stabilizes the structure of the allicin molecule.

Another environmentally friendly extraction method is supercritical fluid extraction (SFE). del Valle et al. [25] studied the extraction of allicin from garlic by SFE using carbon dioxide as the solvent. The optimal extraction conditions was determined to be 323 K, 30 MPa and S/F of 55, and extracts with allicin concentration of 75 μg of allicin.g^{-1} of extract were obtained. The low allicinconcentration in the SFE extracts could be due to the instability of allicin in garlic oil, which forms the SFE extract.

Thus, by comparing of PLE and SFE techniques for obtaining extracts of garlic, it can be concluded that the PLE provides extracts with a higher concentration of allicin (332 ± 5 μg of allicin.g^{-1} of extract and 75 μg of allicin.g^{-1} of extract, respectively) and results in a lower consumption of solvent (S/F of 1.2 and 55, respectively) compared with the SFE.

Table 4. Allicin concentration in the garlic samples

Sample	Concentration of internal standard (mg.cm^{-3})	Retention time of allicin (min)	Peak area of allicin	Retention time of internal standard (min)	Peak area of internal standard	Allicin concentration (μg.g^{-1} sample)	Average allicin concentration (μg.g^{-1} sample)
Fresh garlic[a]	0.5	6.51	1,927,077	9.25	19,261,466	250	248 ± 2
		6.55	1,923,112	9.32	19,357,128	248	
		6.52	1,902,832	9.26	19,349,038	246	
Garlic oil		-	-	9.33	7,593,274	ND	nd
		-	-	9.24	7,551,561	ND	
		-	-	9.24	7,471,950	ND	
Garlic powder		6.51	1,499,071	9.24	19,154,070	196	192 ± 3
		6.52	1,483,502	9.25	19,382,033	191	
		6.52	1,451,215	9.24	19,214,797	189	
Fresh garlic[b]	0.2	6.52	922,974	9.25	4,897,687	188	189 ± 1
		6.50	917,635	9.22	4,881,437	188	
		6.49	912,488	9.20	4,789,298	191	
Garlic oil		-	-	9.23	1,955,068	ND	nd
		-	-	9.20	1,963,760	ND	
Garlic powder		6.49	901,737	9.20	4,745,398	190	179 ± 11
		6.49	794,564	9.20	4,731,269	168	
Garlic PLE extract		6.51	707,641	9.23	2,069,000	337	332 ± 5
		6.50	696,867	9.22	2,097,946	327	

[a,b]Fresh garlic purchased on different days.
nd: not detected

Several other advantages of extracting garlic using the PLE technique are the co-extraction of other polar compounds, such as phenolics, which aresimilar to allicin, have biological activities and the obtaining of extracts with a low content of garlic oil (which is responsible for the garlic odor) due to the low solubility of oil in ethanol, which have applications in the food, cosmetic and pharmaceutical industries.

4. Conclusions

The global extraction yield of garlic PLE at 313 K, 6 MPa and S/F of 1.2 was 1.3 ± 0.3% on a wet basis. The garlic PLE extract had a higher allicin concentration (332 ± 5 μg of allicin.g^{-1} of extract) than fresh garlic and garlic powder samples. PLE is therefore an effective method for obtainingallicin-rich extracts.

ACKNOWLEDGEMENTS

Angela M. Farías-Campomanes is grateful to CAPES (5817-11-0) for the Ph.D. assistantships. M. Angela A. Meireles is thankful to CNPq for a productivity grant (301301/2010-7). Claudia N. Horita and Marise A. R. Pollonio thank FAPESP (2011/51721-4). The authors acknowledge the financial support from CAPES, CNPq and FAPESP. We also would like to thank J. Felipe Osorio-Tobón for assistance with the HPLC analysis.

REFERENCES

[1] P. Mane, R. Mayee, K. Atre, "Medicinal properties of allium sativum (garlic): A review", International Journal of Pharma Research & Development-Online, vol.3, no.2, pp.145-150, 2011.

[2] A. T. Fleischauer, L. Arab, "Garlic and cancer: A critical review of the epidemiologic literature", Journal of Nutrition, vol.131, no.3s, pp.1032S-1040S, 2001.

[3] J. M. Chan, F. Wang, E. A. Holly, "Vegetable and fruit intake and pancreatic cancer in a population-based case-control study in the San Francisco bay area", Cancer Epidemiology Biomarkers & Prevention, vol.14, no.9, pp.2093-2097, 2005.

[4] S. G. Santhosha, P. Jamuna, S. N. Prabhavathi, "Bioactive components of garlic and their physiological role in health maintenance: A review", Food Bioscience, vol.3, pp.59-74, 2013.

[5] J. Han, L. Lawson, G. Han, P. Han, "A spectrophotometric method for quantitative determination of allicin and total garlic thiosulfinates", Analytical Biochemistry, vol.225, no.1, pp.157-160, 1995.

[6] C. J. Cavallito, J. H. Bailey, "Allicin, the antibacterial principle of *Allium sativum*. Isolation, physical properties and antibacterial action", Journal of the American Chemical Society, vol.66, no.11, pp.1950-1951, 1944.

[7] M. T. Murray, Ed., J Pizzorno and M Murray, Allium sativum in: Natural Medicine. Kenmore, WA, USA: Churchill Livingstone, 2012.

[8] D. R. Eagling, S. J. Sterling, "A cholesterol-lowering extract from garlic", Rural Industries Research and Development Corporation, Australia, Tech. Rep. 1-14, 2000.

[9] D. P. Ilić, V. D. Nikolić, L. B. Nikolić, M. Z. Stanković, L. P. Stanojević, M. D. Cakić, "Allicin and related compounds: Biosynthesis, synthesis and pharmacological activity", Physics, Chemistry and Technology, vol.9, no.1, pp.9-20, 2011.

[10] L. Lawson, B. G. Hughes, "Characterization of the formation of allicin and other thiosulfinates for garlic", Planta Medica, vol.58, no.4, pp.345-350, 1992.

[11] L. D. Lawson, Ed., AD Kinghorn and MF Balandrin, Bioactive Organosulfur Compounds of Garlic and Garlic Products in: Human Medicinal Agents from Plants. Washington, DC: American Chemical Society, 1993.

[12] H. Fujisawa, K. Suma, K. Origuchi, H. Kumagai, T. Seki, T. Ariga, "Biological and Chemical Stability of Garlic-Derived Allicin", Journal of Agricultural and Food Chemistry, vol.56, no.11, pp.4229-4235, 2008.

[13] E. Block, "The organosulfur chemistry of the genus *Allium* - Implications for the organic chemistry of sulfur", Angewandte Chemie International Edition, vol.31, no.9, pp.1135-1178, 1992.

[14] M. E. Raybak, E. M. Calvey, J. M. Harnly, "Quantative determination of allicin in garlic: Supercritical fluid extraction and standard addition of alliin", Journal of Agricultural and Food Chemistry, vol.52, no.4, pp.682-687, 2004.

[15] N. Bhagyalakshmi, R. Thimmaraju, L. Venkatachalam, K. N. Chidambara-Murthy, V. Screedhar, "Nutraceutical applications of garlic and the intervention of biotechnology", Critical Reviews in Food Science and Nutrition, vol.45, no.7-8, pp.607-621, 2005.

[16] J. Shi, G. Mazza, M. L. Maguer, Functional Foods: Biochemical and Processing Aspects, 1sted., Boca Raton: CRC Press, 2002.

[17] M. G. Bernardo-Gil, M. A. Ribeiro, M. M. Esquível, "Produção de extractos para aindústriaalimentar: uso de fluidossupercríticos", IndustriaAlimentar, Boletim de Biotecnologia, vol.73, pp.14-21, 2002.

[18] L. M. Rodrigues, S. C. Alcázar-Alay, A. J. Petenate, M. A. A. Meireles, "Bixin extraction from defatted annatto seeds", Comptes Rendus Chimie, vol.17, no.3, pp.268-283, 2014.

[19] D. T. Santos, P. C. Veggi, M. A. A. Meireles, "Optimization and economic evaluation of pressurized liquid extraction of phenolic compounds from jabuticaba skins", Journal of Food Engineering, vol.108, no.3, pp.444-452, 2012.

[20] R. E. González, A. B. Camargo, J. L. Burba, "Obtention of a quantitative secondary standard for allicin synthesis and purification", Revista da la Facultad de Ciencias Agrarias UNCuyo, vol.2, pp.61-70, 2007.

[21] P. A. Pinto Mendes, "Estudo do Teor de AlicinaemAlho", M. Eng. thesis, Instituto Politécnico de Bragança/Escola Superior de Tecnologia e de Gestão, Bragança, 2008.

[22] M. Peleg, Ed., J Blanshard and P Lillford, Glass transition and the physical stability of food powders in: The glassy state of foods. Notthingham, UK: Notthingham University Press, 1993.

[23] J. M. Aguilera, J. M. del Valle, M. Karel, "Caking phenomena in amorphous food powders", Trends in Food Science & Technology, vol.6, no.5, pp.149-155, 1995.

