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RENATA VARDANEGA

The use of clean technologies to obtain biosurfactants and prebiotic carbohydrates from Brazilian ginseng (*Pfaffia glomerata*)

Uso de tecnologias limpas para a obtenção de biossurfactantes e carboidratos prebióticos a partir do ginseng brasileiro (*Pfaffia glomerata)*

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Tese de doutorado apresentada à Faculdade de Engenharia de Alimentos, da Universidade Estadual de Campinas como parte dos requisitos exigidos para obtenção do título de doutora em Engenharia de Alimentos.

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

Orientadora: Prof[a]. Dr[a]. MARIA ANGELA DE ALMEIDA MEIRELES PETENATE

Coorientador: Dr. DIEGO TRESINARI DOS SANTOS

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COMISSÃO EXAMINADORA

Prof^{a}. Dr^{a}. Maria Angela de Almeida Meireles Petenate
ORIENTADORA – DEA/FEA/UNICAMP

Prof. Dr. Guilherme José Máximo
MEMBRO TITULAR – DEA/FEA/UNICAMP

Prof. Dr. Marco Di Luccio
MEMBRO TITULAR – EQA/UFSC

Prof^{a}. Dr^{a}. Priscilla Carvalho Veggi
MEMBRO TITULAR – DCET/UNIFESP

Prof^{a}. Dr^{a}. Rosiane Lopes da Cunha
MEMBRO TITULAR – DEA/FEA/UNICAMP

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Prof. Dr. Julian Martínez
MEMBRO SUPLENTE – DEA/FEA/UNICAMP

Dr. Rodrigo Nunes Cavalcanti
MEMBRO SUPLENTE – POLITÉCNICA/USP

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RESUMO

O ginseng brasileiro (*Pfaffia glomerata*) é uma planta nativa do Brasil que apresenta uma rica composição de compostos bioativos, incluindo a beta-ecdisona que apresenta propriedades estimulantes, as saponinas com atividade surfactante e carboidratos prebióticos. Diante destes aspectos, diferentes processos de extração foram estudados a fim de maximizar a recuperação dos compostos bioativos de interesse, bem como separá-los em diferentes frações, utilizando apenas solventes não tóxicos. Inicialmente, realizou-se um estudo técnico e econômico da extração de beta-ecdisona e carboidratos prebióticos das raízes e partes aéreas do ginseng brasileiro empregando extração com água subcrítica. Os resultados demonstraram que é possível obter extratos das raízes com concentração de beta-ecdisona de 0,7% (base seca, b.s.), enquanto que os extratos das partes aéreas apresentaram apenas 0,3% (b.s.) de beta-ecdisona. Em relação à extração de carboidratos prebióticos, os extratos obtidos das raízes apresentaram um teor de frutooligossacarídeos de até 8,8% (b.s.), o que faz desta matéria-prima uma importante fonte desses compostos. A avaliação econômica do processo de extração com água subcrítica considerou principalmente o teor de beta-ecdisona dos extratos obtidos e demonstrou que o processamento das raízes é técnica e economicamente mais favorável do que o processamento das partes aéreas. Considerando os resultados obtidos neste estudo e dados da literatura sobre extração de compostos bioativos do ginseng brasileiro, um processo de extração intensificado realizado em duas etapas foi proposto com o intuito de aumentar a recuperação de beta-ecdisona, saponinas e carboidratos prebióticos das raízes de ginseng brasileiro. Realizou-se a primeira etapa com etanol e a segunda com água, ambas a 333 K e avaliou-se o efeito da pressão neste processo. Observou-se que para a obtenção de compostos bioativos das raízes de ginseng brasileiro, processos realizados a pressão ambiente apresentam maior recuperação dos compostos do que quando realizados a alta pressão. Nesse processo obteve-se um extrato etanólico com 5,6% (b.s.) de beta-ecdisona e 47% (b.s.) de saponinas com concentração micelar crítica (CMC) = 6 $mg \cdot mL^{-1}$. O extrato etanólico não apresentou carboidratos prebióticos. Já o extrato aquoso apresentou um teor de frutooligossacarídeos de 9% (b.s.), além de 0,5% (b.s.) de beta-ecdisona e 24% (b.s.) de saponinas com CMC = 18 $mg \cdot mL^{-1}$. Desta forma, o processo de extração intensificado permitiu a extração e fracionamento dos compostos bioativos das raízes de ginseng brasileiro, uma vez que foi possível obter um extrato etanólico rico em beta-ecdisona e saponinas e um extrato aquoso rico em carboidratos prebióticos. A partir destes resultados, um estudo econômico foi realizado a fim de comparar diferentes cenários de produção para avaliar a

viabilidade econômica do processo de extração intensificado. Esse estudo confirmou o processo intensificado como sendo a rota de produção de extratos das raízes de ginseng brasileiro economicamente mais viável. Ao final do desenvolvimento deste trabalho demonstrou-se que é possível substituir processos de extração convencionais por processos inovadores que não utilizam solventes tóxicos, minimizam a geração de resíduos através do melhor aproveitamento das matérias-primas e são mais eficientes do ponto de vista energético além de serem altamente promissores do ponto de vista econômico.

PALAVRAS-CHAVE: Ginseng brasileiro, beta-ecdisona, saponinas, carboidratos prebióticos, viabilidade econômica, processos intensificados, processos limpos.

ABSTRACT

Brazilian ginseng (*Pfaffia glomerata*) is a native plant from Brazil that contains a rich composition of bioactive compounds, including beta-ecdysone with stimulating effects, saponins with surfactant activity and prebiotic carbohydrates. In this context, different extraction process were studied to maximize the bioactive compounds recovery, as well as to fractionate them, using only non-toxic solvents. Firstly, a techno-economic evaluation of the extraction of beta-ecdysone from roots and aerial parts of Brazilian ginseng using subcritical water was performed. The results showed that is possible to obtain extracts from Brazilian ginseng roots with 0.7% (dry basis, d.b.) of beta-ecdysone, while the Brazilian ginseng aerial parts extracts yielded 0.3% (d.b.) of beta-ecdysone. In terms of prebiotic carbohydrates, the extracts from Brazilian ginseng roots showed a fructooligosaccharides content of 8.8% (d.b.), which makes this raw material an important source of such compounds. Since to date the beta-ecdysone is the main compound with commercial value obtained from Brazilian ginseng, the economic evaluation of the subcritical water extraction process accounted only the beta-ecdysone content in the extracts. The economic evaluation showed that the manufacturing of roots was a great opportunity of business, while the manufacturing of the aerial parts should not be undertaken. Considering the results obtained in this study and data from literature about extraction of bioactive compounds from Brazilian ginseng, an intensified process was proposed to increase the beta-ecdysone, saponins and prebiotic carbohydrates recovery from Brazilian ginseng roots. The intensified process was performed in two steps: the first step used ethanol as solvent and the second one used water as solvent, both at 333 K. The effect of pressure on this process was evaluated. It was observed that to obtain bioactive compounds from Brazilian ginseng roots, the use of ambient pressure yielded greater results than those obtained at high pressure. In this process, an ethanolic extract containing 5.6% (d.b.) of beta-ecdysone, 47% (d.b.) of saponins with critical micellar concentration (CMC) = 6 $mg \cdot mL^{-1}$ was obtained. No prebiotic carbohydrate was detected in the ethanolic extract, otherwise, the aqueous extract showed a fructooligosaccharides content of 9% (d.b.) besides 0.5% (d.b.) of beta-ecdysone and 24% (d.b.) of saponins with CMC = 18 $mg \cdot mL^{-1}$. In this way, the intensified process allowed the extraction and fractionating of the bioactive compounds from Brazilian ginseng roots, since it was possible to obtain an ethanolic extract rich in beta-ecdysone and saponins and an aqueous extract rich in prebiotic carbohydrates. Based on the experimental results, an economic study was developed aiming to compare different production scenarios to evaluate the economic viability of the intensified process. The study

confirmed that the intensified process is the best way to produce Brazilian ginseng extracts. At the end of the development of this work, it was showed that it is possible replace conventional extraction processes by innovative processes, which use non-toxic solvents, reduces the residues generation and are more efficient from the energetic point of view. Furthermore, the proposed processes showed high economic feasibility.

Keywords: Brazilian ginseng, beta-ecdysone, saponins, prebiotic carbohydrate, process intensification, economic feasibility, clean process.

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INTRODUÇÃO GERAL, OBJETIVOS E ESTRUTURA DA TESE

1.1 INTRODUÇÃO

Nas últimas décadas, os hábitos da população vêm mudando drasticamente devido à sua maior preocupação com os aspectos relacionados à saúde e qualidade de vida. Neste contexto, o interesse por produtos naturais, tanto para fins nutricionais quanto medicinais tem crescido cada vez mais. Esses produtos, também chamados de extratos naturais, têm sua aplicação definida de acordo com a funcionalidade dos compostos bioativos presentes em sua composição e têm como finalidade produzir efeitos benéficos em uma ou mais funções fisiológicas, aumentar o bem-estar e/ou diminuir o risco de doenças [1, 2]. Os produtos naturais podem ser extraídos de diversas fontes, como plantas, resíduos da agricultura e/ou agroindústria, algas e microalgas [3] e encontram diversas aplicações não só na indústria de alimentos, mas também em setores como de fármacos, cosméticos, têxtil, perfumaria, entre outros [2].

Além das mudanças que vêm ocorrendo acerca dos hábitos de consumo da população, outro aspecto que tem ganhado destaque nos últimos anos é a preocupação com os processos envolvidos na obtenção destes produtos, bem como o impacto causado pelos processos de produção no meio ambiente. Os processos convencionais comumente empregados para extração de compostos bioativos possuem diversas limitações por serem demorados e trabalhosos, possuírem baixa seletividade e/ou baixos rendimentos, além de geralmente empregarem grandes quantidades de solventes tóxicos, os quais podem causar danos aos operadores na indústria, aos consumidores e também ao meio ambiente [3].

Essas recentes demandas têm estimulado diversas pesquisas dedicadas ao desenvolvimento de processos que tenham reduzido impacto ambiental e que forneçam produtos naturais de alta qualidade. Neste cenário, os processos envolvendo tecnologias sub- e supercríticas satisfazem bem essas demandas, uma vez que, na maioria dos casos, empregam solventes inofensivos e produzem produtos com alta pureza e qualidade [4]. Dentre estas técnicas, a extração com líquido pressurizado (PLE – *Pressurized Liquid Extraction*) tem se destacado como uma das mais promissoras; quando o solvente empregado na PLE é somente água, o processo pode também ser chamado de extração com água subcrítica (SWE – *Subcritical Water Extraction*) [5]. Água no estado subcrítico é obtida quando esta é aquecida a temperaturas acima do seu ponto de ebulição a pressão ambiente (373 K/ 0,1 MPa) e abaixo do seu ponto crítico (647 K/22,1 MPa) [6]. PLE e SWE têm sido empregadas com sucesso para obtenção de diversos compostos bioativos, os quais já foram extensivamente revisados e

publicados na literatura [1, 5-8]. Dentre as principais classes de compostos extraídas via PLE e SWE estão os polifenóis, carotenoides e alguns óleos essenciais [3, 5]. Além das vantagens relacionadas ao emprego de PLE, estudos relatam que o tempo de extração pode ser ainda mais reduzido quando este processo é assistido por ultrassom [9, 10].

O ginseng brasileiro (*Pfaffia glomerata*), pertencente à família Amaranthaceae, é uma planta arbustiva nativa do Brasil, popularmente difundida e comercializada como substituta do ginseng asiático (*Panax* ssp., Araliaceae) devido à semelhança na morfologia das raízes e, também, devido aos efeitos terapêuticos semelhantes [11]. O ginseng brasileiro é conhecido principalmente devido ao seu efeito adaptógeno e, em função disso, é recomendado para a dieta de atletas a fim de melhorar o seu desempenho durante os treinamentos. Além disso, os benefícios do ginseng brasileiro relatados em estudos farmacológicos são seus efeitos analgésico, anti-inflamatório [12] e antiglicêmico [13], proteção do trato gástrico [14], atividade antimicrobiana [15] e inibição da melanogênese [16]. Além disso, extratos de ginseng brasileiro apresentaram ação depressora do sistema nervoso central [17]. Os efeitos terapêuticos do ginseng brasileiro são atribuídos à presença do composto beta-ecdisona [18].

Comumente, a beta-ecdisona proveniente do ginseng brasileiro é extraída das suas raízes. No entanto, há estudos que relatam a presença de beta-ecdisona também em outras partes da planta, como hastes, inflorescências e folhas [19-21]. O fato de haver beta-ecdisona também em outras partes do ginseng brasileiro além de suas raízes, que já são exploradas comercialmente como fonte deste composto [22], abre a possibilidade de explorar a planta de uma melhor forma, pois, atualmente, as partes aéreas do ginseng brasileiro são descartadas no campo.

Além da beta-ecdisona, as raízes de ginseng brasileiro também são ricas em outros compostos chamados de saponinas, as quais são largamente utilizadas como surfactantes em diversos setores da indústria, tais como alimentos, cosméticos, fármacos, entre outros [23]. As principais fontes comerciais de saponinas de origem vegetal são a *Quillaja saponaria* e a *Yucca schidigera* [23, 24]. No entanto, recentemente outras matérias-primas vegetais vêm sendo investigadas [25], inclusive o ginseng brasileiro [26, 27]. Estudos iniciais já demonstraram que as saponinas do ginseng brasileiro possuem potencial ação surfactante [28, 29]. Desta forma, a extração de saponinas das raízes do ginseng brasileiro representa mais uma alternativa para melhor aproveitar o potencial desta planta e agregar valor aos seus produtos.

Embora haja na literatura estudos dedicados ao entendimento dos efeitos dos compostos provenientes do metabolismo secundário do ginseng brasileiro, como a beta-ecdisona e as saponinas, ainda são os escassos os estudos focados nos metabólitos primários desta matéria-prima, como os polissacarídeos. Recentemente, um estudo demonstrou que extratos provenientes de raízes de ginseng brasileiro contêm majoritariamente polímeros do tipo inulina com potencial efeito prebiótico, embora mais estudos sejam necessários para descrever a fermentabilidade dessa inulina por bactérias benéficas [30].

Uma possível forma de obtenção dos produtos das raízes de ginseng brasileiro pode ser a utilização de dois solventes durante a extração de forma sequencial. Quando esta rota de processamento é realizada utilizando um único equipamento, denomina-se processo intensificado. Os processos intensificados são caracterizados principalmente por visarem à redução do consumo energético e da geração de resíduos para obtenção dos produtos e aumento do rendimento de processo utilizando o mesmo equipamento [31]. A intensificação do processo também pode ser obtida pelo emprego de ultrassom durante a extração a fim de aumentar a recuperação de compostos bioativos devido ao fenômeno de cavitação causado pelo ultrassom [32].

Para que os processos desenvolvidos possam ser viabilizados em escala industrial, além do estudo técnico é preciso que um estudo econômico seja realizado. Diante disso, os processos de extração desenvolvidos para obtenção de compostos bioativos do ginseng brasileiro precisam ser avaliados em relação ao seu potencial econômico. Alguns trabalhos reportam que o custo de produção de extratos vegetais é fortemente influenciado pelo custo de aquisição da matéria-prima [33, 34]. Portanto, o processamento das partes aéreas pode ser promissor, uma vez que esta matéria-prima possui baixo ou nenhum custo de aquisição. Da mesma forma, os processos de extração intensificados podem ser vantajosos, já que estes resultam em maiores rendimentos de compostos bioativos a partir da mesma matéria-prima.

1.2 OBJETIVOS

1.2.1 Objetivo geral

Estudar a viabilidade técnica e econômica da extração de compostos bioativos (beta-ecdisona, saponinas com atividade surfactante e carboidratos prebióticos) das raízes e partes aéreas de ginseng brasileiro utilizando processos limpos.

1.2.2 Objetivos específicos

i) Estudar o processo de extração com água subcrítica (SWE) das raízes e partes aéreas do ginseng brasileiro avaliando o efeito de temperatura, pressão e tempo estático sobre a recuperação de beta-ecdisona e carboidratos prebióticos;

ii) Comparar a viabilidade econômica do processo SWE para obtenção de beta-ecdisona a partir das raízes e partes áreas do ginseng brasileiro;

iii) Fracionar os compostos bioativos das raízes de ginseng brasileiro através do desenvolvimento de um processo de extração intensificado realizado com diferentes solventes de forma sequencial em duas etapas;

iv) Avaliar o efeito da pressão sobre a recuperação dos compostos bioativos das raízes do ginseng brasileiro no processo de extração intensificado;

v) Avaliar o comportamento cinético do processo de extração intensificado através da construção das curvas de extração global (OEC – *Overall Extraction Curve*).

vi) Avaliar a intensificação do processo de extração através do emprego de ultrassom para aumentar a recuperação dos compostos bioativos;

vii) Estimar a viabilidade econômica do processamento das raízes de ginseng brasileiro em diferentes cenários de produção para estabelecer a melhor rota de obtenção dos extratos.

1.3 ESTRUTURA DA TESE

Neste trabalho, as etapas do desenvolvimento do projeto de pesquisa estão apresentadas em 8 capítulos. Nesse **Capítulo 1**- *Introdução, objetivos e estrutura da tese*- são apresentados o tema abordado no estudo, os objetivos pretendidos e as etapas envolvidas durante sua realização. As atividades propostas e realizadas são apresentadas na Figura 1.1.

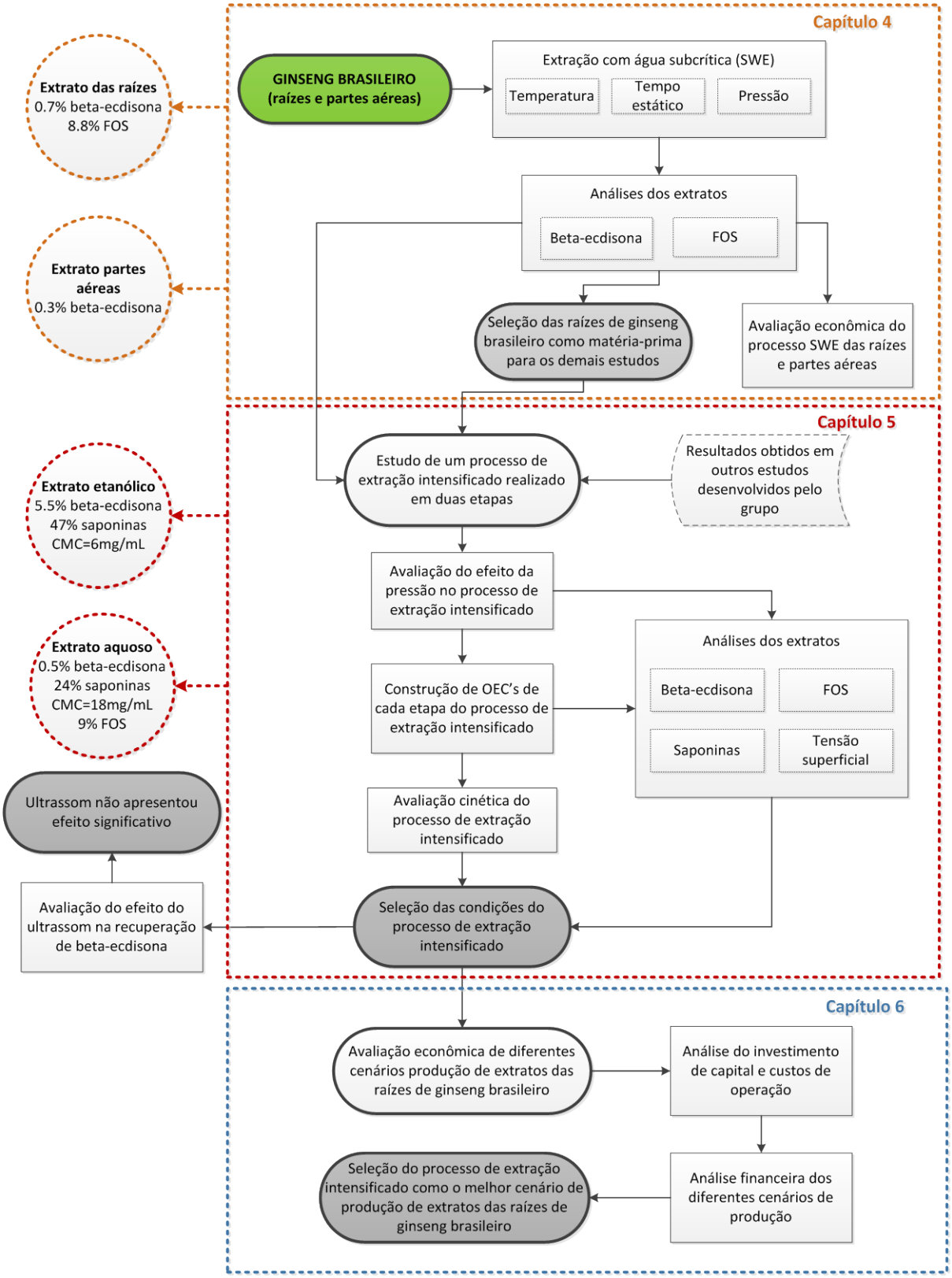

Figura 1.1: Atividades experimentais realizadas na tese.

No **Capítulo 2 –** *Revisão bibliográfica* - é apresentada uma breve contextualização sobre os principais temas abordados no presente estudo. Neste Capítulo são apresentados aspectos relacionados aos compostos bioativos presentes na matéria-prima de estudo, o ginseng brasileiro (*Pfaffia glomerata*), bem como aspectos dos processos de extração empregados, intensificação e avaliação econômica dos processos.

O **Capítulo 3 –** *Intensificação da extração de compostos bioativos pelo emprego de ultrassom* - contempla uma revisão sobre o emprego do ultrassom para intensificação de processos de extração de compostos bioativos. Neste artigo são apresentados os aspectos relacionados ao mecanismo e os parâmetros que influenciam a extração assistida por ultrassom (UAE – *Ultrasound assisted extraction*) e, também, é apresentado o estado da arte da aplicação de ultrassom para intensificação de processos de extração a alta pressão.

No **Capítulo 4** – *Extração de beta-ecdisona e carboidratos prebióticos das raízes e partes aéreas do ginseng brasileiro com água subcrítica* – são apresentados os resultados experimentais do estudo no qual os efeitos de temperatura, pressão e tempo estático foram avaliados sobre a extração com água subcrítica de beta-ecdisona e carboidratos prebióticos a partir das raízes e de beta-ecdisona das partes aéreas do ginseng brasileiro, utilizando o método de planejamento experimental. Além disso, foi realizada uma avaliação econômica dos processos utilizando ambas as matérias-primas para estimar o seu potencial econômico com fonte de extratos ricos em compostos bioativos.

Diante dos resultados obtidos no Capítulo 4 e de dados da literatura, no **Capítulo 5** – *Extração intensificada para obtenção de compostos bioativos das raízes de ginseng brasileiro* – são apresentados os resultados experimentais de um processo de extração intensificafo realizado em duas etapas. Na primeira etapa empregou-se etanol como solvente e na segunda etapa empregou-se água para obtenção de extratos ricos em beta-ecdisona, saponinas e carboidratos prebióticos das raízes de ginseng brasileiro. O efeito da pressão foi avaliado sobre os teores de beta-ecdisona, saponinas e frutooligossacarídeos e também sobre as propriedades surfactantes dos extratos obtidos em cada etapa do processo. Além disso, avaliou-se o comportamento cinético do processo.

Os dados experimentais obtidos no Capítulo 5 permitiram avaliar o potencial econômico de três diferentes cenários operacionais para obtenção de extratos de ginseng brasileiro, os quais são apresentados no **Capítulo 6 –** *Obtenção de extratos de ginseng brasileiro em diferentes cenários de produção: avaliação econômica.* Neste capítulo, os três cenários de produção – I) utilizando apenas etanol como solvente, II) utilizando apenas água

como solvente e; III) utilizando etanol e água como solventes em um processo em duas etapas – foram avaliados em relação ao custo de investimento e ao custo operacional, bem como em relação aos índices econômicos que fornecem as informações necessárias para indicar a viabilidade de implantação dos processos.

O **Capítulo 7** – *Discussão geral* – traz uma discussão integrada de todos os capítulos apresentados anteriormente, bem como os resultados mais relevantes obtidos nos capítulos 4 a 6, melhorando assim o entendimento geral da tese. Por fim, o **Capítulo 8 –** *Conclusões gerais e sugestões para trabalhos futuros* – apresenta, de forma sucinta, as conclusões que puderam ser obtidas durante o desenvolvimento do projeto de pesquisa e traz algumas sugestões para o desenvolvimento de pesquisas futuras.

REFERÊNCIAS

[1] M. Herrero, A. Cifuentes, E. Ibañez, Sub- and supercritical fluid extraction of functional ingredients from different natural sources: Plants, food-by-products, algae and microalgae: A review, Food Chemistry, 98 (2006) 136-148.

[2] R.N. Cavalcanti, T. Forster-Carneiro, M.T.M.S. Gomes, M.A. Rostagno, J.M. Prado, M.A.A. Meireles, Uses and applications of extracts from natural sources, in: M.A. Rostagno, J.M. Prado (Eds.) Natural products extraction: principles and applications, RSC, 2013, pp. 450.

[3] M. Herrero, M. Castro-Puyana, J.A. Mendiola, E. Ibañez, Compressed fluids for the extraction of bioactive compounds, TrAC Trends in Analytical Chemistry, 43 (2013) 67-83.

[4] M.A.A. Meireles, Extraction of Bioactive Compounds from Latin American Plants, in: J. Martinez (Ed.) Supercritical Fluid Extraction of nutraceuticals and bioactive compunds CRC Press - Taylor & Francis Group, Boca Raton, 2008, pp. 243-274.

[5] H. Wijngaard, M.B. Hossain, D.K. Rai, N. Brunton, Techniques to extract bioactive compounds from food by-products of plant origin, Food Research International, 46 (2012) 505-513.

[6] M. Plaza, C. Turner, Pressurized hot water extraction of bioactives, TrAC Trends in Analytical Chemistry.

[7] A. Mustafa, C. Turner, Pressurized liquid extraction as a green approach in food and herbal plants extraction: A review, Analytica Chimica Acta, 703 (2011) 8-18.

[8] M. Herrero, A.d.P. Sánchez-Camargo, A. Cifuentes, E. Ibáñez, Plants, seaweeds, microalgae and food by-products as natural sources of functional ingredients obtained using

pressurized liquid extraction and supercritical fluid extraction, TrAC Trends in Analytical Chemistry.

[9] G. Rocco, C. Toledo, I. Ahumada, B. Sepúlveda, A. Cañete, P. Richter, Determination of polychlorinated biphenyls in biosoils using continuous ultrasound-assisted pressurized solvent extraction and gas chromatrography-mass spectrometry Journal of Chromatography A, 1193 (2008) 32-36.

[10] P. Richter, M. Jimenéz, R. Salazar, A. Maricán, Ultrasound-assisted pressurized solvent extraction for aliphatic and polycyclic aromatic hydrocarbons from soils, Journal of Chromatography A, 1132 (2006) 15-20.

[11] P.M.d. Magalhães, Agrotecnologia para el cultivo de fafia: o ginseng brasileiro., in: J.V.e.a. Martinez (Ed.) Fundamentos de agrotecnologia de cultivo de plantas medicinales iberoamericanas, Convênio Andres Bello/ CYTED, Santafé de Bogotá, 2000, pp. 323-332.

[12] A.G. Neto, J.M.L.C. Costa, C.C. Belati, A.H.C. Vinhólis, L.S. Possebom, A.A. Da Silva Filho, W.R. Cunha, J.C.T. Carvalho, J.K. Bastos, M.L.A. e Silva, Analgesic and anti-inflammatory activity of a crude root extract of Pfaffia glomerata (Spreng) Pedersen, Journal of Ethnopharmacology, 96 (2005) 87-91.

[13] N.R. Sanches, R. Galletto, C.E. Oliveira, R.B. Bazotte, D.A.G. Cortez, Avaliação do potencial anti-hiperglicemiante de *Pfaffia glomerata* (Spreng.) Pedersen (Amaranthaceae), Acta Scientiarum, 23 (2001) 613-617.

[14] C.S. Freitas, C.H. Baggio, J.E. Da Silva-Santos, L. Rieck, C.A. de Moraes Santos, C.C. Júnior, L.C. Ming, D.A. Garcia Cortez, M.C.A. Marques, Involvement of nitric oxide in the gastroprotective effects of an aqueous extract of Pfaffia glomerata (Spreng) Pedersen, Amaranthaceae, in rats, Life Sciences, 74 (2004) 1167-1179.

[15] A.G. Neto, A.A. da Silva Filho, J.M.L.C. Costa, A.H.C. Vinholis, G.H.B. Souza, W.R. Cunha, M.L.A.E. Silva, S. Albuquerque, J.K. Bastos, Evaluation of the trypanocidal and leishmanicidal in vitro activity of the crude hydroalcoholic extract of Pfaffia glomerata (Amarathanceae) roots, Phytomedicine, 11 (2004) 662-665.

[16] S. Nakamura, G. Chen, S. Nakashima, H. Matsuda, Y. Pei, M. Yoshikawa, Brazilian natural medicines. IV. New Noroleanane-type triterpene and ecdysterone-type sterol glycosides and melanogenesis inhibitors from the roots of *Pfaffia glomerata*, Chem. Pharm. Bull., 58 (2010) 690-695.

[17] F. de-Paris, G. Neves, J.B. Salgueiro, J. Quevedo, I. Izquierdo, S.M.K. Rates, Psychopharmacological screening of Pfaffia glomerata Spreng. (Amarathanceae) in rodents, Journal of Ethnopharmacology, 73 (2000) 261-269.

[18] A.R. Zimmer, F. Bruxel, V.L. Bassani, G. Gosmann, HPLC method for the determination of ecdysterone in extractive solution from Pfaffia glomerata, Journal of Pharmaceutical and Biomedical Analysis, 40 (2006) 450-453.

[19] D.F. Felipe, L.Z.S. Brambilla, C. Porto, E.J. Pilau, D.A.G. Cortez, Phytochemical analysis of *Pfaffia glomerata* inflorescences by LC-ESI-MS/MS, Molecules, 19 (2014) 15720-15734.

[20] L.Z. Serra, D.F. Felipe, D.A.G. Cortez, Quantification of beta-ecdysone in different parts of *Pfaffia glomerata* by HPLC, Brazilian Journal of Pharmacognosy, 22 (2012) 1349-1354.

[21] R. Flores, D. Brondani Jr., V. Cezarotto Jr., S.R. Giacomelli, F.T. Nicoloso, Micropropagation and β-ecdysone content of the Brazilian ginsengs Pfaffia glomerata and Pfaffia tuberosa, In Vitro Cell. Dev. Biol.- Plant, 46 (2010) 210-217.

[22] Herbarium, (2015).

[23] O. Guçlu-Ustundag, G. Mazza, Saponins: Properties, Applications and Processing, Critical Reviews in Food Science and Nutrition, 47 (2007) 231-258.

[24] W. Oleszek, A. Hamed, Saponin-Based Surfactant, in: M. Kjellin, I. Johansson (Eds.) Surfactants from Renewable Resources, Wiley, Chennai, India, 2010.

[25] B.D. Ribeiro, D.S. Alviano, D.W. Barreto, M.A.Z. Coelho, Functional properties of saponins from sisal (Agave sisalana) and juá (Ziziphus joazeiro): Critical micellar concentration, antioxidant and antimicrobial activities, Colloids and Surfaces A: Physicochemical and Engineering Aspects, 436 (2013) 736-743.

[26] R.G. Bitencourt, C.L. Queiroga, Í. Montanari Junior, F.A. Cabral, Fractionated extraction of saponins from Brazilian ginseng by sequential process using supercritical CO2, ethanol and water, The Journal of Supercritical Fluids, 92 (2014) 272-281.

[27] R. Vardanega, D.T. Santos, M.A.A. Meireles, Production of biosurfactant from Brazilian ginseng roots by low-pressure solvent extraction with and without the assistance of ultrasound, Recent Patents on Engineering, 8 (2014) 1-13.

[28] D.T. Santos, D.F. Barbosa, R. Vardanega, J.Q. Albarelli, M.A.A. Meireles, Experimental and simulation study on formulation of clove essential oil products using alternative surfactant, Journal of Colloid Science and Biotechnology, 2 (2013) 1-11.

[29] M.T.M.G. Rosa, E.K. Silva, D.T. Santos, A.J. Petenate, M.A.A. Meireles, Obtaining annatto seed oil miniemulsions by ultrasonication using aqueous extract from Brazilian ginseng roots as a biosurfactant, Journal of Food Engineering.

[30] E.R. Caleffi, G. Krausová, I. Hyršlová, L.L.R. Paredes, M.M. dos Santos, G.L. Sassaki, R.A.C. Gonçalves, A.J.B. de Oliveira, Isolation and prebiotic activity of inulin-type fructan extracted from Pfaffia glomerata (Spreng) Pedersen roots, International Journal of Biological Macromolecules, 80 (2015) 392-399.

[31] J.M. Ponce-Ortega, M.M. Al-Thubaiti, M.M. El-Halwagi, Process intensification: New understanding and systematic approach, Chemical Engineering and Processing: Process Intensification, 53 (2012) 63-75.

[32] S.R. Shirsath, S.H. Sonawane, P.R. Gogate, Intensification of extraction of natural products using ultrasonic irradiations- A review of current status, Chemical Engineering and Processing: Process Intensification, 53 (2012) 10-23.

[33] J.F. Osório-Tobón, M.A.A. Meireles, Recent applications of pressurized fluid extraction: curcuminoids extraction with pressurized liquids, Food and Public Health, 3 (2013) 289-303.

[34] I.C.N. Debien, R. Vardanega, D.T. Santos, M.A.A. Meireles, Pressurizes liquid extraction as a promising and economically feasible technique for obtaining beta-ecdysone-rich extracts from Brazilian ginseng roots, Separation Science and Technology, 50 (2015) 1-11.

CAPÍTULO 2

REVISÃO BIBLIOGRÁFICA

2.1 ALIMENTOS FUNCIONAIS E COMPOSTOS BIOATIVOS

O aumento da preocupação da população e do conhecimento científico sobre o impacto dos produtos sintéticos sobre a saúde humana, bem como as evidências sobre os benefícios fisiológicos, nutricionais e medicinais relacionados ao uso de produtos naturais tem estimulado o seu consumo. Os consumidores acreditam cada vez mais que os alimentos estão diretamente relacionados à saúde e, diante disso, há uma crescente demanda para o desenvolvimento de processos para obtenção de alimentos que, além de fornecerem nutrientes, possam prevenir doenças e promover o bem-estar [1]. Neste cenário, os alimentos funcionais desempenham um papel fundamental, uma vez que podem promover efeitos fisiológicos benéficos aos humanos quando consumidos como parte de uma dieta regular e balanceada [2].

O conceito de alimento funcional foi promovido pela primeira vez em 1894 no Japão quando cientistas estudavam a relação entre nutrição, satisfação sensorial, fortificação e modulação do sistema fisiológico e, desde então, uma verdadeira revolução científica e tecnológica tem ocorrido. Com o aumento da disseminação de informações por meio de publicidade na internet, mídias sociais e outros veículos, os consumidores tornaram-se mais conscientes sobre a relação entre os hábitos alimentares e saúde. Desta forma, o desenvolvimento e as vendas de alimentos/bebidas que contêm componentes bioativos específicos têm aumentado consideravelmente. Para atender essa demanda, a regulação destes produtos tem sido aprovada na maioria dos países e diversos novos produtos vêm sendo lançados a cada ano [2]. No Brasil, a definição de alimento funcional é encontrada na Portaria 398 de 30/04/99 da Secretaria de Vigilância Sanitária do Ministério da Saúde que define alimento funcional como “todo aquele alimento ou ingrediente que, além das funções nutricionais básicas, quando consumido como parte da dieta usual, produz efeitos metabólicos e/ou fisiológicos e/ou efeitos benéficos à saúde, devendo ser seguro para consumo sem supervisão médica”.

Desta forma, os alimentos funcionais podem incluir: (i) alimentos usuais que naturalmente contêm compostos bioativos; (ii) alimentos suplementados com compostos bioativos (por exemplo probióticos, antioxidantes) e (iii) ingredientes alimentares introduzidos em alimentos convencionais (por exemplo, prebióticos). É importante salientar que alimentos funcionais não são medicamentos, como comprimidos ou cápsulas, mas sim consumidos como parte da dieta diária normal [3, 4].

Novos alimentos que apresentem alguma propriedade bioativa vêm sendo largamente explorados pela indústria de alimentos [2] e podem ser provenientes das mais variadas fontes, tais como produtos vegetais [5-7], coprodutos do processamento agroindustrial [8, 9], iogurtes, queijos e outras formulações a base de leite [10, 11], produtos cárneos [12], bebidas [13, 14], chás e preparações de ervas [15], biscoitos [16], entre diversos outros. Quando de origem vegetal, os compostos bioativos responsáveis pela funcionalidade desses alimentos são geralmente provenientes do metabolismo secundário de plantas, ou seja, esses compostos não são considerados nutrientes essenciais, mas exercem um papel importante nas funções biológicas das plantas [17, 18]. Sendo assim, compostos bioativos podem ser definidos, de forma sucinta, como compostos que ocorrem na natureza tipicamente em pequenas quantidades, não possuem função nutricional e que exercem efeitos positivos sobre a saúde humana [19].

Os compostos bioativos podem ser divididos em diversas classes, incluindo os polifenóis (flavonoides, ácidos fenólicos, taninos, etc.), carotenoides, fitoesteróis, alcaloides, terpenos, glicosídeos, entre outros [20]. Além dos benefícios à saúde relacionados à ingestão de compostos bioativos na dieta, produtos contendo esses compostos podem encontrar diversas outras aplicações em alimentos e também nas indústrias farmacêutica, cosmética e de perfumes. Dentre as principais funcionalidades associadas aos compostos bioativos, podemos citar: agente corante (ex: carotenoides), antioxidante (ex: antocianinas), aromatizante (ex: óleos essenciais), antimicrobiano (ex: ácidos orgânicos, óleos essenciais), emulsificante (saponinas) e várias outras. É importante ressaltar que os compostos bioativos podem apresentar mais de uma funcionalidade, dependendo da sua composição, como é o caso dos óleos essenciais que além se serem largamente explorados como aromatizantes, também podem apresentar ação antimicrobiana e antioxidante [17, 21].

Além da presença de compostos bioativos, alguns compostos provenientes do metabolismo primário de plantas podem apresentar efeitos benéficos quando ingeridos, como é o caso de carboidratos [22-24]. Os frutanos são carboidratos de reserva das plantas, classificados como inulina ou frutoligossacarídeos (FOS), de acordo com o grau de polimerização (DP- *Degree of Polimerization*) da molécula, sendo que aqueles com DP < 10 são classificados como FOS e os demais como inulina [25]. A inulina e os FOS apresentam diversas propriedades funcionais, agindo como espessantes, substitutos para gorduras em alimentos de baixa caloria e também possuem atividade prebiótica, a qual é grande interesse para a indústria de alimentos [22, 23, 26].

Esses compostos podem ser obtidos a partir de diversas fontes, geralmente naturais, como matrizes vegetais. Além destas, diversos resíduos agroindustriais podem ser utilizados como matéria-prima para obtenção de carboidratos prebióticos, o que representa uma alternativa tanto para agregar valor a coprodutos da indústria quanto para diminuir a quantidade de resíduos dispostos no meio ambiente [27]. Os compostos bioativos obtidos podem ser usados tanto em alimentos funcionais, como também nas indústrias de fármacos e cosméticos [28]. Dados recentes demonstram que mais de 80% dos compostos presentes em alimentos funcionais e mais de 30% dos medicamentos são produzidos a partir de compostos bioativos provenientes de fontes naturais [29]. Desta forma, a exploração de novas matrizes vegetais como fontes de compostos bioativos, bem como o desenvolvimento de processos adequados para obtenção destes é de grande importância para a economia do setor, especialmente em países como o Brasil, que possui uma enorme variedade de matrizes vegetais e precisa alavancar a comercialização de produtos manufaturados ao invés de vender apenas *commodities* [30]. Uma planta nativa brasileira com grande potencial para ser utilizada como matéria-prima para a produção de produtos de alto valor agregado é o ginseng brasileiro, o qual é rico em compostos bioativos com reconhecidos efeitos terapêuticos.

2.2 GINSENG BRASILEIRO (*Pfaffia glomerata*)

O ginseng brasileiro pertence ao gênero *Pfaffia*, da família Amaranthaceae, e recebe este nome popular devido às semelhanças morfológicas de suas raízes e efeitos terapêuticos similares aos do ginseng asiático (*Panax* ssp., Araliaceae) [31]. Dentre as espécies de ginseng brasileiro, as principais são a *Pfaffia glomerata* (Spreng.) Pedersen e a *Pfaffia paniculata* (Mart.) Kuntze [32]. Gosmann et al. [33] investigaram alguns aspectos botânicos e parâmetros químicos para diferenciar as duas espécies e verificaram que o composto beta-ecdisona está presente apenas na *P. glomerata*. Portanto, este composto pode ser utilizado como marcador para diferenciação entre as espécies.

O ginseng brasileiro é uma planta tropical perene, nativa brasileira, que não suporta baixas temperaturas e é encontrado principalmente nos estados do Paraná, Mato Grosso, São Paulo e Goiás [32] (Figura 2.1). As raízes de ginseng brasileiro são popularmente utilizadas como revigorante e para memória [34], além de ser indicado como suplemento na dieta de atletas para melhorar seu desempenho durante os treinamentos. Devido à sua importância na medicina popular, estudos têm sido realizados com o objetivo de elucidar as

propriedades farmacológicas desta planta. Os estudos apontam que os extratos de ginseng brasileiro possuem efeitos analgésico, anti-inflamatório [35] e antiglicêmico [36], proteção do trato gástrico [32], atividade antimicrobiana [37] e inibição da melanogênese [38]. Além disso, extratos de ginseng brasileiro apresentaram ação depressora do sistema nervoso central [39].

Figura 2.1: Ginseng brasileiro (*Pfaffia glomerata*). Fonte: fotografia tirada no campo experimental do CPQBA/UNICAMP, Campinas,SP).

Além da beta-ecdisona, outros compostos presentes nas raízes de ginseng brasileiro já foram identificados, tais como pfaffianol A, pfaffiaglicosídeos A, B, C, D e E, ácido aquebonóico, boussingenosídeo, taxisterona, ptetosterona, pfaffosídeos A, B e C [38]. Além de estudos envolvendo os compostos bioativos provenientes do metabolismo secundário do ginseng brasileiro, como os citados anteriormente, um estudo recente reporta também a presença de carboidratos com atividade prebiótica nas raízes do ginseng brasileiro, o que valoriza ainda mais os efeitos positivos dos produtos obtidos desta planta [26].

Tradicionalmente, apenas as raízes do ginseng brasileiro têm sido utilizadas para fins comerciais em função do seu teor de beta-ecdisona. No entanto, estudos recentes têm demonstrado que outras partes da planta também possuem teores expressivos deste composto. Serra et al. [40] quantificaram beta-ecdisona em diferentes partes do ginseng brasileiro e encontraram o maior teor deste compostos nas inflorescências (3,06 g·100 g^{-1} de extrato seco), seguido das hastes (2,37 g·100 g^{-1} de extrato seco) e raízes (1,63 g·100 g^{-1} de extrato seco). Além da beta-ecdisona, outros compostos bioativos foram identificados nas inflorescências de ginseng brasileiro, tais como flavonoides, saponinas do tipo triterpenoides, ácido oleanólico e ácido glucônico [41]. Em função disso, as partes aéreas do ginseng brasileiro também podem ser consideradas uma fonte de compostos bioativos ao invés de serem descartadas durante o processamento das raízes.

Além das propriedades funcionais dos extratos de ginseng brasileiro reportadas na literatura, estes também podem ser explorados para fins tecnológicos, devido às suas propriedades surfactantes atribuídas à presença de saponinas [28, 42]. Estudos recentes demonstraram que extratos de ginseng brasileiro foram eficientes para estabilização de emulsões contendo óleos essenciais [43, 44]. Emulsões estáveis contendo 25% de óleo de cravo [43] e 3% de óleo de urucum [44] foram obtidas empregando extrato aquoso de raízes de ginseng brasileiro. Assim, o potencial surfactante destes extratos representa mais uma forma de agregar valor aos produtos provenientes desta planta.

2.3 SAPONINAS E BETA-ECDISONA

As saponinas são metabólitos secundários que ocorrem naturalmente em aproximadamente 100 famílias e em mais de 500 espécies de plantas, agindo como barreira no seu sistema defensivo para protegê-las contra patógenos e herbívoros [45, 46]. Quimicamente, as saponinas constituem um vasto grupo de glicosídeos que podem conter de uma a três cadeias de açúcar ligadas a uma aglicona, a qual constitui o núcleo fundamental das saponinas. De acordo com o tipo de núcleo fundamental, as saponinas são classificadas em dois grandes grupos: triterpênicas ou esteroidais (Figura 2.2). Maiores detalhes sobre a classificação e ocorrência das saponinas em plantas foi extensivamente revisado por Vincken et al. [47].

A molécula das saponinas apresenta uma porção com característica hidrofóbica, atribuída à aglicona e outra porção com característica hidrofílica, atribuída às unidades de

açúcar. Estas características conferem caráter anfifílico às saponinas, sendo classificadas como agentes superficiais não-iônicos [45, 48]. Em soluções aquosas, as saponinas se organizam em agregados moleculares chamados de micelas. A massa molecular relativamente baixa das saponinas (~1,67 kDa) faz com que estas sejam rapidamente adsorvidas formando finas camadas interfaciais [49]. A concentração na qual a formação das micelas se inicia é conhecida como concentração micelar crítica (CMC).

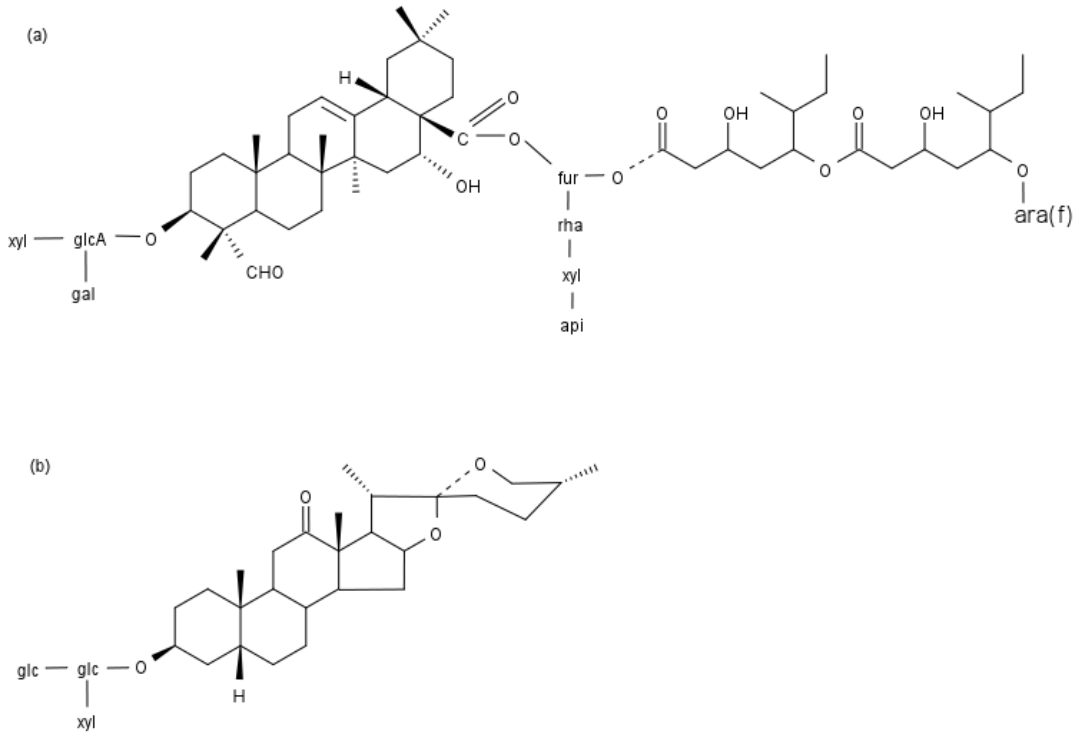

Figura 2.2: Estrutura química de saponinas. (a) saponina triterpênica de *Quillaja saponaria*; (b) saponina esteroidal de *Yucca schidigera*. FONTE: Oleszek & Hamed, [45].

As propriedades superficiais das saponinas são conhecidas há muitos anos, e em função disso elas são tradicionalmente usadas como agentes saponificantes. Mais recentemente, novas aplicações vêm sendo desenvolvidas, tais como bio-remediação de solos contaminados [50, 51], agente hemolítico [52], antioxidante [53, 54] e hipoglicemiante [55], agente plastificante em filmes alimentícios [56]; absorção de lipídeos no trato intestinal [57], solubilização de colesterol em soluções aquosas [58], além de propriedades antimicrobiana, antiviral e antitumoral [59].

O reconhecimento da importância comercial das saponinas, o desenvolvimento de novas aplicações e aumento das evidências de seus benefícios à saúde têm impulsionado as

pesquisas sobre os processos de produção de saponinas em escala comercial a partir de fontes naturais. Atualmente, as principais fontes naturais de saponinas são a *Quillaja saponaria* e a *Yucca sidighera* [45]. No entanto, diversas outras plantas vêm sendo estudadas [57, 60, 61] dentre elas o ginseng brasileiro [42, 62].

A beta-ecdisona, quimicamente definida como 2β,3β,14α,20β,22,25-hexahidroxi-7-colesten-6-one (Figura 2.3), é reconhecida como o principal composto bioativo presente nas raízes de ginseng brasileiro, responsável pelos efeitos benéficos atribuídos ao seu uso [32, 35-39]. O primeiro relato de identificação de beta-ecdisona em plantas é de Takemoto et al. [63] em 1967, que encontrou este composto em raízes de *Achyranthes fauriei*. Apenas alguns anos depois a beta-ecdisona foi encontrada nas raízes de ginseng brasileiro e, desde então, esta planta tem sido considerada uma importante fonte de beta-ecdisona [64]. Até o momento, a beta-ecdisona é considerada o composto de maior valor agregado presente nas raízes e partes aéreas do ginseng brasileiro, uma vez que os estudos que reportam efeitos benéficos associados ao uso de extratos de ginseng brasileiro citam a beta-ecdisona como principal responsável [32, 35-38]. No entanto, o desenvolvimento de processos que permitam explorar também o potencial surfactante das outras saponinas presentes no ginseng brasileiro representa uma possibilidade de agregar valor aos demais produtos provenientes desta planta.

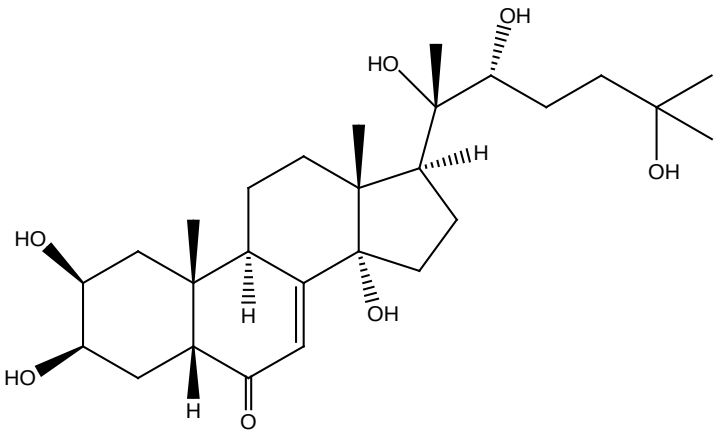

Figura 2.3: Estrutura química da beta-ecdisona.

2.4 INULINA E FRUTOLIGOSSACARÍDEOS

Os frutanos são polímeros constituídos por unidades de frutose ligadas a uma unidade de glicose através de ligações β- (2→1) e são classificados de acordo com o grau de

polimerização (DP) da molécula. Quando a molécula apresenta DP < 10, esta é chamada de frutooligossacarídeos (FOS) e quando 10 < DP < 60, esta é chamada de inulina (Figura 2.4).

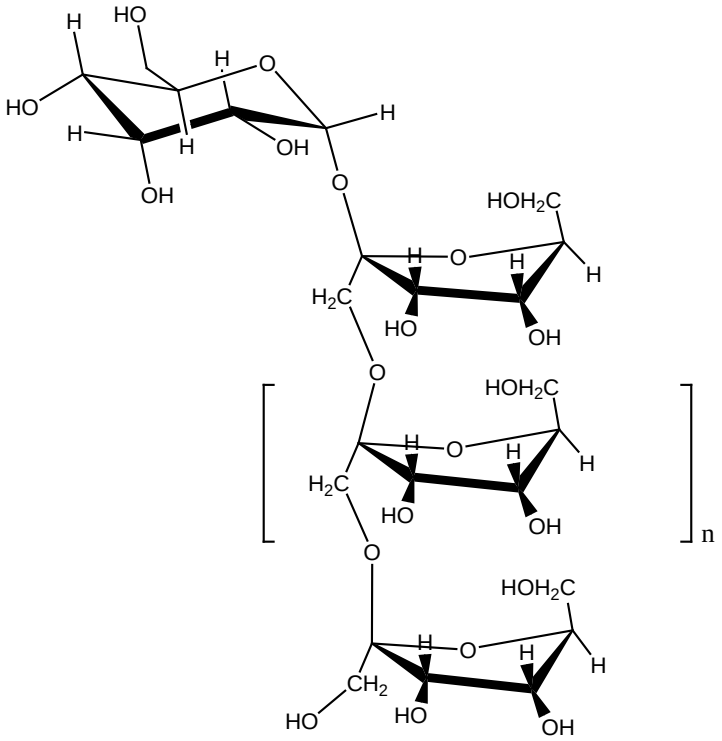

Figura 2.4: Molécula de frutanos do tipo-inulina.

