ÉRICA RESENDE DE OLIVEIRA

EXTRAÇÃO SÓLIDO-LÍQUIDO DOS GRÃOS DE CAFÉ VERDE E DA TORTA DA PRENSAGEM: ESTUDO DE SOLVENTES ALTERNATIVOS

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Profa. Dra. Fabiana Queiroz
Orientadora

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EXTRAÇÃO SÓLIDO-LÍQUIDO DOS GRÃOS DE CAFÉ VERDE E DA TORTA DA PRENSAGEM: ESTUDO DE SOLVENTES ALTERNATIVOS

SOLID-LIQUID EXTRACTION OF GREEN COFFEE BEANS AND ITS PRESS CAKE: ALTERNATIVE SOLVENTS STUDY

Tese apresentada à Universidade Federal de Lavras, como parte das exigências do Programa de Pós-Graduação em Ciência dos Alimentos, área de concentração em Ciência dos Alimentos, para a obtenção do título de Doutora.

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RESUMO

Este trabalho foi realizado com o objetivo de avaliar a viabilidade da extração de óleo, sólidos solúveis e compostos bioativos presentes nos grãos de café verde (*Coffea arabica*) e da torta obtida da prensagem mecânica desses grãos, utilizando solventes verdes (alternativos ou biorenováveis) na forma pura e suas misturas, e métodos alternativos. Os materias, grãos de café verde e torta, foram caracterizados física e quimicamente. Os resultados mostraram que, mesmo após a extração do óleo por prensagem, a torta ainda contém substancial teor de sólidos solúveis e compostos bioativos. Após serem caracterizadas, as matérias-primas foram submetidas à extração em temperatura ambiente, com a finalidade de quantificar compostos bioativos e comparar a performance do solvente etanol e da mistura metanol-acetona para extrair esses compostos. Os resultados obtidos mostraram que a mistura de solvente metanol-acetona apresentou melhor performance para extrair compostos antioxidantes, os quais foram encontrados em maior quantidade na torta. Em seguida, avaliou-se o rendimento da extração de óleo (ou sólidos solúveis) e de compostos bioativos e antioxidantes avaliados, por meio das análises de compostos fenólicos totais, ABTS, DPPH, FRAP e beta-caroteno dos grãos de café verde e da torta, utilizando o extrator tipo Soxhlet; também foram estudados solventes alternativos (acetona, acetato de etila, etanol e isopropanol) e solventes tradicionais (hexano e éter de petroleo), em diferentes tempos de extração (3 e 5 h), no rendimento. Os resultados mostraram que o etanol foi o solvente que apresentou melhor rendimento de sólidos solúveis, de antioxidantes e compostos fenólicos de ambos os materiais, com exceção da análise de antioxidante pelo método de descoloração do beta-caroteno, a qual apresentou melhor resultado quando o acetato de etila foi empregado. Foi também estudado o rendimento da extração sólido-líquido em batelada com solventes puros alternativos (etanol, acetona e acetato de etila) em diferentes temperaturas (35 a 55°C), bem como os parâmetros termodinâmicos do processo. Foi observado que elevadas temperaturas afetaram positivamente o rendimento do processo, e os solventes etanol e acetona apresentaram melhor performance. A análise termodinâmica dos processos mostrou que as ΔH e ΔS foram positivas, e ΔG foram negativas, indicando que os processos são endotérmicos e espontâneos. Uma vez estudado o comportamento dos solventes puros, foram selecionados dois solventes (acetona e acetato de etila) para serem utilizados como cosolventes no processo de otimização da extração etanólica dos grãos e da torta. A extração sólido-líquida foi realizada em banho com agitação e controle de temperatura. Foi utilizado o Delineamento Composto Central Rotacional (DCCR) para planejar o experimento,