[24] P. Patri, C. M. Henrique, C. P. C. C. Martins, "Caracterizaçãofisicoquímica de cultivares de alho", Pesquisa & Tecnologia, vol.7, no.1, 2010.

[25] J. M. del Valle, V. Glatzel, J. L. Martínez, "Supercritical CO_2 extraction of allicin from garlic flakes: Screening and kinetic studies", Food Reviews International, vol.45, no.1, pp.216-224, 2012.

[26] E. Y. Y. da Silva, "Propriedadesfuncionais "*in vitro*" e "*in vivo*" de alhos frescos e processados", D. Health science thesis, Brasilia University/Ciências da Saúde, Brasília, Brazil, 2009.

[27] L. D. Lawson, S. G. Wood, B. G. Hughes, "HPLC analysis of Allicin and other thiosulfinates in garlic clove homogenates", Planta Medica, vol.57, no.3, pp.263-270, 1991.

[28] S. J. Sterling, D. R. Eagling, "Agronomics and allicin yield of Australian grown garlic (Allium sativum)", in Proc. Proceedings of the Second International Symposium on Edible Alliaceae, 2001, (555), p.63-73.

Capítulo 6

Discussão Geral

Discussão geral

De acordo com a revisão bibliográfica que foi apresentada no Capítulo 2, os processos PLE e SFE são técnicas amigáveis com o meio ambiente que permitem a obtenção de polifenóis e tiosulfinatos. Inúmeras propriedades biológicas têm sido atribuídas a estes grupos de compostos, dentre elas a atividade antioxidante, anticancerígena, antiviral, etc. As temperaturas elevadas utilizadas pelo processo de PLE favorece a transferência de massa enquanto a alta pressão mantém o solvente no seu estado liquido aumentando seu poder de solvatação. Por outro lado, no processo de SFE o uso de um fluido a temperatura e pressão acima do seu ponto crítico favorece a solvatação de uma maior gama de compostos que os gases e a difusão através da matriz sólida de modo mais eficiente que os líquidos. PLE e SFE apresentam várias vantagens frente aos métodos convencionais de extração: utilizam menor quantidade de solvente orgânico ou como no caso da SFE o extrato pode ser obtido livre de solvente, são realizados em períodos curtos e evitam a degradação de compostos sensíveis à luz e oxigênio devido que a extração é realizada num extrator fechado. A PLE e SFE têm demonstrado alta eficiência de extração de polifenóis e possui potencial para a obtenção de tiosulfinatos.

Extratos ricos em antocianinas podem ser obtidos a partir de resíduos do processamento de milho roxo via extração com etanol pressurizado, como foi apresentado no **Capítulo 3.** Resíduo inteiro do processamento de milho roxo (*Zea mays* L.), úmido, foi moído e usado como matéria prima. Os efeitos principais dos parâmetros operacionais temperatura (313, 323 e 333 K), pressão (2, 4 e 6 MPa) e tempo de extração estática (5, 10 e 15 min) foram avaliados sobre o rendimento de extração e atividade antioxidante dos extratos. De acordo com os resultados, 333 K e 4 MPa foram as condições que favoreceram rendimento global, enquanto que a atividade antioxidante dos extratos não foi influenciada pela temperatura e seus valores mais altos foram obtidos a 6 MPa. A extração estática de 5 min teve efeito positivo para rendimento de extração e atividade antioxidante dos extratos. A condição operacional de 333 K, 6 MPa e 5 min foi selecionada para a construção das curvas globais de extração. Da análise de determinação de antocianinas, o mesmo perfil antociânico foi observado para os extratos obtidos a partir dos resíduos de milho roxo, porém uma concentração de antocianinas maior foi observada para os extratos obtidos a partir do sabugo. Extratos de sabugo de milho roxo com concentração de antocianinas máxima de 9,4 g per kg de extrato foram obtidos via PLE. Após uma análise econômica utilizando as condições

otimizadas, foi possível determinar que o processo de obtenção de antocianinas a partir de sabugo de milho roxo via PLE é economicamente viável usando unidades de extração de 2 extratores de 0,025 m^3, resultando em extratos com COM que varia de US$ 20 a 30 kg^{-1} quando foram usados 2 extratores de 0,05 a 0,25 m^3.

A seguinte etapa concentrou-se no estudo do uso de tecnologias verdes para a extração de polifenóis a partir de resíduos de plantas Amazônicas ainda pouco exploradas. No **Capítulo 4**, é apresentado pela primeira vez o estudo da obtenção de polifenóis e antioxidantes a partir de semente de assai (*Euterpe precatória*), semente de piquiá (*Caryocar villosum*) e casca de abiú (*Pouteria caimito*) através da extração com líquido pressurizado (PLE) e extração com fluido supercrítico (SFE) usando etanol e dióxido de carbono como solventes de extração, respectivamente. As matérias primas foram separadas manualmente, secadas e moídas. A influência das variáveis de processo pressão, temperatura e razão S/F sobre o rendimento de extrato, rendimento de fenólicos e flavonóides totais e atividade antioxidante dos extratos, foi avaliada. O estudo determinou que o aumento da pressão, temperatura e razão S/F teve influência positiva no rendimento de extrato; porém, estes parâmetros apresentaram efeito diferente sobre sua composição. O rendimento de extração de fenólicos a partir de sementes de assai via PLE diminuiu com o aumento da temperatura e o rendimento foi maior em 4 MPa. A condição de operação de 313 K, 4 MPa e razão S/F de 2,3 levou a produção de extratos de sementes de assai com concentração de fenólicos de 33% e alta atividade antioxidante. Por outro lado, o processo PLE de sementes de piquiá não foi influenciado pela temperatura e os melhores resultados foram obtidos a 8 MPa e razão S/F de 10. Assim, a condição operacional de 313 K, 8 MPa e razão S/F de 10 produziu extratos com alta atividade antioxidante (EC_{50} de 12-16 µg/ml) e concentração de fenólicos de 10% e flavonóides de 5,3%. A temperatura e a pressão influenciaram a atividade antioxidante dos extratos de casca de abiú obtidos via SFE. Maior atividade antioxidante apresentaram os extratos obtidos a 333 K e 15 MPa; porém esta foi menor que as apresentadas pelos extratos de semente de assai e semente de piquiá. Neste trabalho se demonstrou que a técnica de PLE foi eficiente na obtenção de compostos bioativos (fenólicos e flavonóides com atividade antioxidante) a partir dos resíduos do processamento de assai e piquiá; enquanto, a técnica SFE pode ser usada como pré-tratamento para a posterior obtenção de antioxidantes, via PLE, a partir de cascas de abiú.

Extratos ricos em alicina podem ser obtidos a partir de alho através do uso da técnica de PLE, como foi apresentado no **Capítulo 5.** Desde tempos ancestrais, o alho tem

sido utilizado para o tratamento de várias doenças e seus efeitos benéficos têm sido atribuídos ao seu teor de tiosulfinatos, dos quais a alicina representa 70% dos tiosulfinatos no alho. Uma vez que a alicina é um composto altamente instável, que degrada em apenas poucos minutos depois de produzido, a altas temperaturas e em presença de alguns solventes, a PLE é uma técnica com características favoráveis para a extração de compostos bioativos. A PLE foi utilizada como técnica alternativa para a produção de extratos ricos em alicina a partir de alho. Alho *in natura* foi usado como matéria prima e etanol foi usado como solvente de extração. Extratos de alho foram produzidos na condição operacional de 313 K, 6 MPa e razão S/F de 1,2. A concentração de alicina no extrato de alho produzido via PLE e nas amostras comerciais de alho fresco, óleo de alho e alho em pó; foi determinada usando o método do padrão interno. Os resultados indicaram que o processo PLE levou a produção de extratos com concentração de alicina de 332 ± 5 μg de alicina.g^{-1} de extrato, a qual foi maior em até 1.9 vezes às concentrações obtidas para as amostras comerciais de alho fresco e alho em pó. Além disso, alicina não foi detectada nas amostras de óleo de alho.

Em geral, a técnica de PLE mostrou-se eficiente na obtenção de compostos bioativos a partir de matérias primas úmidas e com umidade reduzida. Extratos com alto teor de fenóis, flavonóides e com atividade antioxidante foram obtidos via PLE usando etanol como solvente de extração. Também, a PLE produziu extratos mais concentrados em alicina que os obtidos por outras técnicas de extração apresentadas na literatura incluindo a SFE. Por outro lado, os extratos de casca de abiu obtidos via SFE apresentaram baixa atividade antioxidante em comparação às outras matérias primas estudadas nesta tese, assim outras técnicas de extração tal como a PLE são sugeridas para a obtenção de compostos bioativos.