Devido a sua estrutura, a inulina e os FOS são carboidratos não digeríveis pelo intestino humano e, desta forma, podem ser considerados compostos prebióticos. Os compostos prebióticos são definidos como ingredientes que estimulam seletivamente o crescimento e/ou a atividade de uma ou mais espécies de microrganismos na microbiota, conferindo assim benefícios à saúde e promovendo o bem-estar [22]. Para um alimento ser considerado prebiótico, este deve atender aos seguintes critérios: (i) ser resistente às enzimas salivares, pancreáticas e intestinais; (ii) ser fermentável pela microbiota intestinal e (iii) estimular seletivamente o crescimento e/ou atividade de determinados microrganismos na microbiota [65]. Além disso, esses compostos podem ser usados como substitutos para sacarose, visto que estes possuem em torno de 30-60% da doçura da sacarose e baixo valor calórico (4,2 - 6,3 kJ/g) [66]. Outros benefícios à saúde relatados acerca do consumo de inulina e FOS é o aumento na absorção mineral [67], prevenção de câncer de cólon [68], redução de triglicerídeos e prevenção do aumento dos níveis de colesterol [69].

Esses compostos são encontrados em diversas espécies de plantas, tais como o alho-poró, cebola, alho, aspargos, alcachofra, yacon e chicória, sendo que atualmente a

chicória é a principal fonte industrial de inulina e FOS, uma vez que estes compostos representam mais de 70% da sua composição (em base seca) [25]. Recentemente, novas fontes vêm sendo testadas para obtenção de inulina e FOS, incluindo resíduos agroindustriais [9, 26], tendo em vista o alto potencial destes compostos como ingredientes para alimentos funcionais. Atualmente, inulina e FOS são classificados como alimentos ou ingredientes alimentares (não aditivos) em todos os países da União Europeia e nos Estados Unidos possuem o status GRAS (*Generally Recognized As Safe*). Em todos esses países, inulina e FOS podem ser usados sem limitações específicas em formulações de alimentos e bebidas funcionais [25]. Desta forma, o desenvolvimento de tecnologias adequadas para obtenção destes compostos a partir de matrizes vegetais inovadoras é uma demanda latente da indústria para atender ao mercado cada vez mais preocupado com a qualidade e segurança dos produtos consumidos.

2.5 EXTRAÇÃO DE COMPOSTOS BIOATIVOS COM LÍQUIDOS PRESSURIZADOS

Líquidos pressurizados têm sido amplamente estudados para extração de compostos bioativos a partir de diversas matrizes, principalmente vegetais [70]. O termo PLE (*Pressurized Liquid Extraction*) se refere ao uso de solventes pressurizados sob altas temperaturas (geralmente acima do seu ponto de ebulição e abaixo do seu ponto crítico) e pressão suficientemente alta para manter o solvente no estado líquido durante o processo de extração [71]. Quando o solvente de extração empregado é água, esta técnica pode também ser chamada de SWE (*Subcritical Water Extraction*) [72]. Dentre as razões pelas quais a aplicação de PLE e SWE tem ganhado maior atenção nos últimos anos, podemos destacar o crescente apelo para substituição de técnicas convencionais por outras que utilizem solventes não-tóxicos e em menor quantidade [73]. Embora o princípio do processo de extração e o aparato necessário sejam os mesmos para ambos, PLE e SWE, em SWE há outros parâmetros importantes que serão descritos a seguir.

2.5.1 Extração com água subcrítica (SWE)

Em SWE, utiliza-se como solvente a água em temperaturas acima de seu ponto de ebulição a pressão ambiente (373 K/0,1 MPa) e abaixo do seu ponto crítico (647 K/22,1 MPa)

[74]. Além das vantagens citadas anteriormente, processos SWE são mais rápidos e requerem menor quantidade de solvente do que as técnicas convencionais. Estas características são alcançadas pelo aumento da taxa de transferência de massa e da solubilidade dos compostos devidos ao aumento da temperatura do meio. Plaza e Turner [74] descrevem com detalhes como as propriedades de transferência de massa são afetadas pela viscosidade, difusividade e tensão superficial da água líquida em altas temperaturas. De forma sucinta, sob alta temperatura a água tem sua viscosidade e densidade reduzidas e, portanto, a difusividade de solutos em água é aumentada, favorecendo a penetração do solvente na matriz e a transferência de massa.

Além destas características, a SWE é afetada também pela constante dielétrica (ε) da água, a qual é significativamente reduzida quando a água é aquecida a altas temperaturas e mantida no estado líquido, podendo variar de ε ~80 (temperatura ambiente) até ε ~38-25 (453-523 K) [75]. Assim, quando em menores temperaturas, compostos polares e iônicos podem ser obtidos, enquanto em temperaturas mais altas compostos mais apolares podem ser dissolvidos e extraídos e, desta forma, a SWE pode substituir o emprego de solventes orgânicos [70, 76]. Outra propriedade da água que é fortemente afetada quando em condições subcríticas é a sua constante de dissociação (K_w) que pode aumentar de $1{,}0 \times 10^{-14}$ a 298 K para 1.2×10^{-12} a 623 K, o que implica em uma variação no pH de 7.0 para 5.5. Estas características podem afetar a SWE de várias formas, principalmente favorecendo reações de hidrólise que, em alguns casos, podem ser indesejadas e também alterando o equilíbrio do composto para outras formas (por exemplo, antocianinas podem ocorrer de cinco diferentes formas dependendo do pH do meio) [74, 77].

O aparato experimental necessário para SWE é o mesmo utilizado em PLE e é bastante simples. Consiste basicamente de um reservatório para a água acoplado a uma bomba de alta pressão, uma célula de extração com sistema de aquecimento e válvulas para manter a pressão do sistema (Ilustrações do aparato experimental podem ser encontradas nos Capítulos 4 e 5). Em relação ao modo de operação de SWE, o mais frequente é o modo estático, no qual o equilíbrio é alcançado entre os compostos na matriz vegetal e na fase aquosa; quando o modo dinâmico é utilizado, a água aquecida e pressurizada é alimentada na célula de extração continuamente. Teoricamente, o modo dinâmico é mais favorável para esgotamento dos compostos. Porém, os extratos podem ser mais diluídos, o que representa maior gasto energético para remover a água [72, 73].

A SWE para obtenção de compostos bioativos a partir de matrizes vegetais tem sido extensivamente revisada na literatura [70, 72, 74, 75, 78]. Embora a alta temperatura empregada em SWE também possibilite a extração de compostos de caráter apolar, dependendo da natureza química do composto alvo, o emprego da SWE pode ser limitado devido à baixa solubilidade de alguns compostos em água e à instabilidade sob altas temperaturas, como no caso de compostos termosensíveis que podem ser degradados [79, 80]. Também, recentes trabalhos reportaram que compostos antioxidantes podem ser obtidos via SWE a partir de matrizes que naturalmente não possuem estes compostos, como resultado de reações de Maillard e caramelização, as quais podem ocorrer sob altas temperaturas quando proteínas e açúcares redutores estão presentes na matriz vegetal [81].

O emprego de líquidos pressurizados, especialmente SWE, para extração de saponinas a partir de matrizes vegetais é recente. Embora não haja estudos sobre SWE de ginseng brasileiro reportados na literatura, outros autores demonstraram que a SWE apresenta vantagens em comparação a processos de extração convencionais. Em estudo sobre a extração de saponinas a partir de ginseng americano (*Panax quinquefolium*) empregando diferentes métodos de extração, a SWE apresentou rendimentos de saponinas superiores ao obtido para extração assistida por ultrassom (UAE-*Ultrasound assisted extraction*) a pressão e temperatura ambiente [82]. SWE de saponinas a partir de sementes de berbigão (*Vaccaria segetalis* Garcke) demonstrou que a recuperação de saponinas é fortemente afetada pela temperatura do processo, uma vez que apenas 33% (base seca) das saponinas totais foram recuperadas a 398 K após 3 h de processo, enquanto que 60% (base seca) de saponinas totais foram recuperadas a 448 K nos primeiros 15 min de processo [83].

2.6 INTENSIFICAÇÃO DE PROCESSOS

Nos últimos tempos, o uso mais eficiente das matérias-primas vegetais através da obtenção de diferentes compostos tem sido objeto de diversos estudos, em vista da maior demanda por produtos e energia decorrente do aumento crescente da população [84]. Desta forma, novas plantas vêm sendo delineadas para que diversos processos possam ser realizados no mesmo local onde o resíduo proveniente de um processo é utilizado como matéria-prima para outro. No entanto, há grandes desafios tecnológicos relacionados à integração de diferentes processos que podem incorrer em altos custos de implantação, isto é, a obtenção de diferentes produtos requer processos diferenciados e, consequentemente, requer equipamentos

distintos para cada processo. Neste contexto, a intensificação de processos tem papel fundamental na diminuição de ambos custos de implantação e operação associados à obtenção de diversos produtos [85].

A intensificação de processos pode ser genericamente definida como qualquer desenvolvimento de engenharia de novos equipamentos ou técnicas que resultem em uma tecnologia menor, mais limpa e mais eficiente do ponto de vista energético [86]. Assim, os processos de extração que possibilitem a obtenção de diferentes produtos com alto valor agregado a partir de uma mesma matéria-prima utilizando o mesmo equipamento, e também os desenvolvimentos no processo que representem aumento de sua eficiência são considerados processos intensificados [87].

Recentemente, um processo intensificado foi desenvolvido para obtenção de óleo de cúrcuma (*Curcuma longa* L.) rico em ar-turmerona e um extrato rico em curcuminoides. Inicialmente, os rizomas de cúrcuma foram submetidos à extração com CO_2 supercrítico (SFE) para obtenção do óleo volátil rico em ar-turmerona [88] e imediatamente depois, no mesmo equipamento, o resíduo desta extração foi extraído por PLE utilizando etanol como solvente para obtenção dos curcuminoides [89]. Um processo intensificado similar foi desenvolvido para obtenção de duas frações de extrato de alecrim (*Rosmarinus officinalis*). Inicialmente, óleo volátil rico em terpenoides foi obtido por SFE, e em seguida, extrato rico em compostos fenólicos foi obtido por SWE [90]. Quando o processo SWE é adicionado ao SFE é possível obter uma redução de até 28% no custo anual de operação da planta comparado a quando apenas SFE é realizada, demonstrando que a obtenção de diferentes produtos a partir de uma mesma matéria-prima é promissora para tornar uma planta de extração economicamente viável.

Também, quando ultrassom é utilizado durante a extração a fim de aumentar os rendimentos do processo, este é considerado um processo intensificado [87, 91, 92]. Extração assistida por ultrassom (UAE- *Ultrasound assisted extraction*) tem sido usada para obter diversas classes de compostos bioativos, tais como compostos fenólicos, antioxidantes, óleos, proteínas, saponinas, entre outros [87]. Mais informações acerca do mecanismo e dos aspectos que afetam a extração assistida por ultrassom serão discutidos no Capítulo 3.

2.7 AVALIAÇÃO ECONÔMICA

No cenário comercial atual, novos processos devem apresentar não apenas viabilidade técnica, mas também custos de produção competitivos para fazer frente aos processos já consolidados. Aspectos como rendimento (obtenção da maior quantidade de produto possível), produtividade (obtenção do maior rendimento no menor tempo de processamento possível) e seletividade (obtenção de um produto rico nos compostos de interesse) devem ser considerados para determinar a viabilidade econômica do processo [93].

Para obter uma estimativa do custo de manufatura (COM = *Cost of manufacturing*) mais precisa, é necessário conhecer bem o fluxograma do processo, contendo informações sobre o balanço de massa, energia e tamanho/capacidade estimados de forma apropriada às condições delineadas pelo processo. Além disso, o custo da maior parte dos equipamentos deve ser conhecido, já que a estimativa do COM tem por finalidade avaliar a viabilidade de implantação de um projeto considerando em seus cálculos fatores conhecidos pelo avaliador e estimando fatores variáveis ou desconhecidos [94]. Diversos estudos para estimar o COM de diferentes processos de extração têm sido realizados utilizando o simulador SuperPro Designer® [8, 88, 89, 93, 95, 96], o qual representa uma importante ferramenta de comunicação entre a comunidade científica e industrial [97], já que a estimativa do COM permite encontrar o ponto de equilíbrio entre o melhor rendimento de processo aliado ao custo, considerando a qualidade do produto.

Além da determinação do COM dos processos, também é importante analisar os índices econômicos relacionados ao negócio proposto. Esses índices indicam o potencial de rentabilidade e viabilidade econômica dos processos, baseados na produtividade, custo de operação, custo de investimento e preço de venda de cada produto. Desta forma, é possível comparar diferentes processos para obtenção de um determinado produto e selecionar aquele que trará maior rentabilidade.

Estudos recentes têm demonstrado técnica e economicamente que a proposição de plantas onde se faz uso de processos integrados e/ou intensificados para obtenção de extratos com alto valor agregado a partir de uma mesma matéria-prima pode representar promissoras oportunidades industriais, uma vez que este modo de operação é capaz de reduzir o COM de obtenção destes produtos [89, 90, 98]. Desta forma, processos de extração intensificados, conduzidos com o uso de solventes limpos e tecnologias inovadoras, podem ser uma

alternativa para o processamento do ginseng brasileiro a fim de obter extratos ricos em compostos bioativos com custo de manufatura competitivo.

REFERÊNCIAS

[1] K. Menrad, Market and marketing of functional food in Europe, Journal of Food Engineering, 56 (2003) 181-188.

[2] D. Granato, M.R. Alezandro, F. Nazzaro, Food bioactive compounds: Quality control and functional properties, Food Research International, 77, Part 2 (2015) 73-74.

[3] S.H. Al-Sheraji, A. Ismail, M.Y. Manap, S. Mustafa, R.M. Yusof, F.A. Hassan, Prebiotics as functional foods: A review, Journal of Functional Foods, 5 (2013) 1542-1553.

[4] W. Grajek, A. Olejnik, A. Sip, Probiotics, prebiotics and antioxidants as functional foods: a review, Acta Biochimica Polonica, 52 (2005) 665-671.

[5] A.C. Pedro, D. Granato, N.D. Rosso, Extraction of anthocyanins and polyphenols from black rice (Oryza sativa L.) by modeling and assessing their reversibility and stability, Food Chemistry, 191 (2016) 12-20.

[6] S. Kamiloglu, A.A. Pasli, B. Ozcelik, J. Van Camp, E. Capanoglu, Influence of different processing and storage conditions on in vitro bioaccessibility of polyphenols in black carrot jams and marmalades, Food Chemistry, 186 (2015) 74-82.

[7] C. Sun, S. Fan, X. Wang, J. Lu, Z. Zhang, D. Wu, Q. Shan, Y. Zheng, Purple sweet potato color inhibits endothelial premature senescence by blocking the NLRP3 inflammasome, The Journal of Nutritional Biochemistry, 26 (2015) 1029-1040.

[8] D.T. Santos, P.C. Veggi, M.A.A. Meireles, Extraction of antioxidant compounds from Jabuticaba (Myrciaria cauliflora) skins: Yield, composition and economical evaluation, Journal of Food Engineering, 101 (2010) 23-31.

[9] M.T.C. Machado, K.S. Eça, G.S. Vieira, F.C. Menegalli, J. Martínez, M.D. Hubinger, Prebiotic oligosaccharides from artichoke industrial waste: evaluation of different extraction methods, Industrial Crops and Products, 76 (2015) 141-148.

[10] P.C.B. Lollo, P.N. Morato, C.S. de Moura, M.M. de Oliveira, A.G. Cruz, J.d.A.F. Faria, J. Amaya-Farfan, M. Cristianini, Ultra-high temperature plus dynamic high pressure processing: An effective combination for potential probiotic fermented milk processing which attenuate exercise-induced immune suppression in Wistar rats, Journal of Functional Foods, 14 (2015) 541-548.

[11] S. Verruck, E.S. Prudêncio, C.R.W. Vieira, E.R. Amante, R.D.d.M.C. Amboni, The buffalo Minas Frescal cheese as a protective matrix of Bifidobacterium BB-12 under in vitro simulated gastrointestinal conditions, LWT - Food Science and Technology, 63 (2015) 1179-1183.

[12] L. Marchetti, S.C. Andrés, A.N. Califano, Low-fat meat sausages with fish oil: Optimization of milk proteins and carrageenan contents using response surface methodology, Meat Science, 96 (2014) 1297-1303.

[13] A.E. Oltman, E. Lopetcharat, E. Bastian, M.A. Drake, Identifying key attributes for protein beverages, Journal of Food Science, 80 (2015) 1383-1390.

[14] A. Perna, I. Intaglietta, A. Simonetti, E. Gambacorta, Donkey milk for manufacture of novel functional fermented beverages, Journal of Food Science, 80 (2015) 1352-1359.

[15] D. Granato, R. Grevink, A.A.F. Zielinski, D.S. Nunes, S.M. Ruth, Analytical strategy coupled with response surface methodology to maximize the extraction of antioxidants from green, yellow, and red teas (Camellia sinensis var. sinensis), Journal of Agricultural and Food Chemistry, 62 (2014) 10283-10296.

[16] O. Radočaj, E. Dimić, R. Tsao, Effects of hemp (Cannabis sativa L.) seed oil press-cake and decaffeinated green tea leaves (Camellia sinensis) on functional characteristics of gluten-free crackers, Journal of Food Science, 79 (2014) 318-235.

[17] R.N. Cavalcanti, T. Forster-Carneiro, M.T.M.S. Gomes, M.A. Rostagno, J.M. Prado, M.A.A. Meireles, Uses and applications of extracts from natural sources, in: M.A. Rostagno, J.M. Prado (Eds.) Natural products extraction: principles and applications, RSC, 2013, pp. 450.

[18] J.F. Ayala-Zavala, V. Vega-Vega, C. Rosas-Domínguez, H. Palafox-Carlos, J.A. Villa-Rodriguez, M.W. Siddiqui, J.E. Dávila-Aviña, G.A. González-Aguilar, Agro-industrial potential of exotic fruit byproducts as a source of food additives, Food Research International, 44 (2011) 1866-1874.

[19] M.T.M.S. Gomes, D.T. Santos, M.A.A. Meireles, Trends in particle formation of bioactive compounds using supercritical fluids and nanoemulsions, Food and Public Health, 2 (2012) 142-152.

[20] V. Habauzit, M.-N. Horcajada, Phenolic phytochemicals and bone, Phytochem Rev, 7 (2008) 313-344.

[21] E. Antignac, G.J. Nohynek, T. Re, J. Clouzeau, H. Toutain, Safety of botanical ingredients in personal care products/cosmetics, Food and Chemical Toxicology, 49 (2011) 324-341.

[22] M.B. Roberfroid, G.R. Gibson, L. Hoyles, A.L. McCartney, R. Rastall, I. Rowland, Prebiotics effects: Metabolic and health benefits, British Journal of Nutrition, 104 (2010) 1-63.

[23] D. Campos, I. Betalleluz-Pallardel, R. Chirinos, A. Aguilar-Galvez, G. Noratto, R. Pedreschi, Prebiotic effects of yacon (Smallanthus sonchifolius Poepp. & Endl), a source of fructooligosaccharides and phenolic compounds with antioxidant activity, Food Chemistry, 135 (2012) 1592-1599.

[24] N. Aravind, M.J. Sissons, C.M. Fellows, J. Blazek, E.P. Gilbert, Effect of inulin soluble dietary fibre addition on technological, sensory, and structural properties of durum wheat spaghetti, Food Chemistry, 132 (2012) 993-1002.

[25] A. Franck, Technological functionality of inulin and oligofructose, British Journal of Nutrition, 87 (2002) 287-291.

[26] E.R. Caleffi, G. Krausová, I. Hyršlová, L.L.R. Paredes, M.M. dos Santos, G.L. Sassaki, R.A.C. Gonçalves, A.J.B. de Oliveira, Isolation and prebiotic activity of inulin-type fructan extracted from Pfaffia glomerata (Spreng) Pedersen roots, International Journal of Biological Macromolecules, 80 (2015) 392-399.

[27] J. Bonilla, F.C. Vargas, T.G. de Oliveira, G.L. da Aparecida Makishi, P.J. do Amaral Sobral, Recent patents on the application of bioactive compounds in food: a short review, Current Opinion in Food Science, 5 (2015) 1-7.

[28] R.G. Bitencourt, C.L. Queiroga, Í. Montanari Junior, F.A. Cabral, Fractionated extraction of saponins from Brazilian ginseng by sequential process using supercritical CO2, ethanol and water, The Journal of Supercritical Fluids, 92 (2014) 272-281.

[29] R. Qilong, X. Huabin, B. Zongb, Y. Baogen, Y. Yiwen, Z. Zhiguo, Recent advances in separation of bioactive natural products, Chinese Journal of Chemical Engineering, 21 (2013) 937-952.

[30] M.A.A. Meireles, Extraction of Bioactive Compounds from Latin American Plants, in: J. Martinez (Ed.) Supercritical Fluid Extraction of nutraceuticals and bioactive compunds CRC Press - Taylor & Francis Group, Boca Raton, 2008, pp. 243-274.

[31] A.R. Zimmer, F. Bruxel, V.L. Bassani, G. Gosmann, HPLC method for the determination of ecdysterone in extractive solution from Pfaffia glomerata, Journal of Pharmaceutical and Biomedical Analysis, 40 (2006) 450-453.

[32] C.S. Freitas, C.H. Baggio, J.E. Da Silva-Santos, L. Rieck, C.A. de Moraes Santos, C.C. Júnior, L.C. Ming, D.A. Garcia Cortez, M.C.A. Marques, Involvement of nitric oxide in the gastroprotective effects of an aqueous extract of Pfaffia glomerata (Spreng) Pedersen, Amaranthaceae, in rats, Life Sciences, 74 (2004) 1167-1179.

[33] G. Gosmann, S. Gattuso, R. Fenner, E.F. Pacheco, A. Ferraz, L.A. Savi, C.R.M. Barardi, C.M.O. Simões, M. Sortino, S. Zacchino, C. Gnerre, B. Testa, S.M.K. Rates, Botanical (morphological, micrographic) and pharmacological characteristics of *Pfaffia* species (Amaranthaceae) native to South Brazil, Revista Brasileira de Ciências Farmacêuticas, 39 (2003) 141-147

[34] S.M.K. Rates, G. Gosmann, Gênero *Pfaffia*: aspectos químicos, farmacológicos e implicações para o seu emprego terapêutico, Revista Brasileira de Farmacognosia, 12 (2002) 85-93.

[35] A.G. Neto, J.M.L.C. Costa, C.C. Belati, A.H.C. Vinhólis, L.S. Possebom, A.A. Da Silva Filho, W.R. Cunha, J.C.T. Carvalho, J.K. Bastos, M.L.A. e Silva, Analgesic and anti-inflammatory activity of a crude root extract of Pfaffia glomerata (Spreng) Pedersen, Journal of Ethnopharmacology, 96 (2005) 87-91.

[36] N.R. Sanches, R. Galletto, C.E. Oliveira, R.B. Bazotte, D.A.G. Cortez, Avaliação do potencial anti-hiperglicemiante de *Pfaffia glomerata* (Spreng.) Pedersen (Amaranthaceae), Acta Scientiarum, 23 (2001) 613-617.

[37] A.G. Neto, A.A. da Silva Filho, J.M.L.C. Costa, A.H.C. Vinholis, G.H.B. Souza, W.R. Cunha, M.L.A.E. Silva, S. Albuquerque, J.K. Bastos, Evaluation of the trypanocidal and leishmanicidal in vitro activity of the crude hydroalcoholic extract of Pfaffia glomerata (Amarathanceae) roots, Phytomedicine, 11 (2004) 662-665.

[38] S. Nakamura, G. Chen, S. Nakashima, H. Matsuda, Y. Pei, M. Yoshikawa, Brazilian natural medicines. IV. New Noroleanane-type triterpene and ecdysterone-type sterol glycosides and melanogenesis inhibitors from the roots of *Pfaffia glomerata*, Chem. Pharm. Bull., 58 (2010) 690-695.

[39] F. de-Paris, G. Neves, J.B. Salgueiro, J. Quevedo, I. Izquierdo, S.M.K. Rates, Psychopharmacological screening of Pfaffia glomerata Spreng. (Amarathanceae) in rodents, Journal of Ethnopharmacology, 73 (2000) 261-269.

[40] L.Z. Serra, D.F. Felipe, D.A.G. Cortez, Quantification of beta-ecdysone in different parts of *Pfaffia glomerata* by HPLC, Brazilian Journal of Pharmacognosy, 22 (2012) 1349-1354.

[41] D.F. Felipe, L.Z.S. Brambilla, C. Porto, E.J. Pilau, D.A.G. Cortez, Phytochemical analysis of *Pfaffia glomerata* inflorescences by LC-ESI-MS/MS, Molecules, 19 (2014) 15720-15734.

[42] R. Vardanega, D.T. Santos, M.A.A. Meireles, Production of biosurfactant from Brazilian ginseng roots by low-pressure solvent extraction with and without the assistance of ultrasound, Recent Patents on Engineering, 8 (2014) 1-13.

[43] D.T. Santos, D.F. Barbosa, R. Vardanega, J.Q. Albarelli, M.A.A. Meireles, Experimental and simulation study on formulation of clove essential oil products using alternative surfactant, Journal of Colloid Science and Biotechnology, 2 (2013) 1-11.

[44] M.T.M.G. Rosa, E.K. Silva, D.T. Santos, A.J. Petenate, M.A.A. Meireles, Obtaining annatto seed oil miniemulsions by ultrasonication using aqueous extract from Brazilian ginseng roots as a biosurfactant, Journal of Food Engineering, 168 (2016) 68-78.

[45] W. Oleszek, A. Hamed, Saponin-Based Surfactant, in: M. Kjellin, I. Johansson (Eds.) Surfactants from Renewable Resources, Wiley, Chennai, India, 2010.

[46] J.M. Augustin, V. Kuzina, S.B. Andersen, S. Bak, Molecular activities, biosynthesis and evolution of triterpenoid saponins, Phytochemistry, 72 (2011) 435-457.

[47] J.-P. Vincken, L. Heng, A. de Groot, H. Gruppen, Saponins, classification and occurrence in the plant kingdom, Phytochemistry, 68 (2007) 275-297.

[48] R. Stanimirova, K. Marinova, S. Tcholakova, N.D. Denkov, S. Stoyanov, E. Pelan, Surface rheology of saponin adsorption layers, Langmuir, 27 (2011) 12486-12498.

[49] B. Ozturk, D.J. McClements, Progress in natural emulsifiers for utilization in food emulsions, Current Opinion in Food Science.

[50] V.S. Millioli, Avaliação da potencialidade da utilização de surfactantes na biorremediação de solo contaminado com hidrocarbonetos de petróleo, in: Engenharia Química, Universidade Federal do Rio de Janeiro- UFRJ/EQ, Rio de Janeiro, RJ, 2009, pp. 200.

[51] R. Ghagi, S.K. Satpute, B.A. Chopade, A.G. Banpurkar, Study of functional properties of *Sapindus mukorossi* as a potential bio-surfactant, Indian Journal of Science and Technology, 4 (2011) 530-533.

[52] G.D. Noudeh, F. Sharififar, E. Behravan, E. Mohajeri, V. Alinia, Medical plants as surface activity modifiers, Journal of Medicinal Plants Research, 5 (2011) 5378-5383.

[53] I. Dini, G.C. Tenore, A. Dini, Saponins in Ipomoea batatas tubers: Isolation, chacarterization, quantification and antioxidant properties, Food Chemistry, 113 (2009) 9.

[54] J.H. Lee, J.K. Jeon, S.G. Kim, S.H. Kim, T. Chun, J.Y. Imm, Comparative analyses of total phenols, flavonoids, saponins and antioxidant activity in yellow soy beans and mung beans, International Journal of Food Science and Technology, 46 (2011) 2513-2519.

[55] L. Bi, X. Tian, F. Dou, L. Hong, H. Tang, S. Wang, New antioxidant and antiglycation active triterpenoid saponins from the root bark of Aralia taibaiensis, Fitoterapia, 83 (2012) 234-240.

[56] C. Andreuccetti, R.A. Carvalho, T. Galicia-García, F. Martínez-Bustos, C.R.F. Grosso, Effect of surfactants on the functional properties of gelatin-based edible films, Journal of Food Engineering, 103 (2011) 129-136.

[57] J. Xiong, J. Guo, L. Huang, B. Meng, Q. Ping, Self-micelle formation and the incorporation of lipid in the formulation affect the intestinal absorption of *Panax notoginseng*, International Journal of Pharmaceutics, 360 (2008) 191-196.

[58] S. Mitra, S.R. Dungan, Cholesterol solubilization in aqueous micellar solutions of Quillaja saponin, bile salts or nonionic surfactantes, Journal of Agricultural and Food Chemistry, 49 (2001) 384-394.

[59] S.G. Sparg, M.E. Light, J. van Staden, Biological activities and distribuition of plant saponins, Journal of Ethnopharmacology, 94 (2004) 219-243.

[60] B.D. Ribeiro, D.S. Alviano, D.W. Barreto, M.A.Z. Coelho, Functional properties of saponins from sisal (Agave sisalana) and juá (Ziziphus joazeiro): Critical micellar concentration, antioxidant and antimicrobial activities, Colloids and Surfaces A: Physicochemical and Engineering Aspects, 436 (2013) 736-743.

[61] B.D. Ribeiro, Estratégias de processamento verde de saponinas da biodiversidade brasileira, in: Escola de Química, Universidade Federal do Rio de Janeiro, Rio de Janeiro, 2012, pp. 187.

[62] C.L.S. Vigo, E. Narita, L.C. Marques, Validação da metodologia de quantificação espectrofotométrica das saponinas de *Pfaffia glomerataI* (Spreng.) Pedersen- Amaranthaceae, Revista Brasileira de Farmacognosia, 13 (2003) 46-49.

[63] T. Takemoto, S. Ogawa, N. Nishimoto, Studies on the constituints of *Achyranthis radix* I. Yakigaku Zasshi, 87 (1967) 1463-1468.

[64] Y. Shiobara, S.-S. Inoue, K. Kato, Y. Nishiguchi, Y. Oishi, N. Nishimoto, F. de Oliveira, G. Akisue, M.K. Akisue, G. Hashimoto, A nortriterpenoid, triterpenoids and ecdysteroids from Pfaffia glomerata, Phytochemistry, 32 (1993) 1527-1530.

[65] G.R. Gibson, H.M. Probert, J.A.E. Van Loo, M.B. Roberfroid, Dietary modulation of the human colonic microbiota: Updating the concept of prebiotics, Nutrition Research Reviews, 17 (2004) 257-259.

[66] N. Saad, C. Delattre, M. Urdaci, J.M. Schmitter, P. Bressollier, An overview of the last advances in probiotic and prebiotic field, LWT - Food Science and Technology, 50 (2013) 1-16.

[67] M. Gudiel-Urbano, I. Goñi, Effect of fructooligosaccharide on nutritionalparameters and mineral bioavailability in rats, Journal of Science and Food Agriculture, 82 (2002) 913-917.

[68] B.L. Pool-Zobel, Inulin-type fructans and reduction in colon cancer risk:review of experimental and human data, British Journal of Nutrition, 93 (2005) 73-90.

[69] N.M. Delzenne, C. Daubioul, A. Neyrinck, M. Lasa, H.S. Taper, Inulin andoligofructose modulate lipid metabolism in animals: review of biochemicalevents and future prospects, British Journal of Nutrition, 87 (2002) 255-259.

[70] M. Herrero, A.d.P. Sánchez-Camargo, A. Cifuentes, E. Ibáñez, Plants, seaweeds, microalgae and food by-products as natural sources of functional ingredients obtained using pressurized liquid extraction and supercritical fluid extraction, TrAC Trends in Analytical Chemistry.

[71] R. Vardanega, D.T. Santos, M.A.A. Meireles, Intensification of bioactive compounds extraction from medicinal plants using ultrasonic irradiation, Pharmacognosy Reviews, 8 (2014) 88-95.

[72] M. Herrero, M. Castro-Puyana, J.A. Mendiola, E. Ibañez, Compressed fluids for the extraction of bioactive compounds, TrAC Trends in Analytical Chemistry, 43 (2013) 67-83.

[73] A. Mustafa, C. Turner, Pressurized liquid extraction as a green approach in food and herbal plants extraction: A review, Analytica Chimica Acta, 703 (2011) 8-18.

[74] M. Plaza, C. Turner, Pressurized hot water extraction of bioactives, TrAC Trends in Analytical Chemistry.

[75] M. Herrero, A. Cifuentes, E. Ibañez, Sub- and supercritical fluid extraction of functional ingredients from different natural sources: Plants, food-by-products, algae and microalgae: A review, Food Chemistry, 98 (2006) 136-148.

[76] G. Brunner, Near critical and supercritical water. Part I. Hydrolytic and hydrothermal processes, The Journal of Supercritical Fluids, 47 (2009) 373-381.

[77] K. Chandler, F. Deng, A.K. Dillow, C.L. Liotta, C.A. Eckert, Near-critical water is the absence of acid catalysis, Industrial & Engineering Chemistry Research, 36 (1997) 5175-5179.

[78] H. Wijngaard, M.B. Hossain, D.K. Rai, N. Brunton, Techniques to extract bioactive compounds from food by-products of plant origin, Food Research International, 46 (2012) 505-513.

[79] E.V. Petersson, J. Liu, P.J.R. Sjoberg, R. Danielsson, C. Turner, Pressurized hot water extraction of anthocyanins from red onion: a study on extraction and degradation rates, Analytica Chimica Acta, 663 (2010) 27-32.

[80] M. Co, C. Zettersten, L. Nyholm, P.J.R. Sjöberg, C. Turner, Degradation effects in the extraction of antioxidants from birch bark using water at elevated temperature and pressure, Analytica Chimica Acta, 716 (2012) 40-48.

[81] M. Plaza, M. Amigo-Benavent, M.D. del Castillo, E. Ibáñez, M. Herrero, Facts about the formation of new antioxidants in natural samples after subcritical water extraction, Food Research International, 43 (2010) 2341-2348.

[82] A.S. Engelberth, E.C. Clausen, D.J. Carrier, Comparing extraction methods to recover ginseng saponins from American ginseng (*Panax quinquefolium*), followed by purification using fast centrifugal partition chromatography with HPLC verification, Separation and Purification Technology, 72 (2010) 1-6.

[83] Ö. Güçlü-Üstündağ, J. Balsevich, G. Mazza, Pressurized low polarity water extraction of saponins from cow cockle seed, Journal of Food Engineering, 80 (2007) 619-630.

[84] R. Vardanega, J.M. Prado, M.A.A. Meireles, Adding value to agri-food residues by means of supercritical technology, The Journal of Supercritical Fluids, 96 (2015) 217-227.

[85] K.T. Lee, S. Lim, Y.L. Pang, H.C. Ong, W.T. Chong, Integration of reactive extraction with supercritical fluids for process intensification of biodiesel production: Prospects and recent advances, Progress in Energy and Combustion Science, 45 (2014) 54-78.

[86] A.I. Stankiewicz, J.A. Moulijn, Process intensification: transforming chemical engineering, Chemical Engineering Progress, (2000) 22-34.

[87] S.R. Shirsath, S.H. Sonawane, P.R. Gogate, Intensification of extraction of natural products using ultrasonic irradiations- A review of current status, Chemical Engineering and Processing: Process Intensification, 53 (2012) 10-23.

[88] P.I.N. Carvalho, J.F. Osorio-Tobón, M.A. Rostagno, A.J. Petenate, M.A.A. Meireles, Techno-economic evaluation of the extraction of turmeric (Curcuma longa L.) oil and ar-turmerone using supercritical carbon dioxide, The Journal of Supercritical Fluids.

[89] J.F. Osorio-Tobón, P.I.N. Carvalho, M.A. Rostagno, A.J. Petenate, M.A.A. Meireles, Extraction of curcuminoids from deflavored turmeric (Curcuma longa L.) using pressurized liquids: Process integration and economic evaluation, The Journal of Supercritical Fluids, 95 (2014) 167-174.

[90] G.L. Zabot, Obtenção de compostos bioativos de cravo-da-índia e alecrim utilizando tecnologia supercrítica: influência da geometria de leito, intensificação de processos e custo de manufatura dos

extratos, in: Departamento de Engenharia de Alimentos, Universidade Estadual de Campinas, Campinas, Brasil, 2015, pp. 333.

[91] M.D. Vetal, V.G. Lade, V.K. Rathod, Extraction of ursolic acid from Ocimum sanctum by ultrasound: Process intensification and kinetic studies, Chemical Engineering and Processing: Process Intensification, 69 (2013) 24-30.

[92] P. Santos, A.C. Aguiar, G.F. Barbero, C.A. Rezende, J. Martínez, Supercritical carbon dioxide extraction of capsaicinoids from malagueta pepper (Capsicum frutescens L.) assisted by ultrasound, Ultrasonics Sonochemistry, 22 (2015) 78-88.

[93] J.M. Prado, G.H.C. Prado, M.A.A. Meireles, Scale-up study of supercritical fluid extraction process for clove and sugarcane residue, The Journal of Supercritical Fluids, 56 (2011) 231-237.

[94] J.M. Prado, C.L.C. Albuquerque, R.N. Cavalcanti, M.A.A. Meireles, Use of comercial process simulator to estimate cost of manufaturing (COM) of carotenoids obtained via supercritical technology from palm and buriti trees, in: 9th International Symposium on Supercritical Fluids, Arcachon, France, 2009, pp. 136.

[95] J.M. Prado, I. Dalmolin, N.D.D. Carareto, R.C. Basso, A.J.A. Meirelles, J.V. Oliveira, E.A.C. Batista, M.A.A. Meireles, Supercritical fluid extraction of grape seed: Process scale-up, extract chemical composition and economical evaluation, Journal of Food Engineering, 109 (2012) 249-257.

[96] I.C.N. Debien, R. Vardanega, D.T. Santos, M.A.A. Meireles, Pressurizes liquid extraction as a promising and economically feasible technique for obtaining beta-ecdysone-rich extracts from Brazilian ginseng roots, Separation Science and Technology, 50 (2015) 1-11.

[97] T.M. Takeuchi, P.F. Leal, R. Favareto, L. Cardozo-Filho, M.L. Corazza, P.T.V. Rosa, M.A.A. Meireles, Study of the phase equilibrium formed inside the flash tank used at the separation step of a supercritical fluid extraction unit, The Journal of Supercritical Fluids, 43 (2008) 447-459.

[98] J.F. Osorio-Tobón, P.I.N. Carvalho, M.A. Rostagno, M.A.A. Meireles, Process integration for turmeric products extraction using supercritical fluids and pressurized liquids: Economic evaluation, Food and Bioproducts Processing.

CAPÍTULO 3

INTENSIFICAÇÃO DA EXTRAÇÃO DE COMPOSTOS BIOATIVOS PELO EMPREGO DO ULTRASSOM

INTENSIFICATION OF BIOACTIVE COMPOUNDS EXTRACTION FROM MEDICINAL PLANTS USING ULTRASONIC IRRADIATION

Renata Vardanega[1], Diego T. Santos[1,2], M. Angela A. Meireles[1]

[1]LASEFI/DEA/FEA (School of Food Engineering), UNICAMP (University of Campinas), SP, Brazil,

[2]Industrial Process and Energy Systems Engineering (IPESE), Swiss Federal Institute of Technology Lausanne (EPFL), Lausanne, Switzerland

Address for correspondence:

Dr. Diego T. Santos, LASEFI/DEA/FEA (School of Food Engineering), UNICAMP (University of Campinas), Rua Monteiro Lobato, 80, 13083-862 Campinas, SP, Brazil.

E-mail: diego_tresinari@yahoo.com.br

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ABSTRACT

Extraction processes are largely used in many chemical, biotechnological and pharmaceutical industries for recovery of valuable and bioactive compounds. To replace the conventional extraction techniques, new techniques as high pressure extraction processes that use environment friendly solvents have been developed. However, these techniques, sometimes, are associated with low extraction rate. The ultrasound can be effectively used to improve the extraction rate by the increasing the mass transfer and possible rupture of cell wall due the formation of microcavities leading to higher product yields with reduced processing time and solvent consumption. This review presents a brief survey about the mechanism and aspects that affect the ultrasound assisted extraction focusing on the use of ultrasound irradiation for high pressure extraction processes intensification.

Keywords: process intensification, ultrasound, extraction, high pressure, bioactive compounds

1. INTRODUCTION

Bioactive compounds are largely obtained from medicinal plants. Solid-liquid extraction is used in any chemical, biochemical and pharmaceutical industries for recovery of bioactive compounds. Plants generally contain only a small amount of active compounds, but in most cases its high value justifies the development of high-performance process. The need for effective extraction of bioactive compounds from plants without any loss of activity and high purity has resulted in development of newer process of extraction. [1, 2]

Conventional extraction from plants comprises solid-liquid techniques usually depending upon organic solvents, which present various shortcomings such as toxic residues, chemical transformation of extracts, use of large quantity of organic solvents, which are harmful to human and environment and long-term processing. In recent years, an increase in the development of techniques that overcome these drawbacks with safer solvents have been observed. The use of ultrasound irradiation during extraction procedure presents several advantages in terms of shortening the time of the process, decrease the volume of the extracting solvent, and increasing the yield of the extraction in comparison with conventional methods. [1, 3] In this paper, some principles and factors that influence the ultrasound assisted extraction are presented. The next sections presents some recent applications of ultrasound coupled with extraction techniques under high pressure, as well as results of mathematical modeling.

2. MECHANISM OF ULTRASOUND ASSISTED EXTRACTION

The intensification of extraction process using ultrasound has been attributed to the cavitation phenomena. The effects caused by the ultrasonic waves are compression and expansion cycles during the passage through the fluid. The expansion can create bubbles or cavities in a liquid. This is so when the negative pressure exerted exceeds the local tensile strength of the liquid, which varies depending on its nature and purity. The process by which vapor bubbles form, grow and undergo implosive collapse is known as cavitation. [4] The conditions within these imploding bubbles can be dramatic, with temperatures of 4500 °C and pressures up to 100 MPa, which in turn produces very high shear energy waves and

turbulence in the cavitation zone. The combination of these factors (pressure, heat and turbulence) is used to accelerate mass transfer in extraction process. [5]

Ultrasound also exerts a mechanical effect. In pure liquids, the bubble retains its spherical shape during the collapse, as its surroundings are uniform. However, when the bubble collapses near a solid surface it occurs asymmetrically and produces high-speed jets of solvent towards the cell walls. These jets have a strong impact on the solid surface, therefore, increasing the solvent penetration into the cell and increasing the contact surface area between solid and liquid phase (Figure 1). [4, 6] Another effect caused by the ultrasound wave on the solid material is that the ultrasound waves can facilitate the swelling and hydration and so cause an enlargement in the pores of the cell wall. This will improve the diffusion process and therefore enhancing mass transfer. [7]

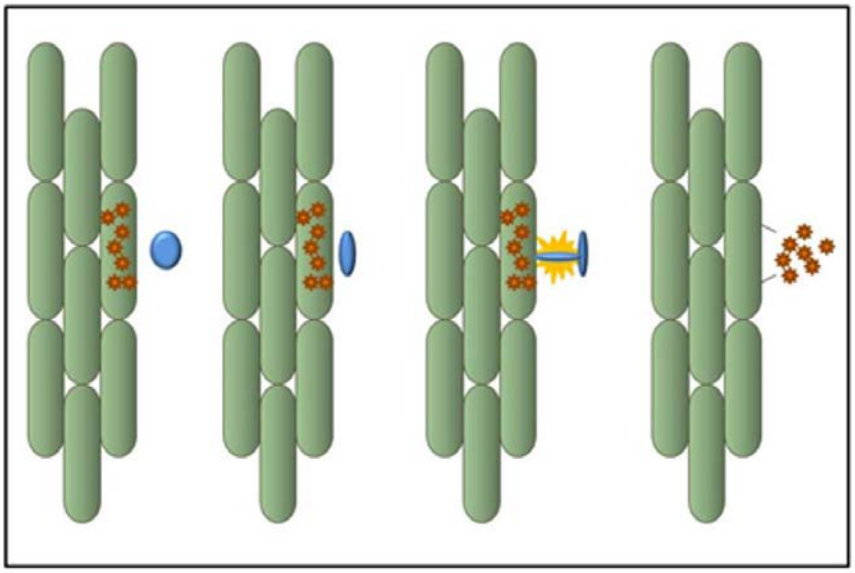

Figure 1: Collapse of cavitation bubble and release of plant content. (Adapted from Pingret et al [3])

Generally, the largest sonochemical effects are observed at lower temperatures, when most of the bubble contents is in the gas. With a decrease in the vapor pressure of the mixture, there is an increase of the implosion intensity, thus increasing the ultrasonic energy produced upon cavitation. [8] The frequency of ultrasound also exerts significant influence on the yield and kinetic extraction. However, this influence depends of the medicinal plant structure and the target compound. [9]

The ultrasonic wave distribution inside an extractor is also a key parameter in the design of an ultrasonic extractor. The maximum ultrasound power is observed in the vicinity of the radiating surface of the ultrasonic horn. Ultrasonic intensity decreases rather abruptly as the distance from the radiating surfaces increases. [9] Also, ultrasound intensity is attenuated with the increase of the presence of solid particles. In order to avoid standing waves or the formation of solid free regions for the preferential passage of the ultrasonic waves, additional agitation or shaking is usually used. [10]

3. FATORS THAT AFFECT ULTRASOUND ASSISTED EXTRACTION

Since the cavitation phenomenon is the principal responsible by the intensification of the extraction process, the parameters that affecting cavitation also affecting the extraction process performed under ultrasound effects. Besides the parameters intrinsically related to the ultrasonic devices (such as the frequency, wavelength, and amplitude of the wave), the ultrasonic power (in $kWhL^{-1}$) and consequently intensity have also an effect on the extraction.

Since the extraction is carried out in a medium, its temperature and pressure, viscosity, surface tension, vapor pressure, besides nature and concentration of dissolved gas and presence of solid particles, if any, also determine the magnitude of the effect caused by the ultrasound in the extraction process and can affect not only the extraction yield but also the composition of the extract and consequently its biological propertiers. [3, 5] We will discuss these factors in the following sections.

3.1 Ultrasonic power, intensity and density

The use of ultrasonics in industrial process has two main requirements; a liquid medium (even if the liquid element forms only 5% of the overall medium) and a source of high energy vibrations (ultrasound). The vibrational energy source is called a transducer which transfers the vibration (after amplification) to the so-called sonotrode or probe, which is in direct or indirect contact with the processing medium. However, the measurement of the actual acoustic energy applied in a sonochemical process is quite difficult. Sometimes, considering the different power level of the device, authors show the values of power applied as, for example, “20% of the total electric power capacity” and this is not as accurate

measurement at all. In fact, in most of the ultrasound devices, the power measured is not proportional to the power step shown, leading to wrong conclusions or irreproducible results. [5, 11]

Even knowing the ultrasonic power actually applied, it is difficult to compare the effects because often the results are not only reported on the different basis, but are also influenced by the geometry of the extractor. For instance, to report data indicating only the power applied is not enough. Indicating the power intensity (W cm^{-2}) or the power density (W cm^{-3}) is more appropriate. [11, 12]

The intensity or amplitude of waves is used to classify the industrial applications: low-intensity ultrasound (LIU) with less than 1 W cm^{-2}, and high-intensity ultrasound (HIU) with 10-1000 W cm^{-2}. [13] The power density takes into account the vessel volume, in which the ultrasound acts and it is very important, especially for the case of ultrasonic baths, where the whole bath volume should be considered. Additionally, when the processing intended to be scalable, power density should be considered, so that is it takes into account extremely different acoustic streams and the corresponding difference of results in the new volume. [5, 11]

3.2 Medium pressure

The cavitation effects in ambient liquids are well known and their application to conventional solvent extraction is well established. However, when a liquid is pressurized, the acoustic intensity required to produce cavitation also increases and this generally places a natural limitation on application of ultrasonics to high pressures processes. In ordinary solvents, cavitation does not occur at high pressures. [14]

To initiate the growth of a cavitation bubble, an acoustic pressure above the so-called Blake thereshold pressure (P_B) has to be applied. [15] Equation 1 assumes that the static gas pressure (P_0), the vapor pressure (P_v), the surface tension (σ) and the equilibrium radius of the bubble (R_0) determine the required negative pressure in the liquid medium to start the explosive growth of a cavity.

$$P_B = P_0 - P_v + \frac{4}{3} \times \sigma \times \sqrt{\frac{2}{3} \times \frac{\sigma}{\left(P_0 + 2 \times \frac{\sigma}{R_0} - P_v\right) \times {R_0}^3}} \quad (1)$$

During pressurization of a liquid, the Blake threshold pressure increases, which implies that higher acoustic pressures are needed to produce cavitation. Obviously, no cavitation occurs when the Blake threshold pressure exceeds the maximum acoustic pressure. [16] Kuijpers et al. [17] showed sonoluminescence evidence for the occurrence of cavitation in CO_2 at 7.5 MPa and 10°C which is well below the critical temperature of CO_2. These authors discuss that the high vapor pressure and low surface tension of the fluid counteracts the external pressure applied. They demonstrated that the threshold pressure of liquid CO2 at 5.82 MPa is equal of the threshold pressure of water at 0.1 MPa and 20°C. The phenomenon was further studied by the same group and published by Kemmere et al. [16] who observed that the cavitation collapse of a bubble was not strong enough to create hot-spots for monomolecular conversion in bulk free-radical polymerization of methyl methacrylate (MMA) using CO_2.

While cavitation has thus been established in near-critical carbon dioxide, the absence of phase boundaries would appear to prohibit bubble formation above the critical point. This would imply that rate enhancement of supercritical fluid extraction process can occur only through the turbulence associated with acoustic streaming or through simple mechanical vibration. [18]

On the other hand, Thompson and Doraiswamy [19] pointed that an increase in the ambient reaction pressure generally results in an overall increase in the sonochemical effects because of the decrease in the vapor pressure of the mixture. Decreasing the vapor pressure increases the intensity of the implosion, thus increasing the ultrasonic energy produced upon cavitation. However, to observe this effect, the threshold pressure should be exceeded.

3.3 Extracting solvent physical properties

The selection of the best extracting solvent for ultrasound assisted extraction normally depends on its physical properties (surface tension, viscosity and vapor pressure) because these properties affect the cavitation intensity in a liquid phase.[1] Although the cavities are more easily formed with a solvent that has a high vapor pressure, low viscosity, and low surface tension, the cavitation intensity increases for solvents with low vapor pressure, high viscosity and high surface tension. [19] The intermolecular forces in the liquid must be overcome in order to form the bubbles. Thus, solvents with high densities, surface

tensions and viscosities generally have higher threshold for cavitation but more harsh conditions once cavitation begins. [20]

Kuijpers et al. [17] calculated that the threshold pressure of the liquid CO_2 equals that of atmospheric water at 5.82 MPa and 20°C. For water at 5.82 MPa, a very high acoustic pressure is required to create cavitation. The threshold pressure in water is determined only by the static pressure and the surface tension of the liquid, because of its low vapor pressure. Because the vapor pressure does not change significantly with increasing temperature, the threshold pressure of water is approximately constant. On the other hand, since CO_2 condenses at a substantially higher pressure, its vapor pressure has a substantial influence.

Moreover, the cavitation phenomenon leads to formation of highly reactive species that lead to chemical reactions. These effects start during the collapse of the cavities in pure aqueous systems, gaseous water molecules entrapped in expanded microbubbles are fragmented as in pyrolysis and the mainly species formed are OH radicals. In aqueous media containing volatile organic gases and solutes, cavitation collapse not only results in the scission of water molecules to hydroxyl and hydrogen radicals, but also in the formation of organic radicals. [20-22]

Furthermore, cavitation can increase the reaction rates of existing process or start new reaction mechanisms by the formation of other reactive radical species. Those statements could suggest dramatic changes in the parameters as temperature or pressure of the bulk surrounding but this is not the case because the time scale for these microreactions is really small to affect cellular structure and enhance mass transport. [11, 23] Balachandran et al. [18] studied the ultrasonic enhancement of the supercritical extraction from ginger and performed some tests for prove the effects of cavitation. As initiation of polymerization reactions by free radicals formed during cavitation is an established technique under ambient conditions, experiments were performed to determine if polymerization could be initiated by sonicating CO_2 at supercritical conditions. The results showed no polymerisation of methyl methacrylate (MMA). The authors concluded either that there could be no cavitation collapse to generate free radicals or the collapse of the cavitational bubble is very weak and unable to create hot spots and induce radical formation.