onde as variáveis independentes foram a concentração de cosolventes (acetona e acetato de etila; 0 a 40%) no etanol e a temperatura (35 a 55°C), e as variáveis respostas, o rendimento e índice de retenção de sólidos solúveis. Observou-se que a temperatura e a adição de acetona tiveram um efeito positivo no rendimento do processo de ambos os materiais, sendo que, para a torta, a adição de acetato também influenciou esta variável resposta. A temperatura também influenciou o índice de retenção do processo de extração de ambos os materiais, no entanto, esta variável resposta não pode ser representada por um modelo empírico. O estudo mostrou que o processo de extração de sólidos solúveis da torta de café é mais viável do que para os grãos. Como método alternativo, foi realizado o processo de extração sólido-líquido dos grãos de café verde assistido por ultrassom (20 kHz, 400 W, ponteira de 3 mm de diâmetro), onde os mesmos parâmetros da otimização realizada para o banho com agitação foram utilizados, exceto a temperatura, no lugar da qual foi utilizado o tempo como variável independente (5 a 60 min). A função desejabilidade foi utilizada para otimizar, simultaneamente, os resultados das variáveis respostas analisadas (rendimento e índice de retenção). As regiões ótimas encontradas foram 16,41 a 31,45% para acetona, 31,45 a 40% para acetato de etila e 54,44 a 60 min para o tempo. Os resultados mostraram que o tempo de extração foi um parâmetro com elevada influência na recuperação de sólidos solúveis e no índice de retenção do processo de extração sólido-líquido de grãos de café verde. Por meio dos resultados obtidos, pode-se demonstrar que o processo de extração sólido-líquido utilizando solventes verdes é viável para a extração de óleo ou sólidos solúveis de grãos de café verde e da torta obtida da prensagem mecânica dos grãos. Assim, a extração sólido-líquido pode ser utilizada como uma segunda etapa de extração para remover os sólidos da torta, uma vez que o processo de prensagem se mostrou com baixa eficiência (< 30%) de rendimento de óleo, bem como da extração de compostos bioativos.

Palavras-chave: Extração sólido-líquido. Sólidos solúveis. Solventes alternativos. DCCR. Termodinâmica. Compostos bioativos. *Coffea arabica*.

ABSTRACT

This work aimed to evaluate the feasibility of the extraction of oil, soluble solids and bioactive compounds from Arabica green coffee beans and its press cake obtained from the mechanical pressing processs, using alternative solvents (pure or mixtures) and methods. The materials, green coffee beans and cake, were physically and chemically characterized. The results showed that even after oil extraction by pressing, the cake still contains substantial content of soluble solids and bioactive compounds. After being characterized, the raw materials were subjected to extraction at room temperature, in order to quantify bioactive compounds and to compare the performance of the solvent ethanol and the methanol-acetone mixture to extract these compounds. The results showed that the solvent mixture methanol-acetone presented better performance to extract antioxidant compounds, which were found in greater quantity in the cake. Further, the oil yield (or soluble solids), bioactive compounds and antioxidants were evaluated through the analysis of total phenolic compounds (TPC), ABTS, DPPH, FRAP and beta-carotene bleaching assay (BCBA) of the green coffee beans and the cake using the Soxhlet type extractor; also, alternative solvents (acetone, ethyl acetate, ethanol and isopropanol) and traditional solvents (hexane and petroleum ether) were studied in different extraction times (3 and 5 h). The results showed that ethanol was the solvent that presented the best yield of soluble solids, antioxidants and phenolic compounds of both materials, except for the BCBA antioxidant analysis, which presented better results when ethyl acetate was employed. It was also studied the yield of the solid-liquid extraction with pure solvents (ethanol, acetone and ethyl acetate) at different temperatures (35 to 55°C), as well as the thermodynamic parameters of the process. It was observed that high temperatures positively affected the yield of the process, and that ethanol and acetone solvents presented better performance. The thermodynamic analysis of the processes showed that the ΔH and ΔS were positive, and ΔG were negative, indicating that the processes were endothermic and spontaneous. Once the behavior of the pure solvents was studied, two solvents (acetone and ethyl acetate) were selected to be used as cosolvents in the process of optimization of the ethanolic extraction of beans and cake. The solid-liquid extraction was carried out in a stirring bath with temperature control. The Rotational Central Composite Design (DCCR) was used to plan the experiment, where the independent variables were the concentration of cosolvents (acetone and ethyl acetate, 0 to 40%)