Capítulo 7

Conclusões Gerais

CONCLUSÕES GERAIS

No **Capítulo 1** foi exposta brevemente uma introdução dos grupos de compostos bioativos polifenóis e tiosulfinatos assim também a tecnologia de extração com líquido pressurizado e fluido supercrítico. Os polifenóis e tiosulfinatos se caracterizam pelas suas propriedades benéficas à saúde (atividade antioxidante, antibacteriana, anticancerígena, entre outras) e pela sua instabilidade em presença de luz, oxigênio e calor. Os processos PLE e SFE são técnicas adequadas para a extração de compostos bioativos por ser realizada em condições amenas de pressão e temperatura e por empregar tempos menores de extração e menores quantidades de solvente.

O estado da arte sobre o processo de extração de polifenóis e tiosulfinatos via PLE e SFE foi apresentado no **Capítulo 2.** PLE e SFE são técnicas ambientalmente amigáveis que permitem a obtenção de extratos ricos em compostos bioativos durante curtos períodos de extração e baixo consumo de solvente. A PLE e SFE são influenciados principalmente pelo tipo de solvente, temperatura e pressão de extração. Também, têm sido aplicadas satisfatoriamente na obtenção de polifenóis e tiosulfinatos.

De acordo aos resultados do **Capítulo 3**, foi possível obter extratos ricos em antocianinas a partir do resíduo do processamento do milho roxo via PLE. Extratos com concentração de antocianinas de até 9,4 g per kg de extrato foram obtidos a partir do sabugo do milho roxo pelo uso de etanol pressurizado. A condição operacional otimizada foi 333 K, 6 MPa e extração estática de 5 min. A concentração de antocianinas no extrato teve um grande impacto sobre o COM, tornando viável o processo quando a matéria prima era resíduo de sabugo de milho roxo (PCC). Extratos com COM de US$ 20 a 30 per kg de extrato foram obtidos pelo uso de 2 extratores de 0,05 a 0,25 m^3 e extração dinâmica de 15 min.

A PLE é uma técnica de extração promissora e ambientalmente amigável para a produção de extratos ricos em polifenóis e com atividade antioxidante a partir do resíduo de frutas Amazônicas. De acordo aos resultados do **Capítulo 4**, o passo de pouca quantidade de etanol (S/F de 2,3) à temperatura e pressão amenas (313 K e 4 MPa) através do leito de extração formado por sementes de assai, por um curto período de tempo (10 min), foi necessário para obter altos rendimentos de extração e produzir extratos de semente de assai com a maior concentração de fenólicos. Da mesma forma, uma razão S/F de 10, 313 K e 8 MPa foram necessários para obter extratos de sementes de piquiá com alta concentração de

fenólicos, flavonóides a antioxidantes. Por outro lado, os extratos de pele de abiu obtidos com dióxido de carbono supercrítico apresentaram menor atividade antioxidante quando comparados aos extratos obtidos por PLE. Porém, SFE se mostrou seletivo na extração de antioxidantes, uma vez que os valores máximos de atividade antioxidante dos extratos foram observados nas condições operacionais nas quais menores rendimentos de extração foram obtidos (333 K e 15 MPa). Nesse sentido, SFE pode ser usado como técnica de pré-tratamento para aumentar a disponibilidade dos antioxidantes presentes nas cascas de abiu para posterior extração pelo uso de técnicas como PLE. Devido às características dos extratos produzidos via PLE, estes podem ser usados na indústria de alimentos como substitutos de antioxidantes sintéticos ou como ingredientes na formulação de cosméticos e fármacos.

Do **Capítulo 5**, a PLE também possui potencial para a produção de extratos ricos em tiosulfinatos tais como a alicina. De acordo aos resultados, extrato rico em alicina produzido a partir de alho, foi obtido através do uso de pouca quantidade de etanol pressurizado em condições amenas de pressão e temperatura. Os extratos de alho produzidos via PLE mostraram-se mais concentrados em alicina que as amostras comerciais de alho fresco, alho em pó e óleo de alho. Além disso, devido às características da extração PLE de alho (tipo e condição do solvente) outras vantagens como a coextração dos polifenóis, os quais apresentam atividade biológica similar aos tiosulfinatos; e a obtenção de extratos de alho com baixo teor de óleo, o qual é responsável pelo odor característico do alho, os extratos PLE de alho possuem potencial para uso na indústria de alimentos, cosmética e farmacêutica.

Em geral, a PLE é uma técnica eficiente na obtenção de polifenóis uma vez que extratos concentrados em polifenóis e com atividade antioxidante foram obtidos à temperatura e pressões de extração amenas, rapidamente e com reduzido consumo de solvente orgânico. A SFE provavelmente não é a técnica de extração mais adequada para a obtenção de extratos ricos em antioxidantes a partir de cascas de abiu.

MEMÓRIA DO PERÍODO DE DOUTORADO

A doutoranda Angela María Farías Campomanes, realizou as atividades de pesquisa apresentadas neste projeto de pesquisa no laboratório LASEFI, com auxílio financeiro da CAPES/ PEC-PG processo 5817110, com vigência de março de 2012 a fevereiro de 2016.

Foram obtidos 26 créditos. As disciplinas cursadas durante o período do doutorado foram: QP832-Tópicos Especiais em Físico-Química VIII, TP121-Tópicos em Engenharia de Alimentos (Estatística), TP159-Tópicos Especiais em Engenharia de Alimentos e TP199-Seminários.

A doutoranda participou do Programa de Estágio Docente grupo C - PED C com atividades de apoio parcial à docência da disciplina TA 331-A Termodinâmica com carga horária de 08 horas semanais, atuando como voluntário no primeiro período letivo de 2013 e como bolsista no segundo período letivo do mesmo ano.

As pesquisas referentes, tanto ao projeto de Doutorado, quanto às parcerias com o Laboratório de Carnes/DTA/UNICAMP, Universidade Federal do Amazonas e a Universidad de Vigo (Espanha), resultaram até o presente momento, em 1 artigo experimental que será submetido no periódico *Industrial Crops and Products,* 1 artigos experimental submetido no periódico *Food and Bioproducts Processing*, 2 artigo experimentais publicados nos periódicos *Food and Public Health* e *Food Reasearch International*, e 3 trabalhos publicados em anais e eventos, sendo 2 trabalhos completos e 1 resumo; com participação no 10 SLACA – Simpósio Latino Americano de Ciência de Alimentos “Impacto da Ciência de Alimentos na Nutrição e Saúde”, realizado em Campinas (SP, Brasil) em 2013 e no evento 14th EMSF (*European Meeting on Supercritical Fluids*), realizado em Marselha, (França) em 2014.

Artigos completos publicados em periódicos

FARÍAS-CAMPOMANES, A. M., HORITA, C. N., POLLONIO, M. A. R. & MEIRELES, M. A. A. 2014. Allicin-Rich Extract Obtained from Garlic by Pressurized Liquid Extraction: Quantitative Determination of Allicin in Garlic Samples. *Food and Public Health*, 4, 272-278.

HORITA, C. N., **FARÍAS-CAMPOMANES**, A. M., BARBOSA, T. S. ESMERINO, E. A., GOMEZ DA CRUZ, A., BOLINI, H. M. A., MEIRELES, M. A. A. & POLLONIO, M. A. R.

2016. The antimicrobial, antioxidant and sensory properties of garlic and its derivatives in Brazilian low-sodium frankfurters along shelf-life. *Food Research International*, 84, 1-8.

Trabalhos completos publicados em anais de congressos

FARÍAS-CAMPOMANES A. M., MEIRELES, M.A.A. Pressurized Liquid Extraction of Antioxidant Compounds from Purple Corn (Zea mays L.) Waste: technic and economic viability studies. In: 14th European Meeting on Supercritical Fluids, 2014, Marselha, França.

FARÍAS-CAMPOMANES A. M., LAMARÃO, C. V. P., BALBOA-ALFAYA, E. M., DOMÍNGUEZ, H., VEIGA JR, V. F., MEIRELES, M. A. A. Obtaining Bioactive Compounds from Amazonian Fruit Wastes by Green Technology. In: 9th Polyphenols World Congress, 2015, St Julian's, Malta.

Resumos publicados em anais de congressos

BALBOA-ALFAYA, E. M., **FARÍAS-CAMPOMANES, A. M.,** LAMARÃO, C. V. P., DOMÍNGUEZ, H., VEIGA JR, V. F., MEIRELES, M. A. M. The Supercritical Fluid Extraction of Antioxidant Compounds from abiu skin. In: Workshop on Supercritical Fluids and Energy, 2013, Campinas, SP, Brazil.

Apêndice

APÊNDICE A

MATERIAL SUPLEMENTAR DO ARTIGO PRESSURIZED LIQUID EXTRACTION OF ANTHOCYANINS FROM PURPLE CORN (Zea Mays L.) WASTE: TECHNICAL AND ECONOMIC VIABILITY

Figura A1. Resíduo de milho roxo usado como matéria prima nos experimentos de extração.