3.4 Presence of dissolved gas in the medium

The type of radicals formed also depends on the presence and gas type dissolved in the medium. The gases act as nucleation sites for cavitation and then bubbling gases through the mixture facilitates the production of cavitation bubbles, but the type of gas used is important. Generally, gases with high specific heat ratio give a greater cavitation effect than one with low specific heat ratio. Monoatomic gases (i.e. argon and helium) convert more energy upon cavitation than diatomic gases (i.e. oxygen) because of the larger ratio of specific heats. Thompson and Doraiswamy [19] and Adewuyi [20] provided these and more information about presence and nature of the dissolved gases on cavitation and reactions
under ultrasound effects.

4. RECENT APPLICATIONS OF ULTRASOUND FOR HIGH PRESSURE EXTRACTION PROCESSES

The combination of techniques which can provide synergistic effects based on the similarity in the controlling mechanisms or supplementary roles can be a viable option with possible commercial applications. This approach meets to the environmentally friendlier concept of saving resources by optimization of process conditions and/or introducing new process technologies to preparations of valuable compounds. [1] Ultrasound-assisted process can be conveniently coupled with other techniques that are performed under high pressure such as extraction process like supercritical fluid extraction and pressurized liquid extraction.

4.1 Ultrasound assisted supercritical fluid extraction (UASFE)

The use of supercritical fluids as solvents is an interesting alternative for obtaining natural products with high quality without generating toxic residues. The usage of this technology increased rapidly, with new applications being developed almost every day. [24] Extraction with supercritical carbon dioxide is also considered as environmentally friendly technology which has gained acceptance as an alternative to conventional solvent extraction because its important advantages such as non-toxic, recyclable, cheap, relatively inert and non-flammable. [25]

Nevertheless, supercritical fluid extraction (SFE) has some drawbacks which caused new researches to overcome them. The requirement of high-pressure equipment and its cost was considered at all times as the main drawback to SFE. However, recent studies have been established that SFE can be economically viable. For obtaining oil from grape seed, the SFE process is economically viable in the 50 L plant, depending on the selling price of products (lower than US$ 100,00/kg). [26] Prado et al [27] also studied the economic viability of SFE of oil and carotenoids from three Amazon palm trees: buriti, pupunha and pressed palm fiber. Under the conditions studied, the prices of SFE oils were higher than selling prices of pressed oils, not because of the investment cost, but because of the raw material cost.

Also, the economics of SFE is affected by slow kinetics of the process. Since high pressures are normally used in SFE, mechanical stirring is difficult to be applied. The use of high-intensity ultrasound represents a potential efficient way to enhancing mass transfer process because of some mechanisms (radiation pressure, streaming, agitation, high amplitude vibrations, etc.). Thus, the application of ultrasound during SFE affects both the kinetics and the yield of extraction once this is probably the unique practical way to produce agitation during SFE. [25, 28-30]

Several studies have shown benefits on the SFE provided by ultrasound irradiation. Therefore, the application of ultrasound during supercritical extraction process has been proposed as a mechanism both for rate acceleration and extraction yield improvement (Table 1). Riera et al [28] firstly developed a pilot-scale ultrasound assisted CO_2 extraction of oil from almonds. The ultrasound power was promoted by a piezoelectric sandwich transducer inside the extractor. The results showed that in the end of the process the kinetics and the extraction yield enhanced by rate of 30% and 20%, respectively, when an ultrasound power of about 50 W was applied. Thereafter, other authors applied different configurations of ultrasound assisted CO_2 extraction to obtain compounds from different medicinal plants with positive results as shown in the Table 1. Figure 2 shows possible configurations of UASFE. The configuration with ultrasonic probe (A) are preferred over that with ultrasonic bath (B) since the transducer is fitted externally in the ultrasonic bath, there is some power attenuation as the ultrasound passes through the extractor walls. Hence, the power density inside the extractor is somewhat lower than that provided by the output controller.

Table 1: Comparison of different benefits on the SFE provided by ultrasound irradiation for selected medicinal plants

Medicinal plant	**Target compound**	**Experimental conditions**						**Results**	**Reference**
		Quantity of raw material	**Particle size**	**Pressure**	**Temperature**	**Ultrasonic power**	**Ultrasonic frequency**		
Almonds	Almond oil	1.5 kg	3-4 and 9-10 mm	28 MPa	55°C	50W	18 kHz	The yield of the oil was increased in 20% when the SFE was ultrasonically assisted. Also, was observed that small particle size favor the ultrasonic action.	[28]
Almonds	Almond oil	1.5 kg	3-4 mm	20-32 MPa	45 and 60°C	85 W	18 kHz	With the new system the yield of oil reach up to 90% with SFE assisted by ultrasound.	[31]
Cocoa cake	Cocoa cake oil	1.5 kg	2-3.5 mm	32 MPa	65°C	85 W	18kHz	The application of ultrasound increases the extracted yield in around 43%.	
Ginger rhizomes	Pungent compounds	Not informed	4-8 mm	16 MPa	40°C	300 W	20 kHz	The yield of pungent compounds from ginger was increased under the influence of ultrasound, with improvements of up to 30% towards the end of the extraction time.	[18]

Table 1 (continued): Comparison of different benefits on the SFE provided by ultrasound irradiation for selected medicinal plants

Adlay seeds	Adlay oil	0.1 kg	0.30-0.45 mm	10-30 MPa	30-55°C	110 W	20kHz	The results showed that the yield extraction of oil from adlay seeds increased 14% with sonication. The operation conditions of SFE with sonication were milder.	[32]
Marigold	Lutein esters	0.1 kg	0. 198-0.245 to 0.350-0.833mm	17.5-32.5 MPa	35-55°C	100-400W	25-33 kHz	The mass transfer coefficient in the solid phase (*ks*) increased from 3.1x10-9 to 4.3x10-9 due to ultrasound. The results showed that the yield of lutein esters increased significantly with the presence of ultrasound (p<0.05).	[33]
Ginseng	Ginsenosides	0.1 kg	Not informed	24 MPa	45°C	7.6 W	20kHz	The ginsenoside extraction yield from supercritical CO2 reverse microemulsion with ultrasound was 2.63 times that without ultrasound.	[34]
Adlay seeds	Adlay oil	0.1 kg	12-20 to 60-80 Mesh	10-25MPa	35-50°C	110 W	20 kHz	Compared with SFE, SFE assisted by ultrasound could give a 14% increase in the yield.	[35]

Table 1 (continued): Comparison of different benefits on the SFE provided by ultrasound irradiation for selected medicinal plants

Malagueta pepper	Oleoresin	0.02 kg	0.177-0.342 and 1.18-1.68 mm	15 MPa	40°C	360 W	20 kHz	The global yield increased when SFE was assisted by ultrasound when compared with only SFE. The highest increase was obtained with particles of 1.18-1.68mm.	[36]

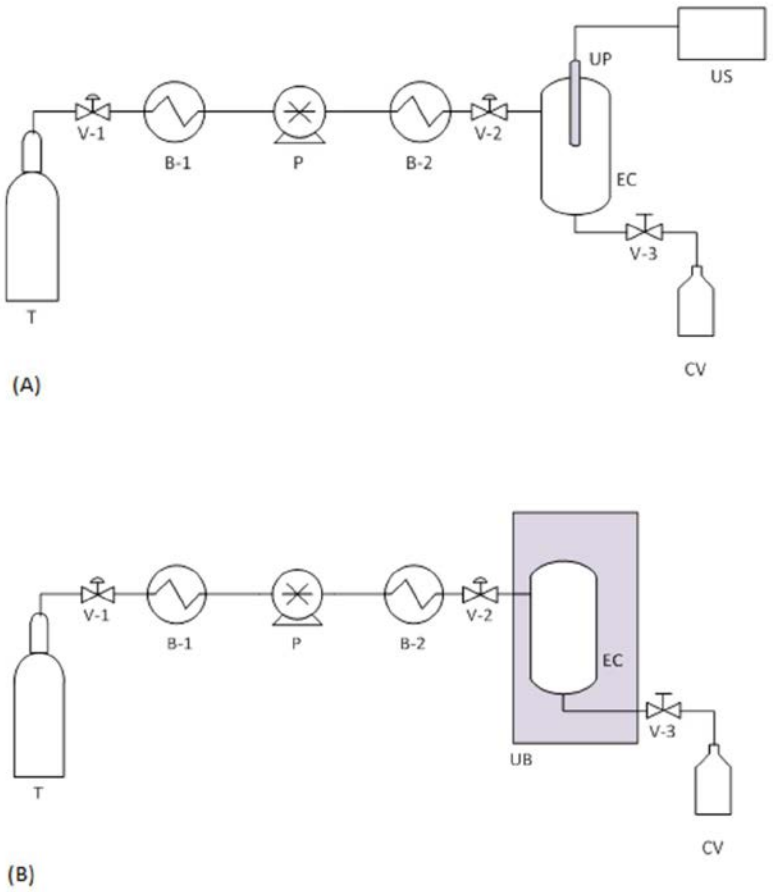

Figure 2: Possible configurations of UASFE. (A) UASFE by ultrasonic probe – T: CO_2 tank; B-1: cooling bath; B-2: heating bath; P: pump; EC: extraction column; UP: ultrasonic probe; US: ultrasonic power supply; CV: collector vessel; V-1, V-2, V-2: control valves. (B) UASFE by ultrasonic bath – T: CO_2 tank; B-1: cooling bath; B-2: heating bath; P: pump; EC: extraction column; UB: ultrasonic bath; CV: collector vessel; V-1, V-2, V-2: control valves.

4.2 Ultrasound assisted pressurized liquid extraction (UAPLE)

Pressurized Liquid Extraction (PLE) has been successfully used for the extraction of several bioactive compounds from different plants. [37] A major advantage of PLE over conventional solvent extraction methods conducted at atmospheric pressure is that pressurized solvents remain in a liquid state well above their boiling points, allowing for high-temperature

extraction. These conditions improve analyte solubility and the kinetics of desorption from matrices. [38]

The use of a pressurized liquid extraction (PLE) technique is an attractive alternative because it allows for fast extraction and reduced solvent consumption. PLE enables the rapid extraction (less than 30 min) of analytes in a closed and inert environment under high pressures (no higher than 20 MPa) and temperatures (25–200 °C). Hence, extracting solvents that are inefficient in extracting at low temperatures, may be much more efficient at the elevated temperatures used in PLE. [39]

Based on positive results obtained by coupling ultrasound with other extraction techniques, the Richter's group in Chile studied the extraction of contaminant compounds from soil using PLE coupled with ultrasound. [40, 41] In the first work [40], the authors observed that when the PLE was assisted by ultrasound, the extraction time can be reduced from 20 min to 10 min obtaining quantitative recoveries of aliphatic and polycyclic aromatic hydrocarbons from soils. When UAPLE was compared with Soxhlet extraction, the results provided were statistically lower than those obtained by the conventional method. However, it is important to point that the extraction time is decreased from 20 h to less than 1h and the organic solvent used in the extraction procedure can be decreased to less than 5% of its initial value. In other work [41], to extraction of polychlorinated biphenyls from biosolids, the recovery of the PLE method was 73%, which was significantly improved (103%) when PLE was assisted with 30 min of ultrasound.

The experimental apparatus used to UAPLE is similar to that used for UASFE presented in the Figure 2, except that the solvent does not need to be pressurized before entering in the system because it stays in the liquid state. Normally, PLE employs generally recognized as safe (GRAS) solvents, such as ethanol and water. [42] However, the use of aqueous surfactant solutions as alternative solvent systems in PLE have been reported for the extraction of ginsenosides from ginseng roots (*Panax quinquefolium*). When compared to the use of pure water or methanol, the presence of a common non-ionic surfactant (Triton X-100) in water at a concentration above its critical micelle concentration was shown to enhance the amount of ginsenosides extracted. The advantages of using aqueous non- ionic surfactant solutions were also demonstrated by comparing performances between ultrasonic-assisted extraction and PLE

methods. These advantages may be provided by the solubility-enhancement effect of the Triton X-100 micelles. For example, certain surfactants are known to increase the mass transfer coefficient during the desorption of pollutants from soil to water, presumably due to the better swelling of the soil organic matters and more complete diffusion of the solvent into the solid matrix.[43]

Thompson and Doraiswamy [19] reported that the addition of surfactants to ultrasonic systems reduces the surface tension of the medium, thus reducing the cavitation threshold and facilitating the generation of bubbles. Based on these aspects, we can expect that using surfactant solutions as solvent in PLE and applying ultrasonic in this system, the results can be promising. Assuming that the addition of surfactant could act to enhance of solubility of the compounds in the extracting solvent and also could reduce the surface tension, the generation of cavitation bubbles consequently will be facilitated. These effects combined could provide good results of mass transfer in extraction process.

Recently, glycol derived solutions, mainly polyethylene glycol (PEG) solutions have attracted increasing interest as novel solvents due their excellent properties and potential application to extraction in analytical chemistry. [44] Owing to their good biocompatibility and low immunogenicity, PEGs are on the Food and Drug Administration's (FDA's) GRAS list and have been approved by FDA for internal consumption. Among several advantages, PEG have good miscibility with water and organic solvents, as well as good solubility for various organic compounds. Therefore, PEGs are used as environmentally friendly solvents.

Moreover, the addition of PEG in solutions of water or other solvents can increase the solution viscosity. PEG has been used as a green solvent in the microwave-assisted extraction of flavones and coumarin compounds from medicinal plants. [45] But to our own known, PEG solutions has no used as extracting solvent to ultrasound-assisted extraction. As discussed previously, the ultrasonic intensity increases for solvents with high viscosity. Therefore, we can expect that the use of solutions with high viscosity as alternative solvent for ultrasound assisted extraction process can enhance the mass transfer producing good results of yield and selectivity of extraction.

4.3 Modeling of ultrasound assisted pressurized fluid extraction

The mass transfer process in solid-liquid extraction involves two chief steps. According to model proposed by Sovová [46] in modeling supercritical fluid extraction, as a result of seed physical manipulation such crushing the extracted solid contains both broken and intact cells. It is then assumed that micro-structurally, a seed particle contain: i) soluble material easily accessible, which is extracted at a rate that is controlled by the external resistance to mass transfer and is located in fractured cells in the particle surface; and ii) "tied" soluble material, which is extracted at a rate that is determined by internal mass transfer mechanisms and is localized in undamaged cells and/or partially damaged cells in the inner portions of the particle. This second step is usually much slower and regarded as limiting step for most solid-liquid systems.

In the literature, some authors affirm that the effective enhancement of extraction with ultrasound should mainly affect the second step. [7, 47] This affirmation is according with the founded by some authors, [33, 36, 48] however, there is no consensus regarding this point. Balachandran et al. [18] reported inverse effect. They observe that when ultrasound is applied during SFE process, the predicted effective diffusivity in the first extraction step approximately doubles, suggesting that the ultrasonic vibration has either increased the number of ruptured cells and/or provided faster access for the solvent to remove solutes from these cells. The effective diffusivity in the second stage also increases when ultrasound is applied, but the enhancement is less significant. Nevertheless, all authors agree that each solid matrix-solvent system have a particular interaction mode and then the ultrasound effect can act by different ways.

To the best of our knowledge, there is few or any work about modeling of UAPLE. However, it is an important field to study.

5. CONCLUSION

The aspects presented in this work established the potentiality of coupling ultrasound with high pressure green extraction techniques to overcome its drawbacks. The major advantages of ultrasound assisted extraction are the less energy requirement, solvent usage and time of process. The variables of the process have a strong influence on the extraction performance and

should be careful studied in laboratory for any process in the pharmaceutical, cosmetic or food industry to obtain bioactive compounds from medicinal plants.

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REFERENCES

1. Shirsath, S.R., S.H. Sonawane, and P.R. Gogate, Intensification of extraction of natural products using ultrasonic irradiations- A review of current status. ChemicalEngineering and Processing: Process Intensification. 2012; 53: 10-23.

2. Palma, M., G.F. Barbero, Z. Piñeiro, A. Liazid, C.G. Barroso, M.A. Rostagno, et al., *Extraction of Natural Products: Principles and Fundamental aspects*, in *Natural Product Extraction: principles and applications*, M.A. Rostagno and J.M. Prado, Editors. 2013, RSC Publishing: Londres. p. 58-88.

3. Pingret, D., A.S. Tixier-Fabiano, and F. Chemat, *Ultrasound-assisted extraction*, in *Natural product extraction: principles and applications*, M.A. Rostagno and J.M. Prado, Editors. 2013, RSC Publishing: Londres. p. 89-112.

4. Luque-García, J.L. and M.D. Luque de Castro, Ultrasound: a powerful tool for leaching. Trends in Analytical Chemistry. 2003; 22: 41-47.

5. Patist, A. and D. Bates, Ultrasonic innovations in the food industry: from the laboratory to commercial production. Innovative Food Science & Emerging Technologies. 2008; 9: 147-154.

6. Rostagno, M.A., A. Villares, E. Guillamón, A. García-Lafuente, and J.A. Martínez, Sample preparation for the analysis of isoflavones from soybeans and soy foods. Journal of Chromatography A. 2009; 1216: 2-29.

7. Vinatoru, M., An overview of the ultrasonically assisted extraction of bioactive principles from herbs. Ultrasonics Sonochemistry. 2001; 8(3): 303-313.

8. Takeuchi, T.M., C.G. Pereira, M.E.M. Braga, J. Maróstica, M. R., P.F. Leal, and M.A.A. Meireles, *Low pressure solvent extraction (solid-liquid extraction, microwave assisted, and ultrasound assisted) from condimentary plants.*, in *Extracting Bioactive Compounds for Food Products*, M.A.A. Meireles, Editor 2009, CRC Press/ Taylor & Francis Group: Boca Ratun, USA. p. 137-218.

9. Wang, L. and C.L. Weller, Recent advances in extraction of nutraceuticals from plants. Trends in Food Science & Technology. 2006; 17(6): 300-312.

10. Vinatoru, M., M. Toma, O. Radu, P.I. Filip, D. Lazurca, and T.J. Mason, The use of ultrasound for the extraction of bioactive principles from plant materials. Ultrasonics Sonochemistry. 1997; 4(2): 135-139.

11. Escaplez, M.D., J.V. García-Pérez, A. Mulet, and J.A. Cárcel, Ultrasound-assisted extraction of natural products. Food Engineering Reviews. 2011; 3: 108-120.

12. Soria, A.C. and M. Villamiel, Effect of ultrasound on the technological properties and bioactivity of food: a review. Trends in Food Science and Technology. 2010; 21: 323- 331.

13. Mulet, A., J.A. Cárcel, J. Benetido, and N. Sanjuan, *Application of Low-Intensity Ultrasonics in Dairy Industry*, in *Engineering and Food for the 21st century*, 2002, CRC Press: Boca Raton, FL. p. e-book.

14. Berlan, J., F. Trabelsi, H. Delmas, A.M. Wilhelm, and J.F. Petrignani, Oxidative degradation of phenol in aqueous media using ultrasound. Ultrasonics Sonochemistry. 1994; 1: S97-102.

15. Hilgenfeldt, S., M.P. Brenner, S. Grossmann, and D. Lohse, Analysis of Rayleighplesset dynamics for sonoluminescing bubbles. Journal of Fluid Mechanics. 1998; 365: 171-204.

16. Kemmere, M., M. kuijpers, L. Jacobs, and J. Keurentjes, Ultrasound-induced polymerization of methyl methacrylate in liquid carbon dioxide: a clean and safe route to produce polymers with controlled molecular weight. Macromolecular Symposia. 2004; 206: 321-331.

17. Kuijpers, M.W.A., D. van Eck, M.F. Kemmere, and J.T.F. Keurentjes, Cavitationinduced reactions in high-pressure carbone dioxide. Science. 2002; 298: 1969-1971.

18. Balachandran, S., S.E. Kentish, R. Mawson, and M. Ashokkumar, Ultrasonic enhancement of the supercritical extraction from ginger. Ultrasonics Sonochemistry.2006; 13: 471-479.

19. Thompson, L.H. and L.K. Doraiswamy, Sonochemistry: Science and Engineering Industrial & Engineering Chemistry Research. 1999; 38: 1215-1249.

20. Adewuyi, Y.G., Sonochesmistry: Environmental science and engineering applications. Industrial & Engineering Chemistry Research. 2001; 40: 4681-4715.

21. Fischer, C.H., E.J. Hart, and A.J. Henglein, Hydrogen/deuterium isotrope exchange in the hydrogen deuteride-water system under the influence of ultrasound. The Journal of Physical Chemistry. 1986; 90: 3059-3060.

22. Seghal, C., T.J. Yu, R.G. Sutherland, and R. Verrall, Use of 2,2-diphenyl-1-picrylhydrazyl to investigate the chemical behavior of free radicals induced by ultrasonic cavitation. The Journal of Physical Chemistry. 1982; 86: 2982-2986.

23. Ince, N.H., G. Tezeanli, R.K. Belen, and P.G. Apikyan, Ultrasound as a catalyzer of aqueous reaction systems: the state of the art and environmentak applications. Applied Catalysis B. 2001; 29: 167-176.

24. Leal, P.F., M.B. Kfouri, F.C. Alexandre, F.H.R. Fagundes, J.M. Prado, M.H. Toyama, et al., Brazilian Ginseng extraction via LPSE and SFE: Global yields, extraction kinetics, chemical composition and antioxidant activity. Journal of Supercritical Fluids. 2010; 54(1): 38-45.

25. Riera, E., A. Blanco, J. García, J. Benedito, A. Mulet, J.A. Gallego-Juárez, et al., High-power ultrasonic system for the enhancement of mass transfer in supercritical CO_2 extraction process. Physics Procedia. 2010; 2: 141-146.

26. Prado, J.M., I. Dalmolin, N.D.D. Carareto, R.C. Basso, A.J.A. Meirelles, J.V. Oliveira, et al., Supercritical fluid extraction of grape seed: Process scale-up, extract chemical composition and economical evaluation. Journal of Food Engineering. 2012; 109(2): 249-257.

27. Prado, J.M., A.R. Assis, M.R. Maróstica-Júnior, and M.A.A. Meireles, Manufacturing cost of supercritical-extracted oils and carotenoids from amazonian plants. Journal of Food Process Engineering. 2010; 33(2): 348-369.

28. Riera, E., Y. Golás, A. Blanco, J.A. Gallego, m. Blasco, and A. Mulet, Mass transfer enhancement in supercritical fluids extraction by means of power ultrasound. Ultrasonics Sonochemistry. 2004; 11: 241-244.

29. Cárcel, J.A., J.V. García-Pérez, J. Benetido, and A. Mulet, Food process innovation through new technologies: use of ultrasound. Journal of Food Engineering. 2012; 110: 200-207.

30. Seidi, S. and Y. Yamini, Analytical sonochemistry: developments, applications, and hyphenations of ultrasound in sample preparation and analytical techniques. Central European Journal of Chemistry. 2012; 10(4): 938-976.

31. Riera, E., A. Blanco, J. García, J. Benedito, A. Mulet, and J.A. Gallego-Juárez, High pressure ultrasonic system for the enhancement of mass transfer in supercritical CO_2 extraction process. Ultrasonics. 2010; 50: 306-309.

32. Hu, A., S. Zhao, H. Liang, T. Qiu, and G. Chen, Ultrasound assisted supercritical fluid extraction of oil and coixenolide from adlay seed. Ultrasonics Sonochemistry. 2007; 14: 219-224.

33. Gao, Y., B. Nagy, X. Liu, B. Simándi, and Q. Wang, Supercritical CO2 extraction of lutein esters from marigold (*Tagetes erecta* L.) enhanced by ultrasound. The Journal of Supercritical Fluids. 2009; 49: 345-350.

34. Luo, D., T. Qiu, and Q. Lu, Ultrasound-assisted extraction of ginsenosides in supercritical CO2 reverse microemulsions. Journal of the Science of Food andAgriculture. 2007; 87: 431-436.

35. Hu, A., Z. Zhang, J. Zheng, Y. Wang, Q. Chen, R. Liu, et al., Optimizations and comparison of two supercritical extractions of adlay oil. Innovative Food Science and Technologies. 2012; 13: 128-133.

36. Santos, P., A.C. Aguiar, C.A. Rezende, and J. Martínez, *Supercritical carbon dioxide extraction of oleoresin from malagueta pepper (Capsicum frutescens L.) enhanced by ultrasound*, in *III Iberoamerican Conference of Supercritical Fluids*2013: Cartagena de Indias, Colombia. p. 1-7.

37. Petersson, E.V., J. Liu, P.J.R. Sjoberg, R. Danielsson, and C. Turner, Pressurized hot water extraction of anthocianins from red onion: a study on extraction and degradation rates. Analytica Chimica Acta. 2010; 663: 27-32.

38. Richter, B.E., B.A. Jones, J.L. Ezzell, and N.L. Porter, Accelerated solvent extraction: a tehcnique for sample preparation. Analytical Chemistry. 1996; 68: 1033-1039.

39. Ju, Z.Y. and L.R. Howard, Effects of Solvent and Temperature on Pressurized Liquid Extraction of Anthocyanins and Total Phenolics from Dried Red Grape Skin. Journal of Agricultural and Food Chemistry. 2003; 51(18): 5207-5213.

40. Richter, P., M. Jimenéz, R. Salazar, and A. Maricán, Ultrasound-assisted pressurized solvent extraction for aliphatic and polycyclic aromatic hydrocarbons from soils. Journal of Chromatography A. 2006; 1132: 15-20.

41. Rocco, G., C. Toledo, I. Ahumada, B. Sepúlveda, A. Cañete, and P. Richter, Determination of polychlorinated biphenyls in biosoils using continuous ultrasound assisted pressurized solvent extraction and gas chromatrography-mass spectrometry Journal of Chromatography A. 2008; 1193: 32-36.

42. Santos, D.T., C.L.C. Albuquerque, and M.A.M. Meireles, Antioxidant dye and pigment extraction using homemade pressurized solvent extracion system. Procedia Food Science. 2011; 1: 1581-1588.

43. Choi, M.P.K., K.K.C. Chan, H.W. Leung, and C.W. Huie, Pressurized liquid extraction of active ingredients (ginsenosides) from medicinal plants using non-ionic surfactant solutions. Journal of Chromatography A. 2003; 983: 153-162.

44. Chen, J., S.K. Spear, J.G. Huddleston, and R.D. Rogers, Polyethylene glycol andsolutions of polyethylene glycol as green reaction media. Green Chemistry. 2005;7(2): 64-82.

45. Zhou, T., X. Xiao, G. Li, and Z. Cai, Study of polyethylene glycol as a green solvent in the microwave-assisted extraction of flavone and coumarin compounds frommedicinal plants. Journal of Chromatography A. 2011; 1218: 3608-3615.

46. Sovová, H., Rate of vegetable oil extraction with supercritical CO2. Modeling of extraction curves. Chemical Engineering Science. 1994; 49: 409-414.

47. Toma, M., M. Vinatoru, L. Paniwnyk, and T.J. Mason, Investigation of the effects of ultrasound on vegetal tissues during solvent extraction. Ultrasonics Sonochemistry. 2001; 8(2): 137-142.

48. Riera, E., Y. Golás, A. Blanco, J.A. Gallego, J. García-Reverter, and S. Subirats, *Effect of high-intensity ultrasound on the particulate almonds oil extraction kinetics using supercritical CO2*, in *6th International Symposium on Supercritical Fluids* 2003:Versailles, France. p. 1-6.

CAPÍTULO 4

EXTRAÇÃO DE BETA-ECDISONA E CARBOIDRATOS PREBIÓTICOS DAS RAÍZES E PARTES AÉREAS DO GINSENG BRASILEIRO UTILIZANDO ÁGUA SUBCRÍTICA

TECHNO-ECONOMIC EVALUATION OF SUBCRITICAL WATER EXTRACTION OF PREBIOTIC CARBOHYDRATES AND BETA-ECDYSONE FROM BRAZILIAN GINSENG ROOTS AND AERIAL PARTS

Renata Vardanega, Pedro I. N. Carvalho, Diego T. Santos, M. Angela A. Meireles*

LASEFI/DEA/FEA (School of Food Engineering)/UNICAMP (University of Campinas), R. Monteiro Lobato, 80, 13083-862, Campinas, São Paulo, Brazil

* Corresponding author: maameireles@gmail.com, meireles@fea.unicamp.br

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ABSTRACT

Brazilian ginseng is a plant widely used as nutraceutical supplement due to human health improvement. Its benefits are mainly attributed to the presence of beta-ecdysone in its composition. More recently prebiotic compounds were found in Brazilian ginseng roots, such as fructooligosaccharides (FOS). Prebiotic compounds have been extensively studied to be added in the human diet to promote well-being and other health benefits. Based on these properties, subcritical water extraction, an environmental friendly process, was performed to obtain the bioactive compounds from Brazilian ginseng roots (BGR) and aerial parts (BGA) aiming to use of the whole plant. For BGR, the effects of temperature (353-453 K) and static extraction time (5-15 min) were evaluated on the extraction yield, beta-ecdysone and FOS contents in the extracts; while for BGA, the effects of temperature (353-453 K), pressure (2-12 MPa) and static extraction time (5-10 min) were evaluated on extraction yield and beta-ecdysone content in the extracts, both using full factorial design and analysis of variance (ANOVA) statistical techniques. The BGR extracts showed a beta-ecdysone content of up to 0.7 $g \cdot 100g^{-1}$ of extract and a FOS content of up to 8.8 $g \cdot 100g^{-1}$ of extract, which means that BGR can be considered a great source of these bioactive compounds. Meanwhile BGA extracts showed a beta-ecdysone content of 0.3 $g \cdot 100g^{-1}$ of extract. The economic evaluation demonstrated that SWE process to obtain beta-ecdysone from BGR is economically feasible and a great opportunity of business, while the manufacturing of BGA should not be undertaken for this purpose, due to the negative economic performance.

Keywords: *Pfaffia glomerata*, beta-ecdysone, prebiotic carbohydrate, subcritical water extraction, economic evaluation

1. INTRODUCTION

The commercial interest in Brazilian ginseng (*Pfaffia glomerata*, Amaranthaceae) as raw material for obtaining natural products has been increased due to its bioactive properties. Among the several benefits associated with Brazilian ginseng consumption, its anabolic effect is more attractive and, therefore, it has been recommended as nutritional supplement for the athletes to improve their performance during the training. Also, Brazilian ginseng extracts can be used as analgesic, anti-inflammatory [1], anti-diabetic [2], gastroprotective [3], antimicrobial [4] and melanogenesis inhibitors [5]. The positive effects of the Brazilian ginseng are mainly attributed to the presence of beta-ecdysone in its composition [6]. Moreover, species from the Amaranthaceae family contain up to 50% of carbohydrates with polymeric fructose as the main component [7] and recently a study confirmed the presence of inulin-type fructans in Brazilian ginseng roots [8]. However, previous studies using species from the Amaranthaceae family have shown that the tuberous roots constitute approximately 50% of carbohydrates with polymeric fructose as the main component and recently, a study confirmed the presence of inulin-type fructan in Brazilian ginseng roots [8].

Fructans are fructose oligomers, which are classified as fructooligosaccharides (FOS) or inulin depending on the degree of polymerization (DP): when DP < 10 they are called FOS and when DP > 10 they are called inulin. Due to their structure, FOS and inulin are non-digestible carbohydrates for the human body and can be considered prebiotics, since they affect the host by selectively stimulating the growth and/or activity of some microorganisms in the colon [9]. Moreover, they can be used as substitutes for sucrose because they have around 30-60% of the sweetness of sucrose and low caloric value [10], among several other health benefits related [11-13]. Because of these properties, industries became interested in the applications of FOS and inulin in functional foods and nutritional composites [14].

Generally, only the roots of Brazilian ginseng (BGR) are commercially used to obtain bioactive compounds while the aerial parts (BGA) are disposed in the field as a waste. However, studies have shown that BGA also contain bioactive compounds including beta-ecdysone [15, 16]. Thus, BGA could be used as a source of bioactive compounds to increase the productivity potential of the whole plant.

There are several established methods in the industry for the extraction of bioactive compounds from vegetable matrices, which generally use ethanol, methanol, acetone, hot water or their mixtures as solvents. However, these methods present some drawbacks because they are not all sustainable, energy effective, fast or even efficient for industrial production [17]. In this way, researches have been mainly focused on the development of innovative techniques that meet the green processes aspects. These green processes generally use non-toxic solvents and consume less time and energy. Among the techniques that meet these demands, the most popular are supercritical fluid extraction (SFE), ultrasound assisted extraction (UAE), microwave assisted extraction (MAE) and pressurized liquid extraction (PLE). When the solvent used in PLE is water, the process is also known as subcritical water extraction (SWE), which refers to water at temperatures between 373 and 647 K and at pressures high enough to maintain the water in the liquid state (above the critical pressure of 22 MPa) [18].

The use of pressurized solvent-based process has several advantages over conventional extraction process. When pressurized, the extracting solvent is at the compressed liquid region and, in this region, the liquids are highly uncompressible; in other words, when the liquid is submitted to pressure variations under constant temperature, its density and solvation power are little affected. The increase in temperature improves the extraction efficiency due to an increase in the mass transfer rates. When water is used as extracting solvent, other important factor to be considered is the variation in the dielectric constant with temperature. At ambient temperature, the water is highly polar with dielectric constant around 80. However, this value can be drastically decreased to 38 when the water is heated at 453 K, and can reach near 27 when the water achieve temperatures around 523 K [19].

Besides the technical study to optimize the operational conditions for a certain process, it is necessary to perform an economical study to verify the feasibility of the developed process in industrial scale. Studies performed to estimate the cost of manufacturing (COM) for extracts from different vegetable matrices demonstrated that the acquisition cost of raw material has a strong impact on the COM [20, 21]. In this way, raw materials with low or zero acquisition cost could be highly promising for utilization as bioactive compounds source from the economic point of view, as is the case of BGA.

In this context, the objective of this work was to assess, in terms of the techno-economical aspects, the production of bioactive compounds-rich extracts from BGR and BGA leftovers in the plantation field after root harvesting by means of the environmentally friendly

SWE process. The SWE operational parameters evaluated were temperature, static extraction time and pressure on the extraction yield, beta-ecdysone and fructooligosaccharides contents in the extracts. In addition, an economic evaluation of the process was carried out.

2. MATERIAL E METHODS

2.1 Raw materials

Brazilian ginseng roots (BGR) were collected and prepared as described in Vardanega et al. [22]. BGR were grown for 7 years in the experimental field of CPQBA (Unicamp, Campinas, Brazil). BGR were washed and dried at 313 K for 5 days in a forced air circulation dryer. Dried BGR (9.2% moisture) were comminuted in a pulse mill (Marconi MA 340, Piracicaba, Brazil) and the larger particles were also comminuted in a knife mill (Tecnal, model TE 631, Piracicaba, Brazil) for 2 s at 18,000 rpm. Milled BGR were separated according to size in Tyler series sieves (W. S. Tyler, Wheeling, IL). The mean diameter of the particles (8.0 μm) was determined by the ASAE method [23]. Brazilian ginseng aerial parts (BGA) were collected in the experimental field of CPQBA (UNICAMP, Campinas, Brazil), washed and dried at 313 K for 5 days in a forced air circulation dryer. The dried BGA were comminuted in a pulse mill (Tecnal, model TE 631, Piracicaba, Brazil) for 2 seconds at 18,000 rpm. Both raw materials were stored in a freezer (Metalfrio, model DA 420, São Paulo, Brazil) at 263 K.

2.2 Raw materials characterization

BGR and BGA were characterized in terms of moisture, ash, proteins, lipids, carbohydrates, soluble and insoluble fiber [24] and starch [25], in triplicate.

2.3 Subcritical water extraction (SWE)

2.3.1 Equipment

The SWE apparatus is shown in Figure 1 and consists of an HPLC pump (Thermoseparation Products, model ConstaMetric 3200 P/F, Florida, USA), a 6.57 mL extraction cell (Waters, serial # 4501374824-10, Pittsburg, USA) containing a sintered metal filter at the bottom and upper parts, an electrical heating jacket and a back pressure regulator

valve (Tescom, 26-1761-24-161, ELK River, USA). More detailed description of the unit can be found elsewhere [26].

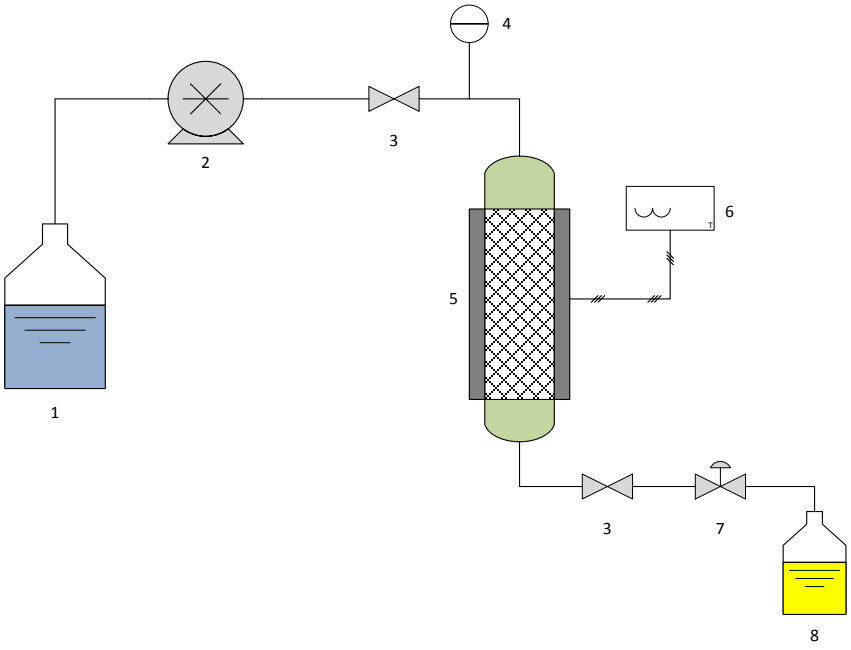

Figure 1: SWE experimental apparatus. (1) Solvent reservoir, (2) Liquid pump, (3) Blocking valves, (4) Pressure gauge; (5) Extraction vessel equipped with jacket for heating; (6) Temperature controller, (7) Back pressure regulator (8) Extract collecting vessel.

2.3.2 Extraction procedure

The experiments with BGR and BGA were performed using 4.5 and 3.0 g (wet basis) of raw material, respectively. For each experiment, the raw material was placed into the extraction cell, which was heated, while distillated water was pumped until the operational pressure was reached. After pressurization, the cell was kept at the work pressure for the desired static extraction time. Thereafter, the valves were carefully opened to start the dynamic period with solvent flow rate of 1.4 mL· min^{-1} during 14 minutes. The aqueous extracts were lyophilized for approximately 96 h at 60 – 100 μHg and 233 K (Liobras, Liotop L101, São Carlos, Brazil). The extraction yield was calculated as the ratio between the mass of dry extract and the initial mass of raw material (dry basis, d.b.) fed into the extraction cell.

2.4 Beta-ecdysone quantification

The beta-ecdysone quantification was performed in high pressure liquid chromatography (HPLC) according to the method described by Rostagno et al [27]. The compounds separation was obtained in a fused-core column (Poroshell 120 EC-C_{18}, 100 × 4.6 mm, 2.7 μm, Agilent Technologies, Little Fall, EUA). The mobile phase was composed by water (A) and acetonitrile (B), both with acetic acid 0.1 %, running at the gradient: 0 min 5% of B; 0.5 min 10% of B; 2 min 12.5 of B; 3 min 15% of B; 4 min 80% of B; 5 min 100% of B; 6 min 100% of B; 7 min 5% of B. The flow rate was 2.0 $mL \cdot min^{-1}$ at 328 K and the injected sample volume was 10μL. The data acquisition was performed at 246 nm using 20-hydroxyecdysone (beta-ecdysone) (Sigma Aldrich, St. Louis, USA) as standard.

2.5 Total sugar quantification

The total sugar content was determined using the Somogyi-Nelson colorimetric method [28, 29]. The extract went through a diluted acid hydrolysis to assure all oligosaccharides were accounted. The dry extract was diluted until a final concentration of 1 $mg \cdot mL^{-1}$ of distilled water was reached.

2.6 FOS quantification

FOS quantification was performed in HPLC system (Alliance, Waters, Milford, USA) connected to an evaporative light scattering detector (2424 ELSD, Waters, Milford, USA). The separation of the compounds was obtained in an Xbridge Amide column (3.5 μm, 4.6 × 250 mm, Waters, Milford, USA). The autosampler and analytical column were maintained at 298 and 313 K, respectively. The mobile phase used to elute the compounds consisted of water (solvent A) and acetonitrile (solvent B), running at the gradient: 0 min 20% of A; 20 min 50% of A; 23 min 40% of A; 32 min 20% of A. The flow rate was 0.5 $mL \cdot min^{-1}$ and the injected volume was 10 μL. Nitrogen (White Martins, Campinas, Brazil) was used as carrier gas to nebulizer. ELSD conditions were gas pressure of 20 psi, drift tube temperature of 348 K and gain of 500. FOS were identified by comparing the retention time with those of the standards. The quantification was performed using the external calibration method. Calibration standard solutions at 7 concentrations levels ranging from 65 to 1000 $mg \cdot L^{-1}$ were prepared in water. The standards 1-kestose, nystose and fructofuranosylnystose were purchased from Wako Pure Chemical Industries (Osaka, Japan).

2.7 Experimental design and statistical analysis

For BGR, the effects of temperature (353, 373, 393, 413, 433 e 453 K) and static extraction time (5, 10 e 15 min) at 12 MPa were evaluated. For BGA, the effects of temperature (353, 393, 413, 433 e 453 K), static extraction time (5 e 10 min) and pressure (2, 7 e 12 MPa) were evaluated. A fully randomized, full factorial design for each raw material was performed, in duplicate (36 experiments for BGR and 60 experiments for BGA). The extracts from both raw materials were analyzed in terms of extraction yield and beta-ecdysone content, while BGR extracts were also analyzed in terms of FOS content. The influence of the parameters was determined by analysis of variance (ANOVA) using Minitab 16® (Minitab Inc., State College, PA, USA) with 95 % of confidence level (p-value ≤ 0.05).

2.8 Economic evaluation

The cost of manufacturing (COM) of the extracts was estimated using the cost tool in the simulator SuperPro Designer® version 8.5 (Intelligen Inc., Scotch Plains, USA). A series of costs influences the COM, which can be related by the following equation:

$$\mathrm{COM} = \mathrm{DMC} + \mathrm{FMC} + \mathrm{GE} \quad (1)$$

where COM is the cost of manufacturing, DMC is the direct manufacturing costs, FMC is the fixed manufacturing costs, and GE is the general expenses.

DMC represents operating expenses that vary with the production rate such as raw materials, supplies, utilities, operators and other operating costs. FMC is independent of variations in the production rate and includes taxes on the land, insurance and depreciation of equipment, which are assumed even when the plant is not in operation. GE represents expenses that are necessary for run the business such as management costs, sales, finance and research and development [30]. Based on these aspects, the economic data fed for the cost model are presented in Table 1. The cost of waste treatment was neglected because the solid generated in the extraction process can be used as raw material to obtain other products [31], for energy production through biomass conversion [32], or even as nutritional source for several agricultural sectors [33]. The cost of acquisition of BGA was also neglected because this raw material is discarded in the field without commercial value [16].

The proposed SWE unit (Figure 2) is composed of two extraction vessels of 0.05 m^3 that are operated in semi-continuous mode, two heat exchangers, a liquid pump, an evaporator and a spray-dryer. A single-effect evaporator was used to concentrate the aqueous extract until reach 60% of moisture prior to spray-dryer due to two reasons: i) for reduction of extract volume and, consequently, reduction of the process time for the spray-dryer operation; ii) to enhance of reuse in the following batch of the water from evaporator condensed compared to that originated from spray dryer output [26]. According to Ravber et al. [17] it was possible to reduce 87% of the water consumption by recycling the water from the evaporator in a subcritical water process to isolate phenolic compounds from larch wood waste. The evaporation temperature in the evaporator was fixed at 318 K. Ambient air was heated to 443 K before it is fed into the spray dryer as the drying medium. The final moisture of the dried extract was fixed at 3% and evaporated water from the spray dryer was considered as water lost during the process. Also, it is assumed that 1% of the extract was lost together with emitted air.

The annual operating time was considered as 7920 h per year, which corresponds to 330 days per year of continuous 24 h per day shifts. To obtain yields in industrial scale, it was assumed that for a given process time, the extraction behavior has the same performance as that obtained experimentally in the laboratory scale unit when the solvent to feed mass ratio and operating parameters (temperature, pressure, density and porosity) are kept constant [34]. The number of operators was defined according to the literature that reported that 1 to 3 operators are required for plants from 0.005 to 0.5 m^3 [34].

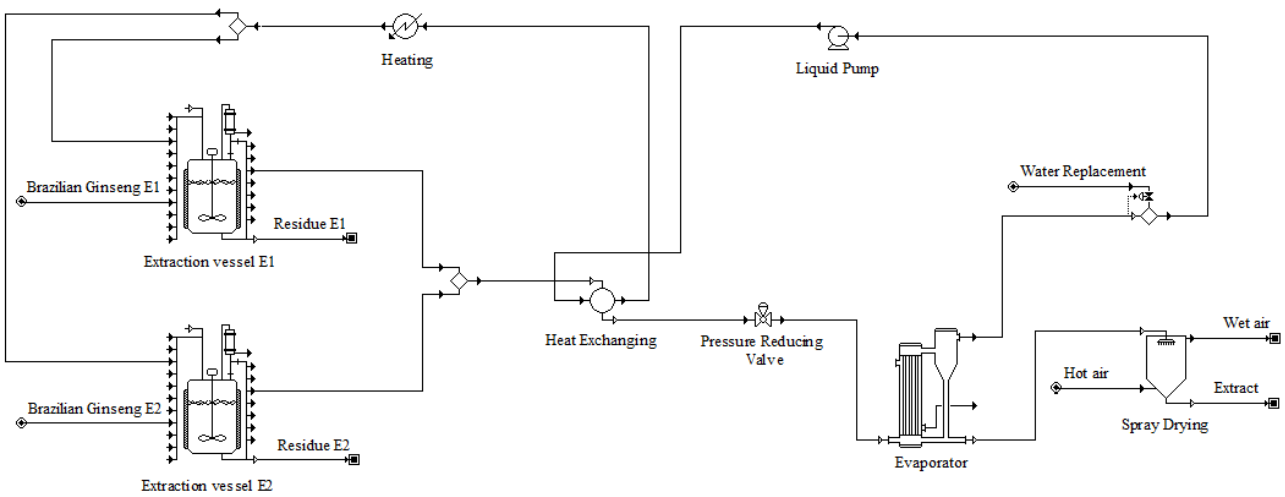

Figure 2: Flowsheet for the subcritical water extraction process (SWE).

Table 1: Economic parameters used for the estimation of cost of manufacturing (COM)

Industrial unit	**Chinese Market**
2 extractors of 0.05 m^3 [a]	US$ 380,000.00
Evaporator [b]	US$ 107,000.00
Spray dryer [b]	US$ 99,000.00
	Other input data
Depreciation rate	10%
Maintenance rate	6%
Labor (base rate)	US$ 6.00/h
Operators	2
Raw materials	
Brazilian ginseng roots [c]	US$ 9.68/kg
Brazilian aerial parts	US$ 0.00/kg
Water [b]	US$ 0.05/ton
Pre-processing	US$ 40.00/ton
Utilities	
Electricity [d]	US$ 0.0954/kW-h
Steam [b]	US$ 12.00/ton

[a]Carvalho et al. [35]

[b]SuperPro Designer® cost database

[c]Santosflora [36]

[d]CPFL [37]

3. RESULTS AND DISCUSSION

3.1 Raw materials characterization

The centesimal composition of Brazilian ginseng roots (BGR) and Brazilian ginseng aerial parts (BGA) are shown in Table 2. To the best or our knowledge no centesimal characterization of BGA was done previously. The values obtained in this study are similar to those found in the literature for BGR [38]. Variations may be due to the environmental conditions of the production region, such as the harvest season and storage. The high total

carbohydrates content for the both raw materials can be highlighted. While the carbohydrates from BGA are mainly constituted by insoluble fibers, the carbohydrates from BGR contain a high content of other carbohydrates. According to Roitsch et al. [39], in mature plants the carbohydrates are mainly accumulated in fruits, tubers and reserve roots. Thus, the other carbohydrates observed in BGR can include reserve carbohydrates, since the BGR were harvested after 7 years of growth. Reserve carbohydrates are mainly comprised of fructans, such as fructooligosaccharides [40].

Table 2: Brazilian ginseng roots (BGR) and aerial parts (BGA) characterization (%, dry basis*).

	BGR	**BGA**
Ash	5.99 ± 0.02	3.84 ± 0.08
Protein	5.8 ± 0.2	3.1 ± 0.8
Lipids	0.020 ± 0.001	0.25 ± 0.02
Total carbohydrates:	78.72 ± 0.06	83.4 ± 0.1
Starch	0.54 ± 0.01	0.29 ± 0.02
Insoluble fiber	18.67 ± 0.01	62.85 ± 0.01
Soluble fiber	3.45 ± 0.05	2.10 ± 0.03
Other carbohydrates	56.09 ± 0.05	18.16 ± 0.03

* BGR moisture: 10.22 ± 0.05%; BGA moisture: 9.8 ± 0.03%.

3.2 Subcritical water extraction (SWE) of Brazilian ginseng roots (BGR) compounds

3.2.1 Extraction yield

The influence of the SWE temperature (353-453 K) and static extraction time (5-15 min) were evaluated for BGR extracts and Figure 3 shows the obtained extraction yields. The extraction yields ranged from 40 ± 3 to 68 ± 3 $g \cdot 100g^{-1}$ of BGR. By means of full factorial design and analysis of variance (ANOVA) statistical techniques the results showed that statistically, for BGR, temperature (p-value < 0.001) and static extraction time (p-value = 0.028) had significant effect on extraction yield. It is possible to observe that the increase of temperature led to higher extraction yields because, generally, the increase of temperature

results in: i) increase in the extracting solvent diffusivity into the vegetable matrix; ii) increase in the solute solubility in the solvent extraction; iii) better breaking of bonds between the solute(s) and vegetable matrix and, iv) decrease of the solvent viscosity and surface tension. Combination of these factors led to higher mass transfer rates that resulted in higher extraction yields [41-43]. This effect was already observed for BGR extraction in other studies at ambient pressure using water and hydroalcoholic mixtures as extracting solvents in the range of temperature from 313 to 333 K [22], and for PLE using ethanol and ethanol:water (80:20, v/v) as extracting solvents in the range of temperature from 353 to 393 K [21]. Also, it was observed that the extraction yield for BGR was favored by the increase of static extraction time. Higher static extraction times provided higher contact time between the vegetable matrix and solvent, resulting in higher mass transfer rates.

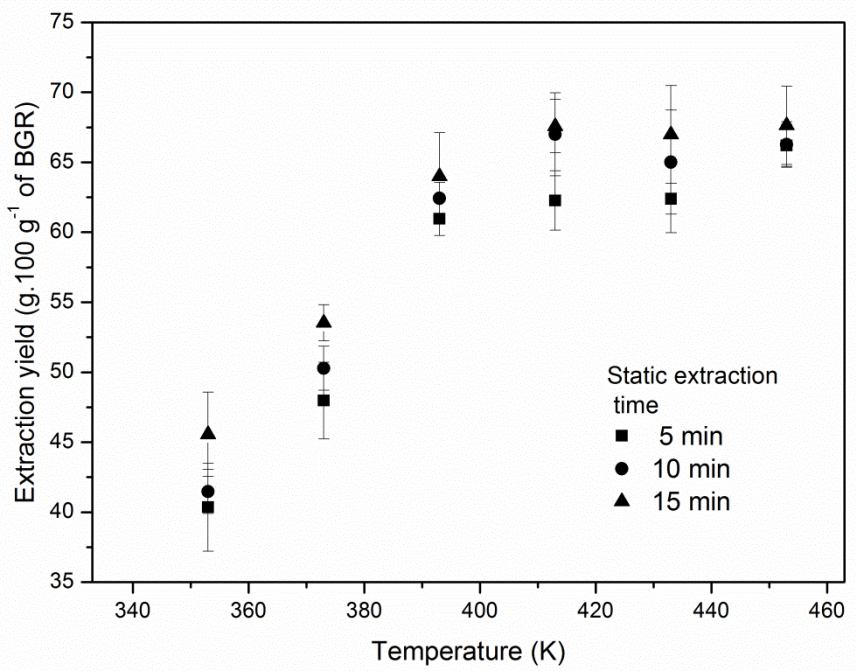

Figure 3: Extraction yields obtained for BGR in different conditions of temperature and static extraction time at 12 MPa.