in ethanol and temperature (35 to 55 °C), and the variables responses were the yield and retention index. It was observed that the temperature and the addition of acetone had a positive effect on the process yield of both materials, and for the cake, the addition of ethyl acetate also influenced this response variable. The temperature also influenced the retention index of the extraction process of both materials, however, this variable response could not be represented by an empirical model. The study showed that the process of extracting soluble solids from the press cake is more viable than for the coffee beans. As an alternative method, the solid-liquid extraction process of the green coffee beans (20 kHz, 400 W, 3 mm diameter tip) was performed, where the same parameters of the optimization performed for the stirred bath were used, except the temperature, instead, the variable time was used as an independent variable (5 to 60 min). The desirability function was used to simultaneously optimize the results of the analyzed response variables (yield and retention index). The optimum regions were 16.41 to 31.45% for acetone, 31.45 to 40% for ethyl acetate and 54.44 for 60 min for the time. The results showed that the extraction time was a parameter with high influence on the recovery of soluble solids and the retention index of the solid-liquid extraction process of green coffee beans. By means of the results obtained, it can be demonstrated that the solid-liquid extraction process using green solvents is feasible for the extraction of oil or soluble solids from green coffee beans and its cake obtained from the mechanical pressing of the beans. Thus, solid-liquid extraction could be used as a second extraction step to remove solids from the cake, since the pressing process was shown to have low efficiency (< 30%) of oil yield as well as for the extraction of bioactive compounds.

Keywords: Solid-liquid extraction. Soluble solids. Green solvents. CCRD. Thermodynamics. Bioactive compounds. *Coffea arabica*.

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PRIMEIRA PARTE

1 INTRODUÇÃO

O Brasil configura-se como o maior produtor de café, sendo responsável por 33% de todo o café comercializado no mercado internacional (incluindo-se os mercados interno e externo), seguido pelo Vietnã, o qual responde por 15% das exportações mundiais, e pela Colômbia, com 7,5% (ICO, 2018).

A maior parte de consumo do café é para a elaboração da bebida café (83 L por habitante ao ano, no Brasil), podendo ser comercializado para esse fim na forma de grãos torrados, torrados e moídos e ainda na forma de pó instantâneo (ABIC, 2018). No entanto, o grão do café verde tem despertado interesse no cenário agroindustrial nacional e internacional, uma vez que o grão possui óleo com características atrativas para a indústria cosmética, além de ser rico em compostos bioativos (AL-HAMAMRE et al., 2012; CAETANO; SILVA; MATA, 2012; VARDON et al., 2013), e que, por sua vez, contém grande quantidade de sólidos solúveis (SOMNUK; EAWLEX; PRATEEPCHAIKUL, 2017), sendo insumo promissor para a agroindústria.

Apesar do óleo de café verde apresentar características como emoliente e ser um quimoprotetor, além de apresentar atividade antioxidativa (RABASCO ALVAREZ et al., 2000), os grãos de café verde não podem ser considerados como fonte de óleo (~15%) (DIAS; BENASSI, 2015; MUSSATTO et al., 2011), o que os torna impróprios para serem destinados ao processo de extração mecânica (prensagem), uma vez que esse processo é adequado à extração de matrizes que contém acima de 20 a 30% de óleo. No entanto, a prensagem mecânica é atualmente, o método empregado pela indústria de café para extrair o óleo dos grãos verdes.

A prensagem mecânica além de ser um processo não indicado à extração de materiais com baixo teor de óleo, gera um subproduto que ainda contém teor substancial do mesmo. Ainda, pode apresentar elevado teor de compostos bioativos hidrofílicos, que ficam retidos nesta biomassa. Esse resíduo, o qual, na maioria das vezes, é descartado, pode, portanto, apresentar relevante potencial econômico para a indústria.