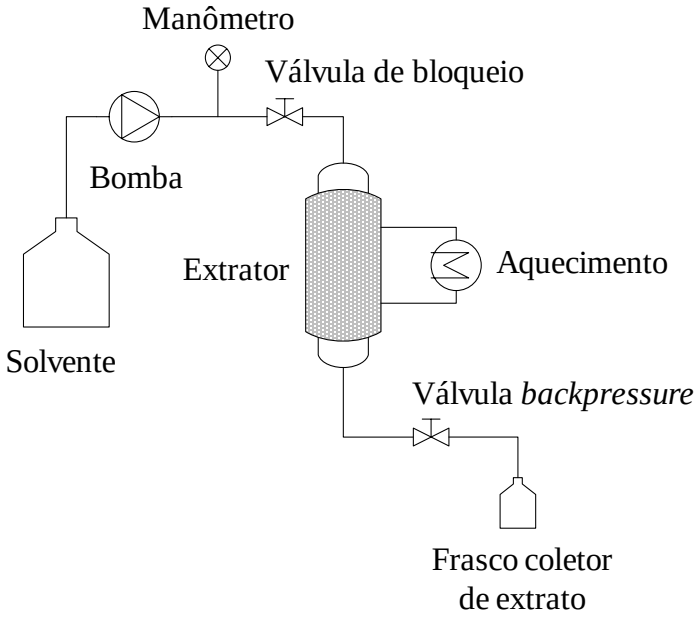


Figura A2. Esquema da unidade de extração com líquido pressurizado.

Tabela A1. Dados experimentais de extração de resíduo de milho roxo via PLE.

T	313								
P	2			4			6		
TES	5	10	15	5	10	15	5	10	15
R	7.3	8.9	7.6	8.2	7.0	7.3	6.8	7.5	8.7
PI	21	21	33.0	26.1	6.7	11.4	29.2	14.6	10.3
DP	1	1	0.3	0.3	0.1	0.2	0.1	0.1	0.2
T	323								
P	2			4			6		
TES	5	10	15	5	10	15	5	10	15
R	8.5	7.4	7.8	7.4	7.5	8.6	7.3	6.7	7.2
PI	31	33	19	13.7	12.2	23.6	27.9	17	25
DP	1	1	1	0.1	0.1	0.1	0.4	1	1
T	333								
P	2			4			6		
TES	5	10	15	5	10	15	5	10	15
R	7.0	8.0	7.3	10.2	8.4	8.2	9.4	7.8	7.9
PI	27.8	14.4	16.0	13.4	26.28	21.5	31.8	20	22.5
DP	0.3	0.2	0.1	0.4	0.01	0.1	0.2	1	0.4

T, temperatura; P, pressão; TES, tempo de extração estática; R, rendimento; PI, atividade antioxidante expressada em porcentagem de inibição; DP, desvio padrão.

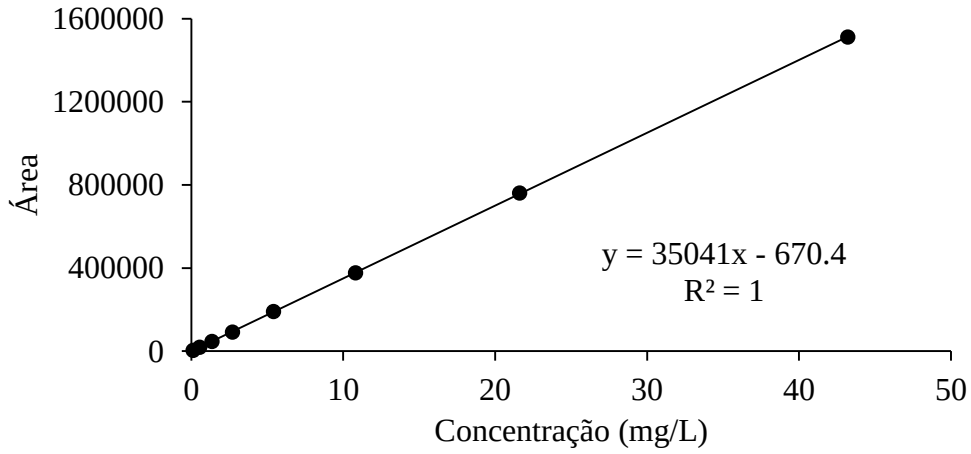


Figura A3. Curva de calibração de cianidina-3-glicosídeo.

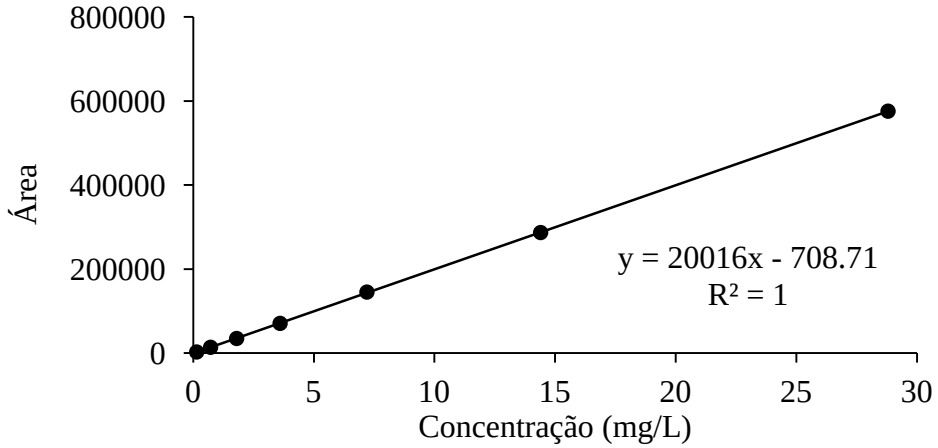


Figura A4. Curva de calibração de pelargonidina-3-glicosídeo.

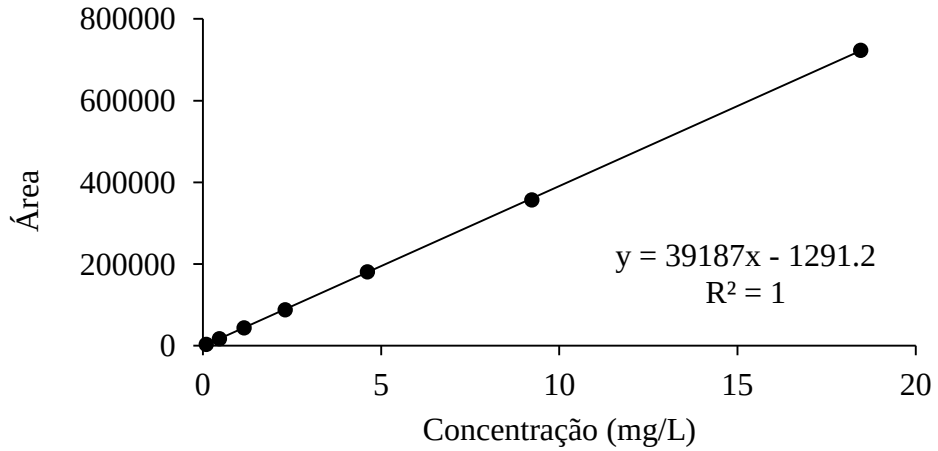


Figura A5. Curva de calibração de peonidina-3-glicosídeo.

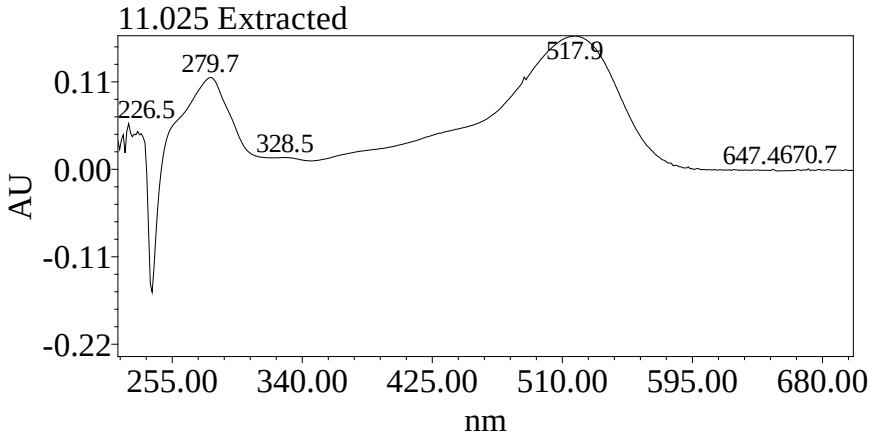


Figura A6. Espectro UV de cianidina-3-glicosídeo.