3.2.2 Beta-ecdysone recovery

The influence of the SWE operational parameters was also evaluated for BGR extracts regarding to beta-ecdysone recovery. The beta-ecdysone content in the extracts obtained from BGR ranged from 0.1 to 0.7 g·100g^{-1} of extract (d.b.), which represents that the highest beta-ecdysone concentration in the extracts was 0.7% (d.b.) (Figure 4). Other authors

studied the beta-ecdysone extraction from BGR by PLE and supercritical fluid extraction (SFE) [21, 44]. The beta-ecdysone content in BGR extracts obtained by PLE with ethanol at 393 K and 8 MPa was 5% (d.b.) [21] and extracts obtained by SFE with CO_2:etanol (90:10, v/v) at 20 MPa and 393 K had 8% of beta-ecdysone (d.b.) [44] , both higher than that obtained in this study. This result might be due to the higher selectivity of these solvents for beta-ecdysone compared to water.

Statistically, the beta-ecdysone content in the BGR extracts was influenced by the parameters temperature (p-value < 0.001) and static extraction time (p-value = 0.001). In Figure 4A, it is observed that the highest beta-ecdysone content in the extract (0.7 $g \cdot 100g^{-1}$ of extract, d.b.) was obtained at 373 K, where the water dielectric constant (ε) is approximately 55 [19]. Literature reports that the decrease in the water dielectric constant caused by increase of temperature can favor the solubilization of more apolar compounds [45, 46]. However, at temperatures above 373 K the beta-ecdysone content decreased, although the water dielectric constant also decreased. Although the melting point of beta-ecdysone is above 513 K, the decrease of beta-ecdysone content observed at elevated temperatures (above 373 K) can be due to changes in the beta-ecdysone molecule caused by chemical reactions and/or due to changes in the interactions between the vegetable matrix and the beta-ecdysone that hampered its extraction. Furthermore, it can be observed that for all studied temperatures the increase of static extraction time decreased the beta-ecdysone content in the BGR extracts, probably because higher static extraction times favor the reactions in the vegetable matrix, as well as in the beta-ecdysone molecule, as mentioned before.

The beta-ecdysone content expressed related to the mass of raw material used during the extraction (Figure 4B) represents the amount of beta-ecdysone recovered from BGR and, therefore, it takes into account the extraction yield obtained in each extraction condition. In the Figure 4B, it can be observed that at 393 K and 15 min of static extraction time the maximum beta-ecdysone recovery (0.4 $g \cdot 100g^{-1}$ of BGR) was achieved. This value is associated with the high extraction yield obtained in this condition. Debien et al. [21] obtained a beta-ecdysone recovery of 0.24 $g \cdot 100\ g^{-1}$ of BGR using PLE with ethanol, while Santos et al. [44], obtained a beta-ecdysone recovery of only 0.022 $g \cdot 100\ g^{-1}$ of BGR by SFE with CO_2:etanol (90:10, v/v), both less than that obtained in this study. This happens since the extraction yield obtained when water is used as extracting solvent is higher than that when using ethanol and/or CO_2, as water is more polar and has higher solvation power. It this way, water can recover greater amounts of beta-ecdysone from BGR, but the extract obtained also

contains other compounds resulting in an extract with low purity in terms of this target compound.

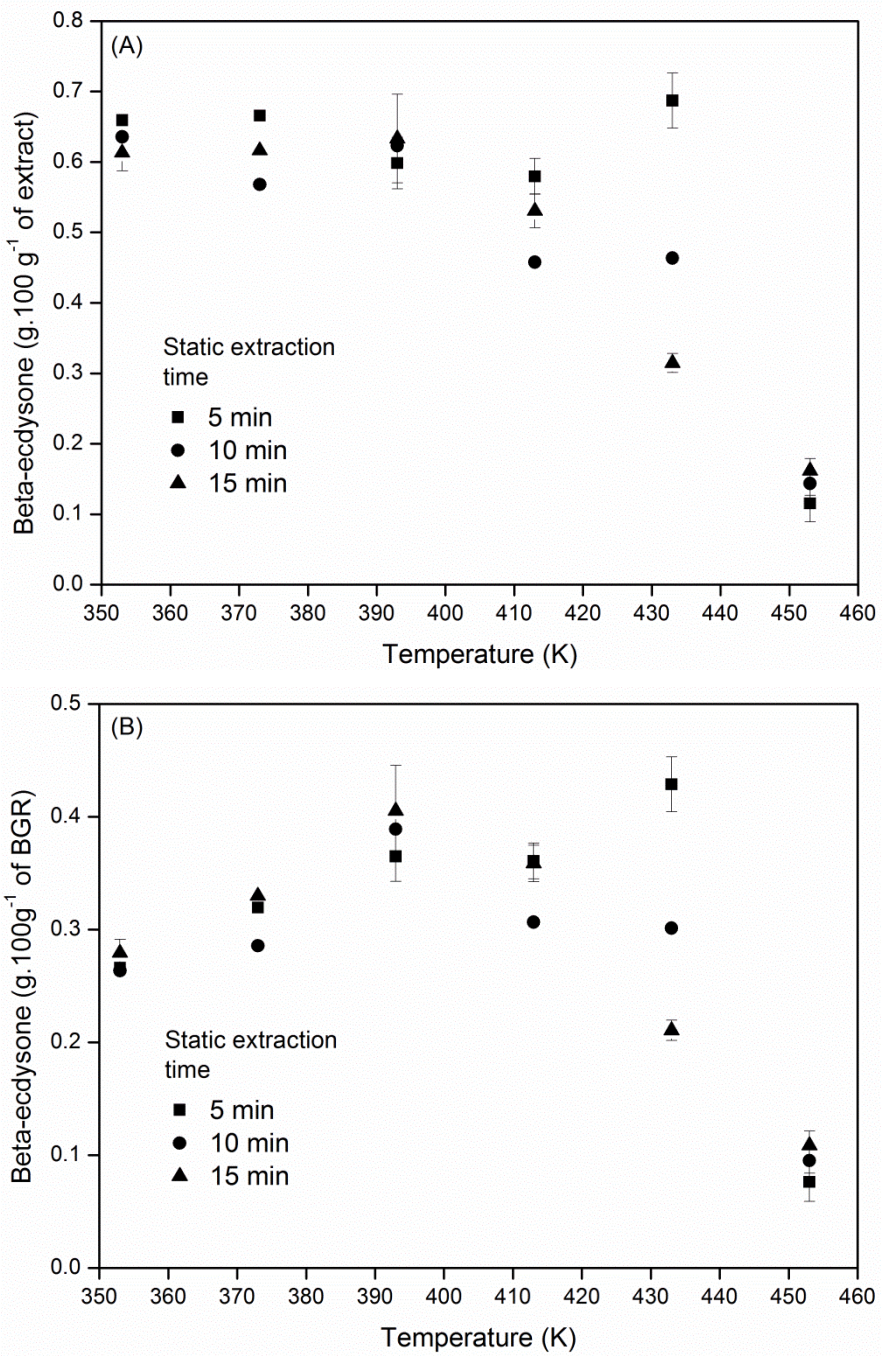

Figure 4: Beta-ecdysone content obtained from BGR in different extraction conditions at 12 MPa. (A) Results expressed in terms of BGR extract mass; (B) Results expressed in terms of BGR mass.

3.2.3 Total sugar content and frutooligosaccharides (FOS) recovery

Figure 5 shows the total sugar content of the extracts obtained from BGR, which ranged from 18 ± 1 to 66 ± 4 g·100 g^{-1} of extract. This result is in agreement with that reported by Caleffi et al. [8] where a total sugar content of 68.86 g·100 g^{-1} of extract was obtained, in which 59.66% was fructose. Statistically, the total sugar content was significantly affected by the temperature (p-value = 0.025). It can be seen that the total sugar content increased up to the temperature of 433 K for the static extraction times of 5 and 10 minutes and for the static extraction time of 15 minutes, the total sugar content increased only up to 413 K. This may be due to the higher contact time between the solvent and plant material under high temperature. Above these temperatures, the total sugar content decreased may due to degradation reactions, such as caramelisation and Maillard reactions. Caramelisation reaction starts at temperatures above 393 K while Maillard reaction proceeds effectively at temperatures above 323 K in the presence of amino acids [47], corroborating with the results obtained in this study. Also, these reactions result in products with colors between yellow and brown, depending on the temperature used [47], as it was observed in the BGR extracts obtained in this study (Supplementary Figure 1).

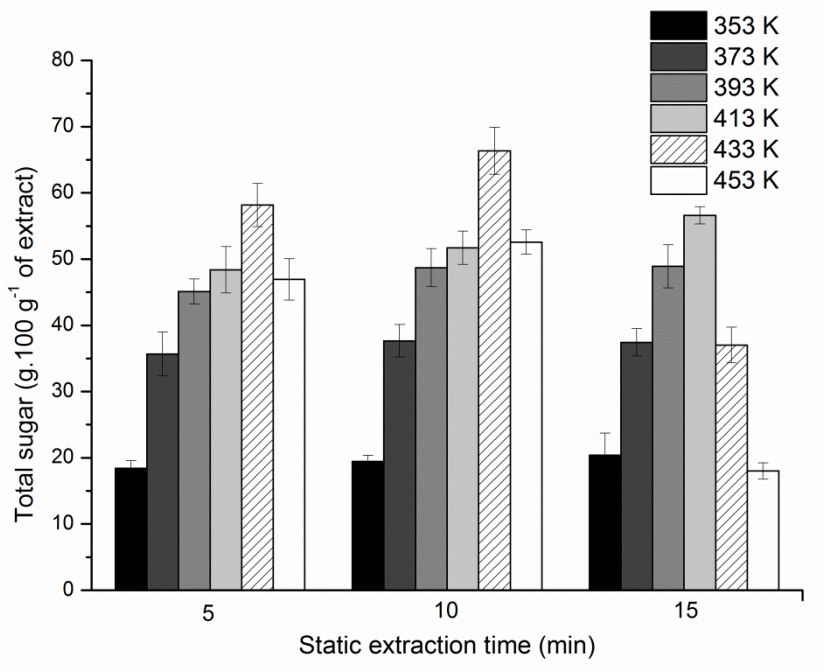

Figure 5: Total sugar content of the BGR extracts (dry basis) obtained in different extraction conditions at 12 MPa.

According to Caleffi et al. [8], inulin-type fructans with prebiotic activity are the major constituents of the carbohydrates of BGR. Based on this statement and on the high total sugar content obtained, the BGR extracts were also investigated regarding to the fructooligosaccharides (FOS) and inulin content. Among the several peaks observed in the chromatograms, three FOS were identified and quantified: 1-kestose (GF2), nystose (GF3) and fructofuranosylnystose (GF4) (Table 3 and Figure 6). The peaks observed from 20.8 to 31.0 min can be FOS with higher degree of polymerization and inulin, however more detailed analyses are necessary to confirm this hypothesis.

The total FOS content in the BGR extracts ranged from 2.0 to 8.8 $g \cdot 100^{-1}$ g of extract (d.b.), where the GF2 content ranged from 0.7 to 2.3 $g \cdot 100^{-1}$ g of extract (d.b.), GF3 content ranged from 0.6 to 2.8 $g \cdot 100^{-1}$ g of extract (d.b.) and GF4 content ranged from 0.8 to 3.7 $g \cdot 100^{-1}$ g of extract (d.b.) (Figure 7A). Temperature (p-value< 0.001) and static extraction time (p-value = 0.041) had significant effect on the total FOS content of BGR extracts. According to the statistical analysis, highest total FOS content in the BGR extract are obtained at 393 K and 5 min of static extraction time.

Figure 7B shows the total FOS content related to the mass of raw material (dry basis), which ranged between 1.5 and 5.5 $g \cdot 100g^{-1}$ of BGR (d.b.). It was also observed that at temperatures above 393 K for the static extraction times of 5 and 10 min and above 413 K for the static extraction time of 15 min the total FOS content decreased. As it was observed for the total sugar content, FOS were degraded in higher temperatures due to caramelisation and Maillard reactions [47]. Other authors reported the extraction of prebiotic carbohydrates from artichoke industrial waste and obtained a FOS content of 0.79 $g \cdot 100\ g^{-1}$ of dry raw material [14]. Jovanovic- Malinovska et al., [48] studied the extraction of prebiotic carbohydrates from several vegetables and fruits and obtained the highest FOS content in scallion (6.2 ± 0.2 $g \cdot 100\ g^{-1}$ of fresh raw material) while the fruit with highest FOS content was nectarine (1.75 ± 0.08 $g \cdot 100\ g^{-1}$ of fresh raw material).

Comparing the prebiotic carbohydrates content obtained from BGR extract with those obtained from other raw materials, BGR can be considered a promising source of these compounds, since it showed a total FOS content 2-fold higher than that obtained from artichoke industrial waste [14]. It is also important to note that the FOS content obtained from BGR can be improved, since there are other compounds that seem to be fructooligosaccharides with higher degree of polymerization (as shown in Figure 5). Then, with further analyses these compounds could be identified and quantified.

Table 3: Parameters of HPLC-ELSD method for the fructooligosaccharides quantified.

Fructooligosaccharide	**Retention time (min)**	**Linearity (mg.L^{-1})**	**R^2**
1- kestose (GF2)	17.848	65-1000	0.981
Nystose (GF3)	19.516	65-1000	0.995
Fructofuranosylnystose (GF4)	20.852	65-1000	0.998

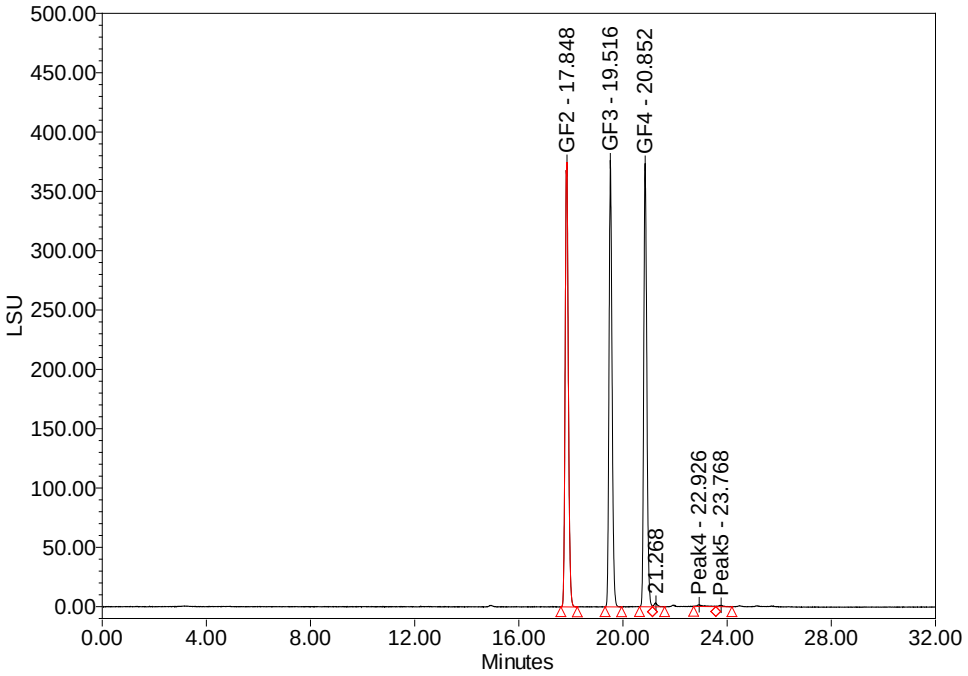

Figure 6: HPLC/ELSD chromatograms of (A) fructooligosaccharides standards and of (B) an extract obtained by SWE.

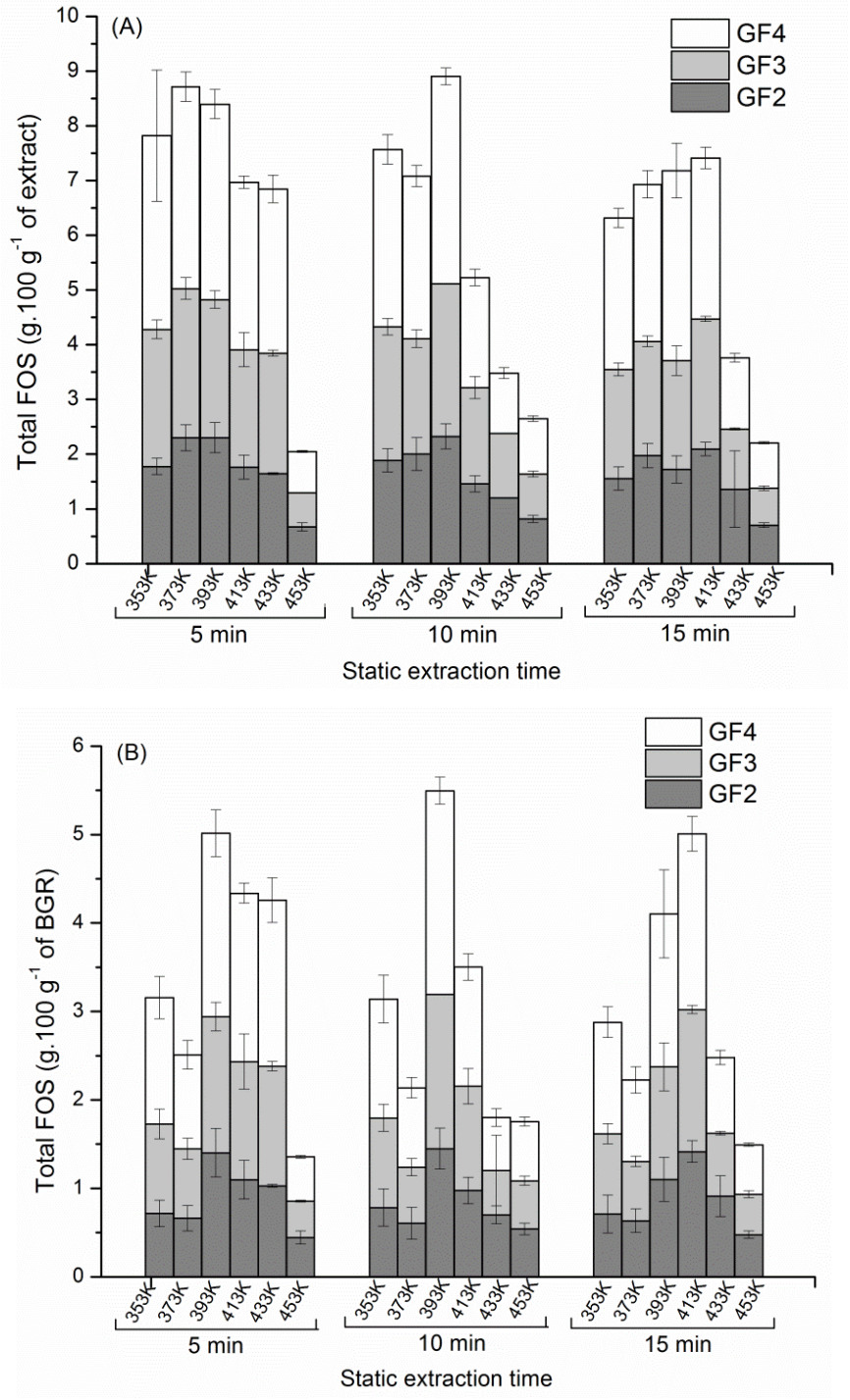

Figure 7: Total FOS content obtained from BGR (dry basis) in different extraction conditions at 12 MPa. (A) Results expressed in terms of BGR extract mass; (B) Results expressed in terms of BGR mass.

3.3 Subcritical water extraction (SWE) of Brazilian ginseng aerial parts (BGA) compounds

3.3.1 Extraction yield

Some authors reported that Brazilian ginseng aerial parts (BGA) contain higher contents of bioactive compounds than BGR, mainly related to beta-ecdysone content [15, 16]. Therefore, the influence of the SWE parameters temperature (353-453 K), static extraction time (5-10 min) and pressure (2-12 MPa) were evaluated on extraction yield and beta-ecdysone recovery from BGA. Figure 8 shows the extraction yield data obtained from BGA, which ranged from 18.3 ± 0.3 to 30.2 ± 0.2 $g \cdot 100g^{-1}$ of BGA (dry basis). The maximum extraction yield obtained from BGA was approximately half that obtained from BGR. It can be due to the high insoluble fibers content present in BGA (Table 2) that could not be extracted by water in the studied conditions.

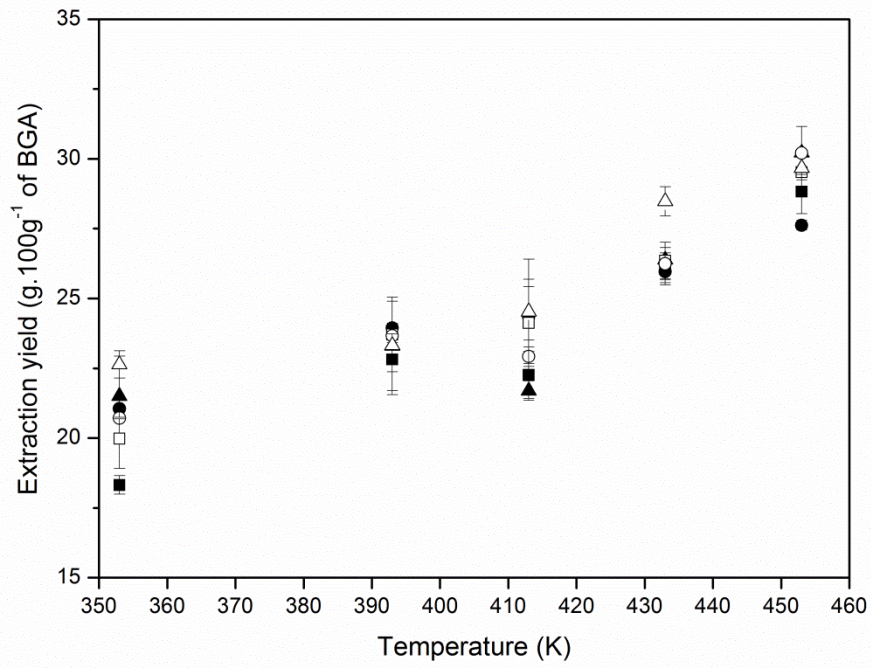

Figure 8: Extraction yields obtained for BGA in different extraction conditions. Pressure: (■,□) 2 MPa; (●,○) 7 MPa; (▲,Δ) 12 MPa. Static extraction time: fill symbols: 5 min; empty symbols: 10 min.

Statistically, temperature (p-value < 0.001), static extraction time (p-value <0.001) and pressure (p-value = 0.004) were significant for extraction yield obtained from

BGA. As discussed previously, the increase in temperature and static extraction time results in higher extraction yields [41-43], as well as the increase of pressure. The pressure could facilitate the solvent penetration into the vegetable matrix porous, which helps the solute solubilization in the solvent [41].

3.3.2 Beta-ecdysone recovery

The highest beta-ecdysone content obtained in BGA extracts was 0.3 $g \cdot 100g^{-1}$ of extract (d.b.) and corresponds to 0.07 $g \cdot 100g^{-1}$ of BGA (d.b.) (Figure 9). Statistically, only temperature had a significant effect (p-value < 0.001) on beta-ecdysone content in the BGA extracts. As well as for BGR, starting at 393 K, the beta-ecdysone content in the BGA extracts decreased (Figure 9A) and, in this case, the same behavior was observed for beta-ecdysone recovery in terms of raw material (Figure 9B). In Figure 9B, it can be observed clearly that the pressure and static extraction time were not significant for obtaining beta-ecdysone-rich extracts, which is in agreement with the statistical analysis. Therefore, the beta-ecdysone recovery from BGA was maximized at 353 K, 2 MPa and 5 min of static extraction time.

The beta-ecdysone content recovered from BGA was 5.9-fold less than that obtained from BGR. Data from literature reported a different behavior from that observed in this study where higher beta-ecdysone contents were obtained from BGA than from BGR [15, 16]. Festucci-Buselli et al. [49] reported that the beta-ecdysone content in the aerial parts of BGR remained constant over time, while the beta-ecdysone content in the roots increased, suggesting that this compounds is accumulated in the roots. In this work the Brazilian ginseng plants were grown for 7 years, while in the study reported by Flores [15] the plants were grown for only 2 years. Thus, the plants used in this work could be accumulated more beta-ecdysone in the roots than in the aerial parts due the longer time of cultivation.

Although the beta-ecdysone recovery from BGA was lower than that obtained from BGR, it can be considered as an alternative source for this compounds, since currently only the BGR is commercially used to obtain beta-ecdysone [16]. Additionally, BGA can be an alternative source of beta-ecdysone when its demand increases without increasing plantation areas, which is very desirable when thinking at the development of sustainable green production processes. Also, a study showed that BGA can be used as fuel to supply the energetic requirements of a process to obtain beta-ecdysone-rich extracts [26].

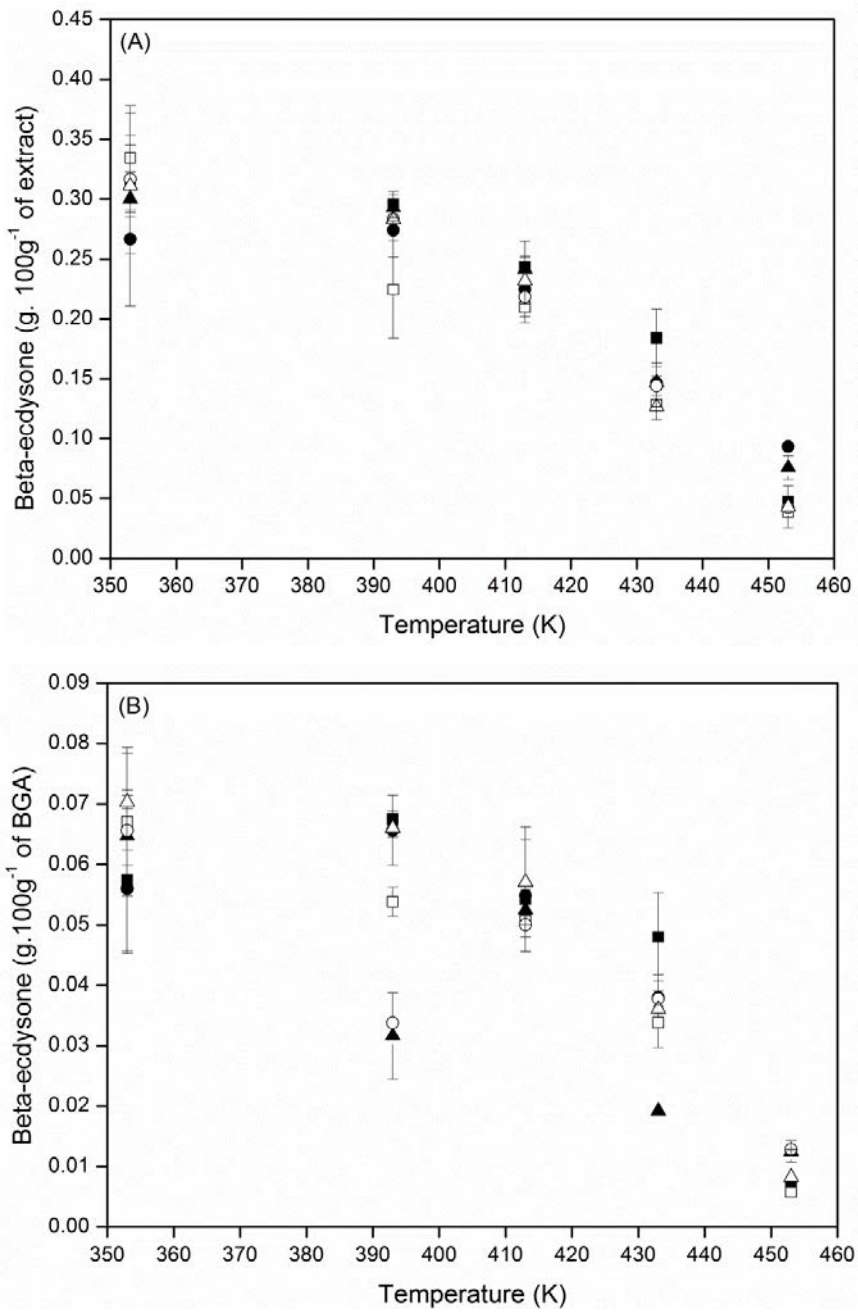

Figure 9: Beta-ecdysone content obtained from BGA in different extraction conditions. Pressure: (■,□) 2 MPa; (●,○) 7 MPa; (▲,Δ) 12 MPa. Static extraction time: fill symbols: 5 min; empty symbols: 10 min. (A) Results expressed in terms of BGA extract mass; (B) Results expressed in terms of BGA mass.

3.4 Economic evaluation

Although both BGR and BGA can be used as raw materials to obtain several bioactive compounds, until this moment the beta-ecdysone is the main compound present in

Brazilian ginseng with stablished commercial value. Therefore, the economic evaluation of the process was carried out to assess the feasibility of utilization of BGR and BGA as raw materials to obtain extracts by SWE taking into account the beta-ecdysone content of the extracts. However, it is extremely important to mention that the feasibility of the processes can be changed, if the other compounds present in the extracts became commercially explored, such as the prebiotic carbohydrates present in the BGR extracts.

Based on this context, the economic evaluation for the beta-ecdysone extraction from BGR and BGA was performed, considering the extraction conditions that maximized the beta-ecdysone recovery from each raw material. In this way, for BGR, the process was simulated using 393 K, 15 min of static extraction time and 12 MPa; for BGA, the process was simulated using 353 K, 5 min of static extraction time and 2 MPa. In these conditions, the annual operating cost (AOC) for BGR extracts was US$ 3.52 million and for BGA extracts was US$ 1.09 million. As it can be seen in Figure 10, the higher AOC obtained for BGR extracts was due to the costs linked to raw material acquisition. Nevertheless, the cost of manufacturing (COM) for BGR extracts was US$ 23.33·kg^{-1} of extract while the COM for BGA extracts was US$ 31.54·kg^{-1} of extract. This result is mainly related to the lower BGA extraction yield, since the COM is obtained as a ratio between the AOC and annual production. The BGR extract production was 150.8 ton·year^{-1} and BGA production was 40.8 ton·year^{-1}, resulting in higher COM for BGA extracts.

Based on the beta-ecdysone content and on the extraction yield obtained from each raw material, the estimated productivity of beta-ecdysone from BGR was 10-fold larger than that from BGA (Figure 10). Using the productivity data, it was possible to estimate the COM regarding to beta-ecdysone. It is important to note that the specific COM of beta-ecdysone does not take into account the purification steps needed to obtain the pure compound. It was observed that using BGR as raw material the COM of beta-ecdysone was US$ 3.48·g^{-1}, while using BGA it was US$ 10.51·g^{-1}. Therefore, the manufacturing of BGR aiming at beta-ecdysone-rich extract production is more adequate than of the processing of BGA. Debien et al. [21] reported that the specific COM for beta-ecdysone obtained from BGR using pressurized ethanol was US$ 9.31·g^{-1} (considering extraction vessels of 0.4 m^3), suggesting that the BGR processing using SWE is economically more attractive. However, it is important to highlight that the aqueous extract contain several other compounds co-extracted with beta-ecdysone. Then the aqueous extract has low beta-ecdysone purity and can require purification steps depending on the further application.

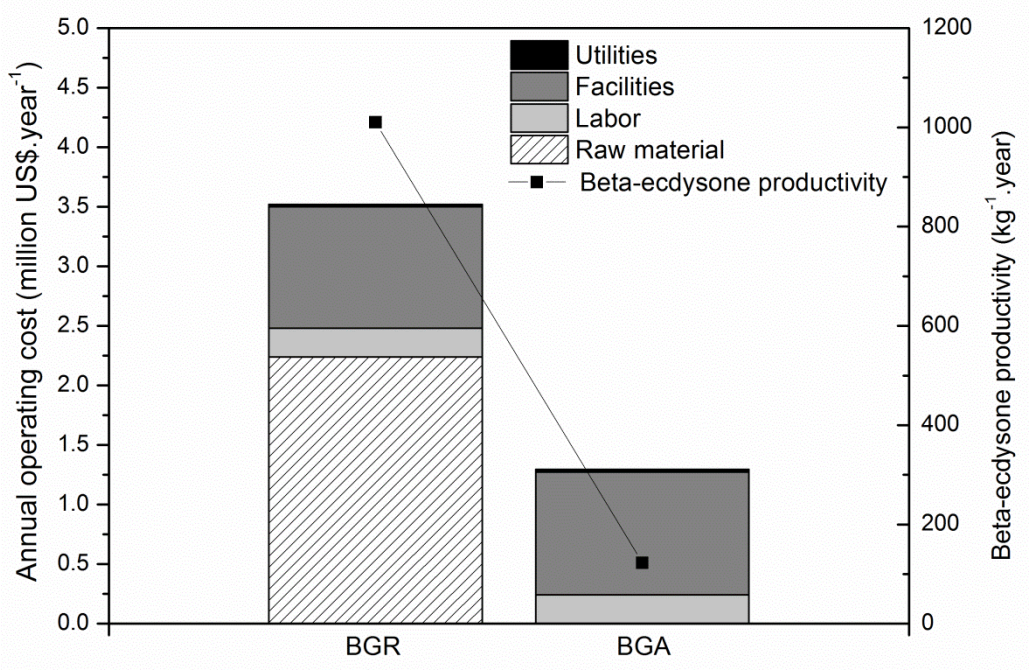

Figure 10: Annual operating cost of extracts from BGR and BGA (bars). Beta-ecdysone productivity of the extracts obtained from BGR and BGA (line).

Considering the composition of the BGR and BGA extracts, it is difficult to stablish selling prices for these products. Thus, a commercial product was used as reference to stablish the selling prices for the BGR and BGA extracts obtained in this work. Pills containing 300 mg of BGR extract standardized to contain 0.96% (w/w) of beta-ecdysone are commercialized in boxes with 45 units which can be sold for US$ 4.74 [50]. Assuming that this selling price is related to a final product, we considered that the selling price for the crude extract containing 0.96% (w/w) of beta-ecdysone represents 25% of the final selling price, i.e., US$ 1.18. Based on these considerations, the selling price considered for a BGR extract containing 0.7% of beta-ecdysone was US$ 61.22·kg^{-1} of extract and the selling price for the BGA extract containing 0.3% of beta-ecdysone was US$ 27.41·kg^{-1} of extract.

To assure the feasibility of the process, some profitability ratios were calculated and are presented in Table 4. Gross margin is an economic indicator that measures the percentage of the annual revenues that is gross profit. In other words, gross margin represents the portion of each dollar of revenue that the company retains as gross profit. The gross margin for BGR extracts was 61% and for BGA extracts the gross margin was a negative value. The return on investment (ROI) is a tool used to evaluate the efficiency of an investment or to compare the efficiency of a number of different investments. ROI measures

the amount of return of an investment relative to the cost of investment. ROI value for BGR extracts was 69.97% and for BGA extracts was 3.71%. According to the literature, a minimum value ROI of 10 to 15% is stablished to accept or discard a project [51, 52]. Additionally, the payback time represents the time required to recover the cost of investment and is calculated based on the ROI. Obviously, the shorter the payback time is, the more attractive is the project. While the payback time for BGR extracts was less than 2 years, for BGA extracts was almost 27 years. Finally, the net present value (NPV) represents the total value of future net cash flows during the life time of the project, i.e. it is the remaining surplus for the investor to have regain in the initial investment. If an investment does not have a positive NPV, or if there are other opportunities with higher NPV, the investment should not be undertaken. Then, based on the values obtained in this work, it enables us to conclude that while the manufacturing of BGR extracts is a great opportunity for business, the manufacturing of BGA extracts is not feasible.

Table 4: Profitability ratios for extracts obtained from Brazilian ginseng roots (BGR) and Brazilian ginseng aerial parts (BGA).

Profitability ratio	**BGR extract**	**BGA extract**
Gross margin (%)	60.70	-18.66
ROI (%)	69.97	3.71
Payback time (years)	1.43	26.98
NPV (million US$)	21.06	-3.59

Although this study demonstrated that BGA is not useful to obtain beta-ecdysone-rich extracts, Santos et al. [26] showed that it can be used as fuel to produce energy to fulfill the energetic requirements of the process. They evaluated the dry BGR extract production in a similar SWE process and observed that use of the solid residue of BGR obtained from the extraction process plus 50% of the total amount of BGA left in the field during the harvest of BGR is enough to supply all the energetic requirements of the proposed process. Therefore, it could improve the revenues of the BGR extracts production, since the utilities costs are reduced. Furthermore, the possibility of utilization of the whole plant in the SWE process meets the green process concepts, since the solvent used is no toxic, the energy supplies are from renewable sources and the waste generation is minimized.

4. CONCLUSIONS

Brazilian ginseng roots (BGR) extracts obtained by subcritical water extraction (SWE) showed high bioactive compounds content, since it contains up to 0.7 $g \cdot 100g^{-1}$ of extract of beta-ecdysone and up to 8.8 $g \cdot 100g^{-1}$ of extract of prebiotic carbohydrates, including 1-kestose (GF2), nystose (GF3) and fructofuranosylnystose (GF4). Moreover, the Brazilian ginseng aerial parts (BGA) extracts showed a lower beta-ecdysone content (0.3 $g \cdot 100g^{-1}$ of extract). The economic evaluation showed that the manufacturing of BGR for beta-ecdysone production had a good profitability with a short payback time of 1.43 years, while the manufacturing of BGA is not feasible from the economic point of view. However, BGA could be used as fuel to supply the energetic requirements of the process and then, enable the utilization of the whole plant.

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REFERENCES

[1] A.G. Neto, J.M.L.C. Costa, C.C. Belati, A.H.C. Vinhólis, L.S. Possebom, A.A. Da Silva Filho, W.R. Cunha, J.C.T. Carvalho, J.K. Bastos, M.L.A. e Silva, Analgesic and anti-inflammatory activity of a crude root extract of Pfaffia glomerata (Spreng) Pedersen, Journal of Ethnopharmacology, 96 (2005) 87-91.

[2] N.R. Sanches, R. Galletto, C.E. Oliveira, R.B. Bazotte, D.A.G. Cortez, Avaliação do potencial anti-hiperglicemiante de *Pfaffia glomerata* (Spreng.) Pedersen (Amaranthaceae), Acta Scientiarum, 23 (2001) 613-617.

[3] C.S. Freitas, C.H. Baggio, J.E. Da Silva-Santos, L. Rieck, C.A. de Moraes Santos, C.C. Júnior, L.C. Ming, D.A. Garcia Cortez, M.C.A. Marques, Involvement of nitric oxide in the gastroprotective effects of an aqueous extract of Pfaffia glomerata (Spreng) Pedersen, Amaranthaceae, in rats, Life Sciences, 74 (2004) 1167-1179.

[4] A.G. Neto, A.A. da Silva Filho, J.M.L.C. Costa, A.H.C. Vinholis, G.H.B. Souza, W.R. Cunha, M.L.A.E. Silva, S. Albuquerque, J.K. Bastos, Evaluation of the trypanocidal and leishmanicidal in vitro activity of the crude hydroalcoholic extract of Pfaffia glomerata (Amarathanceae) roots, Phytomedicine, 11 (2004) 662-665.

[5] S. Nakamura, G. Chen, S. Nakashima, H. Matsuda, Y. Pei, M. Yoshikawa, Brazilian natural medicines. IV. New Noroleanane-type triterpene and ecdysterone-type sterol glycosides and melanogenesis inhibitors from the roots of *Pfaffia glomerata*, Chem. Pharm. Bull., 58 (2010) 690-695.

[6] A.C. Gomes, M. Nicole, J.K. Mattos, S.I.V. Pereira, P. Pereira, D. Silva, R. Vieira, G.d. Capdeville, A. Moita, R. Carneiro, Concentration of β-ecydisone (20E) in susceptible and resistant accessions of *Pfaffia glomerata* infected with *Meloidogyne incognita*and histological characterisation of resistance, Nematology, 12 (2010) 701-709.

[7] C.C.J. Vieira, R.C.L. Figueiredo-Ribeiro, Fructose-containing carbohydrates in the tuberous root of Gomphrena macrocephala St.-Hil. (Amaranthaceae) at different phenological phases, Plant, Cell & Environment, 16 (1993) 919-928.

[8] E.R. Caleffi, G. Krausová, I. Hyršlová, L.L.R. Paredes, M.M. dos Santos, G.L. Sassaki, R.A.C. Gonçalves, A.J.B. de Oliveira, Isolation and prebiotic activity of inulin-type fructan extracted from Pfaffia glomerata (Spreng) Pedersen roots, International Journal of Biological Macromolecules, 80 (2015) 392-399.

[9] M.B. Roberfroid, G.R. Gibson, L. Hoyles, A.L. McCartney, R. Rastall, I. Rowland, Prebiotics effects: Metabolic and health benefits, British Journal of Nutrition, 104 (2010) 1-63.

[10] N. Saad, C. Delattre, M. Urdaci, J.M. Schmitter, P. Bressollier, An overview of the last advances in probiotic and prebiotic field, LWT - Food Science and Technology, 50 (2013) 1-16.

[11] M. Gudiel-Urbano, I. Goñi, Effect of fructooligosaccharide on nutritionalparameters and mineral bioavailability in rats, Journal of Science and Food Agriculture, 82 (2002) 913-917.

[12] B.L. Pool-Zobel, Inulin-type fructans and reduction in colon cancer risk:review of experimental and human data, British Journal of Nutrition, 93 (2005) 73-90.

[13] N.M. Delzenne, C. Daubioul, A. Neyrinck, M. Lasa, H.S. Taper, Inulin andoligofructose modulate lipid metabolism in animals: review of biochemicalevents and future prospects, British Journal of Nutrition, 87 (2002) 255-259.

[14] M.T.C. Machado, K.S. Eça, G.S. Vieira, F.C. Menegalli, J. Martínez, M.D. Hubinger, Prebiotic oligosaccharides from artichoke industrial waste: evaluation of different extraction methods, Industrial Crops and Products, 76 (2015) 141-148.

[15] R. Flores, D. Brondani, V. Cezarotto, S.R. Giacomelli, F.T. Nicoloso, Micropropagation and beta-ecdsysone content of the Brazilian ginsengs *Pfaffia glomerata* and *Pfaffia tuberosa*, In vitro Cellular & Developmental Biology - Plant 46 (2010) 210-217.

[16] L.Z. Serra, D.F. Felipe, D.A.G. Cortez, Quantification of beta-ecdysone in different parts of *Pfaffia glomerata* by HPLC, Brazilian Journal of Pharmacognosy, 22 (2012) 1349-1354.

[17] M. Ravber, Ž. Knez, M. Škerget, Isolation of phenolic compounds from larch wood waste using pressurized hot water: extraction, analysis and economic evaluation, Cellulose, 22 (2015) 3359-3375.

[18] M. Herrero, M. Castro-Puyana, J.A. Mendiola, E. Ibañez, Compressed fluids for the extraction of bioactive compounds, TrAC Trends in Analytical Chemistry, 43 (2013) 67-83.

[19] M. Herrero, A. Cifuentes, E. Ibañez, Sub- and supercritical fluid extraction of functional ingredients from different natural sources: Plants, food-by-products, algae and microalgae: A review, Food Chemistry, 98 (2006) 136-148.

[20] J.F. Osorio-Tobón, P.I.N. Carvalho, M.A. Rostagno, A.J. Petenate, M.A.A. Meireles, Extraction of curcuminoids from deflavored turmeric (Curcuma longa L.) using pressurized liquids: Process integration and economic evaluation, The Journal of Supercritical Fluids, 95 (2014) 167-174.

[21] I.C.N. Debien, R. Vardanega, D.T. Santos, M.A.A. Meireles, Pressurizes liquid extraction as a promising and economically feasible technique for obtaining beta-ecdysone-rich extracts from Brazilian ginseng roots, Separation Science and Technology, 50 (2015) 1-11.

[22] R. Vardanega, D.T. Santos, M.A.A. Meireles, Production of biosurfactant from Brazilian ginseng roots by low-pressure solvent extraction with and without the assistance of ultrasound, Recent Patents on Engineering, 8 (2014) 69-81.

[23] ASAE, Standards methods of determination and spressing fineness of feed materials by sieving, in, 1998, pp. 547.

[24] AOAC, Official Methods of Analysis of AOAC International, 19th ed., AOAC, 2012.

[25] AACC, Aproved Methods, 11th ed., AACC, 2010.

[26] D.T. Santos, J.Q. Albarelli, M.A.A. Meireles, Simulation of an Integrated Sustainable Production of Extract from Brazilian Ginseng Roots with a Cogeneration Plant, Chemical Engineering Transactions, 29 (2012) 91-96.

[27] M.A. Rostagno, I.C.N. Debien, R. Vardanega, G.F. Nogueira, G.F. Barbero, M.A.A. Meireles, Fast analysis of β-ecdysone in Brazilian ginseng (Pfaffia glomerata) extracts by high-performance liquid chromatography using fused-core column, Analytical Methods, 6 (2014) 2452-2459.

[28] N. Nelson, A photometric adaptation of the Somogyi method for the determination of glucose, The Journal of Biological Chemistry, 153 (1944) 375-379.

[29] G.L. Miller, Use of Dinitrosalicylic Acid Reagent for Determination of Reducing Sugar, Analytical Chemistry, 31 (1959) 426-428.

[30] R. Turton, R.C. Bailie, W.B. Whiting, J.A. Shaeiwits, Analysis, synthesis, and design of chemical processes, 3rd ed., Prentice Hall, New Jersey, 2009.

[31] R. Vardanega, J.M. Prado, M.A.A. Meireles, Adding value to agri-food residues by means of supercritical technology, The Journal of Supercritical Fluids, 96 (2015) 217-227.

[32] J.M. Prado, T. Forster-Carneiro, M.A. Rostagno, L.A. Follegatti-Romero, F. Maugeri Filho, M.A.A. Meireles, Obtaining sugars from coconut husk, defatted grape seed, and pressed palm fiber by hydrolysis with subcritical water, J Supercrit Fluid, 89 (2014) 89-98.

[33] M. Odlare, V. Arthurson, M. Pell, K. Svensson, E. Nehrenheim, J. Abubaker, Land application of organic waste - effects in the soil ecosystem, Applied Energy, 88 (2011).

[34] P.C. Veggi, R.N. Cavalcanti, M.A.A. Meireles, Production of phenolic-rich extracts from Brazilian plants using supercritical em subcritical extraction: experimental data and economic evaluation, J Food Eng, 131 (2014) 96-109.

[35] P.I.N. Carvalho, J.F. Osorio-Tobón, M.A. Rostagno, A.J. Petenate, M.A.A. Meireles, Techno-economic evaluation of the extraction of turmeric (Curcuma longa L.) oil and ar-turmerone using supercritical carbon dioxide, The Journal of Supercritical Fluids, 105 (2015) 44-54.

[36] SantosFlora, in, 2015.

[37] CPFL, (2015).

[38] S.C. Alcázar-Alay, F.P. Cardenas-Toro, D.T. Santos, M.A.A. Meireles, Study of an Extraction Process as the Pretreatment Step for Sugar Production from Acid Hydrolysis, Food and Public Health, 5 (2015) 47-55.

[39] T. Roitsch, M.E. Balibrea, M. Hofmann, R. Proels, A.K. Sinha, Extracellular invertases: metabolic enzyme and metabolic protein., Journal of Experimental Botany, 54 (2003) 513-524.

[40] L. Jaime, F. Martínez, M.A. Martín-Cabrejas, E. Mollá, F.J. López-Andréu, K.W. Waldron, R.M. Esteban, Study of total fructan and fructooligosaccharide content in different onion tissues, Journal of the Science of Food and Agriculture, 81 (2001) 177-182.

[41] A. Mustafa, C. Turner, Pressurized liquid extraction as a green approach in food and herbal plants extraction: A review, Analytica Chimica Acta, 703 (2011) 8-18.

[42] H. Wijngaard, M.B. Hossain, D.K. Rai, N. Brunton, Techniques to extract bioactive compounds from food by-products of plant origin, Food Research International, 46 (2012) 505-513.

[43] C.G. Pereira, M.A.A. Meireles, Supercritical fluid extraction of bioactive compounds: fundamentals, applications and economic perspectives., Food Bioprocess Tech, 3 (2010) 340-372.

[44] D.T. Santos, J.Q. Albarelli, M.A. Rostagno, A.V. Ensinas, F. Maréchal, M.A.A. Meirelres, New proposal for production of bioactive compounds by supercritical technology integrated to a sugarcane biorefinery, Clean Techn Environ Policy, 16 (2014) 1455-1468.

[45] L. He, X. Zhang, H. Xu, C. Xu, F. Yuan, Ž. Knez, Z. Novak, Y. Gao, Subcritical water extraction of phenolic compounds from pomegranate (Punica granatum L.) seed residues and investigation into their antioxidant activities with HPLC–ABTS+ assay, Food and Bioproducts Processing, 90 (2012) 215-223.

[46] E. Ibañez, A. Kubátová, F.J. Señoráns, S. Cavero, G. Reglero, S.B. Hawthorne, Subcritical Water Extraction of Antioxidant Compounds from Rosemary Plants, Journal of Agricultural and Food Chemistry, 51 (2003) 375-382.

[47] L.W. Kroh, Caramelisation in food and beverages, Food Chemistry, 51 (1994) 373-379.

[48] R. Jovanovic-Malinovska, S. Kuzmanova, E. Winkelhausen, Application of ultrasound for enhanced extraction of prebiotic oligosaccharides from selected fruits and vegetables, Ultrasonics Sonochemistry, 22 (2015) 446-453.

[49] R.A. Festucci-Buselli, L.A.S. Contim, L.C.A. Barbosa, J.J. Stuart, R.F. Vieira, W.C. Otoni, Level and distribution of 20-hydroxyecdysone during *Pfaffia glomerata* development, Brazilian Journal of plant physiology, 20 (2008) 305-311.

[50] Herbarium, (2015).

[51] M.M. El-Halwagi, Overview of Process Economics, in: M.M. El-Halwagi (Ed.) Sustainable Design Through Process Integration, Butterworth-Heinemann, Oxford, 2012, pp. 15-61.

[52] M.P. Fernández-Ronco, A. de Lucas, J.F. Rodríguez, M.T. García, I. Gracia, New considerations in the economic evaluation of supercritical processes: Separation of bioactive compounds from multicomponent mixtures, The Journal of Supercritical Fluids, 79 (2013) 345-355.

SUPPLEMENTARY MATERIAL

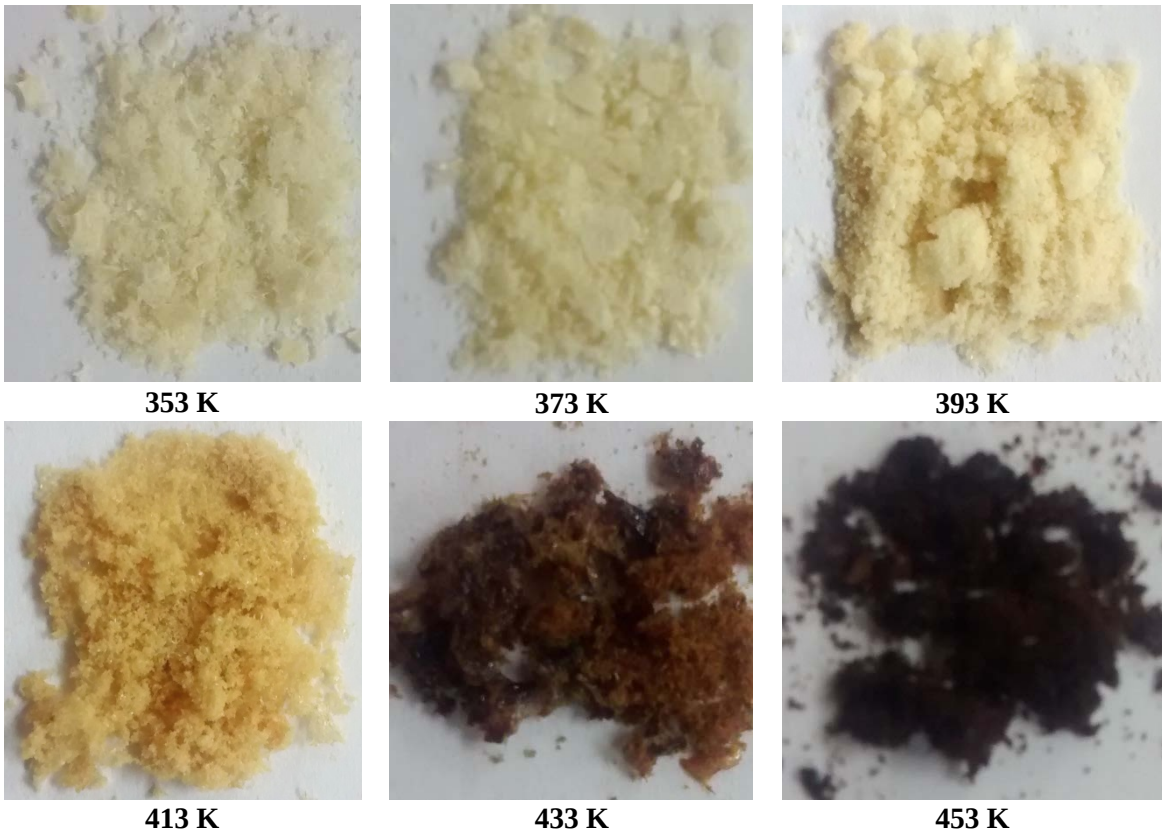

Figure 1: Samples of BGR extracts obtained in different temperatures

CAPÍTULO 5

EXTRAÇÃO INTENSIFICADA PARA OBTENÇÃO DE COMPOSTOS BIOATIVOS DAS RAÍZES DE GINSENG BRASILEIRO

INTENSIFIED PROCESS TO EXTRACT AND FRACTIONATE BIOACTIVE COMPOUNDS FROM BRAZILIAN GINSENG ROOTS

Renata Vardanega, Diego T. Santos, M. Angela A. Meireles*

LASEFI/DEA/FEA (School of Food Engineering)/UNICAMP (University of Campinas), R. Monteiro Lobato, 80, 13083-862, Campinas, São Paulo, Brazil

* Corresponding author: maameireles@gmail.com, meireles@fea.unicamp.br

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ABSTRACT

Brazilian ginseng (*Pfaffia glomerata*) roots contain several bioactive compounds, including beta-ecdysone that shows several therapeutic effects, saponins with surface activity and also fructooligosaccharides (FOS) with prebiotic effects. Regarding to this rich composition, a two-step intensified extraction process that uses the same solid-liquid extraction apparatus was developed to obtain and fractionate the bioactive compounds from Brazilian ginseng roots using ethanol and water as extracting solvents. The intensified process was compared with a conventional extraction process using water as solvent. The beta-ecdysone and saponins were concentrated mainly in the ethanolic extract obtained in the step 1 of the intensified extraction process while the FOS were isolated in the aqueous extract obtained in the step 2. The effect of pressure (0.1, 5 and 10 MPa) was evaluated on extraction yield, beta-ecdysone content, saponins content and reduction rate of water surface tension and FOS content by analysis of variance (ANOVA) statistical method. The highest beta-ecdysone content (5.6%, d.b.) was obtained at ambient pressure (0.1 MPa) while the highest saponins content (55%, d.b,) was obtained at 5 MPa. However, the extracts that had better surface activity also were that obtained at ambient pressure. The kinetic study showed that the suitable process time for the first and second steps were 38 and 110 minutes, respectively.