A fim de explorar esse resíduo (biomassa ou torta) e ainda tornar a extração de grãos de café verde mais eficiente, a extração sólido-líquido pode ser uma alternativa interessante.

A extração sólido-líquido de óleos é comumente realizada utilizando-se o hexano

comercial, que é uma mistura de hidrocarbonetos com um ponto de ebulição em torno 65-69ºC que contém cerca de 65% de n-hexano e 35% de ciclopentano e isómeros do hexano (KEMPER, 2005). Esse solvente apresenta diversas vantagens, tais como baixo calor latente de vaporização, solubilização completa com óleo, baixa corrosividade, fácil recuperação e imiscibilidade na água. Em contrapartida, apresenta desvantagens, como efeitos negativos sobre o meio ambiente, segurança e saúde (DAGOSTIN; CARPINÉ; CORAZZA, 2015), uma vez que é poluente, altamente inflamável e é considerado uma neurotoxina.

Inúmeros solventes alternativos têm sido propostos para substituir o hexano na extração de óleos vegetais, incluindo o tricloroetileno, n-heptano, etanol, acetona, acetato de etila, isopropanol e n-propanol (AQUINO et al., 2011; FRANCO et al., 2007a; GANDHI et al., 2003; JAVED et al., 2015; MANI; JAYA; VADIVAMBAL, 2007; NAVARRO; RODRIGUES, 2016; OLIVEIRA; GARAVAZO; RODRIGUES, 2012; OLIVEIRA; BARROS; GIMENES, 2013; RODRIGUES; ARACAVA; ABREU, 2010; SETH et al., 2007; TIR; DUTTA; AHMED, 2012).

Entre esses, o etanol é um solvente promissor para a extração de óleos vegetais nas indústrias brasileiras e norte americanas. Segundo dados da *Renewable Fuels Association* (RFA, 2017), os Estados Unidos e o Brasil produziram 15.800 e 7.060 milhões de galões de etanol em 2017, respectivamente, o que corresponde a 84% do total produzido mundialmente. Apesar de ser produzido em menor quantidade, o álcool brasileiro tem a vantagem de apresentar menor custo de produção e maior produtividade por acre, em decorrência do uso de cana-de-açúcar como matéria-prima (KOHLHEPP, 2010). Ao que diz respeito à saúde e segurança, o etanol apresenta menor risco do que os hidrocarbonetos (DAGOSTIN; CARPINÉ; CORAZZA, 2015). Ainda do ponto de vista ambiental, ele é produzido por via biotecnológica, não gerando resíduos tóxicos. Do ponto de vista econômico, além de ser produzido em larga escala no Brasil, pode ser facilmente recuperado para posterior reutilização no processo (RODRIGUES, 2011).

Entretanto, em razão da sua natureza polar, o etanol não é um bom solubilizante para o óleo, quando comparado ao hexano. Os triacilgliceróis tem solubilidade limitada no etanol que é capaz de solubilizar não apenas o óleo presente na matriz, mas também água e outros componentes polares. Se etanol hidratado for utilizado como solvente, sua capacidade de solubilizar triacilglicerídeos é reduzida, gradualmente limitando a eficiência do processo (COSTA RODRIGUES, 2011; DAGOSTIN; CARPINÉ; CORAZZA, 2015; NAVARRO; RODRIGUES,

2016).

A fim de aumentar a solubilidade de um componente em outro, alguns recursos termodinâmicos podem ser utilizados, tais como a elevação da temperatura e ainda a adição de cosolventes na mistura. Um cosolvente, por definição, é uma substância que é miscível tanto no soluto como no solvente, e melhora a solubilidade e consequente extração dos compostos de interesse (DAGOSTIN; CARPINÉ; CORAZZA, 2015). Neste contexto, objetivou-se neste trabalho avaliar o processo de extração sólido-líquido dos grãos de café verde e da torta proveniente da extração do óleo de café por prensagem, utilizando diferentes solventes alternativos.