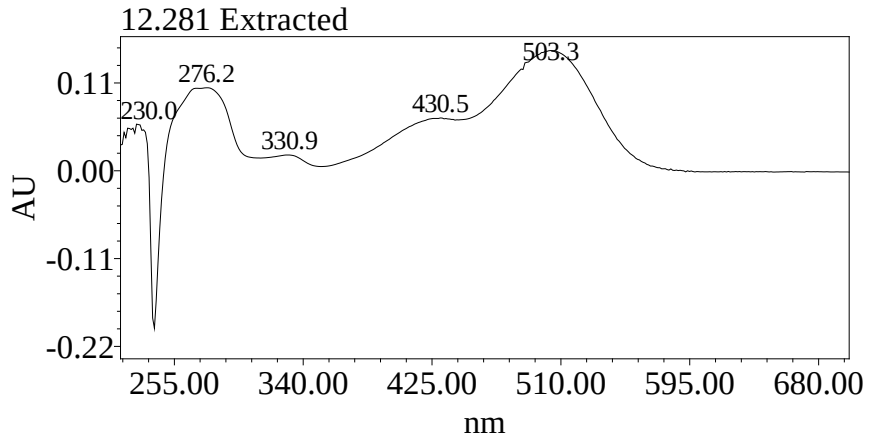


Figura A7. Espectro UV de pelargonidina-3-glicosídeo.

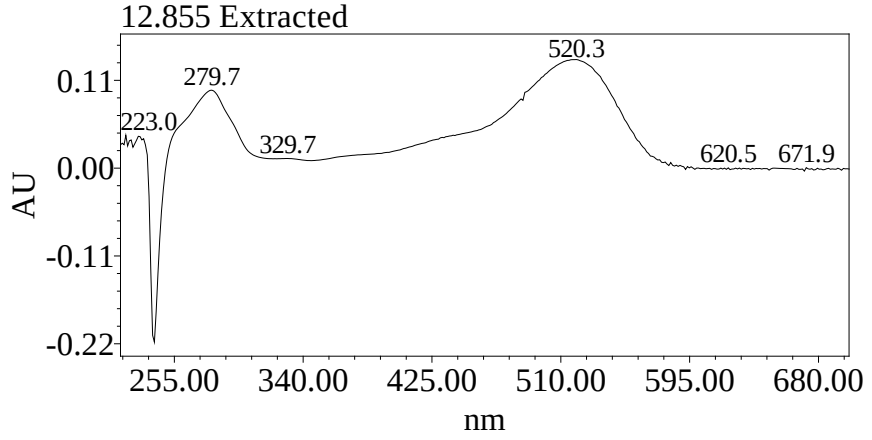


Figura A8. Espectro UV de peonidina-3-glicosídeo

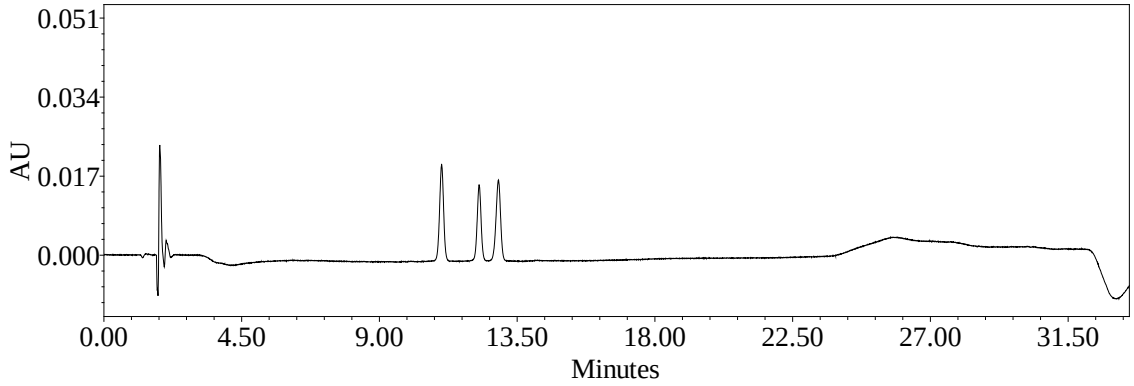


Figura A9. Cromatograma obtido a partir dos padrões de antocianinas.

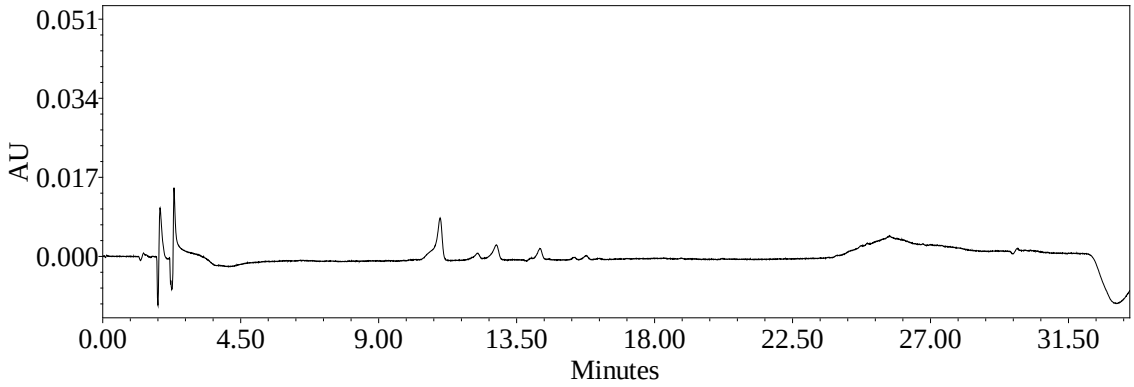


Figura A10. Cromatograma obtido para o extrato de resíduo de milho roxo (PCW) obtido nos primeiros 5 min.

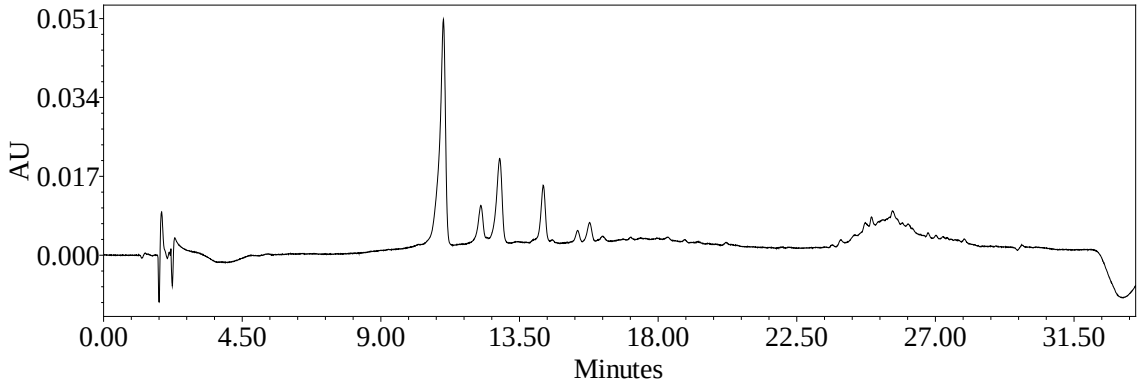


Figura A11. Cromatograma obtido para o extrato de sabugo de milho roxo (PCC) obtido nos primeiros 5 min.

Tabela A2. Rendimento de extração de antocianinas (mg.kg^{-1} de matéria prima) a partir de resíduo de milho roxo (PCW).

T_E		5	10	15	20	30	40	50	60
Cy-3-glu	R	16	26	34	40	51	**	*	*
11,006 min	DP	1	1	1	1	1			
Pl-3-glu	R	5.7	9.4	12	14	20	**	*	*
12,230 min	DP	0.1	0.2	1	1	1			
Pn-3-glu	R	6.6	11.1	14.1	16.5	21.62	**	*	*
12,839 min	DP	0.1	0.1	0.4	0.3	0.01			
U-I	R	3.9	7	8	**	**	*	*	*
14,255 min	DP	0.2	1	1					
U-II 15,376 min		**	*	*	*	*	*	*	*
U-III 15,768 min		**	**	**	*	*	*	*	*

T_E, tempo de extração em min; R, rendimento expressado em mg de antocianina por kg de matéria prima; DP, desvio padrão; U, antocianina não identificada; **traços, abaixo do limite de quantificação (LQ); *não detectado, abaixo de limite de detecção (LD).

Tabela A3. Rendimento de extração de antocianinas (mg.kg^{-1} de matéria prima) a partir de sabugo de milho roxo (PCC).

T_E		5	10	15	20	30	40	50	60
Cy-3-glu	R	158	202	221	229.7	238.9	244.7	**	**
11,006 min	DP	3	1	2	0.5	0.2	0.4		
Pl-3-glu	R	42	57.8	64	68	**	**	*	*
12,230 min	DP	2	0.6	1	1				
Pn-3-glu	R	53	72.1	80.3	84.3	88.8	**	**	*
12,839 min	DP	1	0.1	0.1	0.1	0.1			
U-I	R	28	36	39	**	**	**	*	*
14,255 min	DP	1	2	3					
U-II	R	8.8	**	*	*	*	*	*	*
15,376 min	DP	0.3							
U-III	R	8	**	**	*	*	*	*	*
15,768 min	DP	1							

T_E, tempo de extração em min; R, rendimento expressado em mg de antocianina por kg de matéria prima; DP, desvio padrão; U, antocianina não identificada; **traços, abaixo do limite de quantificação (LQ); *não detectado, abaixo de limite de detecção (LD).