KEYWORDS: *Pfaffia glomerata*, beta-ecdysone, saponins, surfactant, fructooligosaccharides, process intensification.

1. INTRODUCTION

Brazilian ginseng (*Pfaffia glomerata*) is a native plant from Brazil that has been received attention in the last years due to its therapeutic effects and amphiphilic properties. In this context, researches have been concerned on the products development processes and manufacturing chains for this raw material, such as: (i) improvement of cultivation conditions to maximize the production of the bioactive compounds of interest [1-3]; ii) identification of the compounds present in Brazilian ginseng roots (BGR) and aerial parts besides those already known [4-7]; iii) development and optimization the bioactive compounds extraction [8-11]; iv) pharmacological studies involving the use of the BGR extracts [12-16] and, v) application of BGR extracts for obtaining emulsions [17, 18].

The main bioactive compound of BGR is beta-ecdysone, an ecdysteroid that has analgesic, anti-inflammatory [15] and antihyperglycemic properties [19], gastroprotective effects [12], antimicrobial activity [14] and melanogenesis inhibitors ability [4]. Moreover, there is in the Brazilian market a phytotherapic product indicated to memory enhancement that contains beta-ecdysone from BGR as active compound [20]. In addition, BGR is considered the main source of beta-ecdysone [6]. BGR is also a source of saponins, which can be used as natural surfactants due to the amphiphilic characteristics of these molecules [21]. Although *Quillaja saponaria* and *Yucca schidigera* have been considered as the main commercial sources of saponins from vegetable matrices [22], other sources can be explored to obtain these compounds, including the Brazilian ginseng [8, 10], since studies have shown that saponins from BGR extracts have potential to be used in emulsion systems [17, 18].

Furthermore, BGR is constituted by 68% of carbohydrates, of which 59% are inulin-type fructans [7]. Fructans consist of a series of homologous oligo- and polysaccharides of fructose which can be referred to as fructooligosaccharides (FOS). It is generally accepted that FOS is a common name only for fructose oligomers that are mainly composed of 1-kestose (GF2), nystose (GF3) and fructofuranosylnystose (GF4), in which fructosyl units are bound by β – linkage [23]. These compounds play an important role in the human body and can be considered as prebiotic compounds because they are non-digestible and act in the host by stimulating the growth and/or activity of some microorganisms in the colon, then relieving the constipation [24]. They also can decrease the risk of osteoporosis by increasing mineral absorption, especially calcium [25] and atherosclerosis by lowering the synthesis of triglycerides and reducing plasma cholesterol concentrations [26]. The effective intake level

of prebiotic for reducing the risks of osteoporosis was found to be between 8-10 g per day [27] and for relieving constipation was found to be 15-20 g per day [28].

Regarding to the positive effects of compounds obtained from BGR extracts, studies have been developed to determine the optimum extraction conditions to obtain these compounds using several extraction methods, such as: supercritical fluid extraction [10, 29, 30]; pressurized liquid extraction [11] and low pressure solvent extraction [8]. The proposed processes until this moment determined extraction conditions to obtain only beta-ecdysone or only saponins and to our knowledge, there is no study about the extraction conditions to obtain FOS from BGR. Nevertheless, recent studies have shown the need for the development of processes to recover several products from one raw material aiming the whole use of it for improve the process efficiency using green technologies [31]. These technologies are focused on reducing the solid wastes generation and energy consumption and also on increasing the industry efficiency. It meets the process intensification concept, which includes initiatives that increases the production capacity within a given equipment volume, decrease in energy consumption per ton of product and a reduction in residues formation [32]. Based on these aspects, the aim of this study was to propose an intensified process for Brazilian ginseng roots processing that uses the same solid-liquid extraction apparatus to obtain extracts rich in beta-ecdysone, saponins and FOS, using ethanol and water as solvents, in a sequentially manner. The effect of pressure in the intensified extraction was also studied and it was compared with a conventional extraction process using water as extracting solvent.

2. MATERIAL AND METHODS

2.1 Raw material

Brazilian ginseng roots (BGR) were collected and prepared as described in Vardanega et al. [8]. BGR were washed and dried at 313 K for 5 days in a forced air circulation dryer. Dried BGR (9.2% moisture) were comminuted in a pulse mill (Marconi MA 340, Piracicaba, Brazil) and the larger particles were also comminuted in a knife mill (Tecnal, model TE 631, Piracicaba, Brazil) for 2 s at 18,000 rpm. Milled BGR were separated according to size in Tyler series sieves (W. S. Tyler, Wheeling, IL). The mean diameter of the particles (8.0 μm) was determined by the ASAE method [33]. BGR were stored in freezer (Metalfrio, model DA 420, São Paulo, Brazil) at 263 K.

2.2 Intensified extraction process

2.2.1 Equipment

The apparatus used to develop the intensified extraction process is shown in Figure 1 and it consists of an HPLC pump (Thermoseparation Products, model ConstaMetric 3200 P/F, Florida, USA), a 6.57 mL extraction cell (Waters, serial # 4501374824-10, Pittsburg, USA) containing a sintered metal filter at the bottom and upper parts, an electrical heating jacket and a back pressure regulator (Tescom, 26-1761-24-161, ELK River, USA). More detailed description of the unit can be found elsewhere [34].

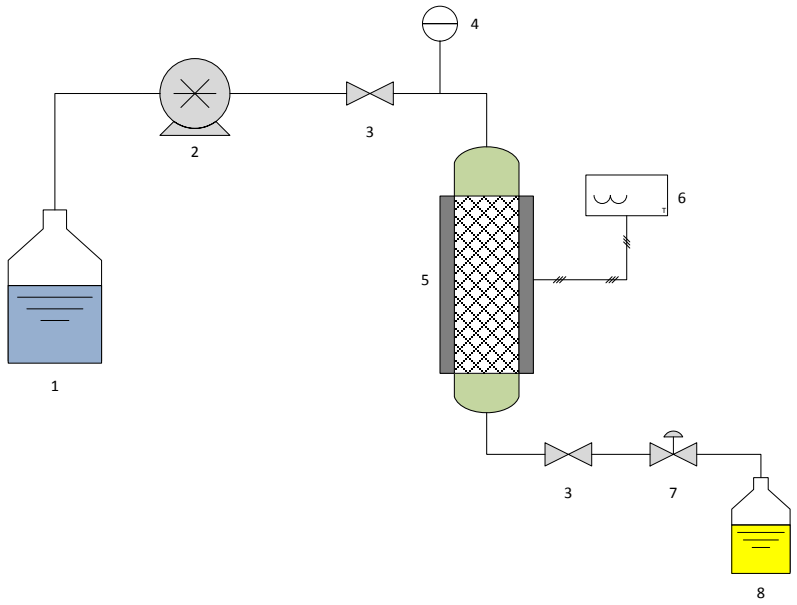

Figure 1: Experimental apparatus used for intensified extraction. (1) Solvent reservoir, (2) Liquid pump, (3) Blocking valves, (4) Pressure gauge; (5) Extraction vessel equipped with jacket for heating; (6) Temperature controller, (7) Back pressure regulator (8) Extract collecting vessel

2.2.2 Extraction procedure

The intensified extraction (IE) was performed in two steps, in a sequentially manner. Ethanol was used as extracting solvent in the first step and water was used in the second one. The effect of the following pressures was evaluated: 0.1 (ambient pressure) (IE-0.1), 5 (IE-5) and 10 (IE-10) MPa. For each experiment 4.5 g (wet basis) of BGR were used. The bed height (H_B) was 2.0 cm and diameter (D_B) was 1.6 cm, resulting in a H_B/D_B ratio of

1.25. The flow rate and temperature were defined as 2.0 mL·min^{-1} and 333 K, respectively, for both steps, as well as the S/F ratio (kg of solvent/kg of raw material, wet basis) of 50. Ethanol was pumped during 113 min in the first step. The second step was started immediately after obtaining the ethanolic extract by replacing the extracting solvent for water. Water was pumped during 144 min to achieve the S/F ratio of 50. A comparative extraction (CE) using only water as solvent at 333 K and ambient pressure was performed until reach S/F=50 for comparison purposes. The S/F value was defined to assure that all extractable compounds were obtained. To eliminate the solvent, the ethanol was eliminated from the ethanolic extracts in a rotaevaporator (LS Scientific, LSRE-52CS-BA, Lagos, Nigeria) at 323 K; and the water was eliminated from the aqueous extracts in a lyophilizer (Liobrás, L101, São Carlos, Brazil) at 233 K. The global extraction yield (X_0) for each step was calculated as the ratio of total extract obtained in the extraction and the amount of raw material used, both in dry basis.

2.3 Extraction kinetics study

The equipment used for the extraction kinetic study is similar to that showed in the Figure 1 with an extraction vessel of 415 mL. In the extraction kinetics study 45 g (wet basis) of BGR were used. The raw material occupied 16 % of the extraction vessel and the empty space was filled with a Teflon column. The H_B/D_B ratio in this case was 2.3. The solvent flow rate was fixed at 10 mL·min^{-1}. The process time was 200 min for the first step and 240 min for the second one, leading to an S/F ratio of 40 and 57 for the first and second steps, respectively. The extraction yield was calculated as described in Section 2.2. The relative beta-ecdysone yield (YBE; g beta-ecdysone ·100g^{-1} of extractable beta-ecdysone) was calculated according to Equation (1):

$$YBE = \frac{m_{BE}/m_{RM}\ (S/F = i,\ \forall\, 0 < i \leq 100)}{m_{BE}/m_{RM}\ (S/F = 100)} \times 100 \quad (1)$$

where m_{BE} is the mass of beta-ecdysone, m_{RM} is the mass of raw material used in the extraction both in dry basis. The term ‘extractable beta-ecdysone’ represents the amount of beta-ecdysone obtained in both steps of the IE-0.1 process.

The spline model of two and three straight lines was fitted to the experimental data of the overall extraction curve (OEC) using Proc Reg and Proc Nlin procedures of SAS 9.2® to estimate: t_{CER} (duration of the constant extraction rate period - CER) and t_{FER} (duration of the falling extraction rate period – FER); M_{CER} (mass transfer rate for the CER period) and M_{FER} (mass transfer rate for FER period); R_{CER} (yield for the CER period) and R_{FER} (yield for the FER period); Y_{CER} (mass ratio of solute in the fluid phase at the extractor outlet for the CER period) and Y_{FER} (mass ratio of solute in the fluid phase at the extractor outlet for the FER period) [35].

2.4 Extracts characterization

2.4.1 Beta-ecdysone quantification

The beta-ecdysone quantification was performed in high pressure liquid chromatography (HPLC) according to the method described by Rostagno et al [36]. The compounds separation was obtained in a fused-core column (Poroshell 120 EC-C_{18}, 100 × 4.6 mm, 2.7 µm, Agilent Technologies, Little Fall, EUA). The mobile phase was composed by water (A) and acetonitrile (B), both with acetic acid 0.1 %, running at the gradient: 0 min 5% of B; 0.5 min 10% of B; 2 min 12.5 of B; 3 min 15% of B; 4 min 80% of B; 5 min 100% of B; 6 min 100% of B; 7 min 5% of B. The flow rate was 2.0 $mL \cdot min^{-1}$ at 328 K and the injected sample volume was 10µL. The data acquisition was performed at 246 nm using 20-hydroxyecdysone (beta-ecdysone) (Sigma Aldrich, St. Louis, USA) as standard.

2.4.2 Saponin quantification

Saponin quantification was performed by spectrophotometric method as described by Vigo et al [37], using commercial saponins (CAS 8047-15-2, lote BCBG4489V, Sigma Aldrich, St Louis, USA) as standard.

2.4.3 Surface tension

The surface tension of the extracts in aqueous solution was measured to evaluate the surfactant capacity of the extracts. The aqueous solutions were prepared with extract concentration ranging between 1 and 40 $mg \cdot mL^{-1}$. To analyze the ethanolic extracts, the ethanol was eliminated from the solid extract and it was diluted in water to prevent the effect

of ethanol in the surface tension. The surface tension measurements were performed at 298 K in tensiometer (Teclis, Tracker-S, Longessaigne, France). The critical micellar concentration (CMC) was determined by the intersection of two straight lines obtained in the surface tension graphic in function of logarithmic concentration [10]. The extracts obtained in the OEC (Overall Extraction Curve) were analyzed in their original concentration.

2.4.4 Fructooligosaccharides (FOS) quantification

FOS quantification was performed in HPLC system (Alliance, Waters, Milford, USA) connected to an evaporative light scattering detector (2424 ELSD, Waters, Milford, USA). The compounds separation was obtained in an Xbridge Amide column (3.5 μm, 4.6 × 250 mm, Waters, Milford, USA). The autosampler and analytical column were maintained at 298 and 313 K, respectively. The conditions for compounds separation were determined in previous assays. The mobile phase used to elute the compounds consisted of water (solvent A) and acetonitrile (solvent B), running at the gradient: 0 min 20% of A; 20 min 50% of A; 23 min 40% of A; 32 min 20% of A. The flow rate was 0.5 $mL \cdot min^{-1}$ and the injected volume was 10 μL. Nitrogen (White Martins, Campinas, Brazil) was used as carrier gas to nebulizer. ELSD conditions were gas pressure of 20 psi, drift tube temperature of 348 K and gain of 500. FOS were identified by comparing the retention time with those of the standards. The quantification was performed using the external calibration method. Calibration standard solutions at 7 concentrations levels ranging from 65 to 1000 $mg \cdot L^{-1}$ were prepared in water. The standards 1-kestose, nystose and fructofuranosylnystose were purchased from Wako Pure Chemical Industries (Osaka, Japan).

2.5 Statistical analysis

The experimental data were analyzed by analysis of variance (one-way ANOVA) and Tukey's test with 95 % of confidence using Minitab 16® (Minitab Inc., State College, PA, USA).

3. RESULTS AND DISCUSSION

3.1 Influence of pressure on the global extraction yield (X_0)

Table 1 shows the global extraction yields (X_0) obtained in the intensified extraction (IE) and in the comparative process (CE) performed in only one step using water as extracting solvent. In this work the process intensification strategy used was the reduction of the number of unit operations or apparatuses involved for bioactive compounds recovery by using the same extraction apparatus to obtain two products: an ethanolic extract and an aqueous extract. The X_0 obtained in the step 1 of the IE ranged between 7.1 ± 0.5 and 9.0 ± 0.7% (dry basis, d.b.) and in the step 2 ranged between 65 ± 2 and 67.5 ± 0.7% (d.b.), totalizing in average 75% (d.b.) of yield after 2 steps, which is similar to the X_0 obtained in the CE (74.9%, d.b.). The pressure did not show statistically significant effect on X_0 for both process steps (p-value for steps 1 and 2 was 0.118 and 0.283, respectively). It can also be observed that the X_0 obtained with water as extracting solvent was expressively higher than that obtained using ethanol. It can be due to the water has higher polarity and solubilization power and, then, more compounds can be extracted from the raw material using this solvent.

Table1: Global extraction yield ($g \cdot 100g^{-1}$ of raw material, d.b.) obtained in the intensified extraction (IE) using ethanol (Step 1) and water (Step 2) as solvents at 333 K in different pressures (IE-0.1 = 0.1 MPa; IE-5= 5 MPa; IE-10= 10 MPa) and in the comparative extraction (CE).

Extraction condition	Step 1	Step 2	Total
IE-0.1	9.0 ± 0.7^{a}	67 ± 1^{a}	76.0 ± 0.7
IE-5	7.1 ± 0.5^{a}	67.5 ± 0.7^{a}	75 ± 1
IE-10	7.7 ± 0.7^{a}	65 ± 2^{a}	72 ± 3
CE	--	--	74.9 ± 0.6

3.2 Influence of pressure on the beta-ecdysone recovery

Table 2 shows the beta-ecdysone content in the extracts obtained in each step of the IE process performed in different pressures. The ethanolic extracts had a beta-ecdysone content of until 3.89 ± 0.18 $g \cdot 100g^{-1}$ of extract (d.b.), while the aqueous extract had only

0.57 ± 0.01 g·100g^{-1} of extract (d.b.), so, the beta-ecdysone concentration in the ethanolic extract is expressively higher than that in the aqueous extracts. Statistically, the beta-ecdysone content in the ethanolic extract was negatively affected by pressure increase (p-value = 0.004), since the extract obtained at 10 MPa had only 2.0 ± 0.11 g·100g^{-1} of extract (d.b.) of beta-ecdysone. The beta-ecdysone content in the aqueous extract was not affected by pressure (p-value = 0.292).

Beta-ecdysone recovery obtained in step 1 of the IE process decreased significantly (p-value < 0.001) by the increase of pressure while the beta-ecdysone recovery obtained in the step 2 of the IE process was not affected by pressure (p-value = 0.118). The total beta-ecdysone recovery was also significantly affected by the pressure (p-value < 0.001) mainly due to the effect of pressure observed in the step 1 of the IE-process. It can be due to a compression effect on the raw material caused by the pressure increase that hampered the solubilization of beta-ecdysone in the ethanol used as extracting solvent in the step 1.

Table 2: Beta-ecdysone content in the extracts from Brazilian ginseng roots obtained in the intensified extraction (IE) process using ethanol (Step 1) and water (Step 2) as solvents at 333 K in different pressures (IE-0.1 = 0.1 MPa; IE-5= 5 MPa; IE-10= 10 MPa) and in the comparative extraction (CE) process.

	(g·100 g^{-1} of extract, d.b.)		
Extraction condition	Step 1	Step 2	
IE-0.1	3.79 ± 0.31^{a}	0.54 ± 0.01^{a}	
IE-5	3.89 ± 0.18^{a}	0.57 ± 0.04^{a}	
IE-10	2.00 ± 0.11^{b}	0.53 ± 0.01^{a}	
CE	--	--	0.95 ± 0.01
	(g·100 g^{-1} of raw material, d.b.)		
Extraction condition	Step 1	Step 2	Total
IE-0.1	0.34 ± 0.01^{a}	0.36 ± 0.01^{a}	0.70 ± 0.01^{ab}
IE-5	0.28 ± 0.01^{b}	0.38 ± 0.02^{a}	0.66 ± 0.01^{b}
IE-10	0.16 ± 0.01^{c}	0.34 ± 0.01^{a}	0.49 ± 0.01^{c}
CE	--	--	0.72 ± 0.01^{a}

While the pressure increase in the IE process affected the total beta-ecdysone recovery, the process carried out at ambient pressure (IE-0.1) showed a similar recovery to

that observed in the CE process. Although the use of ethanol as solvent in the step 1 of the IE process was not able to exhaust the beta-ecdysone content of the BGR because only 50% of the maximum beta-ecdysone content was recovered (0.70 g·100 g^{-1} of raw material, d.b.), it allowed obtaining an ethanolic extract with a beta-ecdysone concentration 7-fold higher than that obtained in the aqueous extract. Probably, the beta-ecdysone that was not extracted using ethanol as solvent was located in an internal part of the vegetable matrix with difficult access; when water was used as solvent it was possible to solubilize other compounds present in the raw material and, then, the remaining beta-ecdysone could be extracted in the step 2. In this way, the utilization of a two-step extraction process is important because in the step 1 using ethanol as solvent it was possible to obtain a more selective extract in terms of beta-ecdysone and, in the step 2 using water as solvent it is possible to recover the remaining beta-ecdysone from BGR that was not extracted in the first one.

In a previous study, Debien et al. [11] produced extracts from Brazilian ginseng roots rich in beta-ecdysone using different temperatures and pressures with ethanol and ethanol:water (80:20, v/v) mixture as solvents obtaining a beta-ecdysone content of 5.0 g·100g^{-1} of extract (d.b.) using ethanol at 393 K and 8 MPa, which represents 0.24 g·100 g^{-1} of raw material. This value is 33% lower than that obtained in the step 1 of IE-0.1. In another study for extraction of beta-ecdysone from BGR performed at 333 K under ambient pressure 0.8 g·100 g^{-1} of raw material was obtained [38], which is similar to the total beta-ecdysone recovered after two steps of the IE-0.1 process.

3.3 Influence of pressure on the saponin recovery

Although the colorimetric method used to quantify saponins has become a popular method because it is simple and inexpensive, it is difficult to compare the results with other studies due to differences in selection of reagent, condition to allow full colour development, standard, and wavelength used [39]. However, it can be used for comparisons between different treatments into the same study. Table 3 shows the saponins content in the extracts obtained after each extraction step. It can be observed that in the step 1 an extract with higher saponin content (up to 55 g·100 g^{-1} of extract, d.b.) was obtained than the extract obtained in the step 2 (up to 35 g·100 g^{-1} of extract, d.b.). In the CE process 49 g·100 g^{-1} of extract (d.b.) was obtained, similarly to that obtained in the step 1, when only ethanol was used. The

pressure had significant effect on saponins content in the ethanolic extract (p-value = 0.027) and in the aqueous extract (p-value = 0.005). In the both steps the highest saponins content was obtained in the IE-5.

Besides quantifying the saponins content in the extracts, it is important to know its surfactant capacity to measure the efficiency of each extract for emulsification purposes. It can be achieved by surface tension measurement and critical micellar concentration determination, which are better ways to compare the surfactant efficiency between different saponins sources.

Table 3: Saponins content in the extracts from Brazilian ginseng roots obtained in the intensified extraction (IE) process process using ethanol (Step 1) and water (Step 2) as solvents at 333 K in different pressures (IE-0.1=0.1 MPa; IE-5= 5 MPa; IE-10= 10 MPa) and in the comparative extraction (CE) process.

	($g \cdot 100\ g^{-1}$ of extract, d.b.)		
Extraction condition	Step 1	Step 2	
IE-0.1	46.5 ± 0.1^{b}	24.9 ± 0.7^{b}	
IE-5	55 ± 2^{a}	35 ± 2^{a}	
IE-10	48 ± 2^{b}	23.1 ± 0.5^{b}	
CE	--	--	49 ± 1
	($g \cdot 100\ g^{-1}$ of raw material, d.b.)		
Extraction condition	Step 1	Step 2	
IE-0.1	4.2 ± 0.3^{a}	16.7 ± 0.1^{b}	
IE-5	3.9 ± 0.4^{a}	24 ± 1^{a}	
IE-10	3.7 ± 0.2^{a}	14.8 ± 0.2^{b}	
CE	--	--	37 ± 1

3.4 Influence of pressure on the reduction rate of water surface tension of the extracts

The surface tension of water is reduced by addition of a surfactant and the lower the surface tension of water is, the higher is the surfactant activity of the extract [38]. Table 4 shows the surface tension of water added of extracts obtained from BGR. The extraction pressure had significant effect (p-value < 0.001) on the surface tension of water when the

ethanolic extracts were added to the solution. However, the surface tension of the water when the aqueous extracts were added was not significantly affected by pressure (p-value = 0.898). Ethanolic extracts obtained were more efficient than the aqueous for reducing the water surface tension, since ethanolic extracts reduced the surface tension of water up to approximately 34 $mN \cdot m^{-1}$, which represents a reduction of 52% in the water surface tension, while the minimum surface tension obtained by adding the aqueous extract in the solution was 42 $mN \cdot m^{-1}$, which represents a reduction of 40% in the water surface tension.

The higher surfactant activity of the extracts obtained in the step 1 than that of extracts obtained in the step 2 could be due to the higher saponins content in the ethanolic extracts (Table 3) and, also, due to the characteristics of the saponins extracted with ethanol. Bitencourt et al. [10] also obtained extracts from BGR in an intensified extraction process using different solvents and observed that the lowest surface tension (25 $mN \cdot m^{-1}$) was obtained when the extract was obtained with supercritical CO_2:ethanol (70:30, v/v) as extracting solvent, suggesting that the extracts obtained with more apolar solvents reduced the water surface tension more effectively.

Table 4: Surface tension ($mN \cdot m^{-1}$) of the extracts obtained in the intensified extraction (IE) using ethanol (Step 1) and water (Step 2) as solvents at 333 K in different pressures (IE-0.1 = 0.1 MPa; IE-5= 5 MPa; IE-10 = 10 MPa) and in the comparative extraction (CE).

Extraction condition	Step 1	Step 2	
IE-0.1	34.3 ± 0.1^{a}	42.8 ± 0.5^{a}	
IE-5	35.8 ± 0.1^{b}	43 ± 2^{a}	
IE-10	38.9 ± 0.2^{c}	42.2 ± 0.6^{a}	
CE	--	--	38.9 ± 0.1

Although the aqueous extracts were less efficient than the ethanolic extracts, the aqueous extracts can also be considered for application as surfactants. In literature, there are reports of using aqueous extracts from BGR as surfactant for obtaining emulsions containing clove oil [40] and annatto seed oil [17]. Also, it can be observed that when the extract obtained in CE process was added into the aqueous solution the water surface tension was lower than that observed by adding the aqueous extracts obtained in the IE process. It suggested that a fractionation of the saponins occurred during the IE process, which additionally resulted in two products that both can be applied for emulsification purposes.

3.4.1 Critical micellar concentration (CMC)

CMC is the concentration value where the formation of micelles begins. After CMC, the formed micelles are dispersed in the solution with no more effect on the surface tension [22]. The extracts from BGR obtained in IE-0.1 and CE processes were selected for comparison purposes, since pressure did not affect the overall quality of the products, being for some selected response variables the best level of pressure. The surface tension values obtained are presented in Figure 2. The ethanolic and aqueous extracts obtained in IE-0.1 process showed CMC values of 6 and 18 $mg \cdot mL^{-1}$, respectively and the extract obtained in CE showed CMC = 20 $mg \cdot mL^{-1}$. Among the tested extracts, the ethanolic extract was the best because it had the lowest CMC value and the highest reduction of surface tension of water (34 $mN \cdot m^{-1}$), corroborating our previous discussion.

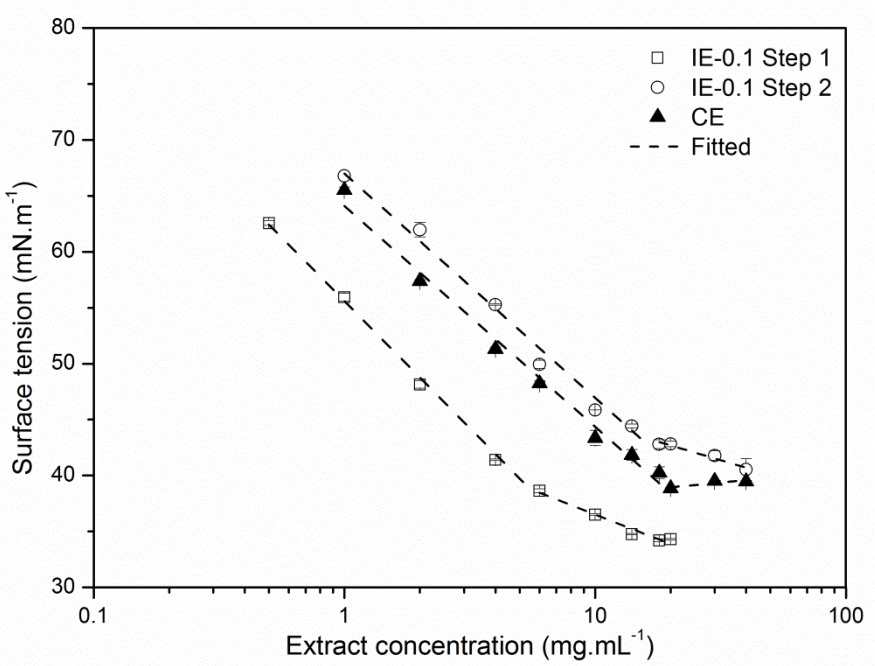

Figure 2: Surface tension of aqueous solutions of extracts from BGR obtained in intensified extraction process performed at ambient pressure (IE-0.1) and in the comparative extraction (CE) process at 333 K as a function of concentration.

Commercial saponins isolated from *Quillaja saponaria* showed CMC values between 0.51 e 0.72 $mg \cdot mL^{-1}$ with minimum values of surface tension around 35 $mN \cdot m^{-1}$ [41], while extracts also obtained from *Quillaja saponaria* that were submitted to purification processes showed CMC values around 0.25 $mg \cdot mL^{-1}$, however with surface tension of

40 $mN \cdot m^{-1}$ [42]. Asian ginseng extracts (*Panax notoginseng*) showed CMC of 0.339 $mg \cdot mL^{-1}$ also with surface tension of 40 $mN \cdot m^{-1}$ [43]. Extracts obtained from *Camellia oleifera* showed a surface tension around 50 $mN \cdot m^{-1}$ with concentration of 5 $mg \cdot mL^{-1}$ [44]. Although the CMCs of the extracts obtained by the IE process developed in this study were higher, it can be observed that both ethanolic and aqueous extracts had similar values of reduction of the surface tension of the water to those reported in the literature.

The BGR extract obtained by Bitencourt et al. [10] using supercritical CO_2:ethanol (70:30, v/v) showed a CMC value of 2 $mg \cdot mL^{-1}$ with surface tension of 25 $mN \cdot m^{-1}$, suggesting that saponins extracted with more apolar solvents has higher surfactant activity than that obtained using ethanol and water. Indeed, the higher reduction of water surface tension verified for extracts obtained with supercritical CO_2:ethanol (70:30, v/v) could be due to the higher extraction selectivity of this solvent. However, the global extraction yield obtained using supercritical CO_2:ethanol (70:30, v/v) was only 0.55 $g \cdot 100\ g^{-1}$ of raw material [10], while the global extraction yield obtained in the IE-0.1 process was 9 and 63 $g \cdot 100\ g^{-1}$ of raw material for the first and second steps, respectively, i.e., up to 115-fold higher than that obtained using supercritical CO_2:ethanol (70:30, v/v).

In this context, to determine the best extraction process to obtain extracts with high surfactant activity it is necessary to perform a study for application of the extracts to verify its efficiency. Also, it is important to evaluate the economics of the processes, because although the extracts obtained in the IE-0.1 process proposed in this work showed lower surfactant activity than that of the extracts obtained with supercritical CO_2:ethanol (70:30, v/v) [10], the cost of manufacturing of the supercritical fluid extraction can be expressively higher than the proposed IE process. Thus, the supercritical fluid extraction could be economically less attractive than the IE-0.1 process.

3.5 Influence of pressure on the fructooligosaccharides (FOS) recovery

In terms of primary metabolites, Brazilian ginseng roots contain a high amount of carbohydrates, mainly inulin-type fructans [7]. Among them, the FOS of low degree of polymerization have been received attention and are also called as prebiotic sugars. In the ethanolic extracts obtained in the step 1 of the IE process any FOS was detected while the total FOS content in the aqueous extracts obtained in the step 2 of the IE process ranged

between 9 ± 1 and 11.2 ± 0.8 g·100 g^{-1} of extract (d.b.). The 1-kestose (GF2) content ranged from 2.4 ± 0.7 to 3.1 ± 0.1g·100 g^{-1} of extract (d.b.), the nystose (GF3) content ranged from 2.8 ± 0.2 to 3.2 ± 0.3 g·100 g^{-1} of extract (d.b.) and the fructofuranosylnystose (GF4) content ranged from 4.0 ± 0.5 to 5.0 ± 0.2 g·100 g^{-1} of extract (d.b.) (Table 5). Statistically, the total FOS content in the aqueous extract was not affected by pressure (p-value = 0.252). The total FOS content in the extract obtained in the CE process was 10.8 ± 0.5 g·100 g^{-1} of extract (d.b.), demonstrating that all soluble FOS present in BGR were recovered only in the step 2 of the IE process.

Total FOS content expressed in terms of raw material ranged between 6.2 ± 0.7 and 7.6 ± 0.5 g·100 g^{-1} of raw material (d.b.). Due to the increase of the industrial interest on adding prebiotic components in their products and also due to the advantages of using fruits and vegetables as source of these compounds, several raw materials have been reported to obtain prebiotic sugars. Thus, the use of BGR as a source of prebiotic sugars is promising, since the detected total FOS content obtained from BGR is similar to that obtained from other raw materials. Jovanovic-Malinovska et al. [45] studied several raw materials, including vegetable and fruits, to extract FOS. The highest FOS content observed among the vegetables tested was obtained from scallion (3.3 ± 0.1 g·100^{-1} of fresh raw material) and among the fruits tested, the highest FOS content was obtained from nectarine (0.89 ± 0.03 g·100^{-1} of fresh raw material). Other raw materials were also tested, including by-products from the food industry, such as artichoke industrial waste that provided an extract with a FOS content of 0.79 g·100 g^{-1} of dry raw material [46] and onion outer fleshy layers that showed a FOS content of 10 g·100 g^{-1} of dry raw material [47].

Table 5: FOS content of the extracts from Brazilian ginseng roots (BGR) obtained in the second step of the intensified extraction (IE) process using water (Step 2) as solvent at 333 K in different pressures (IE-0.1 = 0.1 MPa; IE-5= 5 MPa; IE-10= 10 MPa) and in the comparative extraction (CE) process.

	($g \cdot 100\ g^{-1}$ of extract)			
Extraction condition	GF2	GF3	GF4	Total FOS
IE-0.1	2.4 ± 0.7	2.9 ± 0.1	4.0 ± 0.5	9 ± 1[a]
IE-5	3.1 ± 0.1	3.2 ± 0.3	5.0 ± 0.4	11.2 ± 0.8[a]
IE-10	2.8 ± 0.1	2.8 ± 0.2	4.8 ± 0.2	10.4 ± 0.4[a]
CE	2.6 ± 0.5	3.8 ± 0.4	4.4 ± 0.3	10.8 ± 0.5 [a]
	($g \cdot 100\ g^{-1}$ of raw material)			
Extraction condition	GF2	GF3	GF4	Total FOS
IE-0.1	1.6 ± 0.5	1.9 ± 0.1	2.7 ± 0.3	6.2 ± 0.7 [a]
IE-5	2.1 ± 0.1	2.2 ± 0.2	3.4 ± 0.2	7.6 ± 0.5 [a]
IE-10	1.8 ± 0.1	1.8 ± 0.2	3.1 ± 0.2	6.7 ± 0.5 [a]
CE	2.0 ± 0.2	2.8 ± 0.4	3.3 ± 0.1	8.1 ± 0.3 [a]

The extraction of FOS from Brazilian ginseng roots showed a different behavior from the beta-ecdysone and saponins, since the FOS were not soluble in ethanol. Also, it is important to mention that various peaks other than GF2, GF3 and GF4 were detected, which might correspond to oligosaccharides of higher degree of polymerization; therefore, the FOS content obtained from Brazilian ginseng roots can be increased.

3.6 Kinetic study

3.6.1 Extraction yield

Figure 3 shows the overall extraction curve (OEC) of the proposed IE process performed at 333 K and ambient pressure. The graphical is in logarithmic scale to facilitate the data visualization. In the step 1 the maximum yield obtained was 5 ± 1 % (d.b.) and, in the step 2 was 63 ± 6 % (d.b.), totalizing 68 % (d.b.). The experimental data from the first step was fitted to a spline of two straight lines and the data from the second step was fitted to a spline of three straight lines. From these fitted data it was possible to estimate the process parameters showed in Table 6.

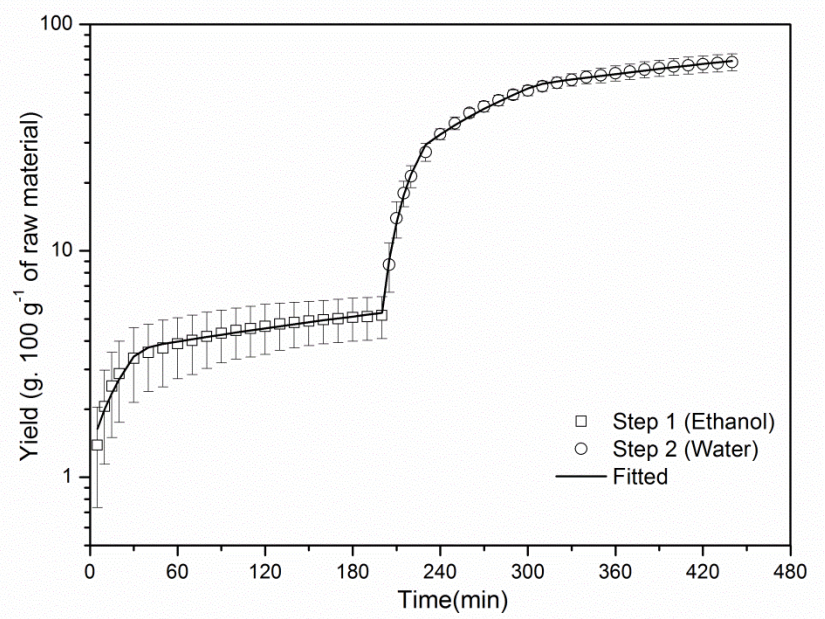

Figure 3: Experimental and fitted data of the overall extraction curve (OEC) of the proposed intensified extraction (IE) process performed at 333 K and ambient pressure.

The t_{CER} (duration of the constant extraction rate period - CER) estimated for the step 1 was 36 min and at this time it was possible to obtain an extraction yield of 3.6% (d.b.), which corresponds to 70% of the total extraction yield obtained after 200 min of process. In the step 2, the t_{CER} estimated was 29 min and the extraction yield obtained was 29% (d.b.), which represents only 46% of the total extraction yield obtained at the end of process after 240 min; otherwise, the step 2 should be performed until reach 108 min, which is the t_{FER} (duration of the falling extraction rate period – FER), because at this time the extraction yield was 55% (d.b.) and represents 87% of the total extraction yield obtained.

In the pressurized liquid extraction of BGR reported by Debien et al. [11] carried out using ethanol at 393 K and 8 MPa, the estimated t_{CER} was 26 min and 50% of the total extraction yield was recovered (3.5%, d.b.) after 130 min of extraction. Vardanega et al. [38] reported another extraction process of BGR performed with water at 333 K and ambient pressure. In that study, the t_{CER} was 8 min, the R_{CER} (yield for the CER period) was 65% (d.b.) and the M_{CER} (mass transfer rate for the CER period) was 2.8×10^{-2} kg of extract·min^{-1}. It can be observed that the R_{CER} obtained in the present work was only 28.9% (d.b.) and it is expressively lower than that reported in the literature using a similar process [38] due to the lower M_{CER} estimated for the IE process (8.4×10^{-4} kg of extract·min^{-1}).

The M_{CER} estimated for the IE process was lower because a hydroalcoholic mixture was formed in the beginning of the step 2 of the IE process. The hydroalcoholic mixture was formed because the ethanol used in the step 1 was not eliminated from the bed extraction before starting the step 2 since our focus was in the development of a process that embrace the process intensification strategy.

Table 6: Estimated parameters obtained by using the spline model for the intensified extraction (IE) of Brazilian ginseng roots (BGR)

	Step 1	Step 2
t_{CER} (min)	36 ± 8	28.6 ± 0.3
R_{CER} (%)	3.6 ± 0.2	28.9 ± 0.2
$M_{CER} \times 10^4$ (kg·min^{-1})	0.7 ± 0.3	8.40 ± 0.08
Y_{CER} (g ext·g solvent)	0.012 ± 0.006	0.084 ± 0.001
S/F_{CER} (kg solvent. kg^{-1} raw material)	7 ± 1	6.3 ± 0.3
t_{FER} (min)	--	108 ± 7
R_{FER} (%)	--	55 ± 3
$M_{FER} \times 10^4$ (kg·min^{-1})	--	3.20 ± 0.04
$Y_{FER} \times 10^2$ (g ext·g solvent)	--	3.20 ± 0.04
S/F_{FER} (kg solvent. kg^{-1} raw material)	--	25.2 ± 0.6

t_{CER} = duration of the constant extraction rate period – CER); R_{CER} = yield for the CER period; M_{CER} = mass transfer rate for the CER period; Y_{CER} = mass ratio of solute in the fluid phase at the extractor outlet for the CER period; t_{FER} = duration of the falling extraction rate period – FER; R_{FER} = yield for the FER period; M_{FER} = mass transfer rate for FER period; Y_{FER} = mass ratio of solute in the fluid phase at the extractor outlet for the FER period.

Another aspect to mention is that both steps of the OEC did not achieve the diffusional stage, however, when the step 2 started, the OEC showed an abrupt increase on the extraction rate due to the use of water, a polar solvent that can extract more compounds present in BGR than ethanol. A similar behavior was observed in an intensified extraction process performed to recover rosemary compounds using supercritical CO_2 and pressurized

water. In this case, the extraction yield of the fraction obtained with pressurized water was 7.4-fold higher than that obtained with supercritical CO_2 and, also, when the pressurized water step started an increase on the extraction rate was observed [48].

3.6.2 Beta-ecdysone recovery

Figure 4 shows the accumulated beta-ecdysone obtained in the overall extraction curve. The ethanolic extract obtained in the end of the step 1 (after 200 min and S/F = 40) showed a beta-ecdysone content of 5.6 ± 0.9 g·100 g^{-1} of extract (d.b.) and the aqueous extract obtained in the step 2 after 240 min and S/F = 55 showed a beta-ecdysone content of 0.51 ± 0.01 g·100 g^{-1} of extract (d.b.). It means that the ethanolic extract presented a 11-fold higher beta-ecdysone concentration that the aqueous extract. In spite of that, the ethanolic extract showed a relative beta-ecdysone yield (YBE) of 40% (d.b.) and the aqueous extracts had an YBE of 45% (d.b.) which represents the amount of beta-ecdysone recovered from the total extractable beta-ecdysone of the BGR.

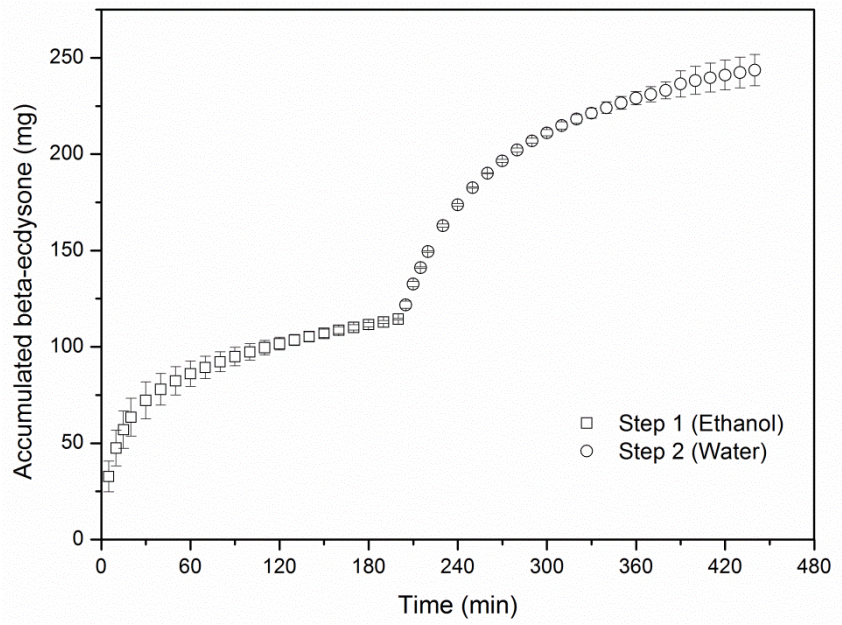

Figure 4: Accumulated beta-ecdysone obtained in the overall extraction curve at 333 K and ambient pressure.

During 36 min of process in the step 1 (estimated t_{CER} value, S/F=7) it was recovered 76 mg of beta-ecdysone, which correspond to 66% of the total beta-ecdysone

obtained in this step. On other hand, at the t_{CER} of the step 2, 59 mg of beta-ecdysone were recovered, which represent only 47% of the total beta-ecdysone obtained in this step. After 108 min of process in the step 2 (estimated t_{FER} value, S/F = 25) 100 mg of beta-ecdysone (80% of the total beta-ecdysone extracted in the step 2) were recovered. The beta-ecdysone recoveries obtained in the first and second steps suggest that the bioactive compounds are extracted along all extraction process.

3.6.3 Reduction rate of water surface tension

In step 1 of the IE process, the surface tension of the extracts ranged between 35 and 65 $mN{\cdot}m^{-1}$ and in the step 2 ranged between 42 $mN{\cdot}m^{-1}$ and 57 $mN{\cdot}m^{-1}$ (Figure 5). After 20 min of process in the step 1, the extract concentration was drascticaly reduced, which caused an abrupt increase in the surface tension. In the step 2, it can be observed that up to 60 min the surface tension kept constant around 42 $mN{\cdot}m^{-1}$ and, after this showed an crescent behavior, probably due to the decrease in the extract concentration to lower than the CMC for the aqueous extract (18 $mg{\cdot}mL^{-1}$). Therefore, regarding to the surfactant activity, the step 1 could be conducted until 20 min and the step 2 until 60 min.

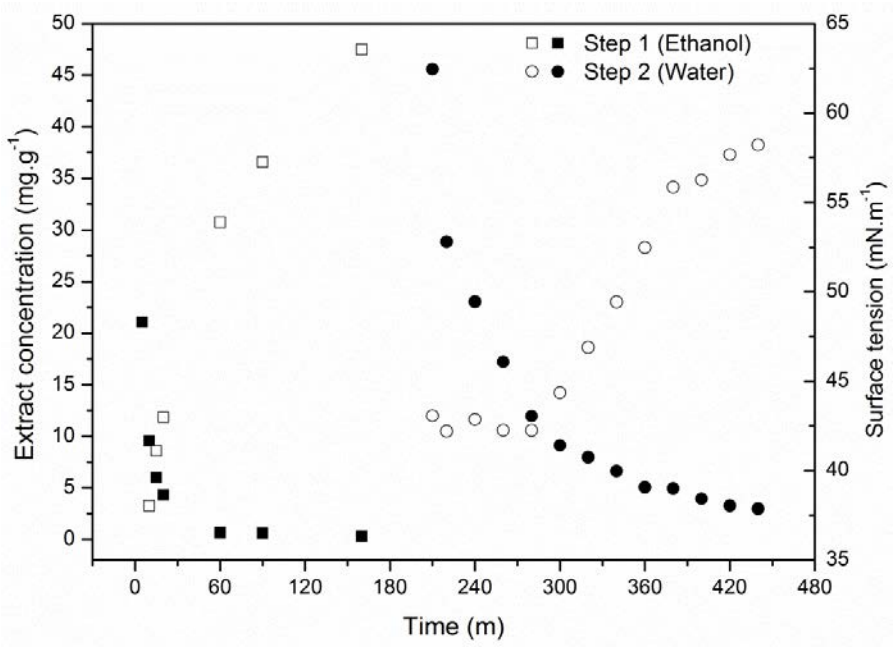

Figure 5: Surface tension (open symbols) and extract concentration (filled symbols) of the overall curve extraction obtained in the intensified extraction performed at 333 K and ambient pressure.

3.6.4 FOS recovery

Figure 6 shows the accumulated mass of FOS obtained in the second step of the IE process step after 240 min of extraction and S/F=55. The GF2, GF3 and GF4 portions corresponds to 20, 26 and 54% of the total FOS recovered, respectively, which represents a total FOS content of 7.9 ± 0.6 g·100g^{-1} of extract. It can be observed that these compounds were extracted along all extraction process as observed for the beta-ecdysone recovery and after 108 min of process 80% of the total FOS was recovered.

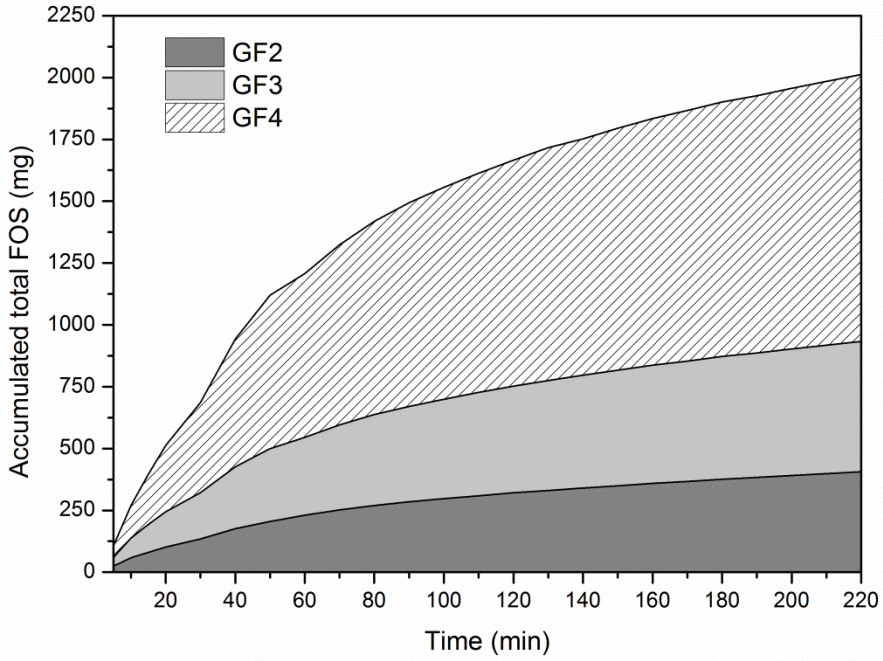

Figure 6: Accumulated fructooligosaccharides (GF2, GF3 and GF4) of the aqueous extract obtained in the second step of the IE process performed at 333 K and ambient pressure.

In the ethanolic extract obtained in the step 1 no FOS were detected. Thus, these compounds were isolated in the aqueous extract obtained in the step 2. Beta-ecdysone and saponins were obtained in both steps of the IE process. However, the ethanolic extract showed a beta-ecdysone concentration 7-fold higher than the aqueous extract. Also, the surfactant activity of ethanolic extract (CMC = 6 mg·mL^{-1}) was more effective than that of the aqueous extract (CMC = 18 mg·mL^{-1}). Therefore, the use of ethanol as extracting solvent in the first step was more selective to obtain beta-ecdysone and saponins with surface activity while the use of water in the second step was more selective to obtain FOS.

4. CONCLUSIONS

By performing the two-step intensified extraction process, it was possible to obtain two extract fractions rich in bioactive compounds from BGR using ethanol and water as solvents at 333 K and ambient pressure. The beta-ecdysone and saponins were obtained in the both steps of the IE process. However, the beta-ecdysone concentration in the ethanolic extract was 7-fold higher than that obtained in the aqueous extract. Also, the surfactant properties of the ethanolic extract were better than the aqueous extract due to its higher saponins concentration. Moreover, the FOS were isolated in the aqueous extract. Then, the intensified extraction process can be considered a suitable alternative to maximize the yields obtained from BGR, although an economic evaluation is necessary to stablish the best route for BGR processing.

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REFERENCES

[1] R. Flores, D. Brondani, V. Cezarotto, S.R. Giacomelli, F.T. Nicoloso, Micropropagation and beta-ecdsysone content of the Brazilian ginsengs *Pfaffia glomerata* and *Pfaffia tuberosa*, In vitro Cellular & Developmental Biology - Plant 46 (2010) 210-217.

[2] N.S. Calgaroto, G.Y. Castro, D. Cargnelutti, L.B. Pereira, J.F. Goncalves, L.V. Rossato, F.G. Antes, V.L. Dressler, E.M.M. Flores, M.R.C. Schetinger, F.T. Nicoloso, Antioxidant system activation by mercury in *Pfaffia glomerata* plantlets, Biometals, 23 (2010) 295-305.