2. REFERENCIAL TEÓRICO

2.1 Café

A planta de café é originária da Etiópia, centro da África, onde ainda hoje faz parte da vegetação natural. Os manuscritos mais antigos que mencionam a cultura do café datam de 575 D.C. no Iêmen, onde os frutos eram consumidos *in natura*. Na Pérsia, no século XVI, os grãos passam a ser torrados e posteriormente consumidos na forma de bebida, como conhecemos atualmente (NEVES, 1974; TAUNAY, 1939).

O cafeeiro é considerado uma árvore pequena ou um arbusto perene. É caracterizado por frutos ovoides com cores vermelhas ou amarelas, contendo duas sementes cartilaginosas, envolvidas por uma polpa adocicada e comestível. Sua semente é responsável, quase que exclusivamente, pelo alto valor desta planta (SILVA, 2005). Pertence à família Rubiacea e gênero *Coffea*, sendo que as duas espécies de maior relevância econômica são a *Coffea arábica – conhecida como arábica* - e a *Coffea canephora*. A espécie arábica é a mais apreciada, pois é reconhecida por produzir uma bebida de melhor qualidade (bebida mais delicada e com aroma acidífero) (FIGURA 1). A *Coffea arabica* representa cerca de dois terços da produção mundial, enquanto o terço restante é de *Coffea canephora* (SMITH, 1985; ILLY, 2002). A outra espécie, é originária da região do Congo, e não menos importante que a arábica em função do alto teor de cafeína (2,2% - o dobro da variedade arábica) e sólidos solúveis verificados nos seus grãos, representa aproximadamente 34% da produção mundial de café, e possui diversas variedades,

dentre elas a robusta ou conilon, ou ainda *kouillou*, em francês (FIGURA 2). Além da arábica, esta é a outra variedade cultivada no Brasil (RONCHI, 2009). Os cafés brasileiros são na maioria misturas (*blend*) dos dois grãos, resultando em uma bebida de qualidade razoável e com baixo custo (LIMA FILHO et al., 2015).

Figura 1 – Cafeeiro e grão de café do tipo Arábica.

Fonte: Do autor (2017).

Figura 2 - Cafeeiro e grão de café do tipo Robusta.

Fonte: Café (2015).

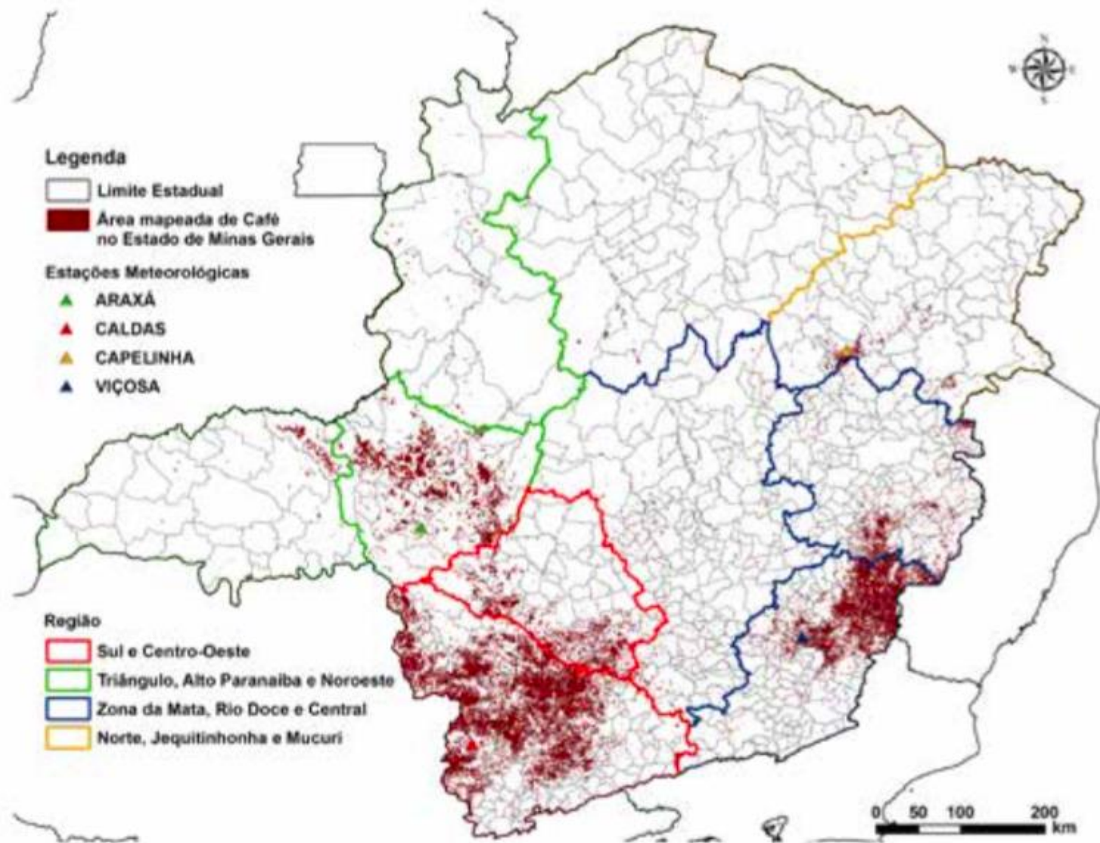
O café chegou ao continente europeu em 1615, trazido por viajantes em suas constantes passagens pelo Oriente. Apesar do imenso interesse de países como Alemanha, França e Itália em