Tabela A4. Concentração de antocianinas (mg.kg^{-1} extrato) nos extratos de resíduo de milho roxo (PCW).

T_E		5	10	15	20	30	40	50	60
Cy-3-glu	R	844	698	680	689	675	**	*	*
11,006 min	DP	28	24	19	16	16			
Pl-3-glu	R	298	249	242	249	158	**	*	*
12,230 min	DP	8	7	11	12	16			
Pn-3-glu	R	347	294	286	286	285.7	**	*	*
12,839 min	DP	3	3	9	6	0.2			
U-I	R	206	172	166	**	**	*	*	*
14,255 min	DP	12	16	16					
U-II 15,376 min		**	*	*	*	*	*	*	*
U-III 15,768 min		**	**	**	*	*	*	*	*

T_E, tempo de extração em min; R, rendimento expressado em mg de antocianina por kg de matéria prima; DP, desvio padrão; U, antocianina não identificada; **traços, abaixo do limite de quantificação (LQ); *não detectado, abaixo de limite de detecção (LD).

Tabela A5. Concentração de antocianinas (mg.kg^{-1} extrato) nos extratos de sabugo de milho roxo (PCC).

T_E		5	10	15	20	30	40	50	60
Cy-3-glu	R	4967	4627	4571	4512	4393	4230	**	**
11,006 min	DP	82	32	31	11	3	7		
Pl-3-glu	R	1330	1326	1335	1332	**	**	*	*
12,230 min	DP	50	13	19	29				
Pn-3-glu	R	1679	1654	1663.1	1656	1633	**	**	*
12,839 min	DP	37	3	0.1	2	3			
U-I	R	880	815	799	**	**	**	*	*
14,255 min	DP	39	55	63					
U-II	R	276	**	*	*	*	*	*	*
15,376 min	DP	8							
U-III	R	263	**	**	*	*	*	*	*
15,768 min	DP	44							

T_E, tempo de extração em min; R, rendimento expressado em mg de antocianina por kg de matéria prima; DP, desvio padrão; U, antocianina não identificada; **traços, abaixo do limite de quantificação (LQ); *não detectado, abaixo de limite de detecção (LD).

T_E (min)	5	10	15	20	30	40	50	60

Figura A12. Extratos PLE de resíduo de milho roxo (PCW) obtidos durante a cinética de extração realizada a 333 K, 6 MPa e 5 min de extração estática.

T_E (min)	5	10	15	20	30	40	50	60

Figura A13. Extratos PLE de sabugo de milho roxo (PCC) obtidos durante a cinética de extração realizada a 333 K, 6 MPa e 5 min de extração estática.

APÊNDICE B

MATERIAL SUPLEMENTAR DO ARTIGO RECOVERY OF BIOACTIVE COMPOUNDS FROM AMAZONIAN FRUIT WASTES VIA GREEN TECHNIQUES

Tabela B1. Dados suplementares de rendimento global para PLE de semente de açaí.

Condição operacional	R_1 (%)	R_2 (%)	Média	DP
313 K/2 MPa	0.706	0.635	0.67	0.04
313 K/4 MPa	0.678	0.608	0.64	0.03
313 K/6 MPa	0.618	0.653	0.64	0.02
323 K/2 MPa	0.619	0.788	0.70	0.08
323 K/4 MPa	0.667	0.750	0.71	0.04
323 K/6 MPa	0.705	0.745	0.72	0.02
333 K/2 MPa	0.669	0.768	0.72	0.05
333 K/4 MPa	0.695	0.818	0.76	0.06
333 K/6 MPa	0.785	0.847	0.82	0.03

R, rendimento; DP, desvio padrão.

Tabela B2. Dados suplementares do teor fenólico (g GAE.100g^{-1} extrato) e atividade antioxidante dos extratos de semente de açaí obtidos via PLE.

Condição	Teor fenólico				Atividade antioxidante			
operacional	TP_1	TP_2	M	DP	PI_1	PI_2	M	DP
313 K/2 MPa	28	25	26	1	41.3	43.7	43	1
313 K/4 MPa	28	38	33	5	41.7	42.9	42	1
313 K/6 MPa	28	30	29	1	46.6	45.4	46	1
323 K/2 MPa	26	28	27	1	47.4	45.9	47	1
323 K/4 MPa	31	26	29	3	53	39.1	46	7
323 K/6 MPa	28	18	23	5	27.8	26.7	27	1
333 K/2 MPa	18	22	20	2	38.1	36.6	37	1
333 K/4 MPa	26	26	25.6	0.1	40.3	35.4	38	2
333 K/6 MPa	15	28	21	6	15.7	35.8	26	10

TP, teor fenólico; M, média; DP, desvio padrão; PI, atividade antioxidante expressada em porcentagem de inibição.

Tabela B3. Dados suplementares de rendimento global para PLE de semente de piquiá realizado a 313 K.

S/F		5		10		15		20		25
MPa	R (%)	DP	R (%)	DP	R (%)	DP	R (%)	DP	R (%)	DP
2	17.3	1.5	9.1	0.8	5.2	0.4	2.3	0.3	1.8	0.6
4	16.1	0.7	10.5	0.3	5.4	0.6	3.0	0.6	1.6	0.7
6	16.9	2.4	10.6	1.7	11.8	4.3	4.4	2.9	3.0	1.0
8	16.8	1.7	10.1	0.8	3.3	0.4	2.27	0.04	1.7	0.1
10	15.5	0.9	6.9	0.8	2.4	0.3	1.38	0.01	0.9	0.1

R, rendimento; DP, desvio padrão; S/F, razão entre a massa de solvente e massa de matéria prima.

Tabela B4. Dados suplementares de rendimento global para PLE de semente de piquiá realizado a 323 K.

S/F		5		10		15		20		25
MPa	R (%)	DP	R (%)	DP	R (%)	DP	R (%)	DP	R (%)	DP
2	12.9	4.6	9.8	0.2	3.2	0.4	1.3	0.3	1.6	1.1
4	13.6	1.6	11.2	3.0	4.6	2.8	1.3	0.8	2.4	1.7
6	13.4	2.7	12.4	1.1	5.2	0.9	2.9	1.7	3.0	0.4
8	14.7	1.8	10.7	1.7	7.7	0.5	3.3	1.1	3.4	0.1
10	16.8	0.6	7.8	2.7	4.1	2.1	2.1	1.4	2.1	1.6

R, rendimento; DP, desvio padrão; S/F, razão entre a massa de solvente e massa de matéria prima.

Tabela B5. Dados suplementares de rendimento global para PLE de semente de piquiá realizado a 333 K.

S/F		5		10		15		20		25
MPa	R (%)	DP	R (%)	DP	R (%)	DP	R (%)	DP	R (%)	DP
2	24	4	10	3	3	2	3	1	2	1
4	24	2	12.3	0.5	4	1	3.1	0.4	2	2
6	15	1	11.6	0.1	5	1	3	1	1.4	0.1
8	18	3	11	4	6	4	2	2	4	2
10	19	1	8	3	2	1	1	1	0.6	0.1

R, rendimento; DP, desvio padrão; S/F, razão entre a massa de solvente e massa de matéria prima.

Tabela B6. Dados suplementares de teor fenólico (g GAE100.g^{-1} de extrato) nos extratos de semente de piquiá obtidos via PLE a 313 K.

S/F	5		10		15		20		25	
MPa	TP	DP	TP	DP	TP	DP	TP	DP	TP	DP
2	8.4	1.2	6.5	0.6	5.5	0.4	5.1	0.4	4.9	0.3
4	9.6	1.5	7.6	0.7	6.4	0.5	5.9	0.4	5.6	0.4
6	10.5	1.6	8.4	0.8	6.2	0.4	5.6	0.3	5.3	0.3
8	12.0	1.8	10.2	0.9	9.3	0.8	8.7	0.7	8.3	0.6
10	11.3	1.8	9.6	1.1	8.9	0.9	8.4	0.8	8.1	0.8

TP, teor fenólico nos extratos; DP, desvio padrão; S/F, razão entre a massa de solvente e massa de matéria prima.

Tabela B7. Dados suplementares de teor fenólico (g GAE.100g^{-1} de extrato) nos extratos de semente de piquiá obtidos via PLE a 323 K.

S/F	5		10		15		20		25	
MPa	TP	DP	TP	DP	TP	DP	TP	DP	TP	DP
2	9.2	1.8	6.9	0.8	6.1	0.6	5.8	0.5	5.5	0.5
4	9.7	1.8	7.4	0.8	6.4	0.5	6.1	0.5	5.6	0.4
6	10.7	2.0	8.2	0.8	7.0	0.6	6.4	0.5	5.9	0.4
8	12.5	2.1	10.5	1.0	8.6	0.7	7.9	0.5	7.3	0.5
10	11.5	1.7	9.7	1.0	8.6	0.8	8.0	0.7	7.5	0.6

TP, teor fenólico nos extratos; DP, desvio padrão; S/F, razão entre a massa de solvente e massa de matéria prima.