[3] N.S. Calgaroto, D. Cargnelutti, L.V. Rossato, J.G. Farias, S.T. Nunes, L.A. Tabaldi, F.G. Antes, E.M.M. Flores, M.R.C. Schetinger, F.T. Nicoloso, Zinc alleviates mercury-induced oxidative stress in *Pfaffia glomerata* (Spreng.) Pedersen, Biometals, 24 (2011) 959-971.

[4] S. Nakamura, G. Chen, S. Nakashima, H. Matsuda, Y. Pei, M. Yoshikawa, Brazilian natural medicines. IV. New Noroleanane-type triterpene and ecdysterone-type sterol

glycosides and melanogenesis inhibitors from the roots of *Pfaffia glomerata*, Chem. Pharm. Bull., 58 (2010) 690-695.

[5] D.F. Felipe, L.Z.S. Brambilla, C. Porto, E.J. Pilau, D.A.G. Cortez, Phytochemical analysis of *Pfaffia glomerata* inflorescences by LC-ESI-MS/MS, Molecules, 19 (2014) 15720-15734.

[6] L.Z. Serra, D.F. Felipe, D.g.A.G. Cortez, Quantification of ²-ecdysone in differents parts of Pfaffia glomerata by HPLC, Revista Brasileira de Farmacognosia, 22 (2012) 1319-1354.

[7] E.R. Caleffi, G. Krausová, I. Hyršlová, L.L.R. Paredes, M.M. dos Santos, G.L. Sassaki, R.A.C. Gonçalves, A.J.B. de Oliveira, Isolation and prebiotic activity of inulin-type fructan extracted from Pfaffia glomerata (Spreng) Pedersen roots, International Journal of Biological Macromolecules, 80 (2015) 392-399.

[8] R. Vardanega, D.T. Santos, M.A.A. Meireles, Production of biosurfactant from Brazilian ginseng roots by low-pressure solvent extraction with and without the assistance of ultrasound, Recent Patents on Engineering, 8 (2014) 69-81.

[9] D.T. Santos, R. Vardanega, J.Q. Albarelli, A.V. Ensinas, F. Maréchal, M.A.A. Meireles, Energy consumption versus antioxidant activity of pressurized fluids extracts from *Pfaffia glomerata* roots, Chemical Engineering Transactions, 35 (2013) 1099-1104.

[10] R.G. Bitencourt, C.L. Queiroga, Í. Montanari Junior, F.A. Cabral, Fractionated extraction of saponins from Brazilian ginseng by sequential process using supercritical CO2, ethanol and water, The Journal of Supercritical Fluids, 92 (2014) 272-281.

[11] I.C.N. Debien, R. Vardanega, D.T. Santos, M.A.A. Meireles, Pressurizes liquid extraction as a promising and economically feasible technique for obtaining beta-ecdysone-rich extracts from Brazilian ginseng roots, Separation Science and Technology, 50 (2015) 1-11.

[12] C.S. Freitas, C.H. Baggio, J.E. Da Silva-Santos, L. Rieck, C.A. de Moraes Santos, C.C. Júnior, L.C. Ming, D.A. Garcia Cortez, M.C.A. Marques, Involvement of nitric oxide in the gastroprotective effects of an aqueous extract of Pfaffia glomerata (Spreng) Pedersen, Amaranthaceae, in rats, Life Sciences, 74 (2004) 1167-1179.

[13] C.S. Freitas, C.H. Baggio, A. Twardowschy, A.C.d. Santos, B. Mayer, A.P. Luiz, C.A.M.d. Santos, M.C.A. Marques, A.R.S.d. Santos, Involvement of glutamate and cytokine pathways on antinociceptive effect of Pfaffia glomerata in mice, Journal of Ethnopharmacology, 122 (2009) 468-472.

[14] A.G. Neto, A.A. da Silva Filho, J.M.L.C. Costa, A.H.C. Vinholis, G.H.B. Souza, W.R. Cunha, M.L.A.E. Silva, S. Albuquerque, J.K. Bastos, Evaluation of the trypanocidal and leishmanicidal in vitro activity of the crude hydroalcoholic extract of Pfaffia glomerata (Amarathanceae) roots, Phytomedicine, 11 (2004) 662-665.

[15] A.G. Neto, J.M.L.C. Costa, C.C. Belati, A.H.C. Vinhólis, L.S. Possebom, A.A. Da Silva Filho, W.R. Cunha, J.C.T. Carvalho, J.K. Bastos, M.L.A. e Silva, Analgesic and anti-inflammatory activity of a crude root extract of Pfaffia glomerata (Spreng) Pedersen, Journal of Ethnopharmacology, 96 (2005) 87-91.

[16] F. de-Paris, G. Neves, J.B. Salgueiro, J. Quevedo, I. Izquierdo, S.M.K. Rates, Psychopharmacological screening of Pfaffia glomerata Spreng. (Amarathanceae) in rodents, Journal of Ethnopharmacology, 73 (2000) 261-269.

[17] M.T.M.G. Rosa, E.K. Silva, D.T. Santos, A.J. Petenate, M.A.A. Meireles, Obtaining annatto seed oil miniemulsions by ultrasonication using aqueous extract from Brazilian ginseng roots as a biosurfactant, Journal of Food Engineering, 168 (2016) 68-78.

[18] D.T. Santos, D.F. Barbosa, R. Vardanega, M.T.M.S. Gomes, M.A.A. Meireles, Novel method to produce emulsions containing essential oils from saponin-rich pressurized aqueous plant extracts, Journal of Colloid Science and Biotechnology, 2 (2013) 1-7.

[19] N.R. Sanches, R. Galletto, C.E. Oliveira, R.B. Bazotte, D.A.G. Cortez, Avaliação do potencial anti-hiperglicemiante de *Pfaffia glomerata* (Spreng.) Pedersen (Amaranthaceae), Acta Scientiarum, 23 (2001) 613-617.

[20] H.L.B. LTDA, in, Colombo - PR, Brazil, http://www.herbarium.net/pt/Default.aspx.

[21] W. Oleszek, A. Hamed, Saponin-Based Surfactant, in: M. Kjellin, I. Johansson (Eds.) Surfactants from Renewable Resources, Wiley, Chennai, India, 2010.

[22] O. Guçlu-Ustundag, G. Mazza, Saponins: Properties, Applications and Processing, Critical Reviews in Food Science and Nutrition, 47 (2007) 231-258.

[23] W.Y. Yun, Fructooligosaccharides: occurrence, preparation, and application, Enzyme and Microbial Technology, 19 (1996) 107-117.

[24] M.B. Roberfroid, G.R. Gibson, L. Hoyles, A.L. McCartney, R. Rastall, I. Rowland, Prebiotics effects: Metabolic and health benefits, British Journal of Nutrition, 104 (2010) 1-63.

[25] E. Van den Heuvel, T. Muys, W. Van Dokkum, G. Schaafsma, Oligofructose stimulates calcium absorption in adolescents, American Journal of Clinical Nutrition, 69 (1999) 544-548.

[26] M.H. Davidson, K.C. Maki, Effects of dietary inulin on serum lipids, Journal of Nutrition, 129 (1999) 1474-1477.

[27] S.A. Abrams, I.J. Griffin, K.M. Hawthorne, L. Liang, S.K. Gunn, G.A. Darlington, A combination of prebiotic short- and long-chain inulin-type fructans enhances calcium absorption and bone mineralization in young adolescents, American Journal of Clinical Nutrition, 82 (2005) 471-476.

[28] G.R. Gibson, E.R. Beatty, X. Wang, J.H. Cummings, Selective stimulation of bifidobacteria in the human colon by oligofructose and inulin, Gastroenterology, 108 (1995) 975-982.

[29] P.F. Leal, M.B. Kfouri, F.C. Alexandre, F.H.R. Fagundes, J.M. Prado, M.H. Toyama, M.A.A. Meireles, Brazilian Ginseng extraction via LPSE and SFE: Global yields, extraction kinetics, chemical composition and antioxidant activity, Journal of Supercritical Fluids, 54 (2010) 38-45.

[30] I.C.N. Debien, M.A.A. Meireles, Supercritical fluid extraction of beta-ecdysone from Brazilian ginseng (*Pfaffia glomerata*) roots, Food and Public Health, 4 (2014) 67-73.

[31] R. Vardanega, J.M. Prado, M.A.A. Meireles, Adding value to agri-food residues by means of supercritical technology, The Journal of Supercritical Fluids, 96 (2015) 217-227.

[32] A.I. Stankiewicz, J.A. Moulijn, Process intensification: transforming chemical engineering., Chemical Engineering Progress, 96 (2000) 22-34.

[33] ASAE, Standards methods of determination and spressing fineness of feed materials by sieving, in, 1998, pp. 547.

[34] D.T. Santos, J.Q. Albarelli, M.A.A. Meireles, Simulation of an Integrated Sustainable Production of Extract from Brazilian Ginseng Roots with a Cogeneration Plant, Chemical Engineering Transactions, 29 (2012) 91-96.

[35] S.P. Jesus, M.N.C. Calheiros, H. Hense, M.A.A. Meireles, A simplified model to describe the kinetic behavior of supercritical fluid extraction from a rice bran oil byproduct, Food and Public Health, 3 (2013) 215-222.

[36] M.A. Rostagno, I.C.N. Debien, R. Vardanega, G.F. Nogueira, G.F. Barbero, M.A.A. Meireles, Fast analysis of β-ecdysone in Brazilian ginseng (Pfaffia glomerata) extracts by

high-performance liquid chromatography using fused-core column, Analytical Methods, 6 (2014) 2452-2459.

[37] C.L.S. Vigo, E. Narita, L.C. Marques, Validação da metodologia de quantificação espectrofotométrica das saponinas de *Pfaffia glomerataI* (Spreng.) Pedersen- Amaranthaceae, Revista Brasileira de Farmacognosia, 13 (2003) 46-49.

[38] R. Vardanega, D.T. Santos, M.A.A. Meireles, Production of biosurfactant from Brazilian ginseng roots by low-pressure solvent extraction with and without the assistance of ultrasound, Recent Patents on Engineering, 8 (2014) 1-13.

[39] C.Y. Cheok, H.A.K. Salman, R. Sulaiman, Extraction and quantification of saponins: A review, Food Research International, 59 (2014) 16-40.

[40] D.T. Santos, D.F. Barbosa, R. Vardanega, J.Q. Albarelli, M.A.A. Meireles, Experimental and simulation study on formulation of clove essential oil products using alternative surfactant, Journal of Colloid Science and Biotechnology, 2 (2013) 1-11.

[41] S. Mitra, S.R. Dungan, Micellar Properties of Quillaja saponin. 1. Effect of temperature, salt and pH on solution properties, Journal of Agricultural and Food Chemistry, 45 (1997) 1587-1595.

[42] R. Stanimirova, K. Marinova, S. Tcholakova, N.D. Denkov, S. Stoyanov, E. Pelan, Surface rheology of saponin adsorption layers, Langmuir, 27 (2011) 12486-12498.

[43] J. Xiong, J. Guo, L. Huang, B. Meng, Q. Ping, Self-micelle formation and the incorporation of lipid in the formulation affect the intestinal absorption of *Panax notoginseng*, International Journal of Pharmaceutics, 360 (2008) 191-196.

[44] Y.-F. Chen, C.-H. Yang, M.-S. Chang, Y.-P. Ciou, Y.-C. Huang, Foam properties and detergent abilities of the saponins from Camellia oleifera, International Journal Molecular Science, 11 (2010) 4417-4425.

[45] R. Jovanovic-Malinovska, S. Kuzmanova, E. Winkelhausen, Application of ultrasound for enhanced extraction of prebiotic oligosaccharides from selected fruits and vegetables, Ultrasonics Sonochemistry, 22 (2015) 446-453.

[46] M.T.C. Machado, K.S. Eça, G.S. Vieira, F.C. Menegalli, J. Martínez, M.D. Hubinger, Prebiotic oligosaccharides from artichoke industrial waste: evaluation of different extraction methods, Industrial Crops and Products, 76 (2015) 141-148.

[47] L. Jaime, F. Martínez, M.A. Martín-Cabrejas, E. Mollá, F.J. López-Andréu, K.W. Waldron, R.M. Esteban, Study of total fructan and fructooligosaccharide content in different onion tissues, Journal of the Science of Food and Agriculture, 81 (2001) 177-182.

[48] G.L. Zabot, M.N. Moraes, P.I.N. Carvalho, M.A.A. Meireles, New proposal for extracting rosemary compounds: Process intensification and economic evaluation, Industrial Crops and Products, 77 (2015) 758-771.

CAPÍTULO 6

OBTENÇÃO DE EXTRATOS DE GINSENG BRASILEIRO EM DIFERENTES CENÁRIOS DE PRODUÇÃO: AVALIAÇÃO ECONÔMICA

OBTAINING BRAZILIAN GINSENG EXTRACTS IN DIFFERENT PRODUCTION SCENARIOS: ECONOMIC EVALUATION

Renata Vardanega, Pedro I. N. Carvalho, Juliana Q. Albarelli, Diego T. Santos, M. Angela A. Meireles*

LASEFI/DEA/FEA (School of Food Engineering)/UNICAMP (University of Campinas), R. Monteiro Lobato, 80, 13083-862, Campinas, São Paulo, Brazil

* Corresponding author: maameireles@gmail.com, meireles@fea.unicamp.br

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ABSTRACT

The present study aims at studying a two-step intensified extraction process that operates at ambient pressure in order to recover Brazilian ginseng roots bioactive compounds. The intensified process consists in a sequential extraction system using ethanol followed by water, envisioning the improvement of the overall extraction yield of the process. A technical-economic analysis of the proposed process was assessed through the use computational simulation tools. The intensified process was compared to a simple extraction process using only ethanol or water as extracting solvents. The results showed that the lowest payback time for the investment is achieved not by minimizing process cost but at maximizing beta-ecdysone production. Economic performance indicators of income statement and profitability ratios showed that, even with higher investment cost, the intensified process presented higher economic attractiveness than the single step extraction processes using ethanol or water.

KEYWORDS: *Pfaffia glomerata*, economic evaluation, process intensification, financial ratios, beta-ecdysone.

1. INTRODUCTION

Brazilian ginseng (*Pfaffia glomerata*) is a plant native from the countries of South America, especially is some states of Brazil, such as São Paulo, Paraná, Mato Grosso and Goiás. It is used commercially as a substitute for Asian ginseng (*Panax* ssp.) due to its similar pharmacological effects [1, 2] . The Brazilian ginseng roots (BGR) are traditionally used in folk medicine as anti-inflammatory, analgesic, tonic, aphrodisiac, anti-diabetic and antiulcer-gastric, with a number of studies demonstrating its effectiveness [3-7].

Due to the reported bioactive activities of BGR products, it can be considered as a functional ingredient to promote health benefits. In fact, BGR powdered and it extracts, which are sold as capsules or tablets, have been used as nutritional supplement even for athletes to assist them in their training and development due to its adaptogen effect, i.e., promotes endurance and help the body to adapt to external stresses. BGR products are also indicated for women's health to promote some benefits such as improving the hormone balance [8] and protecting the skin against the aging process [9]. These effects of BGR are mainly attributed to the presence of the steroid beta-ecdysone among their bioactive compounds [10, 11].

Until this moment, beta-ecdysone rich-extracts are considered the main commercial products obtained from BGR. However, recent studies have been demonstrated that BGR extracts contain also saponins that can act as natural surfactant for obtaining emulsions containing essential oils, such as clove oil [12] and annatto seed oil [13]. Furthermore, Caleffi et al., [14] reported that BGR contain an important fraction of inulin-type polysaccharides, which presented a potential prebiotic effect.

Based on these aspects, some efforts have been done to develop green processes focused on obtaining BGR extracts rich in beta-ecdysone as well as saponins and prebiotic compounds [15-18]. Although high-pressure extraction processes are recognized as more selective processes, it was observed that the use of high pressure during the extraction process was not necessary for obtaining bioactive compounds from BGR, as demonstrated in the Chapter 5.

The current market demands not only high quality products, but also processes that have competitive costs. Recent studies have been demonstrated that the use of integrated and intensified processes were able to reduce the costs of manufacturing (COM) of curcuminoids from turmeric [19] and volatile oil and terpenoids from rosemary [20]. In this context, the present paper aims at evaluating a two-step intensified extraction process that operates at

ambient pressure in order to recover BGR bioactive compounds. In this intensified process, the first step consist in a ethanolic extraction to obtain high beta-ecdysone and saponins content and a second extraction step using water. In the second extraction, an aqueous extract is obtained which allowed the extraction of the remained bioactive compounds of BGR that could not be extracted with ethanol, improving the overall extraction yield of the process. The process was evaluated using simulation tools and compared to the processes using only ethanol or water as extracting solvents. The process was analyzed from an economic point of view in order to establish the best extraction process to obtain bioactive compounds from BGR.

2. MATERIAL AND METHODS

2.1 Description of the proposed process for valorization route for Brazilian ginseng roots

In the present study, the extraction of bioactive compounds from Brazilian ginseng roots (BGR) using ambient pressure was evaluated. The required mass and energy balances of the processes were estimated by the commercial simulator SuperPro Designer® version 8.5 (Intelligen Inc., Scotch Plains, USA).

Three different operational scenarios were suggested: Scenario I represents the process carried out using only ethanol as extracting solvent producing one ethanolic extract; Scenario II represents the process carried out using only water as solvent producing one aqueous extract; and Scenario III represents the intensified process carried out in a sequential two steps mode using both ethanol and water as extracting solvents, respectively, producing two products: an ethanolic extract and an aqueous extract. The processes layout proposed for each operational scenario is presented in Figure 1. Gantt charts are also provided to illustrate the start and finish of each operation for the proposed production scenarios (Figure 2).

For the Brazilian ginseng roots extraction, it was considered a prior preparation of the material step in which the roots were cleaned, air dried and milled. This preprocessing unit was not simulated and a cost of 40.00 US$/ton of raw-material was assumed [21]. The prepared roots were then sent to a low-pressure solvent extraction process.

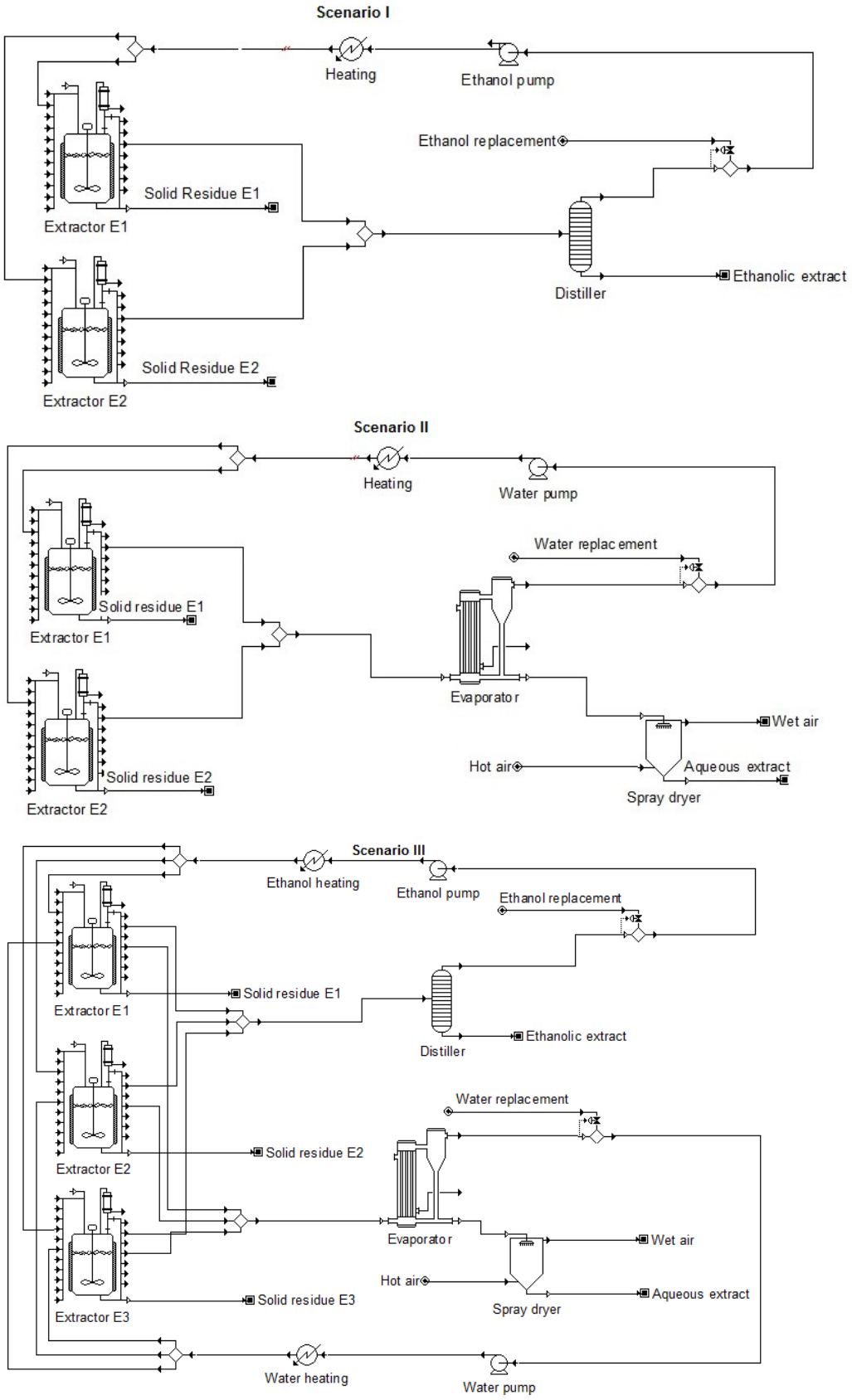

Figure 1: Processes layout of the BGR extracts production in different operational scenarios

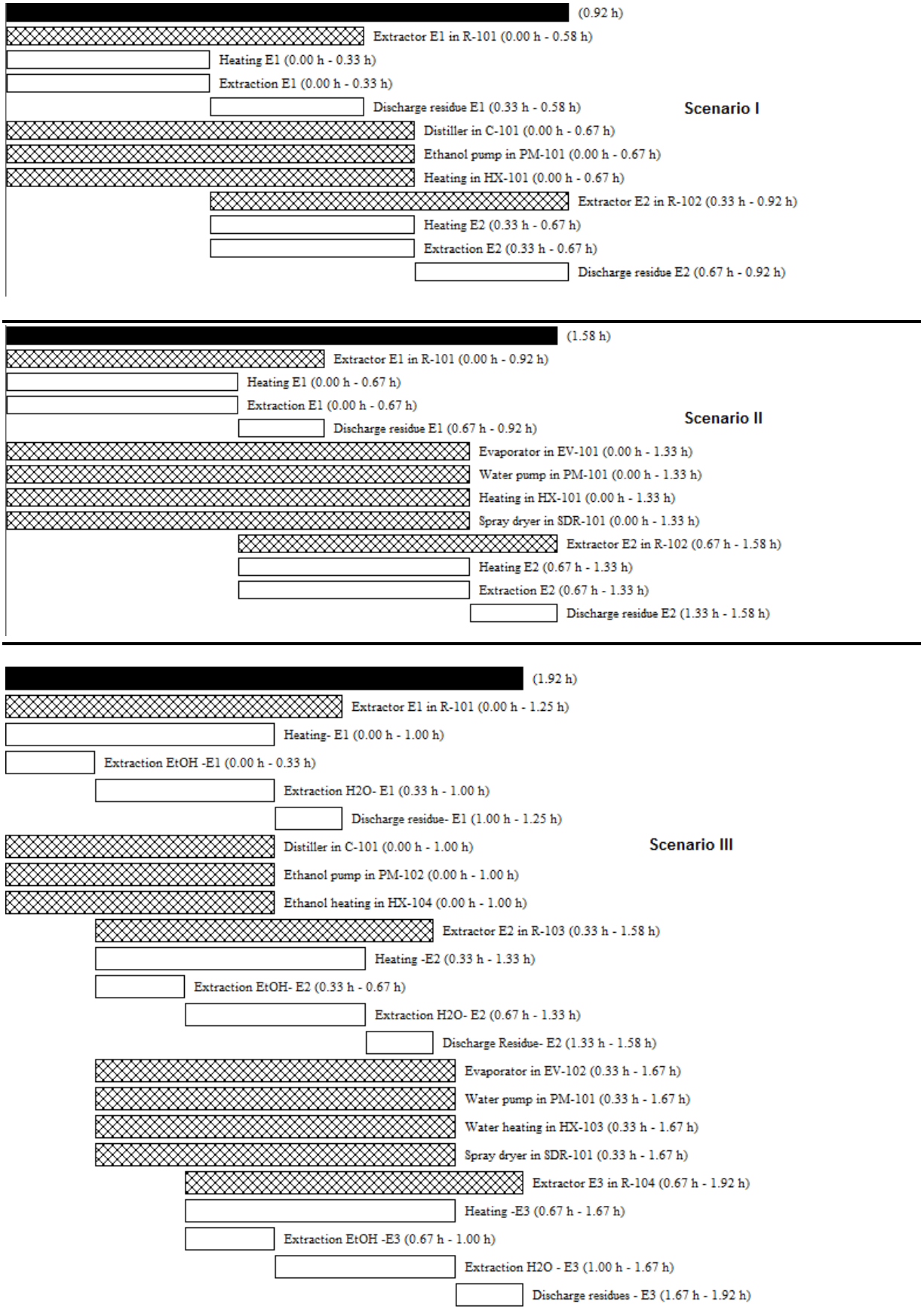

Figure 2: Gantt chart for one batch of the processes in each scenario.

For both Scenarios I and II, the extraction reactor consisted in a single batch reactor in which the solvent, ethanol or water, was pumped at the desired proportions and temperature. In the Scenario III, at first, the extraction using ethanol as solvent was carried out until reach the pre-determined solvent to feed ratio (S/F), then the ethanol stream was closed and the extracted raw material remained in the extractor to start the second step by opening the water stream. In all scenarios, the extraction temperature was fixed at 333 K. As, in general, the raw materials used for obtaining bioactive compounds are no commodities and farmed in small quantities, manufacturing plants with too big capacity are not mandatory needed and it is recommended to start with extractors of 10 to 100L, and if necessary, increase the capacity by adding more extractors [20]. Based on this, extractors with capacity of 50 L were used in this study.

After extraction, the used solvent was separated from the extracted compounds by evaporation and recycled to the process. When ethanol was used as solvent, a distillation column operating at 0.1 MPa, 323 K and 13.85 stages was considered. For water recuperation and recycling, it was needed an evaporator to concentrate the aqueous extract until 60% of moisture and then a spray-dryer to obtain the dried extract [22]. The recycling system counts with losses of solvent, therefore, up to 98% of ethanol and 95% of water was recovered and recycled.

The extraction process was modeled based on experimental results performed by the authors and reported in the Chapter 5. To obtain yields in industrial scale, it was assumed that for a given process time, the extraction behavior has the same performance as that obtained experimentally in the laboratory scale unit when the solvent to feed mass ratio and operating parameters (temperature, pressure, density and porosity) are kept constant [23]. The operational conditions admitted are shown in Table 1.

2.2 Strengths-Weaknesses-Opportunities-Threats (SWOT) matrix evaluation

Usually, the first step in the evaluation of any project should be the analysis of the Strengths-Weaknesses-Opportunities-Threats (SWOT) matrix. The SWOT analysis is a structured planning method used to take the information from an objective of the business venture or project and separate it into internal (strengths and weaknesses) and external issues (opportunities and threats). As a whole, the evaluation of the SWOT matrix determines what

may assist the firm in accomplishing its objectives, and what obstacles must be overcome for the project to become feasible. If the conclusion of the SWOT analysis is positive, the following steps comprises the detailed economic evaluation of the process[24].

Table 1: Main parameters adopted for simulating the evaluated scenarios

Scenario	**I**	**II**	**Unit**
Information to set the raw materials inlet flow			
Raw material processed	34	34	kg/batch
Raw material moisture content	10	10	%
Solvent	Ethanol	Water	
Solvent mass to Feed mass ratio (S/F)	0.5 – 40	0.5 – 57	kg solvent/kg feed
Information to set the equipment			
Extraction pressure	0.1	0.1	MPa
Extraction temperature	333	333	K
Extraction time	5 – 200	5 – 240	Min
Extraction yield	1.4 – 5.2	3.5 – 62.9	% (dry basis)
β-ecdysone content in the extract	5.6 – 6.2	0.51 – 0.52	%
Ethanol recovery pressure	0.016	--	MPa
Ethanol recovery temperature	323	--	K
Water recovery pressure		0.0096	MPa
Water recovery temperature		318	K
Final extract moisture after concentration	--	60	%
Air inlet temperature	--	380	K
Final extract moisture after drying	1.5	3	%

2.3 Technical-economic evaluation

From the data obtained in the simulations it was possible to determine the beta-ecdysone productivity, which represents the total amount of beta-ecdysone produced per year (Eq. 1).

$$BEP = EY \times BEC \times \text{number of batches} \quad \text{(Eq. 1)}$$

Where BEP is the beta-ecdysone productivity, EY is the extraction yield (Eq. 2), BEC is the beta-ecdysone concentration in the extract (Eq. 3) and number of batches is the number of batches carried out in a year.

EY = mass of dry extract/ mass of dry raw material (Eq. 2)

BEC = mass of beta-ecdysone/ mass of dry extract (Eq. 3)

The economic analysis firstly evaluated the total capital investment of each scenario and the operating costs. The total capital investment refers to the fixed costs that are associated with the process and includes the direct fixed capital, working capital and startup costs. Direct fixed capital (DFC) represents the fixed assets of the project, such as plant and equipment and it is calculated as the sum of direct, indirect and miscellaneous costs that are associated with a plant's capital investment. The direct cost includes cost elements that are directly related to an investment, such as cost of equipment, process piping, instrumentation, buildings, facilities, etc. The indirect cost includes costs that are indirectly related to an investment, such as costs of engineering and construction. Additional costs such as the contractor's fee and contingencies are included in the miscellaneous costs. By default, the DFC was estimated in the SuperPro simulator using cost correlations to estimate the purchase cost of all major process equipment (Table 2) and cost factors with respect to purchase cost to generate estimates to all other cost factors.

The working capital was calculated by multiplying the number of days covered by the corresponding unit cost per day. The number of the days considered to estimate the working capital was 30 days. Finally, the startup cost includes pre-opening, one-time expenditures incurred to prepare a new plant for operation and it was calculated by the simulator based on specified percentage (5%) of the DFC.

Table 2: Equipment cost assumed in each scenario

	Price	**Scenario I**	**Scenario II**	**Scenario III**
Industrial unit	(thousand US$)		(number of equipment)	
Extractors of 50 L [a]	190.00	2	2	3
Distiller [b]	23.00	1		1
Evaporator [b]	116.00		1	1
Spray dryer [b]	107.00		1	1

[a] Carvalho et al. (2015); [b]SuperPro Designer® cost database;

The operating cost includes costs related to the demand of a number of resources (raw materials, consumables, labor, heating/cooling utilities and power), as well as additional operating costs (waste treatment, facilities, transportation, selling costs, running royalties, etc). The cost of waste treatment was neglected because the solid generated in the extraction process can be used as raw material to obtain other products [25], for energy production through biomass conversion [26], as biosorbent to remove heavy metal ions [27], or even as nutritional source for several agricultural sectors [28]. Supervisory and administrative costs as well as labor benefits were estimated by the simulator, taking the base salary as a reference. The cost of raw material included the acquisition, transport and preparation costs of the Brazilian ginseng roots. The costs of utilities are due to the energy consumption involved in the heat exchangers, evaporators, spray-dryer and distiller and the electricity consumed during the process.

The annual operating time was considered as 7920h per year, which corresponds to 330 days per year of continuous 24 h per day shifts. The selling price of the produced extracts was calculated based on the amount of beta-ecdysone produced as, although BGR are a rich source of different bioactive compounds, until this moment, the beta-ecdysone is the most well-known and already commercialized bioactive compound. The price of beta-ecdysone was calculated based on the price of commercialized as pills of BGR extract. A box of BGR extract pills containing 45 units is sold for US$ 4.74, each pill contains 300 mg of dry BGR extract with a beta-ecdysone content of 0.96% (w/w) [29]. As the selling price for BGR extracts corresponds to a final product, it was assumed that the selling price for the crude extract (discounting the costs related to the pills manufacturing and selling costs) would be 25% of this total, i.e., US$ 1.19. Therefore the price of beta-ecdysone would be 9.14 US$/g. Table 3 shows the main input data used for the economic analysis.

The income statement and profitability ratios were calculated for the evaluated scenarios. The income statement was analyzed considering a period of 10 years. In this extend, it was evaluated the gross profit (Eq. 4) and the net profit (Eq. 5) for each scenario.

Gross profit = annual operating cost – annual revenue (Eq. 4)

Net profit = (gross profit + annual depreciation) – annual income taxes (Eq. 5)

Table 3: Main input data used for the economic analysis.

	Value	
Annual operating time	7920	H
Depreciation rate	10	%
Maintenance rate	6	%
Labor (base rate)	6.00	US$/h
Operators	2	for single step extraction process
	5	for intensified process
Utilities		
Electricity [c]	0.0954	US$/kW-h
Steam [a]	12.00	US$/ton
Chilled water [a]	0.4	US$/ton
Raw materials		
Brazilian ginseng roots [b]	9.68	US$/kg
Ethanol [a]	0.75	US$/kg
Water [a]	0.05	US$/ton
Pre-processing[d]	40.00	US$/ton
Product		
Beta-ecdysone	9.14	US$/g

[a]SuperPro Designer® cost database; [b]Santosflora (2013); [c]CPFL (http://www.cpfl.com.br/Paginas/default.aspx); [d]Veggi et al. [21]

The annual depreciation is an income tax deduction that represents a fixed capital loss which is mostly due to equipment wear out and obsolescence. As default, the simulator used the straight-line method to calculate the annual depreciation where a constant annual depreciation is calculated during the period accounted (10 years).

The profitability ratios selected in this study to evaluate the economic feasibility of the scenarios were the gross margin of the process (Eq. 6), the return on investment ratio (ROI) (Eq. 7), breakeven point (Eq. 8), payback time (Eq. 9), present value (NPV) and internal rate of return (IRR).

Gross margin = gross profit/ annual revenue (Eq. 6)

ROI = annual net profit/total capital investment (Eq. 7)

Breakeven point = annual operating cost – annual revenue = 0 (Eq. 8)

Payback = total capital investment/ annual net profit (Eq. 9)

Gross margin is a measure of profit that directly tells us what percentage of the annual revenues is gross profit. The return on investment ratio (ROI) measures the amount of return of an investment relative to the cost of investment. It is used to evaluate the efficiency of an investment or to compare the efficiency of a number of different investments. In a general way, a minimum value of ROI of 10 to 15% is established to accept or cancel a project [24, 30]. The payback time represents the time needed for the total capital investment to be recovered by the cumulative net profits. The shorter is the payback time, more attractive the project appears to be, since the initial investment is more quickly recovered, although projects with payback times between 2 and 5 years are considered feasible.

Net present value (NPV) represents the total value of future net cash flows during the life of the project, discounted to reflect the time value of money at the beginning of the project (i.e., at time zero). If an investment does not have a positive NPV, or if there are other opportunities with higher NPV, the investment should not be undertaken. The internal rate of return (IRR) represents the average intrinsic profitability of a project and it is a discounted rate that makes the NPV of all cash flows from a particular project equal to zero.

Two sensitivity analyses were accomplished in order to explore the uncertainties related to the prices and cost assumed for the evaluation of the process. The first sensitive analysis evaluated the effect of the beta-ecdysone selling price on the required sales. It was analyzed the extract selling price in the range between 46.59 and 372.69 US$/kg of extract (corresponding to a range between 4.85 and 38.82 US$/g of beta-ecdysone). The second sensitivity analysis consisted in assuming underestimations in the fixed capital investment and in the annual operating costs, therefore it was evaluated the process payback time adding overestimations by up 10% for positive shift in these costs.

3. RESULTS AND DISCUSSION

3.1 SWOT analysis

Table 4 shows the SWOT matrix for Brazilian ginseng roots (BGR) processing that reports its strengths, weakness, opportunities and threats. This tool assists investors to make decisions about invest or not in a project and it should be based on a wide knowledge of the present situation and of the future trends of the market [24, 31].

Table 4: SWOT matrix for the Brazilian ginseng roots processing

Opportunities	**Threats**
• Existence of a stablished market for BGR products based on its adaptogen effect • Opening a new market based on the surfactant properties of BGR extracts • General growing concern for healthy food	• Competition with the BGR products well-stablished in the market • Development of the market for the new BGR products • Customer's capacity to differentiate the higher quality products from the conventional products in the market
Strengths	**Weakness**
• Great potential of BGR as bioactive compounds resource • Employment of only green solvents to obtaining the bioactive compounds • No generation of toxic residues in the process • Process easily scalable • Possibility of manufacturing other raw materials to diversify the products	• Low agricultural production of BGR • Dependency on the quality of raw material • Need of increase the knowledge about the BGR composition to improve the isolation of the compounds • Need of application studies for the new BGR products

The opportunities and strengths analysis demonstrated that BGR products, such as BGR powdered commercialized as powder or tablets and capsules and even it extracts, are already stablished in the market justifying the production of new added-value products from BGR. Likewise, the recent studies reporting other applications for the BGR extracts as biosurfactant [12, 13] and prebiotic [14] are a great opportunity to expand the market for BGR products. Also, all the technologies evaluated in the present study use only non-toxic solvents,

which meet the green process concept and can be applied to obtain bioactive compounds from other raw materials to diversify the products of the company.

On the other hand, analyzing the threats and weakness the small agricultural production of BGR is an important aspect that should be taken in to consideration, as there is not enough raw material to operate the process around the year. A possible solution would be to use the same equipment for other extraction processes using other raw materials during the year. Another weakness of the process is the lack of information on the BGR composition, necessary in order to optimize the bioactive compounds isolation. It will require the development of strategies to incorporate the new BGR products in the market focusing in the benefits and differences of the new bioactive products.

The general analysis of the SWOT matrix permits a global comprehension of the situation of the project and it can be concluded that there is commercial interest on the BGR products. So, the choice of the best operational scenario is important and should be further studied from the economic point of view.

3.2 Determination of simulation conditions for different scenarios

The simulation of the productivity in terms of beta-ecdysone and operating costs performed based on the experimental data obtained in the extraction process carried out with ethanol (scenario I) and water (scenario II) as extracting solvents is shown in Figure 3.

For scenario I, a range of S/F ratio from 0.8 to 3.6 kg ethanol/ kg dry BGR is the region where higher beta-ecdysone productivity was achieved, while for scenario II, higher beta-ecdysone productivity was achieved in the S/F ratio range from 6.5 to 8.8 kg water/kg dry BGR. Scenario I required lower amount of solvent to achieve the higher beta-ecdysone productivity than the Scenario II due to the different kinetic behavior observed when ethanol or water was used as extracting solvent. At these points, the yearly beta-ecdysone production was of 1.55 ton of beta-ecdysone/year (S/F of 3.6) and 1.02 ton of beta-ecdysone/year (S/F of 8.8), for Scenarios I and II respectively. The beta-ecdysone productivity obtained in the Scenario I was 33% higher than in the Scenario II. Since the beta-ecdysone recovery obtained in the region of higher beta-ecdysone productivity was 50 and 40% for the scenarios I and II, respectively, and the quantity of solvent required to achieve it in the Scenario I was lower, it allowed performing more production batches per year in this scenario, thus resulting in the higher beta-ecdysone productivity.

However, in the S/F ratio ranges where highest beta-ecdysone productivities were achieved, the operating costs does not correspond to the lowest value. Otherwise, the operating costs obtained in the scenario I at S/F of 3.6 kg ethanol/ kg dry BGR was US$ 9.58 million/year and the operating costs obtained in the scenario II at S/F of 8.8 kg water/ kg dry BGR was US$ 6.47 million/year. The operating costs of the scenario II was lower because, in this case, the longer process time required to achieve the S/F ratio of 8.8 kg water/ kg dry BGR resulted in less production batches, which in turn required less raw material consumption, which represent the main cost that contributes to the total cost of manufacturing of BGR extracts [16].

The beta-ecdysone productivity decreases in longer process times as well as the operating costs associated, which implies directly on the revenues of the process. Analyzing the payback time when the industrial plant operates in the region of higher productivity (HP) and when it operates in the region of lower operating costs (LOC), it is possible to evaluate the impact of this two variables in the economic factors of the process. Scenario I presented a payback time of 2.92 years and 4.74 years, operating at LH and LOC respectively, and Scenario II presented payback time of 5.46 years and 5.88 years, operating at LH and LOC respectively. Payback time increased with the increase of operating time, showing that the variable that had higher impact in this analysis was the increase in beta-ecdysone productivity. Even tough, higher costs are associated with higher beta-ecdysone production, mainly due to the higher raw material input, the impact of the increase in the revenue for selling the product is higher than the increase in the expenses generating a better payback time.

Based on the results, the S/F ratio of 3.6 kg ethanol/kg dry BGR was selected for Scenario I and the S/F ratio of 8.8 kg water/kg dry BGR was selected for Scenario II. According to the experimental data, the extraction time required to achieve these S/F ratios was 20 and 40 min for Scenarios I and II, respectively. The ethanol flow rate was equal to 14.4 kg/min and water flow rate was equal to 16.5 kg/min. Thereby, these were the input data used to simulation of the Scenario III.

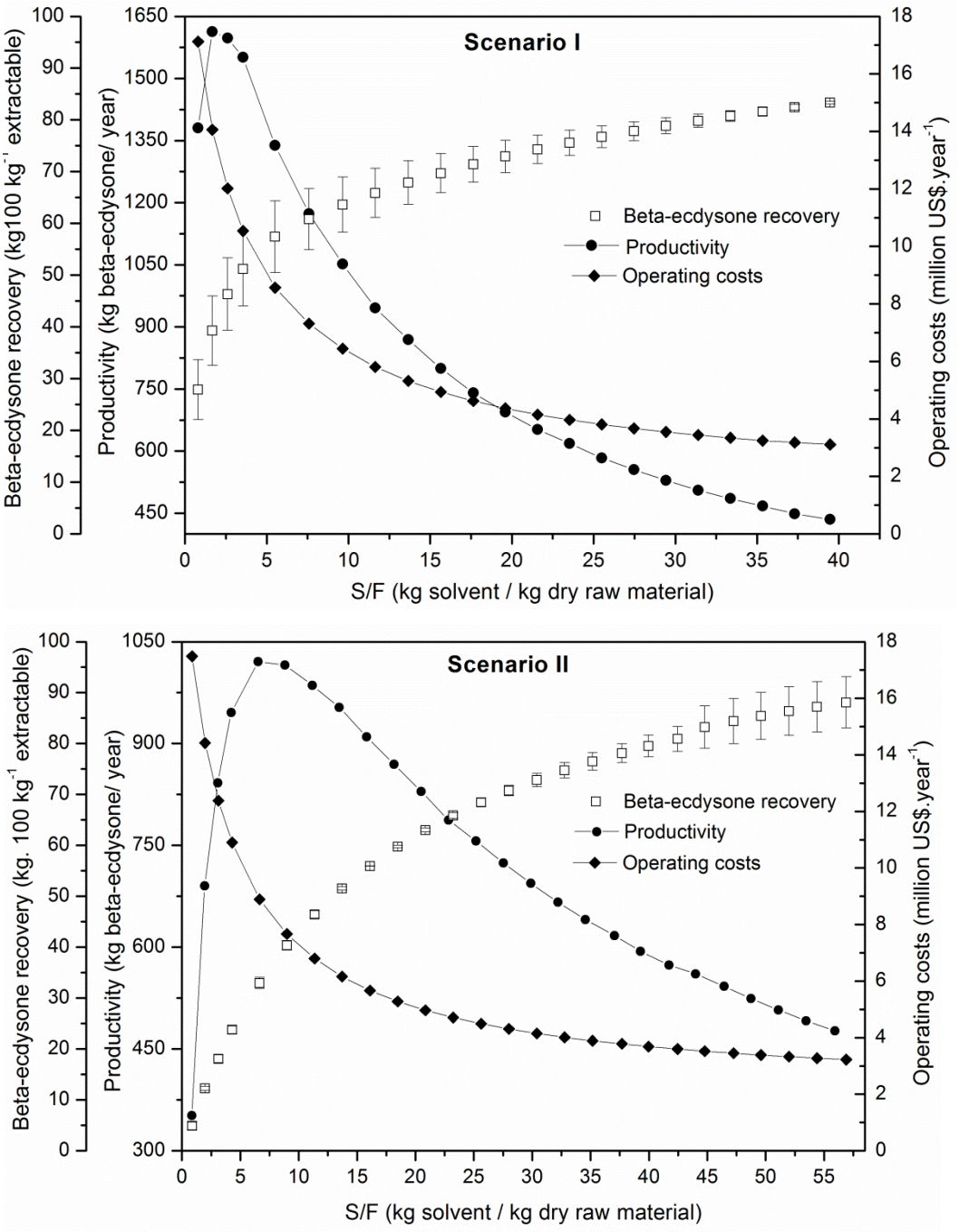

Figure 3: Beta-ecdysone productivity, operating cost and beta-ecdysone recovery (obtained from Chapter 5) for scenario I (ethanol as solvent) and scenario II (water as solvent).

3.3 Economic evaluation of the studied scenarios

Table 5 summarizes the investment and operating costs as well as the beta-ecdysone productivity for the different scenarios. The higher capital investment was obtained for the Scenario III, because according to the process times established for each step (20 min

in the first step and 40 min for the second step) three extractors were needed to operate the plant in the semi-continuous mode. Also, to obtain the dried extract in the Scenario I, only one distiller was used, while in the Scenario III, it was also needed an evaporator to concentrate the aqueous extract until 60% of moisture and then a spray-dryer to obtain the dried extract.

Table 5: Capital investment, operating costs and beta-ecdysone productivity.

Economic parameters	**Scenario I (million US$)**	**Scenario II (million US$)**	**Scenario III (million US$)**
Total capital investment	***4.26***	***5.71***	***7.32***
Direct fixed capital	3.11	4.70	6.39
Working capital	0.84	0.54	0.61
Startup cost	0.31	47	0.32
Operating costs	***10.54***	***7.03***	***7.90***
Raw materials	8.94	5.69	5.86
Labor	0.16	0.20	0.68
Facility	0.70	1.06	1.99
Utilities	0.11	0.09	0.15
Productivity parameters	**(ton/year)**	**(ton/year)**	**(ton/year)**
Beta-ecdysone	***1.54***	***1.05***	***1.91***
Ethanolic extract	1.54	--	1.01
Aqueous extract	--	1.05	0.90

The main partial cost that contributes to the operating cost was the raw material price, corresponding to around 80% of the total operating cost in average. A similar behavior was also found in other simulations studies, where different extraction process were performed to obtain BGR extracts, such as pressurized liquid extraction [16] and subcritical water extraction, as showed in the Chapter 4. Therefore, operating cost is directly dependent of the amount of raw material required in each scenario, which is dependent of the number of batches performed per year. The required time for each batch was 0.92, 1.58 and 1.92 h for the scenarios I, II and III, respectively (Figure 2). The lower is the time of each batch, the

higher is the number of batches performed per year and, consequently, higher amount of raw material is required to be manufactured.

Furthermore, the operating costs can be reduced by means of using the solid residue from the extraction process as a fuel to electricity and steam production in a cogeneration system [22, 32]. Santos et al. [22] demonstrated that the solid residue from the extraction process (BGR previously extracted) and also 49.3% of the total amount of aerial parts from Brazilian ginseng left in the field during the harvest of the roots are enough to fulfill the energy requirements to produce dry BGR extracts by pressurized water extraction at 333 K and 12 MPa. In addition, if not used for cogeneration, the BGR leftover can be sold as animal feed or even to be used as adsorbent to remove heavy metal ions, what could increase the revenues of the process [32].

Regarding to the beta-ecdysone productivity, Scenario III produced higher amount of the target bioactive compound. Biomass raw material could be better used, since it allowed to recover the remained beta-ecdysone present in BGR that were not recovered by using ethanol as extracting solvent in the first step.

Table 6 summarizes the results of income statement and profitability ratios for the different scenarios. It was possible to observe that all scenarios had a positive performance in all indicators, demonstrating the feasibility of the production of beta-ecdysone-rich extracts in all evaluated scenarios. However, Scenario III showed a net profit 2.3-fold higher than Scenario I and 3.5-fold higher than Scenario II, which means that performing two extraction-steps to obtain two different products (an ethanolic and an aqueous extract) increased the revenues from the process.

The gross margin for all scenarios was positive and for Scenario III it was 88% and 122% higher than those for Scenarios I and II, respectively. For all evaluated scenarios the calculated ROI was positive and higher than the healthy value, payback time was inside the recommended range, between 2 and 5 years, and the NPV was positive. Considering these parameters Scenario III showed better economic performance than the other scenarios. The higher breaking-even price was found for Scenario II, while Scenario III showed a value only 10% higher than Scenario I. Only Scenario III showed an IRR higher than its own calculated ROI. According to El-Hawagi [30], if the IRR value in higher or equal to the ROI value, the project is recommended. These findings enable us to conclude that Scenario III is the most feasible operation mode for obtaining BGR products.

Table 6: Income statement and profitability ratios for the different scenarios.

	Scenario I	**Scenario II**	**Scenario III**
Income statement			
Gross profit (million US$)	3.99	2.25	8.41
Taxes (40%) (million US$)	1.60	0.90	3.36
Net profit (million US$)	2.66	1.75	6.05
Profitability ratios			
Gross margin (%)	28.72	24.24	53.89
ROI (%)	62.44	30.69	84.01
Payback time (years)	1.60	3.26	1.19
NPV (million US$)	15.25	7.15	35.28
IRR (%)	48.36	24.14	115.86
Break-even point (million US$)	10.83	19.37	11.86

3.4 Sensitivity analysis

As beta-ecdysone is a bioactive compound that can be used as nutraceutical ingredient in healthy food and in the pharmaceutical industry, the selling prices for the extracts with high content of this compound can be fixed at high values. However, the variability of the beta-ecdysone content in the extract, and also the variability in the market demand cause fluctuations in the selling prices, thus, affecting the feasibility of the project. Also, the other bioactive compounds present in BGR extracts, such as saponins and inulin-type polysaccharides, can contribute to increase the selling price of these extracts.

To evaluate how the uncertainties on the extract selling price affects the project in terms of required sales, the price curve for the Scenario III was determined. Considering the annual productivity of each extract (17.6 ton of ethanolic extract and 170 ton of aqueous extract) and its beta-ecdysone content, an average selling price for the BGR products was defined as US$ 93.17/kg. Based on this average selling price, the price curve was determined in the range between 46.59 and 372.69 US$/kg as shown in Figure 4. Each point of this curve represents for a specific extract price the required sales to meet the Break-even point. The lowest value represents a discount of 50% of the average selling price and the highest one represents an increase of 200% on the average selling price. The required sales are strongly affected by the selling price of the extracts. For an extract price of US$186.34/kg which

corresponds to an increase of 100% on the average selling price, the required sales was 44 ton. This value represents only 24% of the annual production and it means that the whole investment could be recovered after less than one year. Nevertheless, if the selling price was reduced to US$ 46.59/kg, it would be necessary to sell 1800 ton of extract and it would require 9.59 years to reach the break-even point, i.e. the project would be infeasible.

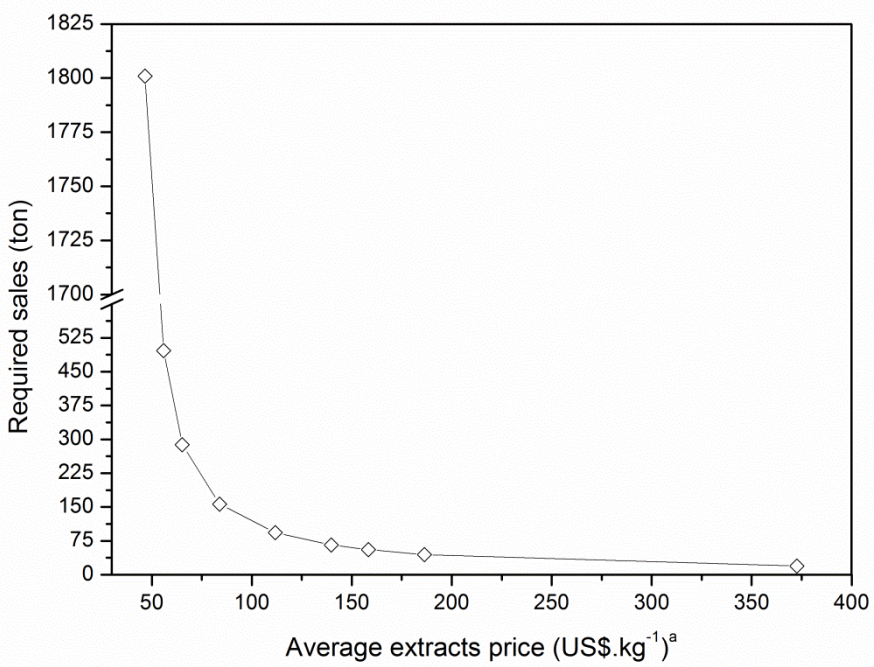

Figure 4: Price curve for the BGR products obtained in the scenario III. [a] The average extracts price was calculated as a weighted average based on the annual productivity (0.9% of ethanolic extract and 91% of aqueous extract) of the extracts.