desenvolver o plantio do café em suas terras, foram os holandeses que introduziram o cultivo da planta na Europa (NEVES, 1974; TAUNAY, 1939). Apostando no produto, com mudas procedentes de Moka, os holandeses deram início ao cultivo sistemático do café, no ano de 1658, em Java, a leste da Índia – origem da famosa mistura de grãos Mocha-Java, e prosseguiram no Sri Lanka, ilha do oceano Índico. Em 1699, levaram mais mudas para as colônias insulares da Indonésia, Sumatra, Timor, Bali, Malabar e Celebes, e, em 1718, plantaram no Suriname, na América do Sul. Sempre sob controle da Holanda, essas regiões figuram como as primeiras exportadoras de café comercial do mundo. Amsterdã tornou-se poderoso centro difusor do produto (MARTINS; AQUINO, 2008). Ingleses e espanhóis completaram, posteriormente, no decurso dos séculos XVIII e XIX, a propagação do café arábica no continente americano (CARDOSO, 1994). O café foi então trazido ao norte do Brasil, em 1727, proveniente da Guiana Francesa, pelo sargento-mor Francisco de Mello Palheta a pedido do governador do estado do Maranhão (NEVES, 1974; TAUNAY, 1939).

O café é uma das bebidas mais consumidas no mundo. Desde 1820, o café passou gradativamente a ser o principal produto brasileiro de exportação, superando o algodão, o açúcar e outros produtos agrícolas. Originalmente cultivados no Estado do Rio de Janeiro, os cafezais se espalharam, alcançando terras paulistas ao longo do vale do rio Paraíba. Em 1836, São Paulo já respondia por 25 % de toda a produção cafeeira do Brasil e, em 1850, o café representava quase metade de todas as exportações brasileiras. Logo, o ciclo de produção do café é responsável por uma importante movimentação financeira que afeta diversos setores da economia em todo o país (MAPA, 2015a). A área total plantada no país com a cultura de café (arábica e conilon) é equivalente a 2,2 milhões de hectares. A área plantada do café arábica no país é de 1.78 milhão de hectares, o que equivale a 81% da área existente com lavouras de café. Para o café conilon, a área estimada é de 417,93 mil hectares. O café arábica representa, aproximadamente, 76% da produção total (arábica e conilon) de café do país (~ 43 milhões de sacas). Já a produção do conilon representa, aproximadamente, 24% da produção total (~ 13 milhões de sacas) (CONAB, 2018b). O Brasil tem condições climáticas que favorecem o cultivo do café em 15 regiões produtoras. Essa diversidade garante cafés variados de Norte a Sul do país (MAPA, 2015b). Contudo, o maior produtor nacional de café é o Estado de Minas Gerais, onde concentra-se a maior área plantada, de 975,27 mil de hectares, predominando a espécie arábica, com 98,64% do total no estado. Isso