Tabela B8. Dados suplementares de teor fenólico (g GAE.100g^{-1} de extrato) nos extratos de semente de piquiá obtidos via PLE a 333 K.

S/F	5		10		15		20		25	
MPa	TP	DP	TP	DP	TP	DP	TP	DP	TP	DP
2	8.1	0.8	6.5	0.5	6.0	0.4	5.5	0.3	5.3	0.3
4	9.4	1.0	7.6	0.5	7.0	0.4	6.5	0.4	6.2	0.3
6	10.2	1.7	7.8	0.7	6.8	0.6	6.2	0.5	6.0	0.4
8	11.8	1.6	9.9	0.8	8.6	0.6	8.1	0.5	7.4	0.4
10	11.1	1.5	9.5	0.9	8.8	0.8	8.4	0.7	8.3	0.7

TP, teor fenólico nos extratos; DP, desvio padrão; S/F, razão entre a massa de solvente e massa de matéria prima.

Tabela B9. Dados suplementares de teor de flavonóides (g CE.100g^{-1} de extrato) nos extratos de semente de piquiá obtidos via PLE a 313 K.

S/F		5		10		15		20		25
MPa	TF	DP	TF	DP	TF	DP	TF	DP	TF	DP
2	4.2	0.1	3.0	0.1	2.5	0.1	2.3	0.1	2.2	0.1
4	4.8	0.2	3.3	0.1	2.8	0.1	2.6	0.1	2.5	0.1
6	5.5	0.1	3.9	0.1	2.9	0.1	2.6	0.1	2.5	0.1
8	7.07	0.01	5.33	0.03	4.85	0.03	4.53	0.03	4.31	0.03
10	6.20	0.01	4.94	0.02	4.54	0.02	4.31	0.02	4.16	0.02

TF, teor de flavonóides nos extratos; DP, desvio padrão; S/F, razão entre a massa de solvente e massa de matéria prima.

Tabela B10. Dados suplementares de teor de flavonóides (g CE.100g^{-1} de extrato) nos extratos de semente de piquiá obtidos via PLE a 323 K.

S/F		5		10		15		20		25
MPa	TF	DP	TF	DP	TF	DP	TF	DP	TF	DP
2	4.3	0.2	2.8	0.1	2.5	0.1	2.4	0.1	2.2	0.1
4	5.0	0.2	3.2	0.1	2.8	0.1	2.7	0.1	2.5	0.1
6	5.80	0.04	3.85	0.02	3.30	0.02	3.03	0.02	2.79	0.02
8	7.7	0.3	5.6	0.2	4.7	0.2	4.3	0.2	3.9	0.2
10	6.41	0.05	5.08	0.05	4.46	0.04	4.17	0.04	3.90	0.03

TF, teor de flavonóides nos extratos; DP, desvio padrão; S/F, razão entre a massa de solvente e massa de matéria prima.

Tabela B11. Dados suplementares de teor de flavonóides (g CE.100g^{-1} de extrato) nos extratos de semente de piquiá obtidos via PLE a 333 K.

S/F		5		10		15		20		25
MPa	TF	DP	TF	DP	TF	DP	TF	DP	TF	DP
2	3.95	0.01	2.98	0.02	2.73	0.02	2.53	0.02	2.41	0.02
4	4.6	0.2	3.3	0.1	3.1	0.1	2.8	0.1	2.7	0.1
6	5.2	0.2	3.5	0.1	3.1	0.1	2.8	0.1	2.7	0.1
8	6.82	0.04	5.1	0.1	4.4	0.1	4.14	0.05	3.78	0.04
10	6.00	0.04	4.81	0.03	4.46	0.03	4.26	0.03	4.18	0.03

TF, teor de flavonóides nos extratos; DP, desvio padrão; S/F, razão entre a massa de solvente e massa de matéria prima.

Tabela B12. Dados suplementares de atividade antioxidante (EC_{50}, $\mu g.mL^{-1}$) dos extratos de semente de piquiá obtidos via PLE a 313 K, determinados pelo método do radical DPPH.

S/F		5		10		15		20		25
MPa	EC_{50}	DP	EC_{50}	DP	EC_{50}	DP	EC_{50}	DP	EC_{50}	DP
2	32	1	35	1	36	1	36	1	43	1
4	26	1	30	2	33	1	32	1	39	1
6	20	1	25	1	26.9	0.4	28	1	35	1
8	11	1	16	1	17.0	0.1	19.2	0.4	25	2
10	15.2	0.4	19.9	0.4	20.66	0.02	22.4	0.5	29	2

EC_{50}, atividade antioxidante expressada em EC_{50}, DP, desvio padrão; S/F, razão entre a massa de solvente e massa de matéria prima.

Tabela B13. Dados suplementares de atividade antioxidante (EC_{50}, $\mu g.mL^{-1}$) dos extratos de semente de piquiá obtidos via PLE a 323 K, determinados pelo método do radical DPPH.

S/F		5		10		15		20		25
MPa	EC_{50}	DP	EC_{50}	DP	EC_{50}	DP	EC_{50}	DP	EC_{50}	DP
2	30	1	34	1	35	1	35	1	42	1
4	25	1	29	2	31	1	31	1	38	1
6	18.4	0.3	24	1	24	1	26	1	33	1
8	11	1	15	1	16.0	0.3	18	1	24	2
10	13.4	0.3	18.0	0.3	18.9	0.2	21.1	0.4	27	2

EC_{50}, atividade antioxidante expressada em EC_{50}, DP, desvio padrão; S/F, razão entre a massa de solvente e massa de matéria prima.

Tabela B14. Dados suplementares de atividade antioxidante (EC_{50}, $\mu g.mL^{-1}$) dos extratos de semente de piquiá obtidos via PLE a 333 K, determinados pelo método do radical DPPH.

S/F		5		10		15		20		25
MPa	EC_{50}	DP	EC_{50}	DP	EC_{50}	DP	EC_{50}	DP	EC_{50}	DP
2	34	1	37	1	36	1	37	1	45	2
4	27.3	0.3	32	2	34	1	33	1	41	1
6	23	1	27	2	29	1	30	1	37	1
8	12	1	17	1	17.9	0.4	20.0	0.4	26	2
10	17	1	22.0	0.2	22.68	0.01	24	1	31	1

EC_{50}, atividade antioxidante expressada em EC_{50}, DP, desvio padrão; S/F, razão entre a massa de solvente e massa de matéria prima.

Tabela B15. Dados suplementares de atividade antioxidante (EC_{50}, $\mu g.mL^{-1}$) dos extratos de semente de piquiá obtidos via PLE a 313 K, determinados pelo método do radical ABTS.

S/F		5		10		15		20		25
MPa	EC_{50}	DP	EC_{50}	DP	EC_{50}	DP	EC_{50}	DP	EC_{50}	DP
2	27.1	0.2	28	1	32	2	34.9	0.3	37	1
4	22	1	24	1	27	2	30.2	0.1	33	1
6	18	1	19.3	0.4	22	2	25.8	0.2	29	1
8	9.9	0.4	12.1	0.2	15	1	17.1	0.2	21	1
10	13	1	15.0	0.3	18	1	20.3	0.1	24	1

EC_{50}, atividade antioxidante expressada em EC_{50}, DP, desvio padrão; S/F, razão entre a massa de solvente e massa de matéria prima.

Tabela B16. Dados suplementares de atividade antioxidante (EC_{50}, $\mu g.mL^{-1}$) dos extratos de semente de piquiá obtidos via PLE a 323 K, determinados pelo método do radical ABTS.

S/F		5		10		15		20		25
MPa	EC_{50}	DP	EC_{50}	DP	EC_{50}	DP	EC_{50}	DP	EC_{50}	DP
2	25.15	0.04	26	1	30	2	33	1	35	1
4	20	1	23	1	25	2	28.1	0.1	32	1
6	15	1	18.2	0.3	21	2	23.9	0.2	28	1
8	9.2	0.2	11.3	0.1	14	1	15.9	0.3	20	1
10	12	1	13.6	0.2	17	1	18.9	0.2	23	1

EC_{50}, atividade antioxidante expressada em EC_{50}, DP, desvio padrão; S/F, razão entre a massa de solvente e massa de matéria prima.

Tabela B17. Dados suplementares de atividade antioxidante (EC_{50}, $\mu g.mL^{-1}$) dos extratos de semente de piquiá obtidos via PLE a 333 K, determinados pelo método do radical ABTS.

S/F		5		10		15		20		25
MPa	EC_{50}	DP	EC_{50}	DP	EC_{50}	DP	EC_{50}	DP	EC_{50}	DP
2	28.7	0.4	28	1	32	2	34.9	0.3	38	2
4	23.2	0.1	24	1	28	2	31.8	0.2	34	1
6	19	1	21	1	24	2	27.0	0.4	31	1
8	10	1	13.0	0.1	16	1	18.0	0.3	22	1
10	14	1	15.9	0.1	19	1	21.5	0.3	25	1

EC_{50}, atividade antioxidante expressada em EC_{50}, DP, desvio padrão; S/F, razão entre a massa de solvente e massa de matéria prima.