Assuming underestimations in the fixed capital investment (FCI) and in the annual operating costs (AOC), a sensitivity analysis was carried out adding overestimations by up 10% for positive shift in these costs. Figure 5 shows that the payback time increases from 1.19 years in the original situation to 1.40 and 2.39 years by increasing the FCI and AOC, respectively. In that way, the original situation was not considerably affected by the previous underestimations, since the payback time remained in the profitability region.

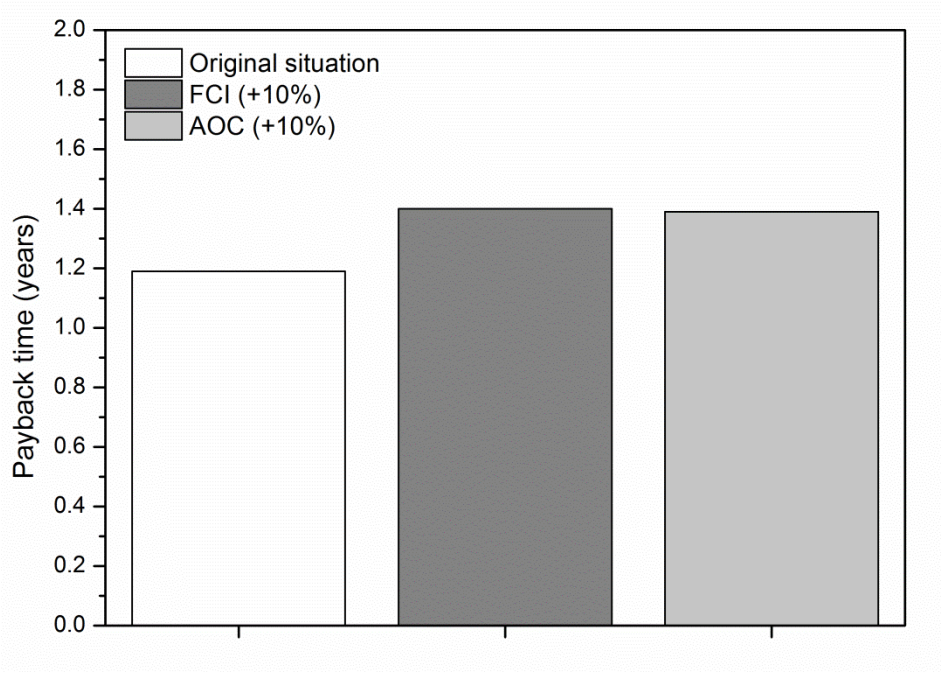

Figure 5: Sensitivity analysis for payback time for scenario III

It is important to mention that to operate the Scenario III at an industrial plant using 3 extractors of 50 L, it is required 606 ton of BGR/year. As the Brazilian ginseng is a regional raw material and it is farmed in small or medium quantities, even assuming that all of the estimated amount of BGR exported from Brazil (232 ton of BGR containing 7% of moisture [22]) could be used for this purpose, the Brazilian ginseng production would be not enough to run an industrial plant during a whole year. The total BGR produced per year could be processed in 4.5 months. Considering that the plant would run only during 4.5 months and it would be closed during the other months, the payback time increases to 3.22 years, the gross margin decreases to 42.46%, ROI is 31.09% and NPV is US$ 8.89 million. Even so, the project could be considered feasible. Nevertheless, one alternative could be the use of the process equipment during the other months to manufacture extracts from other vegetable sources instead to leave the process stopped. It would allow the increase on the revenues of the business by means of increasing the range of bioactive compounds to be sold.

4. CONCLUSIONS

The two-step intensified extraction process that operates at ambient pressure in order to recover Brazilian ginseng roots bioactive compounds demonstrated to be the best economic alternative under the evaluated parameters. The SWOT matrix showed the

potentiality of increasing the current market for the BGR extract but the necessity to overcome the small agricultural production and characterization of this raw material. Evaluating the simulation of the one-step extraction using ether ethanol or water the best solvent to feed ratio evaluated was not set at the lowest operational cost but at the higher beta-ecdysone production as it was the point in which the payback time was minimized. Higher beta-ecdysone productivity implicated in a higher operational cost due to the expressive impact of the raw material cost but at the same time in a higher revenue for the process, leading the processes with higher beta-ecdysone productivity, the one-step ethanolic extraction and the intensified extraction, to present the best profitability ratios. The economic evaluation showed that, even though the intensified process lead to higher initial investments due to the larger number of equipment, the process demonstrated to be the best feasibility option when considering the income statement and profitability ratios calculated. The price of the extract demonstrated to affect greatly the viability of the process.

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REFERENCES

[1] L.Z. Serra, D.F. Felipe, D.A.G. Cortez, Quantification of beta-ecdysone in different parts of *Pfaffia glomerata* by HPLC, Brazilian Journal of Pharmacognosy, 22 (2012) 1349-1354.

[2] C.S. Freitas, M.F.R. De Paula, L. Rieck, M.C.A. Marques, Actions of crude hydroalcoholic extract of Pfaffia sp on gastrointestinal tract, Brazilian Archives of Biology and Technology, 46 (2003) 355-360.

[3] A.G. Neto, J.M.L.C. Costa, C.C. Belati, A.H.C. Vinhólis, L.S. Possebom, A.A. Da Silva Filho, W.R. Cunha, J.C.T. Carvalho, J.K. Bastos, M.L.A. e Silva, Analgesic and anti-

inflammatory activity of a crude root extract of Pfaffia glomerata (Spreng) Pedersen, Journal of Ethnopharmacology, 96 (2005) 87-91.

[4] N.R. Sanches, R. Galletto, C.E. Oliveira, R.B. Bazotte, D.A.G. Cortez, Avaliação do potencial anti-hiperglicemiante de *Pfaffia glomerata* (Spreng.) Pedersen (Amaranthaceae), Acta Scientiarum, 23 (2001) 613-617.

[5] C.S. Freitas, C.H. Baggio, J.E. Da Silva-Santos, L. Rieck, C.A. de Moraes Santos, C.C. Júnior, L.C. Ming, D.A. Garcia Cortez, M.C.A. Marques, Involvement of nitric oxide in the gastroprotective effects of an aqueous extract of Pfaffia glomerata (Spreng) Pedersen, Amaranthaceae, in rats, Life Sciences, 74 (2004) 1167-1179.

[6] N.R. Alvim, K.C.T. Cunha, L.E.R. Cortez, R.B. Bazotte, L.C. Marques, D.A.G. Cortez, Efeitos biológicos de *P.glomerata* (Spreng.) Pedersen e da *P. paniculata* (Martiuz) Kuntze (Amaranthaceae), Acta Scientiarum, 21 (1999) 349-352.

[7] F. de-Paris, G. Neves, J.B. Salgueiro, J. Quevedo, I. Izquierdo, S.M.K. Rates, Psychopharmacological screening of Pfaffia glomerata Spreng. (Amarathanceae) in rodents, Journal of Ethnopharmacology, 73 (2000) 261-269.

[8] M. Oshima, Y. Gu, Pfaffia paniculata-induced changes in plasma estradiol-17beta, progesterone and testosterone levels in mice, Journal of Reproduction and Development, 49 (2003) 175-180.

[9] S. Eberlin, M.D.C.V. Pereda, G.C. Dieamant, C. Nogueira, R.M. Werka, M.L.S. Queiroz, Effects of a Brazilian herbal compound as a cosmetic eyecare for periorbital hyperchromia ("dark circles"), Jounal of Cosmetic Dermatology, 8 (2009) 127-135.

[10] A.R. Zimmer, F. Bruxel, V.L. Bassani, G. Gosmann, HPLC method for the determination of ecdysterone in extractive solution from Pfaffia glomerata, Journal of Pharmaceutical and Biomedical Analysis, 40 (2006) 450-453.

[11] A.C.M.M. Gomes, N. M., J.K. Mattos, S.I.V. Pereira, P. Pereira, D.B. Silva, R. Vieira, G. Capdeville, A.W. Moita, R.M.D.G. Carneiro, Concentration of β-ecydisone (20E) in susceptible and resistant accessions of Pfaffia glomerata infected with Meloidogyne incognita and histological characterisation of resistance, Nematology, 12 (2010) 701-709.

[12] D.T. Santos, D.F. Barbosa, R. Vardanega, M.T.M.S. Gomes, M.A.A. Meireles, Novel method to produce emulsions containing essential oils from saponin-rich pressurized aqueous plant extracts, Journal of Colloid Science and Biotechnology, 2 (2013) 1-7.

[13] M.T.M.G. Rosa, E.K. Silva, D.T. Santos, A.J. Petenate, M.A.A. Meireles, Obtaining annatto seed oil miniemulsions by ultrasonication using aqueous extract from Brazilian ginseng roots as a biosurfactant, Journal of Food Engineering, 168 (2016) 68-78.

[14] E.R. Caleffi, G. Krausová, I. Hyršlová, L.L.R. Paredes, M.M. dos Santos, G.L. Sassaki, R.A.C. Gonçalves, A.J.B. de Oliveira, Isolation and prebiotic activity of inulin-type fructan extracted from Pfaffia glomerata (Spreng) Pedersen roots, International Journal of Biological Macromolecules, 80 (2015) 392-399.

[15] P.F. Leal, M.B. Kfouri, F.C. Alexandre, F.H.R. Fagundes, J.M. Prado, M.H. Toyama, M.A.A. Meireles, Brazilian Ginseng extraction via LPSE and SFE: Global yields, extraction kinetics, chemical composition and antioxidant activity, Journal of Supercritical Fluids, 54 (2010) 38-45.

[16] I.C.N. Debien, R. Vardanega, D.T. Santos, M.A.A. Meireles, Pressurizes liquid extraction as a promising and economically feasible technique for obtaining beta-ecdysone-rich extracts from Brazilian ginseng roots, Separation Science and Technology, 50 (2015) 1-11.

[17] R. Vardanega, D.T. Santos, M.A.A. Meireles, Production of biosurfactant from Brazilian ginseng roots by low-pressure solvent extraction with and without the assistance of ultrasound, Recent Patents on Engineering, 8 (2014) 1-13.

[18] R.G. Bitencourt, C.L. Queiroga, Í. Montanari Junior, F.A. Cabral, Fractionated extraction of saponins from Brazilian ginseng by sequential process using supercritical CO2, ethanol and water, The Journal of Supercritical Fluids, 92 (2014) 272-281.

[19] J.F. Osorio-Tobón, P.I.N. Carvalho, M.A. Rostagno, A.J. Petenate, M.A.A. Meireles, Extraction of curcuminoids from deflavored turmeric (Curcuma longa L.) using pressurized liquids: Process integration and economic evaluation, The Journal of Supercritical Fluids, 95 (2014) 167-174.

[20] G.L. Zabot, M.N. Moraes, P.I.N. Carvalho, M.A.A. Meireles, New proposal for extracting rosemary compounds: Process intensification and economic evaluation, Industrial Crops and Products, 77 (2015) 758-771.

[21] P.C. Veggi, R.N. Cavalcanti, M.A.A. Meireles, Production of phenolic-rich extracts from Brazilian plants using supercritical and subcritical fluid extraction: Experimental data and economic evaluation, Journal of Food Engineering, 131 (2014) 96-109.

[22] D.T. Santos, J.Q. Albarelli, M.A.A. Meireles, Simulation of an Integrated Sustainable Production of Extract from Brazilian Ginseng Roots with a Cogeneration Plant, Chemical Engineering Transactions, 29 (2012) 91-96.

[23] P.C. Veggi, R.N. Cavalcanti, M.A.A. Meireles, Production of phenolic-rich extracts from Brazilian plants using supercritical em subcritical extraction: experimental data and economic evaluation, J Food Eng, 131 (2014) 96-109.

[24] M.P. Fernández-Ronco, A. de Lucas, J.F. Rodríguez, M.T. García, I. Gracia, New considerations in the economic evaluation of supercritical processes: Separation of bioactive compounds from multicomponent mixtures, The Journal of Supercritical Fluids, 79 (2013) 345-355.

[25] R. Vardanega, J.M. Prado, M.A.A. Meireles, Adding value to agri-food residues by means of supercritical technology, The Journal of Supercritical Fluids, 96 (2015) 217-227.

[26] J.M. Prado, T. Forster-Carneiro, M.A. Rostagno, L.A. Follegatti-Romero, F. Maugeri Filho, M.A.A. Meireles, Obtaining sugars from coconut husk, defatted grape seed, and pressed palm fiber by hydrolysis with subcritical water, J Supercrit Fluid, 89 (2014) 89-98.

[27] J.Q. Albarelli, R.B. Rabelo, D.T. Santos, M.M. Beppu, M.A.A. Meireles, Effects of supercritical carbon dioxide on waste banana peels for heavy metal removal, The Journal of Supercritical Fluids, 58 (2011) 343-351.

[28] M. Odlare, V. Arthurson, M. Pell, K. Svensson, E. Nehrenheim, J. Abubaker, Land application of organic waste - effects in the soil ecosystem, Applied Energy, 88 (2011).

[29] Herbarium, (2015).

[30] M.M. El-Halwagi, Overview of Process Economics, in: M.M. El-Halwagi (Ed.) Sustainable Design Through Process Integration, Butterworth-Heinemann, Oxford, 2012, pp. 15-61.

[31] J. Terrados, G. Almonacid, L. Hontoria, Regional energy planning through SWOT analysis and strategic planning tools.: Impact on renewables development, Renewable and Sustainable Energy Reviews, 11 (2007) 1275-1287.

[32] D.T. Santos, J.Q. Albarelli, M.A. Rostagno, A.V. Ensinas, F. Maréchal, M.A.A. Meirelres, New proposal for production of bioactive compounds by supercritical technology integrated to a sugarcane biorefinery, Clean Techn Environ Policy, 16 (2014) 1455-1468.

CAPÍTULO 7

DISCUSSÃO GERAL

DISCUSSÃO GERAL

Diante da rica e complexa composição de compostos bioativos encontrados no ginseng brasileiro, incluindo beta-ecdisona, saponinas, carboidratos prebióticos e outros, neste trabalho foram estudados diferentes processos de extração a fim de maximizar a recuperação dos compostos de interesse, bem como separá-los em diferentes frações. Os processos de extração estudados abrangeram técnicas convencionais desenvolvidas a pressão ambiente e também técnicas emergentes como extração assistida por ultrassom (UAE – *Ultrasound assisted extracticon*) e a extração com água subcrítica (SWE – *Subcritical water extraction*), sempre com a preocupação de desenvolver processos que não empregassem solventes tóxicos e que apresentassem alto rendimento. Inicialmente, o desenvolvimento do **Capítulo 3** permitiu ampliar o conhecimento acerca da extração assistida por ultrassom, onde foi possível verificar que o emprego de ultrassom pode auxiliar na recuperação de compostos bioativos reduzindo o tempo de processo e consumo de energia, bem como aumentando os rendimentos de extração. No entanto, é necessário que as variáveis do processo (potência e densidade do ultrassom, pressão do meio, propriedades físicas do solvente, presença de partículas sólidas, etc) sejam cuidadosamente estudadas, visto que exercem grande influência na extração dos compostos bioativos a partir de matrizes vegetais. As informações obtidas neste estudo permitiram inferir que os rendimentos dos compostos bioativos de interesse presentes nas raízes de ginseng brasileiro poderiam ser aumentados ao realizar a extração assistida por ultrassom.

O estudo de extração com água subcrítica das raízes e partes aéreas do ginseng brasileiro apresentado no **Capítulo 4** demonstrou que é possível obter altos rendimentos de extração (até 68% para as raízes e 30% para as partes aéreas). No entanto, o emprego de água como solvente é pouco seletivo para a recuperação de beta-ecdisona, visto que sua concentração no extrato não superou 0,7 e 0,3% para os extratos obtidos das raízes e partes aéreas, respectivamente. Entretanto, os extratos obtidos a partir das raízes apresentaram elevados teores de carboidratos prebióticos (até 8,8%), compostos pelos frutooligossacarídeos (FOS) conhecidos como 1-cestose (GF2), nistose (GF3) e frutofunarosilnistose (GF4). A avaliação econômica do processo SWE para obtenção de extratos das raízes e partes aéreas do ginseng brasileiro demonstrou que embora as partes aéreas não tenham nenhum custo de aquisição, o seu processamento como matéria-prima para obtenção de compostos bioativos não é viável, visto que todos os indicadores financeiros obtidos foram desfavoráveis. Por

outro lado, a utilização das raízes como matéria-prima pode ser considerada promissora, pois apresentou indicadores altamente positivos, enquanto que as partes aéreas podem ser utilizadas como fonte de energia para suprir a demanda da planta industrial.

Diante dos resultados obtidos neste estudo, optou-se por utilizar apenas as raízes do ginseng brasileiro para dar continuidade ao estudo. Como o extrato obtido por SWE apresentou uma concentração de beta-ecdisona significativamente inferior ao reportado na literatura quando etanol foi usado como solvente em um processo de extração com líquido pressurizado (PLE- *Pressurized liquid extraction*), houve a necessidade de estudar o desenvolvimento de um processo de extração intensificado a fim de extrair seletivamente os compostos das raízes de ginseng brasileiro. Para isso, um processo realizado em duas etapas, empregando etanol e água como solventes de forma sequencial, foi desenvolvido a fim de extrair a beta-ecdisona e saponinas na primeira etapa realizada com etanol como solvente e, na sequência obter os carboidratos prebióticos utilizando água como solvente. Além disso, como nos dados de extração das raízes de ginseng brasileiro obtidos em processos realizados a alta pressão reportados na literatura não foi observado efeito significativo da pressão sobre a recuperação de compostos bioativos nos níveis estudados, avaliou-se o seu efeito em diferentes níveis no processo de extração intensificado. Os resultados apresentados no **Capítulo 5** demonstraram que a extração dos compostos bioativos do ginseng brasileiro pode ser realizada a pressão ambiente, uma vez que a pressão não exerceu influência significativa no processo. Também verificou-se que é possível extrair seletivamente os compostos bioativos das raízes de ginseng brasileiro, pois o extrato etanólico obtido apresentou concentração de beta-ecdisona 7 vezes maior do que o extrato aquoso, além de melhores propriedades surfactantes, o que pode ser verificado pela concentração micelar crítica (CMC) que foi de 6 $mg \cdot mL^{-1}$ para o extrato etanólico e 18 $mg \cdot mL^{-1}$ para o extrato aquoso. Além disso, os carboidratos prebióticos foram extraídos apenas na etapa com água, ou seja, foi possível fracioná-los do extrato etanólico e, desta forma, obter dois produtos com características distintas.

Como verificou-se que o etanol utilizado como solvente na primeira etapa do processo de extração intensificado não foi capaz de exaurir a beta-ecdisona presente nas raízes de ginseng brasileiro, um estudo envolvendo a extração assistida por ultrassom foi realizado. O objetivo desse estudo foi aumentar a recuperação de beta-ecdisona no extrato etanólico e, assim, permitir um fracionamento mais eficiente dos compostos, pois, conforme verificado no **Capítulo 3**, a ocorrência de cavitação causada pelo emprego de ultrassom pode aumentar as

taxas de transferência de massa e também causar um efeito mecânico de rompimento da parede celular na matriz vegetal, facilitando assim a liberação dos compostos intracelulares para o meio. Todavia, os resultados obtidos no estudo realizado demonstraram que o ultrassom não apresentou efeito significativo sobre a recuperação de beta-ecdisona no extrato etanólico e, desta forma, seu uso foi descartado para este fim.

Apesar de os dados experimentais obtidos demonstrarem a viabilidade técnica do processo de extração intensificado, fez-se necessário realizar um estudo para verificar também a viabilidade econômica do processo. Para isso, três cenários de produção foram estabelecidos para avaliar qual a melhor rota de obtenção de extratos das raízes de ginseng brasileiro. No cenário I apenas etanol foi empregado como solvente, no cenário II apenas água e o cenário III correspondeu ao processo de extração intensificado realizado em duas etapas. Esse estudo demonstrou com êxito que embora o custo de investimento necessário para o processo de extração intensificado (cenário III) tenha sido maior, este apresentou maior produtividade e, por conseguinte, melhor desempenho do ponto de vista econômico. Também foi possível verificar que mesmo a planta industrial operando durante apenas 4,5 meses e ficando parado durante os outros meses do ano, o processamento das raízes de ginseng brasileiro através do processo de extração proposto é uma excelente oportunidade de negócio. Todavia, a versatilidade da planta tradicional proposta permite que outras matérias-primas sejam selecionadas e processadas para obtenção de extratos vegetais, e desta forma, aumentem a diversidade de produtos e rentabilidade do negócio.

Desta forma, os dados aqui reportados reiteram os esforços feitos para demonstrar que é possível substituir processos de extração convencionais por processos inovadores que não utilizam solventes tóxicos, minimizam a geração de resíduos através do melhor aproveitamento das matérias-primas e são mais eficientes do ponto de vista energético, além de serem altamente promissores do ponto de vista econômico. Também, contribuem para a desmistificação da crença de que processos de extração a alta pressão são impraticáveis devido ao seu alto custo de investimento, pois conforme demonstrado no Capítulo 4, o tempo de retorno simulado para o investimento em uma planta de extração com água subcrítica para obtenção de compostos bioativos do ginseng brasileiro foi menor do que dois anos, mesmo considerando apenas a comercialização da beta-ecdisona presente no extrato.

Além destes aspectos, é importante ressaltar que dados inéditos foram reportados acerca da identificação e quantificação dos frutooligossacarídeos GF2, GF3 e GF4 provenientes das raízes de ginseng brasileiro, os quais são altamente desejáveis pela indústria

de alimentos devido à sua atividade prebiótica no organismo humano. Os teores destes compostos encontrados nos extratos das raízes de ginseng brasileiro podem ser considerados expressivos quando comparados aos extratos provenientes de outras matérias-primas. Sendo assim, em virtude da vasta gama de compostos biotivos obtidos a partir do ginseng brasileiro, este pode ser uma excelente opção tanto para agricultores que pretendem diversificar sua produção, quanto para a indústria de produtos naturais que busca por novas fontes de compostos bioativos.

CAPÍTULO 8

CONCLUSÕES GERAIS E SUGESTÕES PARA TRABALHOS FUTUROS

8.1 CONCLUSÕES GERAIS

O processo de SWE das raízes e partes aéreas permite obter altos rendimentos de extração, tanto para as raízes quando para as partes aéreas do ginseng brasileiro, porém com baixa seletividade em relação ao teor de beta-ecdisona dos extratos, visto que os extratos das raízes e das partes aéreas apresentaram um teor de até 0,7 e 0,3%, respectivamente. Em contrapartida, esse processo foi eficiente para extração de carboidratos prebióticos a partir das raízes, obtendo teores de até 8,8%. A temperatura exerceu influência significativa sobre o teor de beta-ecdisona dos extratos obtidos tanto a partir das raízes quando das partes aéreas, já o tempo estático foi significativo apenas para as raízes, enquanto que a pressão não teve efeito significativo. Em relação ao teor de carboidratos prebióticos dos extratos, mais especificamente frutooligossacarídeos (FOS), obtidos das raízes do ginseng brasileiro, ambos temperatura e tempo estático exerceram influência significativa.

O estudo de viabilidade econômica do processo SWE demonstrou que apenas o processamento das raízes é economicamente favorável, visto que as partes aéreas apresentaram indicadores econômicos negativos. Todavia, estas podem ser utilizadas como combustível para suprir a demanda energética da planta de SWE para obtenção dos extratos das raízes.

O processo de extração intensificado realizado a pressão ambiente permitiu obter e fracionar diferentes compostos bioativos das raízes de ginseng brasileiro, uma vez que o extrato etanólico apresentou um teor de beta-ecdisona 7 vezes maior do que o extrato aquoso e, também, melhor capacidade surfactante devido à maior concentração de saponinas verificada. Além disso, foi possível recuperar os FOS apenas no extrato aquoso. Também, verificou-se que a pressão não exerce influência significativa neste processo e, portanto, o processo pode ser realizado a pressão ambiente.

A extração assistida por ultrassom empregando etanol como solvente não foi capaz de aumentar a concentração de beta-ecdisona do extrato etanólico, desta forma a possibilidade de empregar o ultrassom durante a primeira etapa do processo de extração intensificado para aumentar a recuperação de beta-ecdisona no extrato etanólico foi descartada.

O estudo da viabilidade econômica da produção de extratos das raízes de ginseng brasileiro em diferentes cenários de produção demonstrou que o processo de extração

intensificado é o mais favorável, embora os demais cenários estudados (apenas empregando etanol ou água como solventes) também podem ser viáveis. Além disso, observou-se que mesmo que a planta opere apenas durante 4,5 meses do ano e fique parada nos demais, o processo pode ser factível. Todavia, durante os meses que a planta não estiver processando as raízes de ginseng brasileiro, esta pode processar outras matérias-primas e, assim, aumentar a diversidade de produtos e também a rentabilidade do negócio.

8.2 SUGESTÕES PARA TRABALHOS FUTUROS

i. Identificar e quantificar as saponinas com propriedades surfactantes presentes nas raízes de ginseng brasileiro, através de métodos analíticos específicos, tais como espectrometria de massa e similares;
ii. Estudar o comportamento interfacial do extrato etanólico e do extrato aquoso quando aplicados em sistemas emulsionados e avaliar a sua eficiência como agente estabilizante;
iii. Identificar os demais possíveis frutooligossacarídeos presentes no extrato aquoso das raízes de ginseng brasileiro;
iv. Avaliar a atividade prebiótica dos frutooligossacarídeos presentes no extrato aquoso das raízes de ginseng brasileiro;
v. Estudar técnicas de separação para melhorar o fracionamento dos compostos bioativos presentes nos extratos etanólico e aquoso obtidos das raízes de ginseng brasileiro;
vi. Realizar o aumento de escala do processo de extração intensificado das raízes de ginseng brasileiro.

MEMÓRIA DO PERÍODO DE DOUTORADO

MEMÓRIA DO PERÍODO DE DOUTORADO

A doutoranda Renata Vardanega ingressou no programa de doutorado em Engenharia de Alimentos (DEA/FEA/UNICAMP) em março de 2013. Durante 8 meses usufruiu de bolsa de doutorado concedida pelo CNPq (processo 140282/2013-0); em 2013 a bolsa de doutorado passou a ser financiada pela FAPESP (processo 2013/17260-5) com vigência de novembro de 2013 a fevereiro de 2016. Durante o período de doutorado cursou 4 disciplinas: TP 199- Seminários (2 créditos); IQ 323 - Equilíbrio de fases (2 créditos); TP 143 – Reologia (3 créditos) e TP 159 – Tópicos especiais em Engenharia de Alimentos – (2 créditos). Além das disciplinas cursadas, outros 4 créditos foram cumpridos através da participação no Programa de Estágio Docente grupo C (PED C) com atividades de apoio parcial à docência da disciplina TA 331 – Termodinâmica, atuando como voluntária entre ago/2013 a dez/2013 e como bolsista entre set/2014 a jan/2015. Para atingir o número de créditos exigido pelo programa, outras duas disciplinas cursadas durante o período de mestrado foram convalidadas: TP 121 – Tópicos em Engenharia de Alimentos - Ciclo de aprendizado PDSA (2 créditos) e TP 121 – Tópicos em Engenharia de Alimentos - Métodos estatísticos (2 créditos).

A doutoranda participou do XXI Congresso de Iniciação Científica da Unicamp na qualidade de avaliadora de trabalhos inscritos na área de Tecnológicas em 2013 e 2015. Também, em 2013 participou no SFE'13 (Workshop on Supercritical Fluids and Energy), realizado em Campinas – SP. Em 2014 participou no evento EMSF 2014 (European Meeting on Supercritical Fluids), realizado em Marselha – França. Em 2014, a doutoranda participou do I Congresso Sul Brasileiro de Engenharia de Alimentos realizando em Pinhalzinho - SC, como palestrante do tema "Compostos bioativos: agregando valor à matérias-primas vegetais através de processos limpos".

As atividades referentes ao presente projeto de pesquisa e realizadas em cooperação resultaram até o momento em 9 artigos, sendo 1 artigo de revisão publicado no periódico *Pharmacognosy Reviews*, 1 artigo de revisão publicado no periódico *The Journal of Supercritical Fluids* e 1 artigo de revisão publicado no periódico *Green Chemistry Letters and Reviews*, 2 artigos experimentais publicados no periódico *Chemical Engineering Transactions*, 1 artigo experimental publicado no periódico *Analytical Methods*, 1 artigo experimental publicado no periódico *Separation Science and Technology* e 3 manuscritos que

correspondem aos Capítulos 4 a 6. Também, durante este período foi publicado 1 capítulo no livro *Food Waste Recovery* e 7 trabalhos científicos em anais de eventos, sendo 2 trabalhos completos e 6 resumos.

Durante o desenvolvimento experimental do projeto de pesquisa realizou-se uma parceria com o grupo de pesquisa Laboratório de Biotecnologia/ELL/USP – Lorena, SP, a qual foi realizada sob supervisão do doutorando Paulo Marcelino Franco sob coordenação do Prof. Dr. Silvio Silvério da Silva para treinamento sobre a metodologia para quantificação de saponinas totais. Também, realizou-se uma parceria com o grupo de pesquisa ThoMSon – Laboratório de Espectrometria de Massas/IQ/UNICAMP, coordenado pelo Prof. Dr. Marcon N. Eberlin. Esta parceria foi realizada com o intuito de identificar as saponinas presentes nos extratos das raízes e partes aéreas de ginseng brasileiro e os experimentos foram realizados sob supervisão do doutorando Marcos Franco. No entanto, as técnicas de espectrometria de massas utilizadas não permitiram a identificação das saponinas presentes.

ARTIGOS PUBLICADOS EM PERIÓDICOS

Debien, I. C. N., **Vardanega, R.**, Santos, D. T., Meireles, M. A. A. Pressurized liquid extraction as a promissing and economically feasible technique for obtaining beta-ecdysone-rich extracts from Brazilian ginseng (*Pfaffia glomerata*) roots, *Separation Science and Technology*, v.50, p.1-11, 2015.

Prado, J. M., **Vardanega, R.**, Rostagno, M. A., Foster-Carneiro, T. Meireles, M. A. A. The study of model systems subjected to sub- and supercritical water hydrolysis for the production of fermentable sugars, *Green Chemistry Letters and Reviews*, v.8, p. 16-30, 2015.

Vardanega, R., Prado, J. M., Meireles, M. A. A. Adding value to agri-food residues by means of supercritical technology, *The Journal of Supercritical Fluids*, v. 96, p. 217-227, 2015.

Vardanega, R., Santos, D. T., Meireles, M. A. A. Intensification of bioactive compounds extraction from medicinal plants using ultrasonic irradiation –A review, *Pharmacognosy Reviews*, v. 8, p. 88-95, 2014**.**

Rostagno, M. A., Debien, I. C. N., **Vardanega, R.**, Nogueira, G. C., Barbero, G. F. Fast analysis of β-ecdysone in Brazilian ginseng (*Pfaffia glomerata*) extracts by high-performance liquid chromatography using fused-core column, *Analytical Methods*, v. 6, p.2452-2459, 2014.

Rostagno, M. A., Prado, J. M., **Vardanega, R.**, Forster-Carneiro, T., Meireles, M. A. A. Study of recovery of fermentable sugars with subcritical water and carbon dioxide from palm fiber and grape seed, *Chemical Engineering Transactions*, v. 37, p. 403-408, 2014.

Forster-Carneiro, T., Prado, J. M., **Vardanega, R.**, Perez, D. L., Meireles, M. A. A. Subcritical water hydrolysis of sugarcane bagasse and coconut husk assisted by carbon dioxide, *Chemical Engineering Transactions*, v. 37, 391-396, 2014.

CAPÍTULOS DE LIVRO PUBLICADOS

Prado, J. M., **Vardanega, R.**, Debien, I. C. N., Meireles, M. A. A., Gerschenson, L. N., Sowbhagya, H. B., Chemat, S. Conventional Extraction, In: *Food Waste Recovery*. Galanakis, C (Editor), 1 ed., Elsevier, 2013, v.1, 127-148.

TRABALHOS PUBLICADOS EM ANAIS DE CONGRESSOS

Santos, D. T., **Vardanega, R.**, Meireles, M. A. A. Avaliação do potencial de resíduos do cultivo de ginseng brasileiro como fonte de compostos bioativos. In: Simpósio Brasileiro de Compostos Bioativos (SBCB), 2014, Campinas, 1-2.

Vardanega, R., Santos, D. T., Meireles, M. A. A. Obtaining bioactive compounds from Brazilian ginseng roots using pressurized water. 14th European Meeting on Supercritical Fluids (14th EMSF), Marseille- França, Maio, 2014.

Rostagno, M. A., Debien, I. C. N., **Vardanega, R.**, Nogueira, G. C., Barbero, G. F. Fast analysis of β-ecdysone in Brazilian ginseng (*Pfaffia glomerata*) extracts by high-performance liquid chromatography using fused-core column. Pittsburgh Conference on Analytical Chemistry and Applied Spectrometry, Chicago – EUA, 2014.

Vardanega, R., Santos, D. T.; Meireles, M. A. A. Extraction process intensification of saponins from Brazilian ginseng (*Pfaffia glomerata*) using ultrasound and hyphenized processes for integral use of the plant. Workshop on Supercritical Fluids and Energy (SFE'13), Campinas-Brasil, 2013.

Santos, D. T., **Vardanega, R.**, Albarelli, J. Q., Ensinas, A. V., Maréchal, F., Meireles, M. A. A. Design of a future biorefinery based on the use of sub/supercritical fluids using nontraditional biomass: Brazilian ginseng case study. Workshop on Supercritical Fluids and Energy (SFE'13), Campinas-Brasil, 2013.

Debien, I. C. N., **Vardanega, R.**, Santos, D. T., Meireles, M. A. A. Optimization of pressurized liquid extraction of ecdysteroids from Brazilian ginseng roots. III Iberoamerican Conference on Supercritical Fluids (PROSCIBA), Cartagena de Índias - Colômbia, 2013.

SANTOS, D. T., **Vardanega, R.**, PRADO, J. M., Meireles, M. A. A. Two-step subcritical water extraction method for integral use of Brazilian ginseng roots. 9th International conference on renewable resources and biorefineries (RRB-9), Antwerp – Bélgica, p. 128-129, 2013.

APÊNDICES

APÊNDICE A

O Apêndice A contém informações suplementares referentes ao ***Capítulo 4***, incluindo dados de análise dos extratos por espectrometria de massas LC-MS/MS, tabelas de análise de variância (ANOVA) geradas para os planejamentos experimentais realizados para a extração com água subcrítica das raízes e partes aéreas do ginseng brasileiro, curvas de calibração para quantificação de beta-ecdisona e frutoolissacarídeos.

Apêndice A.1: Análise dos extratos das raízes e partes aéreas do ginseng brasileiro por Espectrometria de massas – LC-MS/MS

Para a análise dos extratos por LC-MS e LC-MS/MS, as amostras foram solubilizadas em água até a concentração de 250 $\mu g \cdot mL^{-1}$. As análises foram realizadas em um sistema LC-MS/MS (Agilent Technologies,1290 Series Liquid Chromatography, Santa Clara, USA). A separação dos compostos foi realizada em uma coluna de fase reversa (Poroshell 120 EC-C_{18}, 100 × 4,6 mm, 2,7 μm, Agilent Technologies, Santa Clara, USA). A fase móvel foi composta por água com 0,1% de ácido fórmico (A) e acetonitrila com 0,1% de ácido fórmico (B), e o gradiente empregado foi de 0 min: 75% A; 7 min: 70% A; 10 min: 70% A; 12 min: 0% A; 15 min 0% A; 17 min: 75% A; 20 min 75% A. A vazão foi 0,5 $mL \cdot min^{-1}$ a 308 K e o volume de amostra injetado foi 10 μL. A aquisição dos dados foi realizada usando um Agilent ifunnel (Q-TOF 6550 LC-MS) com fonte Dual Agilent Jet Stream ESI (Dual AJS-ESI) nas seguintes condições: gás de secagem a 523 K, vazão do gás de secagem 11 $L \cdot min^{-1}$, nebulização a 35 psi, gás de bainha a 598 K com fluxo de 10 $L \cdot min^{-1}$, voltagem do capilar de 3500 V, fragmentador de 100 V e voltagem no octapolo de 750 V. A faixa de aquisição foi entre 100 a 1500 *m/z* para MS e fragmentação MS/MS.

A Figura A.1 apresenta o scan do padrão de beta-ecdisona no tempo de eluição entre 1,3 e 2 min. Observa-se que além do íon com razão massa/carga (*m/z*) 481, o qual corresponde à beta-ecdisona, há também fragmentos com *m/z* 463, 445 e 427 que correspondem a perdas neutras de 1 a 3 moléculas de água da beta-ecdisona (Figura 1 B). A presença destes íons associados com a perda de água da molécula de beta-ecdisona já foi

reportada anteriormente. O íon 503 corresponde à um aduto de sódio da molécula de beta-ecdisona.

Nas Figuras A.2 e A.3, observa-se que ambos os extratos obtidos a partir das raízes e das partes aéreas de ginseng brasileiro também apresentaram uma dispersão de íons em torno do *m/z* 481. Essa dispersão de íons não representa um conjunto de diferentes substâncias presentes nos extratos e sim perdas neutras de água da mesma molécula (beta-ecdisona), conforme observado nos espectros do padrão de beta-ecdisona (Figura A.1).

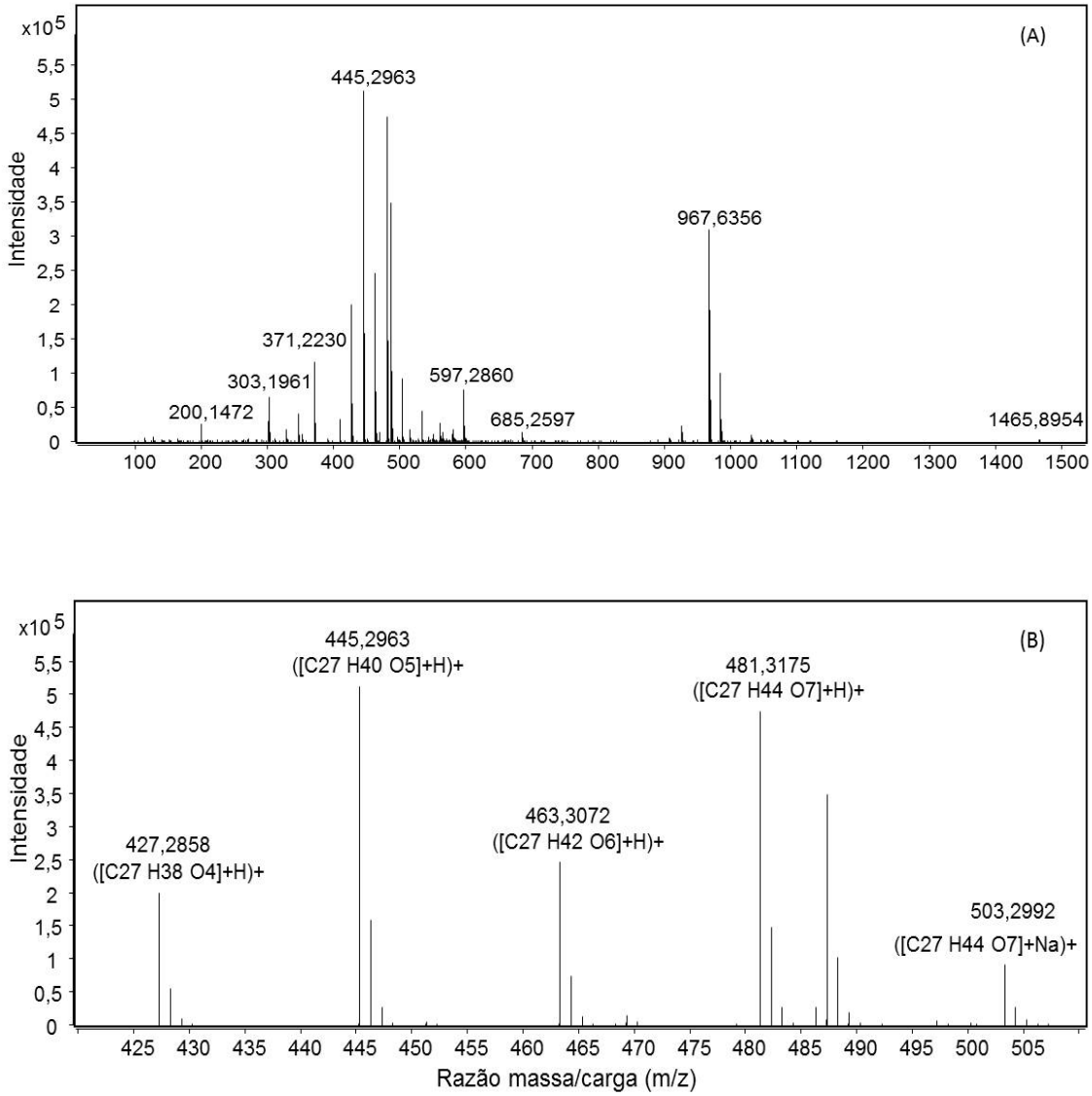


Figura A.1: (A) *Scan* completo do padrão de beta-ecdisona e (B) Ampliação do *scan* do padrão de beta-ecdisona na região de 420 a 510 *m/z*.

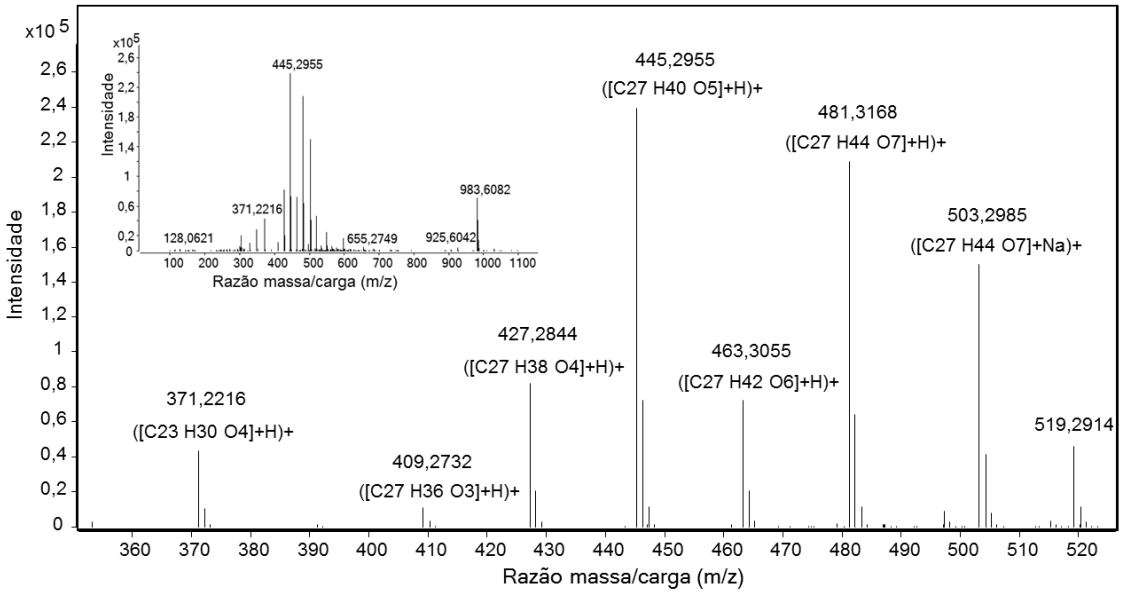


Figura A.2: Ampliação do *scan* do extrato das raízes de ginseng brasileiro na faixa de 355 a 525 *m/z*. No detalhe, *scan* completo do extrato das raízes de ginseng brasileiro.

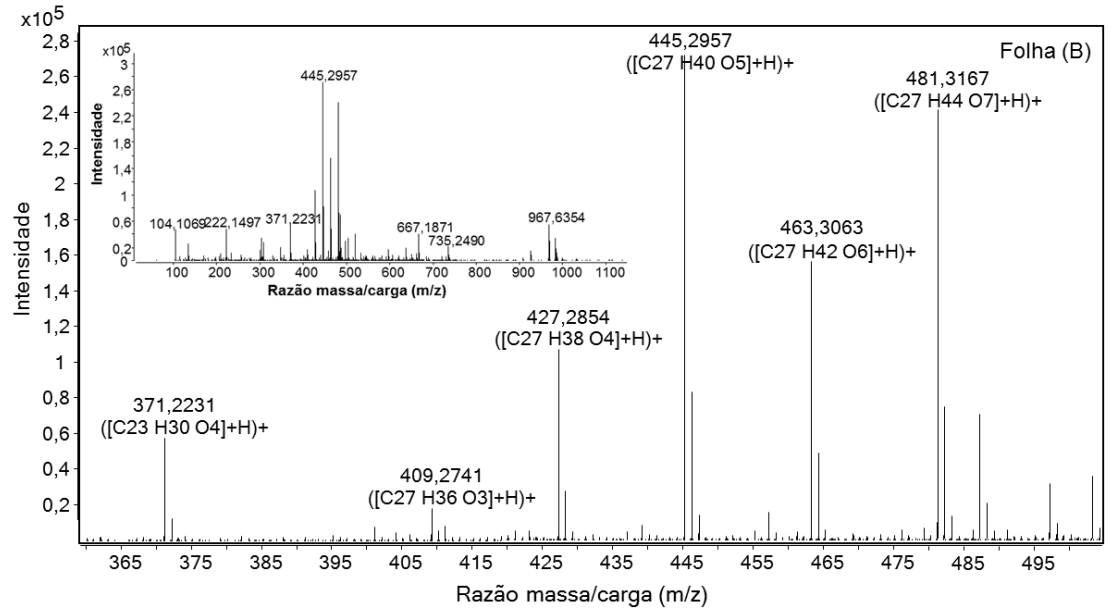


Figura A.3: Ampliação do *scan* do extrato das partes aéreas de ginseng brasileiro na faixa de 355 a 515 *m/z*. No detalhe, *scan* completo do extrato das partes aéreas de ginseng brasileiro.

Apêndice A.2: Tabelas de análise de variância (ANOVA) geradas para o planejamento experimental das raízes de ginseng brasileiro

General Linear Model: Rendimento versus Temperature; Static extraction time

```
Factor                  Type   Levels  Values
Temperature             fixed       6  353; 373; 393; 413; 433; 453
Static extraction time  fixed       3  5; 10; 15

Analysis of Variance for Rendimento, using Adjusted SS for Tests

Source                                DF   Seq SS   Adj SS  Adj MS      F      P
Temperature                            5  2954,68  2954,68  590,94  49,64  0,000
Static extraction time                 2   105,14   105,14   52,57   4,42  0,028
Temperature*Static extraction time    10    22,93    22,93    2,29   0,19  0,994
Error                                 18   214,26   214,26   11,90
Total                                 35  3297,01
S = 3,45012   R-Sq = 93,50%   R-Sq(adj) = 87,36%
```

General Linear Model: b-ecdysone versus Temperature; Static extraction time

```
Factor                  Type   Levels  Values
Temperature             fixed       6  353; 373; 393; 413; 433; 453
Static extraction time  fixed       3  5; 10; 15

Analysis of Variance for b-ecdysone, using Adjusted SS for Tests

Source                                DF    Seq SS    Adj SS    Adj MS       F
Temperature                            5  0,951054  0,930467  0,186093  182,49
Static extraction time                 2  0,034700  0,029890  0,014945   14,66
Temperature*Static extraction time    10  0,124817  0,124817  0,012482   12,24
Error                                 10  0,010197  0,010197  0,001020
Total                                 27  1,120769
Source                                    P
Temperature                           0,000
Static extraction time                0,001
Temperature*Static extraction time    0,000
Error
```

General Linear Model: FOS extrato versus Temperature; Static extraction time

```
Factor                  Type   Levels  Values
Temperature             fixed       6  353; 373; 393; 413; 433; 453
Static extraction time  fixed       3  5; 10; 15

Analysis of Variance for FOS extrato, using Adjusted SS for Tests
Source                 DF   Seq SS   Adj SS  Adj MS      F      P
Temperature             5  113,351  114,731  22,946  15,70  0,000
Static extraction time  2   10,957   10,957   5,479   3,75  0,041
Error                  20   29,237   29,237   1,462
Total                  27  153,545
S = 1,20906   R-Sq = 80,96%   R-Sq(adj) = 74,29%
```

Apêndice A.3: Tabelas de análise de variância (ANOVA) geradas para o planejamento experimental das partes aéreas de ginseng brasileiro

General Linear Model: Yield versus Temperature; Pressure; Static time

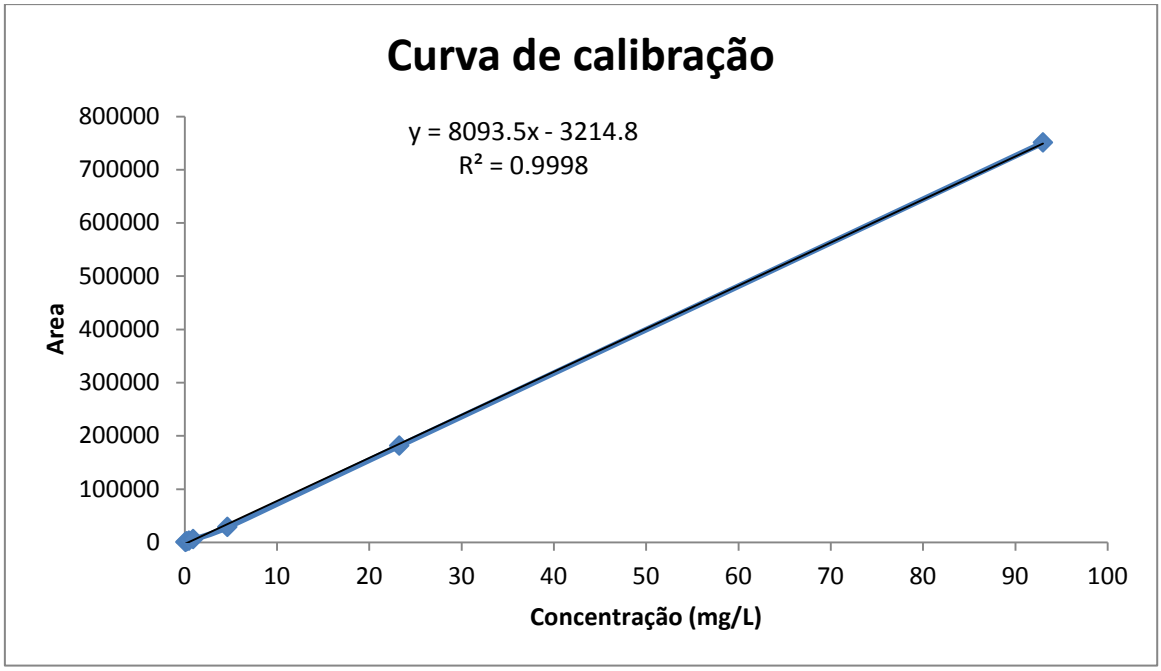
```
Factor       Type   Levels  Values
Temperature  fixed       5  80; 120; 140; 160; 180
Pressure     fixed       3  20; 70; 120
Static time  fixed       2  5; 10


Analysis of Variance for Yield, using Adjusted SS for Tests

Source                            DF   Seq SS   Adj SS   Adj MS       F      P
Temperature                        4  534,664  534,664  133,666  188,38  0,000
Pressure                           2    9,585    9,585    4,793    6,75  0,004
Static time                        1    9,238    9,238    9,238   13,02  0,001
Temperature*Pressure               8   15,747   15,747    1,968    2,77  0,020
Temperature*Static time            4    1,349    1,349    0,337    0,48  0,753
Pressure*Static time               2    2,870    2,870    1,435    2,02  0,150
Temperature*Pressure*Static time   8   17,056   17,056    2,132    3,00  0,013
Error                             30   21,287   21,287    0,710
Total                             59  611,797


S = 0,842360   R-Sq = 96,52%   R-Sq(adj) = 93,16%
```

General Linear Model: Beta-ecdisone versus Temperature; Pressure; ...

```
Factor       Type   Levels  Values
Temperature  fixed       5  80; 120; 140; 160; 180
Pressure     fixed       3  20; 70; 120
Static time  fixed       2  5; 10


Analysis of Variance for Beta-ecdisone, using Adjusted SS for Tests

Source                            DF    Seq SS    Adj SS    Adj MS       F
Temperature                        4  0,490211  0,490211  0,122553  154,80
Pressure                           2  0,000190  0,000190  0,000095    0,12
Static time                        1  0,002557  0,002557  0,002557    3,23
Temperature*Pressure               8  0,005721  0,005721  0,000715    0,90
Temperature*Static time            4  0,006420  0,006420  0,001605    2,03
Pressure*Static time               2  0,002283  0,002283  0,001142    1,44
Temperature*Pressure*Static time   8  0,005192  0,005192  0,000649    0,82
Error                             30  0,023751  0,023751  0,000792
Total                             59  0,536325

Source                               P
Temperature                      0,000
Pressure                         0,888
Static time                      0,082
Temperature*Pressure             0,527
Temperature*Static time          0,116
Pressure*Static time             0,252
Temperature*Pressure*Static time 0,591
Error
Total


S = 0,0281370   R-Sq = 95,57%   R-Sq(adj) = 91,29%
```

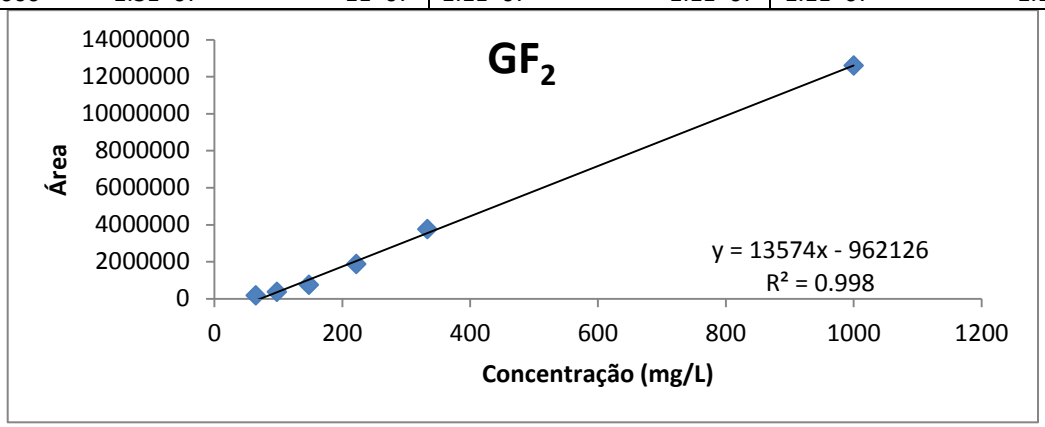
Apêndice A.4: Curva de calibração para quantificação de beta-ecdisona dos extratos de ginseng brasileiro

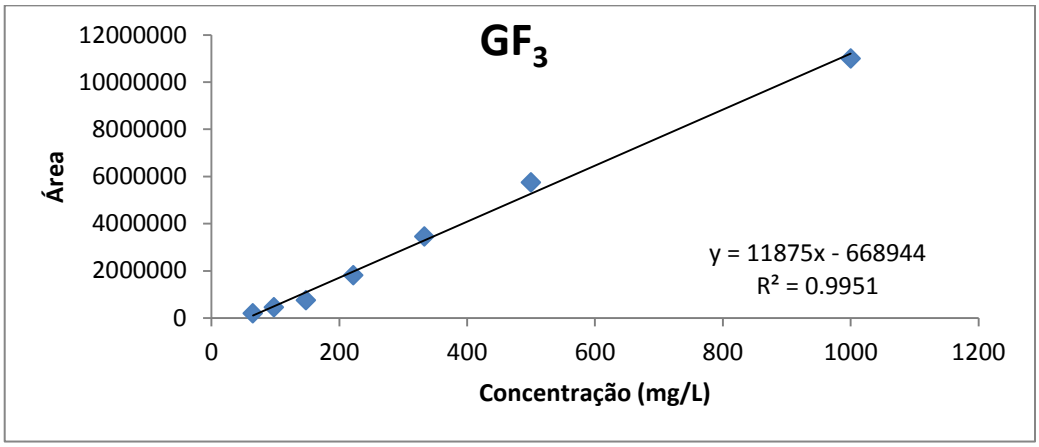
Concentração (mg/L)	Concentração real (mg/L)	Area 1	Area 2	Media
0.1	0.093	454	419	436.5
0.25	0.2325	1299	1222	1260.5
0.5	0.465	2652	2626	2639
1	0.93	5402	5183	5292.5
5	4.65	28478	28461	28469.5
25	23.25	181514	180726	181120
100	93	751318	750106	750712

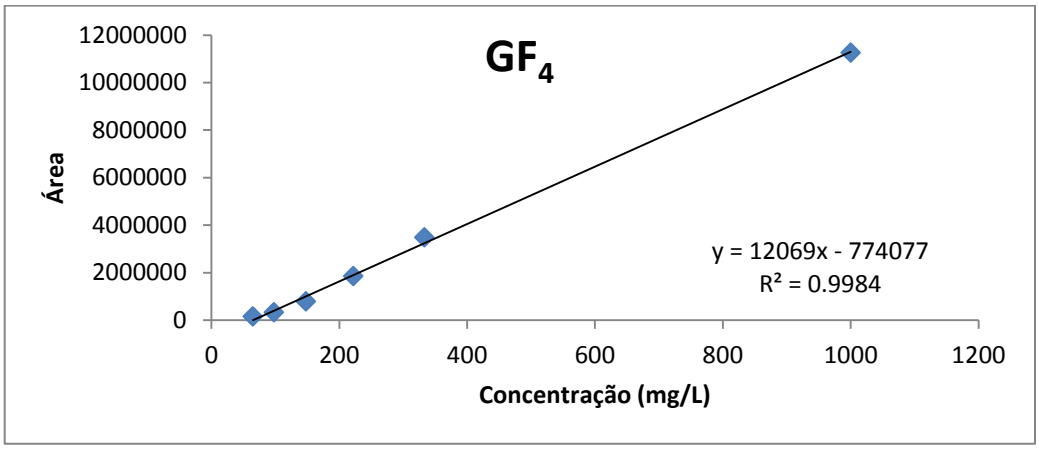


Apêndice A.5: Curvas de calibração para quantificação de frutooligossacarídeos.