Tabela B18. Dados suplementares de rendimento global para SFE de casca de abiú.

T		313		323		333
MPa	R (%)	DP	R (%)	DP	R (%)	DP
15	2.8	0.2	1.96	0.04	1.4	0.1
20	3.5	0.3	3.2	0.2	3.2	0.1
25	4.0	0.2	4.3	0.2	4.25	0.01
30	4.28	0.04	4.7	0.3	4.4	0.3
35	4.3	0.1	5.5	0.3	5.18	0.03

R, rendimento; DP, desvio padrão; T, temperatura expressada em K.

Tabela B19. Dados suplementares de atividade antioxidante dos extratos de casca de abiu obtidos via SFE.

T		313		323		333
MPa	PI (%)	DP	PI (%)	DP	PI (%)	DP
15	4.7	0.4	6	1	8.8	0.2
20	8	2	4.1	0.2	4.9	0.3
25	4	1	6.0	0.1	5.2	0.2
30	6	1	4.70	0.01	5	1
35	4.7	0.1	4.24	0.01	5	1

PI, atividade antioxidante expressada em porcentagem de inibição, DP, desvio padrão; T, temperatura expressada em K.

APÊNDICE C

MATERIAL SUPLEMENTAR DO ARTIGO ALLICIN-RICH EXTRACT OBTAINED FROM GARLIC BY PRESSURIZED LIQUID EXTRACTION: QUANTITATIVE DETERMINATION OF ALLICIN IN GARLIC SAMPLES.

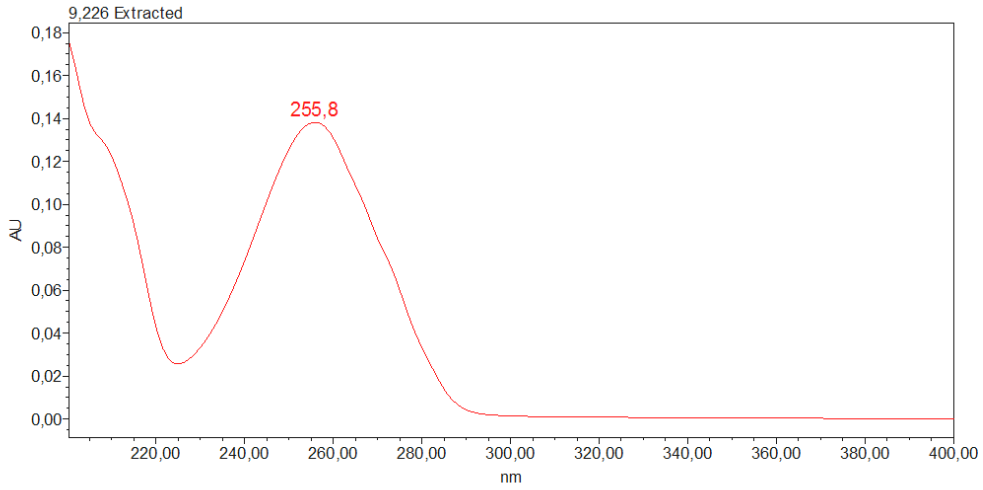


Figura C1. Espectro UV do padrão interno identificado nas amostras de alho.

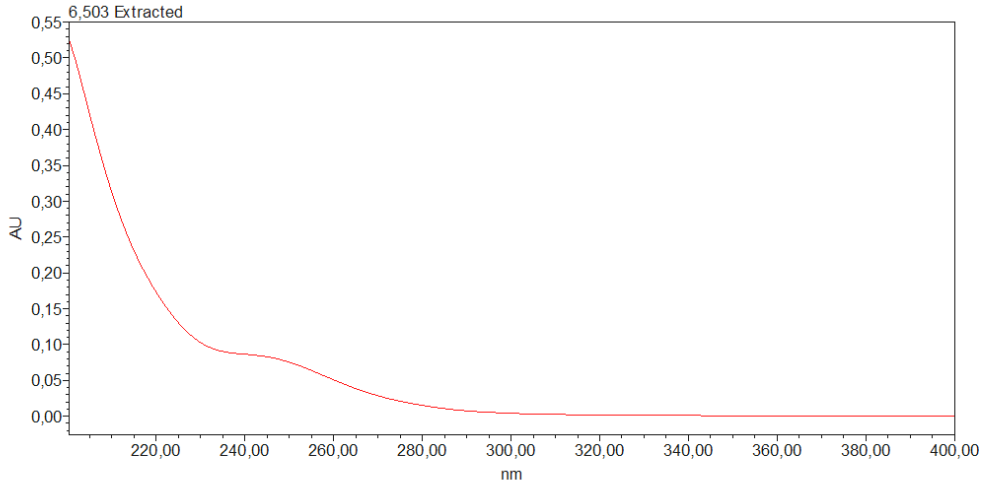


Figura C2. Espectro UV da alicina identificada nas amostras de alho.

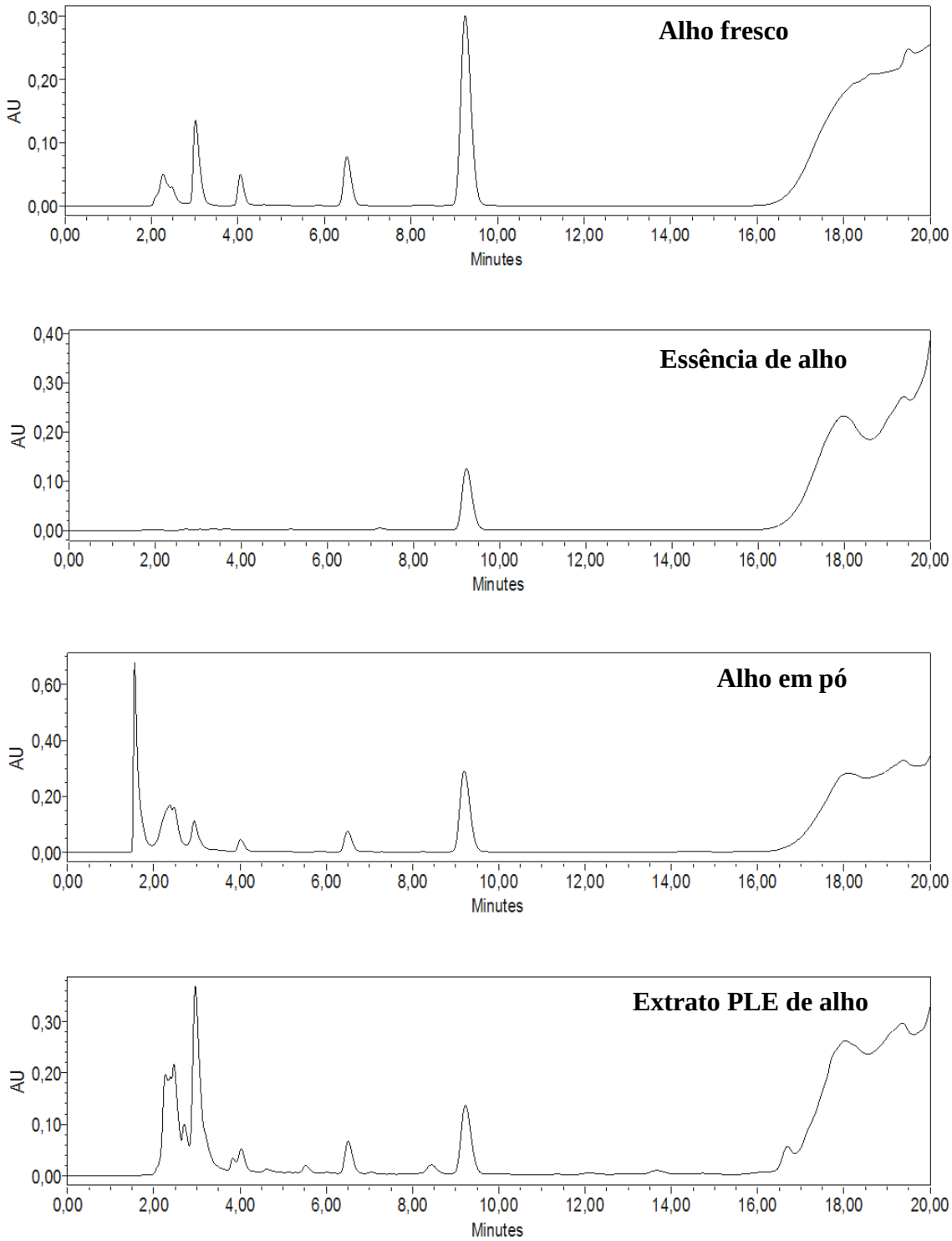


Figura C3. Cromatogramas das amostras de alho. Alicina, t_R: 6.5 min; padrão interno, t_R: 9.2 min