	GF_2 (1-cestose)			GF_3 (nistose)			GF_4 (frutofuranosilnistose)		
Concentração	**Área 1**	**Área 2**	**Média**	**Área 1**	**Área 2**	**Média**	**Área 1**	**Área 2**	**Média**
65	174644	65117	174644	197053	86957	197053	159788	76021	159788
98	354333	405594	379964	423560	482367	452964	295604	373996	334800
148	677141	826357	751749	672141	837836	754989	689310	887513	788412
222	1743327	2007195	2E+06	1686744	1939301	1813023	1713561	1985591	1849576
333	3172297	4363802	4E+06	3023660	3891333	3457497	3025230	3946075	3485653
500	--	--	--	5747268	--	5747268	--	--	
1000	1.3E+07		1E+07	1.1E+07		1.1E+07	1.1E+07		1.1E+07







APÊNDICE B

O Apêndice B contém dados suplementares referentes ao ***Capítulo 5***, incluindo dados sobre a extração de beta-ecdisona das raízes de ginseng brasileiro assistida por ultrassom, tabelas de análise estatística, rotinas de ajuste e parâmetros ajustados pelo modelo *spline* e curva de tensão superficial dos extratos de raízes de ginseng brasileiro.

Apêndice B.1: Extração de beta-ecdisona das raízes de ginseng brasileiro assistida por ultrassom

Os resultados obtidos no Capítulo 5 mostraram que na primeira etapa do processo de extração intensificado que utilizou etanol como solvente apenas 40% do total de beta-ecdisona das raízes de ginseng brasileiro foram recuperados. Como uma alternativa para aumentar a recuperação de beta-ecdisona no extrato etanólico obtido na primeira etapa do processo realizou-se um processo de extração assistido por ultrassom, conforme descrito por Torres et al. [1]. O aparato experimental é compost por dois components principais: a primeira é um dispersor de fase múltipla (Ultra-turrax®, IKA, Lelystad, Holanda) e a segunda é uma sonda ultrassônica (Unique, Indaiatuba, Brasil). Também, um banho termostático (Marconi, modelo MA184, Piracicaba, Brasil) é utilizado para manter a temperatura de extração constante. Esse equipamento permite a circulação do solvente sob alta velocidade através da matéria-prima. Em função disso, o processo é chamado *High Turbulence Extraction – HTE* e quando o processo é assistido por ultrassom este é chamado de *Ultrasound Assisted High Turbulence Extraction – UAHTE*.

Inicialmente, 70 g de BGR (base úmida) foram colocados no funil do Ultra-Turrax® e 490 g de etanol foram adicionados para alcançar o S/F de 7, de acordo com o que foi definido no Capítulo 5. O solvente circulou a uma vazão de 2L/min (24000 rpm) durante 40 min [1]. Para os ensaios que foram assistidos por ultrassom, a sonda ultrassônica foi acoplada ao Ultra-Turrax® e operou a uma potência de 800 W. A temperatura de extração foi fixada em 333 K.

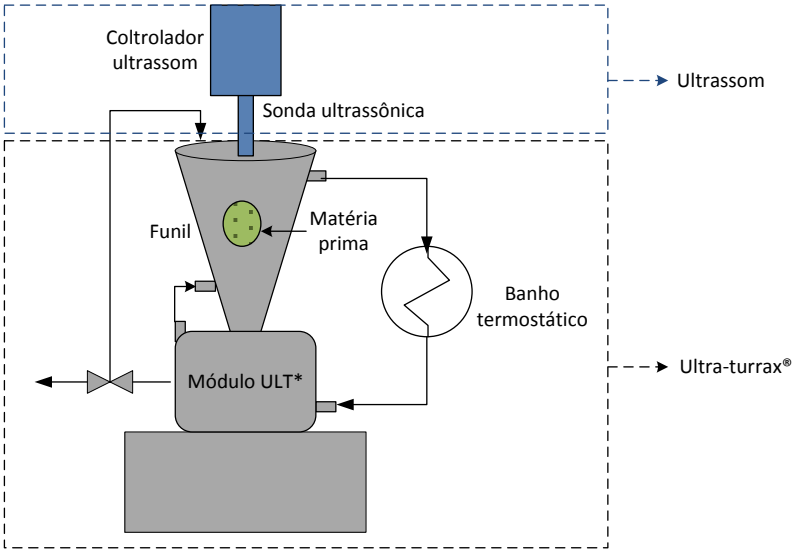


Figure B.1: Aparato experimental do sistema de Ultra-turrax® acoplado ao ultrassom utilizado para extração [1]. * O módulo ULT contém o sistema de rotor-estacionário utilizado para promover cisalhamento.

O rendimento de extração obtido nos processos HTE e UAHTE foi 7,1 ± 0,3 g·100 g^{-1} de matéria-prima (base seca) para ambos os processos. O rendimento de extração obtido no processo de extração intensificado realizado à pressão ambiente e 333 K e S/F = 7 foi 3,6 ± 0,3 g·100 g^{-1} de matéria-prima. A alta velocidade de circulação do solvente através da matéria-prima promovida pelo processo HTE foi suficiente para aumentar a taxa de transferência de massa do processo de extração e, desta forma, a assistência do ultrassom não apresentou nenhum efeito.

O teor de beta-ecdisona dos extratos obtidos nos processos HTE e UAHTE foi 3,41 ± 0,02 e 3,35 ± 0,03 g·100 g^{-1} de extrato, respectivamente, o que representa uma recuperação de 0,24 g·100 g^{-1} de matéria-prima. Observa-se que o emprego de ultrassom também não exerceu efeito sobre a recuperação de beta-ecdisona das raízes de ginseng brasileiro. Portanto, o uso de ultrassom pode ser descartado quando pretende-se aumentar a recuperação de beta-ecdisona.

REFERENCES

[1] R.A.C. Torres, D.T. Santos, M.A.A. Meireles, Novel extraction method to produce active compounds solutions from plant materials, Food and Public Health, 5 (2015) 38-46.

Apêndice B.2: Análise estatística para verificar a influência da pressão no processo intensificado.

One-way ANOVA: Rendimento etapa 1 versus Pressão

```
Source   DF     SS     MS     F      P
Pressão   2  3,943  1,972  4,75  0,118
Error     3  1,245  0,415
Total     5  5,188

S = 0,6442   R-Sq = 76,00%   R-Sq(adj) = 60,01%

                       Individual 95% CIs For Mean Based on
                       Pooled StDev
Level  N   Mean  StDev  ---+---------+---------+---------+------
  1    2  9,000  0,707              (---------*---------)
 50    2  7,050  0,495  (---------*---------)
100    2  7,700  0,707       (--------*---------)
                       ---+---------+---------+---------+------
                        6,0       7,5       9,0      10,5
Pooled StDev = 0,644


Grouping Information Using Tukey Method


Pressão  N    Mean  Grouping
  1      2  9,0000  A
100      2  7,7000  A
 50      2  7,0500  A
```

One-way ANOVA: Rendimento etapa 2 versus Pressão

```
Source   DF     SS     MS     F      P
Pressão   2  3,203  1,602  1,98  0,283
Error     3  2,430  0,810
Total     5  5,633

S = 0,9   R-Sq = 56,86%   R-Sq(adj) = 28,11%

                        Individual 95% CIs For Mean Based on
                        Pooled StDev
Level  N    Mean  StDev  -----+---------+---------+---------+----
  1    2  66,950  1,344         (------------*-------------)
 50    2  67,500  0,707            (-------------*-------------)
100    2  65,750  0,354  (------------*-------------)
                        -----+---------+---------+---------+----
                          64,5      66,0      67,5      69,0
Pooled StDev = 0,900

Grouping Information Using Tukey Method

Pressão  N     Mean  Grouping
 50      2  67,5000  A
  1      2  66,9500  A
100      2  65,7500  A
```

One-way ANOVA: Beta-ecdisona extrato etapa 1 versus Pressão

```
Source   DF      SS      MS      F      P
Pressão   2  4,5733  2,2867  57,17  0,004
Error     3  0,1200  0,0400
Total     5  4,6933

S = 0,2   R-Sq = 97,44%   R-Sq(adj) = 95,74%


                          Individual 95% CIs For Mean Based on
                          Pooled StDev
Level  N    Mean   StDev   -+---------+---------+---------+--------
  1    2  3,8000  0,2828                        (----*-----)
 50    2  3,9000  0,1414                         (-----*----)
100    2  2,0000  0,1414   (-----*-----)
                          -+---------+---------+---------+--------
                         1,60      2,40      3,20      4,00

Pooled StDev = 0,2000


Grouping Information Using Tukey Method

Pressão  N    Mean  Grouping
 50      2  3,9000  A
  1      2  3,8000  A
100      2  2,0000    B
```

One-way ANOVA: Beta-ecdisona. MP etapa 1 versus Pressão

```
Source   DF         SS         MS       F      P
Pressão   2  0,0347363  0,0173682  498,61  0,000
Error     3  0,0001045  0,0000348
Total     5  0,0348408

S = 0,005902   R-Sq = 99,70%   R-Sq(adj) = 99,50%


                            Individual 95% CIs For Mean Based on
                            Pooled StDev
Level  N     Mean    StDev  ------+---------+---------+---------+---
  1    2  0,33850  0,00212                                (-*--)
 50    2  0,27500  0,00707                       (-*-)
100    2  0,15500  0,00707  (-*-)
                            ------+---------+---------+---------+---
                                0,180     0,240     0,300     0,360

Pooled StDev = 0,00590


Grouping Information Using Tukey Method

Pressão  N     Mean  Grouping
  1      2  0,33850  A
 50      2  0,27500    B
100      2  0,15500      C
```

One-way ANOVA: Beta-ecdisona extrato etapa 2 versus Pressão

```
Source   DF        SS        MS     F      P
Pressão   2  0,001656  0,000828  1,90  0,292
Error     3  0,001304  0,000435
Total     5  0,002961

S = 0,02085   R-Sq = 55,94%   R-Sq(adj) = 26,57%


                          Individual 95% CIs For Mean Based on
                          Pooled StDev
Level  N     Mean    StDev  ---+---------+---------+---------+------
  1    2  0,53850  0,00212     (-------------*------------)
 50    2  0,56500  0,03536               (------------*-------------)
100    2  0,52500  0,00707  (------------*------------)
                          ---+---------+---------+---------+------
                           0,490     0,525     0,560     0,595

Pooled StDev = 0,02085


Grouping Information Using Tukey Method

Pressão  N     Mean  Grouping
 50      2  0,56500  A
  1      2  0,53850  A
100      2  0,52500  A
```

One-way ANOVA: Beta-ecdisona MP etapa 2 versus Pressão

```
Source   DF        SS        MS     F      P
Pressão   2  0,001941  0,000971  4,73  0,118
Error     3  0,000616  0,000205
Total     5  0,002557

S = 0,01433   R-Sq = 75,91%   R-Sq(adj) = 59,85%


                          Individual 95% CIs For Mean Based on
                          Pooled StDev
Level  N     Mean    StDev  --------+---------+---------+---------+-
  1    2  0,36100  0,00566          (---------*----------)
 50    2  0,38100  0,02263                (----------*----------)
100    2  0,33700  0,00849  (---------*----------)
                          --------+---------+---------+---------+-
                                0,330     0,360     0,390     0,420

Pooled StDev = 0,01433


Grouping Information Using Tukey Method

Pressão  N     Mean  Grouping
 50      2  0,38100  A
  1      2  0,36100  A
100      2  0,33700  A
```

One-way ANOVA: Beta-ecdisonaTotal MP versus Pressão

```
Source   DF        SS        MS       F      P
Pressão   3  0,064573  0,021524  172,03  0,000
Error     4  0,000500  0,000125
Total     7  0,065073

S = 0,01119   R-Sq = 99,23%   R-Sq(adj) = 98,65%


                          Individual 95% CIs For Mean Based on
                          Pooled StDev
Level  N     Mean    StDev  ---+---------+---------+---------+------
  1,0  2  0,69974  0,00784                                (--*--)
  1,1  2  0,71950  0,00354                                   (--*--)
 50,0  2  0,65465  0,01480                          (---*--)
100,0  2  0,49104  0,01441  (--*--)
                          ---+---------+---------+---------+------
                           0,490     0,560     0,630     0,700

Pooled StDev = 0,01119


Grouping Information Using Tukey Method

Pressão  N     Mean  Grouping
  1,1    2  0,71950  A
  1,0    2  0,69974  A B
 50,0    2  0,65465    B
100,0    2  0,49104      C
```

One-way ANOVA: Saponinas etapa 1 versus Pressão

```
Source   DF     SS     MS      F      P
Pressão   2  75,87  37,93  15,14  0,027
Error     3   7,51   2,50
Total     5  83,38

S = 1,583   R-Sq = 90,99%   R-Sq(adj) = 84,98%


                        Individual 95% CIs For Mean Based on
                        Pooled StDev
Level  N    Mean  StDev  ---+---------+---------+---------+------
  1    2  46,450  0,085  (--------*--------)
 50    2  54,665  1,817                     (--------*--------)
100    2  48,050  2,051      (--------*--------)
                        ---+---------+---------+---------+------
                        44,0      48,0      52,0      56,0

Pooled StDev = 1,583


Grouping Information Using Tukey Method

Pressão  N    Mean  Grouping
 50      2  54,665  A
100      2  48,050    B
  1      2  46,450    B
```

One-way ANOVA: Saponinas etapa 2 versus Pressão

```
Source   DF      SS     MS      F      P
Pressão   2  165,49  82,75  53,21  0,005
Error     3    4,66   1,55
Total     5  170,16

S = 1,247   R-Sq = 97,26%   R-Sq(adj) = 95,43%


                         Individual 95% CIs For Mean Based on Pooled StDev
Level  N    Mean  StDev     +---------+---------+---------+---------
  1    2  24,900  0,707         (-----*----)
 50    2  35,000  1,980                              (-----*-----)
100    2  23,050  0,495     (-----*-----)
                            +---------+---------+---------+---------
                         20,0      25,0      30,0      35,0

Pooled StDev = 1,247


Grouping Information Using Tukey Method

Pressão  N    Mean  Grouping
 50      2  35,000  A
  1      2  24,900    B
100      2  23,050    B
```

One-way ANOVA: Saponinas MP etapa 1 versus Pressão

```
Source   DF      SS      MS     F      P
Pressão   2  0,2342  0,1171  1,19  0,415
Error     3  0,2941  0,0980
Total     5  0,5284

S = 0,3131   R-Sq = 44,33%   R-Sq(adj) = 7,22%


                          Individual 95% CIs For Mean Based on Pooled StDev
Level  N    Mean   StDev    +---------+---------+---------+---------
  1    2  4,1693  0,3195              (-------------*-------------)
 50    2  3,8586  0,3988         (-------------*-------------)
100    2  3,6926  0,1819    (-------------*-------------)
                            +---------+---------+---------+---------
                          3,00      3,50      4,00      4,50

Pooled StDev = 0,3131


Grouping Information Using Tukey Method

Pressão  N    Mean  Grouping
  1      2  4,1693  A
 50      2  3,8586  A
100      2  3,6926  A
```

One-way ANOVA: Saponinas MP etapa 2 versus Pressão

```
Source   DF      SS      MS      F      P
Pressão   2  87,880  43,940  58,46  0,004
Error     3   2,255   0,752
Total     5  90,135

S = 0,8670   R-Sq = 97,50%   R-Sq(adj) = 95,83%


                        Individual 95% CIs For Mean Based on
                        Pooled StDev
Level  N    Mean  StDev  ---+---------+---------+---------+------
  1    2  16,650  0,071       (-----*----)
 50    2  23,650  1,485                          (-----*----)
100    2  14,750  0,212  (----*-----)
                        ---+---------+---------+---------+------
                        14,0      17,5      21,0      24,5

Pooled StDev = 0,867


Grouping Information Using Tukey Method

Pressão  N    Mean  Grouping
 50      2  23,650  A
  1      2  16,650    B
100      2  14,750    B
```

One-way ANOVA: Tensão superficial etapa 1 versus Pressao

```
Source   DF       SS       MS       F      P
Pressao   2  22,1966  11,0983  689,34  0,000
Error     3   0,0483   0,0161
Total     5  22,2449

S = 0,1269   R-Sq = 99,78%   R-Sq(adj) = 99,64%


                        Individual 95% CIs For Mean Based on
                        Pooled StDev
Level  N    Mean  StDev  ---+---------+---------+---------+------
  1    2  34,300  0,071  (-*-)
 50    2  35,835  0,035            (-*-)
100    2  38,925  0,205                                (*-)
                        ---+---------+---------+---------+------
                        34,5      36,0      37,5      39,0

Pooled StDev = 0,127


Grouping Information Using Tukey Method

Pressao  N     Mean  Grouping
100      2  38,9250  A
 50      2  35,8350    B
  1      2  34,3000      C
```

One-way ANOVA: Tensão superficial etapa 2 versus Pressao

```
Source   DF    SS    MS     F      P
Pressao   2  0,35  0,17  0,11  0,898
Error     3  4,65  1,55
Total     5  5,00

S = 1,246   R-Sq = 6,92%   R-Sq(adj) = 0,00%


                        Individual 95% CIs For Mean Based on
                        Pooled StDev
Level  N    Mean  StDev  ---+---------+---------+---------+------
  1    2  42,825  0,502     (-----------------*----------------)
 50    2  42,620  2,022   (----------------*-----------------)
100    2  42,245  0,559  (----------------*-----------------)
                        ---+---------+---------+---------+------
                        40,0      41,6      43,2      44,8

Pooled StDev = 1,246


Grouping Information Using Tukey Method

Pressao  N    Mean  Grouping
  1      2  42,825  A
 50      2  42,620  A
100      2  42,245  A
```

One-way ANOVA: FOS etapa 2 versus Pressão

```
Source   DF     SS     MS     F      P
Pressão   2  3,656  1,828  2,26  0,252
Error     3  2,423  0,808
Total     5  6,078

S = 0,8986   R-Sq = 60,15%   R-Sq(adj) = 33,58%


                        Individual 95% CIs For Mean Based on
                        Pooled StDev
Level  N    Mean  StDev   -+---------+---------+---------+--------
  1    2   9,323  1,303   (------------*-------------)
 50    2  11,230  0,762               (-------------*------------)
100    2  10,393  0,381          (------------*-------------)
                        -+---------+---------+---------+--------
                        7,5       9,0      10,5      12,0

Pooled StDev = 0,899


Grouping Information Using Tukey Method

Pressão  N     Mean  Grouping
 50      2  11,2301  A
100      2  10,3933  A
  1      2   9,3228  A
```

One-way ANOVA: FOS-MP etapa 2 versus Pressão

```
Source   DF     SS     MS     F      P
Pressão   2  1,910  0,955  2,95  0,196
Error     3  0,971  0,324
Total     5  2,881

S = 0,5689   R-Sq = 66,30%   R-Sq(adj) = 43,83%


                          Individual 95% CIs For Mean Based on Pooled StDev
Level  N    Mean   StDev    +---------+---------+---------+---------
  1    2  6,2363  0,7428    (-----------*-----------)
 50    2  7,5875  0,4577                (------------*------------)
100    2  6,6601  0,4580        (------------*-----------)
                              +---------+---------+---------+---------
                             5,0       6,0       7,0       8,0

Pooled StDev = 0,5689


Grouping Information Using Tukey Method

Pressão  N    Mean  Grouping
 50      2  7,5875  A
100      2  6,6601  A
  1      2  6,2363  A
```

Apêndice B.3: Modelo de rotina de ajusta de duas retas – SAS

```
/* ---------------------------------------------------------------------- */
/* Departamento de Engenharia de Alimentos - DEA / Unicamp                */
/* Ajuste das curvas experimentais no SAS                                 */
/* Renata Vardanega  - LASEFI                                             */
/* Campinas - 27 de julho de 2015                                         */
/* ---------------------------------------------------------------------- */

/* --[Cabeçalho]------------------------------------------------------- */
   Options NoDate NoNumber PS=100 LS=100 FormDLim='-';
   Title'Processo Intensificado - Ginseng Brasileiro';
   FootNote;

 /*----Leitura interna dos dados utilizando o Proc Import]------------- */

Data Dados;
input Tempo Rend1Etapa1Reta1 Rend1Etapa1Reta2 Rend2Etapa1Reta1
Rend2Etapa1Reta2 Rend1Etapa2Reta1 Rend1Etapa2Reta2 Rend1Etapa2Reta3
Rend2Etapa2Reta1 Rend2Etapa2Reta2 Rend2Etapa2Reta3;
Cards;
5     1.8   .     0.9   .     10.2  .     .     7.2   .     .
10    2.7   .     1.4   .     15.7  .     .     12.1  .     .
15    3.3   .     1.8   .     19.6  .     .     16.3  .     .
20    3.7   .     2.1   .     23.0  .     .     19.7  .     .
30    .     4.2   .     2.5   29.1  .     .     25.6  .     .
40    .     4.4   .     2.7   .     34.0  .     .     31.5  .
50    .     4.6   .     2.9   .     38.2  .     .     34.9  .
60    .     4.7   .     3.1   .     41.9  .     .     39.4  .
70    .     4.9   .     3.2   .     45.0  .     .     41.7  .
80    .     5.0   .     3.4   .     48.0  .     .     44.4  .
90    .     5.1   .     3.5   .     50.6  .     .     47.2  .
100   .     5.3   .     3.7   .     52.9  .     .     49.2  .
110   .     5.3   .     3.7   .     55.3  .     .     51.4  .
120   .     5.5   .     3.8   .     .     57.4  .     .     53.0
130   .     5.5   .     4.0   .     .     59.4  .     .     54.4
140   .     5.6   .     4.0   .     .     61.2  .     .     55.7
150   .     5.7   .     4.1   .     .     62.8  .     .     56.4
160   .     5.7   .     4.2   .     .     64.1  .     .     57.5
170   .     5.8   .     4.2   .     .     65.4  .     .     58.4
180   .     5.9   .     4.3   .     .     66.7  .     .     59.5
190   .     5.9   .     4.3   .     .     67.8  .     .     60.5
200   .     6.0   .     4.4   .     .     69.0  .     .     61.2
210   .     .     .     .     .     .     69.9  .     .     61.9
220   .     .     .     .     .     .     70.8  .     .     62.6
230   .     .     .     .     .     .     71.6  .     .     63.3
240   .     .     .     .     .     .     72.3  .     .     64.0
;

Comment Rend1Etapa1Reta1 ------------------------------------------------
-----------------------------------------------;

PROC PRINT DATA=Dados;
RUN;
ODS OUTPUT PARAMETERESTIMATES=parms1;
PROC GLM DATA=Dados;
      Title "Rend1Etapa1Reta1";
      MODEL Rend1Etapa1Reta1=Tempo/SS1;
```

```
RUN;
proc print noobs data=parms1;
title "parms_Rend1Etapa1Reta1";
run;

ODS OUTPUT PARAMETERESTIMATES=parms2;
PROC GLM DATA=Dados;
      Title "Rend1Etapa1Reta2";
      MODEL Rend1Etapa1Reta2=Tempo/ noint SS1;
RUN;
proc print noobs data=parms2;
title "parms_Rend1Etapa1Reta2";
run;

Comment Rend2Etapa1Reta1 ------------------------------------------------
-------------------------------------------------;

ODS OUTPUT DATA=DADOS PARAMETERESTIMATES=parms1;
PROC GLM DATA=Dados;
      Title "Rend2Etapa1Reta1";
      MODEL Rend2Etapa1Reta1=Tempo/SS1;
RUN;
proc print noobs data=parms1;
title "parms_Rend2Etapa1Reta1";
run;

ODS OUTPUT PARAMETERESTIMATES=parms2;
PROC GLM DATA=Dados;
      Title "Rend2Etapa1Reta2";
      MODEL Rend2Etapa1Reta2=Tempo/ noint SS1;
RUN;
proc print noobs data=parms2;
title "parms_Rend2Etapa1Reta2";
run;

quit;

Data DadosNlin;
input Tempo Rend1Etapa1 Rend2Etapa1 Rend1Etapa2 Rend2Etapa2;
Cards;
5     1.8   0.9   10.2  7.2
10    2.7   1.4   15.7  12.1
15    3.3   1.8   19.6  16.3
20    3.7   2.1   23.0  19.7
30    4.2   2.5   29.1  25.6
40    4.4   2.7   34.0  31.5
50    4.6   2.9   38.2  34.9
60    4.7   3.1   41.9  39.4
70    4.9   3.2   45.0  41.7
80    5.0   3.4   48.0  44.4
90    5.1   3.5   50.6  47.2
100   5.3   3.7   52.9  49.2
110   5.3   3.7   55.3  51.4
120   5.5   3.8   57.4  53.0
130   5.5   4.0   59.4  54.4
140   5.6   4.0   61.2  55.7
150   5.7   4.1   62.8  56.4
160   5.7   4.2   64.1  57.5
170   5.8   4.2   65.4  58.4
```

```
180   5.9   4.3   66.7  59.5
190   5.9   4.3   67.8  60.5
200   6.0   4.4   69.0  61.2
210   .     .     69.9  61.9
220   .     .     70.8  62.6
230   .     .     71.6  63.3
240   .     .     72.3  64.0
;
PROC PRINT DATA=DadosNlin;
RUN;

Comment Rend1Etapa1 ------------------------------------------------------
-----------------------------------------;

PROC NLIN DATA=DadosNlin;
TITLE 'Rend1Etapa1';
PARMS             b0 = 1.6568       /*----termo independente da equaçao do
periodo tcer---*/
                  b1 = 0.0927       /*----termo de primeira ordem do período
tcer---*/
                  b2 = -0.0831                  /*----termo de primeria
ordem do período difusional (com mext x AL1)---*/
                  knot1 = 30.5885;
                  AL1 = MAX(Tempo-knot1,0);

MODEL Rend1Etapa1 = b0 + b1*Tempo + b2*AL1;
      Output out = a p=Rend1Etapa1_hat r= Mres;
      Axis order = (0 to 100 by 10);
      run;

Comment Rend2Etapa1 ------------------------------------------------------
-----------------------------------------;
PROC NLIN DATA=DadosNlin;
TITLE 'Rend2Etapa1';
PARMS             b0 = 0.9000       /*----termo independente da equaçao do
periodo tcer---*/
                  b1 = 0.0500             /*----termo de primeira ordem do
período tcer---*/
                  b2 = -0.0404                  /*----termo de primeria
ordem do período difusional (com mext x AL1)---*/
                  knot1 = 42.0430;
                  AL1 = MAX(Tempo-knot1,0);

MODEL Rend2Etapa1 = b0 + b1*Tempo + b2*AL1;
      Output out = a p=Rend2Etapa1_hat r= Mres;
      Axis order = (0 to 100 by 10);
      run;
```

Apêndice B.4: Modelo de rotina de ajusta de três retas – SAS

```
/* ---------------------------------------------------------------------- */
/* Departamento de Engenharia de Alimentos - DEA / Unicamp                */
/* Ajuste das curvas experimentais no SAS                                 */
/* Renata Vardanega  - LASEFI                                             */
/* Campinas - 27 de julho de 2015                                         */
/* ---------------------------------------------------------------------- */
/* --[Cabeçalho]---------------------------------------------------------- */
   Options NoDate NoNumber PS=100 LS=100 FormDLim='-';
   Title'Processo Intensificado - Ginseng Brasileiro';
   FootNote;

 /*----Leitura interna dos dados utilizando o Proc Import]------------- */
Data Dados;
input Tempo Rend1Etapa1Reta1 Rend1Etapa1Reta2 Rend2Etapa1Reta1
Rend2Etapa1Reta2 Rend1Etapa2Reta1 Rend1Etapa2Reta2 Rend1Etapa2Reta3
Rend2Etapa2Reta1 Rend2Etapa2Reta2 Rend2Etapa2Reta3;
Cards;
5     1.8   .     0.9   .     10.2  .     .     7.2   .     .
10    2.7   .     1.4   .     15.7  .     .     12.1  .     .
15    3.3   .     1.8   .     19.6  .     .     16.3  .     .
20    3.7   .     2.1   .     23.0  .     .     19.7  .     .
30    .     4.2   .     2.5   29.1  .     .     25.6  .     .
40    .     4.4   .     2.7   .     34.0  .     .     31.5  .
50    .     4.6   .     2.9   .     38.2  .     .     34.9  .
60    .     4.7   .     3.1   .     41.9  .     .     39.4  .
70    .     4.9   .     3.2   .     45.0  .     .     41.7  .
80    .     5.0   .     3.4   .     48.0  .     .     44.4  .
90    .     5.1   .     3.5   .     50.6  .     .     47.2  .
100   .     5.3   .     3.7   .     52.9  .     .     49.2  .
110   .     5.3   .     3.7   .     55.3  .     .     51.4  .
120   .     5.5   .     3.8   .     .     57.4  .     .     53.0
130   .     5.5   .     4.0   .     .     59.4  .     .     54.4
140   .     5.6   .     4.0   .     .     61.2  .     .     55.7
150   .     5.7   .     4.1   .     .     62.8  .     .     56.4
160   .     5.7   .     4.2   .     .     64.1  .     .     57.5
170   .     5.8   .     4.2   .     .     65.4  .     .     58.4
180   .     5.9   .     4.3   .     .     66.7  .     .     59.5
190   .     5.9   .     4.3   .     .     67.8  .     .     60.5
200   .     6.0   .     4.4   .     .     69.0  .     .     61.2
210   .     .     .     .     .     .     69.9  .     .     61.9
220   .     .     .     .     .     .     70.8  .     .     62.6
230   .     .     .     .     .     .     71.6  .     .     63.3
240   .     .     .     .     .     .     72.3  .     .     64.0
;

Comment Rend1Etapa1Reta1 ------------------------------------------------
-----------------------------------------------;

PROC PRINT DATA=Dados;
RUN;
ODS OUTPUT PARAMETERESTIMATES=parms1;
PROC GLM DATA=Dados;
      Title "Rend1Etapa1Reta1";
/* ---------------------------------------------------------------------- */
/* Departamento de Engenharia de Alimentos - DEA / Unicamp                */
/* Ajuste das curvas experimentais no SAS                                 */
```

```
/* Renata Vardanega  - LASEFI                                          */
/* Campinas - 27 de julho de 2015                                      */
/* ------------------------------------------------------------------- */

/* --[Cabeçalho]------------------------------------------------------- */
   Options NoDate NoNumber PS=100 LS=100 FormDLim='-';
   Title'Processo Intensificado - Ginseng Brasileiro';
   FootNote;

 /*----Leitura interna dos dados utilizando o Proc Import]------------ */

Data Dados;
input Tempo Rend1Etapa1Reta1 Rend1Etapa1Reta2 Rend2Etapa1Reta1
Rend2Etapa1Reta2 Rend1Etapa2Reta1 Rend1Etapa2Reta2 Rend1Etapa2Reta3
Rend2Etapa2Reta1 Rend2Etapa2Reta2 Rend2Etapa2Reta3;
Cards;
5     1.8   .     0.9   .     10.2  .     .     7.2   .     .
10    2.7   .     1.4   .     15.7  .     .     12.1  .     .
15    3.3   .     1.8   .     19.6  .     .     16.3  .     .
20    3.7   .     2.1   .     23.0  .     .     19.7  .     .
30    .     4.2   .     2.5   29.1  .     .     25.6  .     .
40    .     4.4   .     2.7   .     34.0  .     .     31.5  .
50    .     4.6   .     2.9   .     38.2  .     .     34.9  .
60    .     4.7   .     3.1   .     41.9  .     .     39.4  .
70    .     4.9   .     3.2   .     45.0  .     .     41.7  .
80    .     5.0   .     3.4   .     48.0  .     .     44.4  .
90    .     5.1   .     3.5   .     50.6  .     .     47.2  .
100   .     5.3   .     3.7   .     52.9  .     .     49.2  .
110   .     5.3   .     3.7   .     55.3  .     .     51.4  .
120   .     5.5   .     3.8   .     .     57.4  .     .     53.0
130   .     5.5   .     4.0   .     .     59.4  .     .     54.4
140   .     5.6   .     4.0   .     .     61.2  .     .     55.7
150   .     5.7   .     4.1   .     .     62.8  .     .     56.4
160   .     5.7   .     4.2   .     .     64.1  .     .     57.5
170   .     5.8   .     4.2   .     .     65.4  .     .     58.4
180   .     5.9   .     4.3   .     .     66.7  .     .     59.5
190   .     5.9   .     4.3   .     .     67.8  .     .     60.5
200   .     6.0   .     4.4   .     .     69.0  .     .     61.2
210   .     .     .     .     .     .     69.9  .     .     61.9
220   .     .     .     .     .     .     70.8  .     .     62.6
230   .     .     .     .     .     .     71.6  .     .     63.3
240   .     .     .     .     .     .     72.3  .     .     64.0
;

Comment Rend1Etapa2 ------------------------------------------------------
-----------------------------------------;

PROC PRINT DATA=Dados;
RUN;
ODS OUTPUT PARAMETERESTIMATES=parms1;
PROC GLM DATA=Dados;
      Title "Rend1Etapa2Reta1";
      MODEL Rend1Etapa2Reta1=Tempo/SS1;
RUN;
proc print noobs data=parms1;
title "parms_Rend1Etapa2Reta1";
run;

ODS OUTPUT PARAMETERESTIMATES=parms2;
```

```
PROC GLM DATA=Dados;
      Title "Rend1Etapa2Reta2";
      MODEL Rend1Etapa2Reta2=Tempo/ noint SS1;
RUN;
proc print noobs data=parms2;
title "parms_Rend1Etapa2Reta2";
run;

ODS OUTPUT PARAMETERESTIMATES=parms3;
PROC GLM DATA=Dados;
      Title "Rend1Etapa2Reta3";
      MODEL Rend1Etapa2Reta3=Tempo/ noint SS1;
RUN;
proc print noobs data=parms3;
title "parms_Rend1Etapa2Reta3";
run;
Comment Rend2Etapa2 ------------------------------------------------------
-----------------------------------------;
ODS OUTPUT DATA=DADOS PARAMETERESTIMATES=parms1;
PROC GLM DATA=Dados;
      Title "Rend2Etapa2Reta1";
      MODEL Rend2Etapa2Reta1=Tempo/SS1;
RUN;
proc print noobs data=parms1;
title "parms_Rend2Etapa2Reta1";
run;

ODS OUTPUT PARAMETERESTIMATES=parms2;
PROC GLM DATA=Dados;
      Title "Rend2Etapa2Reta2";
      MODEL Rend2Etapa2Reta2=Tempo/ noint SS1;
RUN;
proc print noobs data=parms2;
title "parms_Rend2Etapa2Reta2";
run;

ODS OUTPUT PARAMETERESTIMATES=parms3;
PROC GLM DATA=Dados;
      Title "Rend2Etapa2Reta3";
      MODEL Rend2Etapa2Reta3=Tempo/ noint SS1;
RUN;
proc print noobs data=parms3;
title "parms_Rend2Etapa2Reta3";
run;
quit;
Data DadosNlin;
input Tempo Rend1Etapa1 Rend2Etapa1 Rend1Etapa2 Rend2Etapa2;
Cards;
5     1.8   0.9   10.2  7.2
10    2.7   1.4   15.7  12.1
15    3.3   1.8   19.6  16.3
20    3.7   2.1   23.0  19.7
30    4.2   2.5   29.1  25.6
40    4.4   2.7   34.0  31.5
50    4.6   2.9   38.2  34.9
60    4.7   3.1   41.9  39.4
70    4.9   3.2   45.0  41.7
80    5.0   3.4   48.0  44.4
90    5.1   3.5   50.6  47.2
```

```
100   5.3   3.7   52.9  49.2
110   5.3   3.7   55.3  51.4
120   5.5   3.8   57.4  53.0
130   5.5   4.0   59.4  54.4
140   5.6   4.0   61.2  55.7
150   5.7   4.1   62.8  56.4
160   5.7   4.2   64.1  57.5
170   5.8   4.2   65.4  58.4
180   5.9   4.3   66.7  59.5
190   5.9   4.3   67.8  60.5
200   6.0   4.4   69.0  61.2
210   .     .     69.9  61.9
220   .     .     70.8  62.6
230   .     .     71.6  63.3
240   .     .     72.3  64.0
;
PROC PRINT DATA=DadosNlin;
RUN;
Comment Rend1Etapa1 ------------------------------------------------------
-----------------------------------------;
PROC NLIN DATA=DadosNlin;
TITLE 'Rend1Etapa2';
PARMS             b0 = 6.5500       /*----termo independente da equaçao do
periodo tcer---*/
                  b1 = 0.8460       /*----termo de primeira ordem do período
tcer---*/
                  b2 = -0.5253                  /*----termo de primeria
ordem do período difusional (com mext x AL1)---*/
                  b3 = -0.1985             /*----termo de primeria ordem do
período difusional---*/
            C1 = 28.34                 /*----tcer---*/
            C2 = 113.9;               /*----tfer---*/

                                    AL1 = MAX(Tempo-C1,0);
                                    AL2 = MAX(Tempo-C2,0);

MODEL Rend1Etapa2 = b0 + b1*Tempo + b2*AL1 + b3*AL2;
      Output out = a p=Rend1Etapa2_hat r= Mres;
      Axis order = (0 to 100 by 10);
      run;
      Comment Rend2Etapa1 ------------------------------------------------
-------------------------------------------------;
PROC NLIN DATA=DadosNlin;
TITLE 'Rend2Etapa2';
PARMS             b0 = 3.4000       /*----termo independente da equaçao do
periodo tcer---*/
                  b1 = 0.8340             /*----termo de primeira ordem do
período tcer---*/
                  b2 = -0.5072                  /*----termo de primeria
ordem do período difusional (com mext x AL1)---*/
                  b3 = -0.2326             /*----termo de primeria ordem do
período difusional---*/
            C1 = 28.7776                /*----tcer---*/
            C2 = 103.5;               /*----tfer---*/

                                    AL1 = MAX(Tempo-C1,0);
                                    AL2 = MAX(Tempo-C2,0);

MODEL Rend2Etapa2 = b0 + b1*Tempo + b2*AL1 + b3*AL2;
```

```
Output out = a p=Rend2Etapa2_hat r= Mres;
Axis order = (0 to 100 by 10);
run;
```

Apêndice B.5: Modelo de resultados obtidos pelo ajuste realizado no SAS

```
Rend1Etapa1Reta1

                                    The GLM Procedure

                                Number of observations    26

NOTE: Due to missing values, only 4 observations can be used in this analysis.

---------------------------------------------------------------------------------------------------

                                     Rend1Etapa1Reta1

                                    The GLM Procedure

Dependent Variable: Rend1Etapa1Reta1

        Source                      DF         Sum of Squares     Mean Square    F Value    Pr > F

        Model                        1      1.98450000      1.98450000      63.00    0.0155

        Error                        2      0.06300000      0.03150000

        Corrected Total              3      2.04750000

                   R-Square     Coeff Var      Root MSE    Rend1Etapa1Reta1 Mean

                   0.969231      6.173301      0.177482                2.875000


        Source                      DF       Type I SS     Mean Square    F Value    Pr > F

        Tempo                        1      1.98450000      1.98450000      63.00    0.0155


                                                  Standard
                  Parameter        Estimate           Error    t Value    Pr > |t|

                  Intercept      1.300000000      0.21737065       5.98      0.0268
                  Tempo          0.126000000      0.01587451       7.94      0.0155

---------------------------------------------------------------------------------------------------

                                  parms_Rend1Etapa1Reta1

             Dependent        Parameter        Estimate          StdErr     tValue      Probt

          Rend1Etapa1Reta1    Intercept     1.300000000      0.21737065       5.98     0.0268
          Rend1Etapa1Reta1    Tempo         0.126000000      0.01587451       7.94     0.0155

---------------------------------------------------------------------------------------------------

                                     Rend1Etapa1Reta2

                                    The GLM Procedure

                                Number of observations    26

NOTE: Due to missing values, only 18 observations can be used in this analysis.
```

Rend1Etapa1Reta2

The GLM Procedure

Dependent Variable: Rend1Etapa1Reta2

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	455.5245236	455.5245236	148.85	<.0001
Error	17	52.0254764	3.0603221		
Uncorrected Total	18	507.5500000			

R-Square	Coeff Var	Root MSE	Rend1Etapa1Reta2 Mean
0.897497	33.11125	1.749378	5.283333

NOTE: No intercept term is used: R-square is not corrected for the mean.

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Tempo	1	455.5245236	455.5245236	148.85	<.0001

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Tempo	0.0398743455	0.00326830	12.20	<.0001

---

parms_Rend1Etapa1Reta2

Dependent	Parameter	Estimate	StdErr	tValue	Probt
Rend1Etapa1Reta2	Tempo	0.0398743455	0.00326830	12.20	<.0001

Rend2Etapa1Reta1

The GLM Procedure

Number of observations 26

NOTE: Due to missing values, only 4 observations can be used in this analysis.

Rend2Etapa1Reta1

The GLM Procedure

Dependent Variable: Rend2Etapa1Reta1

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	0.80000000	0.80000000	160.00	0.0062
Error	2	0.01000000	0.00500000		
Corrected Total	3	0.81000000			

R-Square	Coeff Var	Root MSE	Rend2Etapa1Reta1 Mean

```
                0.987654      4.561979      0.070711                1.550000

       Source                     DF       Type I SS     Mean Square    F Value    Pr > F

       Tempo                       1      0.80000000      0.80000000     160.00    0.0062

                                                 Standard
                Parameter         Estimate           Error    t Value    Pr > |t|

                Intercept     0.5500000000      0.08660254       6.35      0.0239
                Tempo         0.0800000000      0.00632456      12.65      0.0062

-----------------------------------------------------------------------------------------------

                                    parms_Rend2Etapa1Reta1

           Dependent        Parameter        Estimate          StdErr     tValue     Probt

        Rend2Etapa1Reta1    Intercept    0.5500000000      0.08660254       6.35    0.0239
        Rend2Etapa1Reta1    Tempo        0.0800000000      0.00632456      12.65    0.0062

-----------------------------------------------------------------------------------------------

                                     Rend2Etapa1Reta2

                                    The GLM Procedure

                                  Number of observations    26

NOTE: Due to missing values, only 18 observations can be used in this analysis.

-----------------------------------------------------------------------------------------------

                                     Rend2Etapa1Reta2

                                    The GLM Procedure

Dependent Variable: Rend2Etapa1Reta2

                                           Sum of
       Source                     DF         Squares     Mean Square    F Value    Pr > F

       Model                       1     229.5710297     229.5710297     213.39    <.0001

       Error                      17      18.2889703       1.0758218

       Uncorrected Total          18     247.8600000

                  R-Square     Coeff Var      Root MSE    Rend2Etapa1Reta2 Mean

                  0.926212      28.28777      1.037218                3.666667

NOTE: No intercept term is used: R-square is not corrected for the mean.

       Source                     DF       Type I SS     Mean Square    F Value    Pr > F

       Tempo                       1     229.5710297     229.5710297     213.39    <.0001

                                                 Standard
                Parameter         Estimate           Error    t Value    Pr > |t|

                Tempo         0.0283071553      0.00193780      14.61      <.0001

-----------------------------------------------------------------------------------------------

                                    parms_Rend2Etapa1Reta2
```

Dependent	Parameter	Estimate	StdErr	tValue	Probt
Rend2Etapa1Reta2	Tempo	0.0283071553	0.00193780	14.61	<.0001

---

parms_Rend2Etapa1Reta2

Obs	Tempo	Rend1Etapa1	Rend2Etapa1	Rend1Etapa2	Rend2Etapa2
1	5	1.8	0.9	10.2	7.2
2	10	2.7	1.4	15.7	12.1
3	15	3.3	1.8	19.6	16.3
4	20	3.7	2.1	23.0	19.7
5	30	4.2	2.5	29.1	25.6
6	40	4.4	2.7	34.0	31.5
7	50	4.6	2.9	38.2	34.9
8	60	4.7	3.1	41.9	39.4
9	70	4.9	3.2	45.0	41.7
10	80	5.0	3.4	48.0	44.4
11	90	5.1	3.5	50.6	47.2
12	100	5.3	3.7	52.9	49.2
13	110	5.3	3.7	55.3	51.4
14	120	5.5	3.8	57.4	53.0
15	130	5.5	4.0	59.4	54.4
16	140	5.6	4.0	61.2	55.7
17	150	5.7	4.1	62.8	56.4
18	160	5.7	4.2	64.1	57.5
19	170	5.8	4.2	65.4	58.4
20	180	5.9	4.3	66.7	59.5
21	190	5.9	4.3	67.8	60.5
22	200	6.0	4.4	69.0	61.2
23	210	.	.	69.9	61.9
24	220	.	.	70.8	62.6
25	230	.	.	71.6	63.3
26	240	.	.	72.3	64.0

Rend1Etapa1

The NLIN Procedure
Dependent Variable Rend1Etapa1
Method: Gauss-Newton

Iterative Phase

Iter	b0	b1	b2	knot1	Sum of Squares
0	1.6568	0.0927	-0.0831	30.5885	0.4070
1	1.6568	0.0927	-0.0831	30.5885	0.4067

NOTE: Convergence criterion met.

Estimation Summary

Method	Gauss-Newton
Iterations	1
R	1.128E-8
PPC	6.86E-10
RPC(b2)	0.000528
Object	0.000725
Objective	0.406709
Observations Read	26
Observations Used	22
Observations Missing	4

	Sum of	Mean	Approx

Source	DF	Squares	Square	F Value	Pr > F
Model	3	25.7278	8.5759	379.55	<.0001
Error	18	0.4067	0.0226		
Corrected Total	21	26.1345			

Parameter	Estimate	Approx Std Error	Approximate 95% Confidence Limits	
b0	1.6568	0.1420	1.3585	1.9550
b1	0.0927	0.00781	0.0763	0.1091
b2	-0.0831	0.00785	-0.0996	-0.0667
knot1	30.5885	1.8348	26.7337	34.4432

Approximate Correlation Matrix

	b0	b1	b2	knot1
b0	1.0000000	-0.8807710	0.8768043	0.4495329
b1	-0.8807710	1.0000000	-0.9954963	-0.7473048
b2	0.8768043	-0.9954963	1.0000000	0.7025903
knot1	0.4495329	-0.7473048	0.7025903	1.0000000

Rend2Etapa1

The NLIN Procedure
Dependent Variable Rend2Etapa1
Method: Gauss-Newton

Iterative Phase

Iter	b0	b1	b2	knot1	Sum of Squares
0	0.9000	0.0500	-0.0404	42.0430	0.3101
1	0.9000	0.0500	-0.0404	42.0430	0.3100

NOTE: Convergence criterion met.

Estimation Summary

Method	Gauss-Newton
Iterations	1
R	3.599E-9
PPC	2.95E-10
RPC(b2)	0.000437
Object	0.000145
Objective	0.310029
Observations Read	26
Observations Used	22
Observations Missing	4

Source	DF	Sum of Squares	Mean Square	F Value	Approx Pr > F
Model	3	21.0227	7.0076	406.85	<.0001
Error	18	0.3100	0.0172		
Corrected Total	21	21.3327			

Parameter	Estimate	Approx Std Error	Approximate 95% Confidence Limits	
b0	0.9000	0.1048	0.6799	1.1201
b1	0.0500	0.00450	0.0405	0.0595
b2	-0.0404	0.00456	-0.0500	-0.0308
knot1	42.0430	3.2551	35.2042	48.8817

Approximate Correlation Matrix

	b0	b1	b2	knot1
b0	1.0000000	-0.8593378	0.8487934	0.4402294
b1	-0.8593378	1.0000000	-0.9877296	-0.7548560
b2	0.8487934	-0.9877296	1.0000000	0.6754440
knot1	0.4402294	-0.7548560	0.6754440	1.0000000

Apêndice B.6: Exemplo de curva de tensão superficial obtida para os extratos etanólicos e aquosos de raízes de ginseng brasileiro