representa 50,2% da área cultivada no país (MAPA, 2015a). Sendo que as principais regiões produtoras no estado de Minas Gerais são o Sul e Centro-Oeste (FIGURA 3).

Figura 3 - Mapeamento do café no estado de Minas Gerais.



Fonte: CONAB (2017).

Em decorrência da diversidade de regiões ocupadas pela cultura do café, o Brasil produz tipos variados do produto, fato que possibilita atender às diferentes demandas mundiais. Essa diversidade também permite o desenvolvimento dos mais variados *blends*, tendo como base o café de terreiro ou natural, o despolpado, o descascado, o de bebida suave, os ácidos, os encorpados, além de cafés aromáticos, especiais e de outras características (MAPA, 2015b).

Os principais motivos do consumo da bebida de café têm sido seu efeito estimulante e o seu aroma e sabor. Além disso, o café exibe atividade antioxidante e acredita-se que seu consumo moderado reduza o desenvolvimento de alguns tipos de câncer como o de intestino grosso. Sua

atividade antioxidante é atribuída à presença de compostos fenólicos, como ácidos clorogênicos, à cafeína e a alguns produtos resultantes da reação de Maillard (melanoidinas) (ABIC, 2017), a qual ocorre durante a torrefação dos grãos.

2.1.1 Composição química do café

Um dos constituintes mais importantes dos grãos de café são os carboidratos, já que representam quase metade da massa do grão cru, em base seca. A proporção de carboidratos em grãos de café é diretamente afetada pela espécie, local de cultivo, estádio de maturação dos frutos, entre outros. Carboidratos de baixa e alta massa molar são encontrados no café. O principal carboidrato de baixa massa molar presente nos grãos de cafés crus é a sacarose, com valores variando entre 2 e 5% para a variedade robusta e 5 e 8,5% para a variedade arábica. Frutose, glicose, manose, arabinose, ramnose, rafinose, galactose e ribose também estão presentes em quantidades inferiores a 1 % da massa do grão (base seca). A fração de polissacarídeos, ou carboidratos de alta massa molar, presentes no café cru, é constituída, principalmente, de três polímeros: arabinogalactanos, mananos e celulose. Os teores de mananos e celulose nos grãos de cafés crus não variam entre as variedades arábica e robusta e são de 22 e 7%, respectivamente. Já os arabinogalactanos representam cerca de 14% do grão de café arábica, e 17 % do grão de café robusta. O teor de celulose varia com a espécie e pode chegar a 7,8% para arábica ou 8,7% para robusta (TRUGO; MACRAE, 1986; BRADBURY, 2001; FERNANDES et al., 2003).

Os ácidos orgânicos, que também estão presentes nos grãos de café, são produtos secundários do metabolismo celular e, parcialmente, responsáveis pela coloração do grão cru. O teor de ácidos no café também é dependente de alguns fatores, como a idade, o tipo de processamento e fermentação. Grande parte dos ácidos encontrados no café são os ácidos clorogênicos, que são esteres do ácido quínico. E, em minoria, estão presentes os ácidos quínico, málico, cítrico e fosfórico. Em média, o café arábica (5,5 g/kg) contém mais ácido quínico que o robusta (3,5 g/kg). Para os ácidos málico e cítrico, os teores são superiores em arábica, 5,6 g/kg e 12,3 g/kg, respectivamente, do que em robusta, 3,0 g/kg e 8,6 g/kg. O teor de ácido fosfórico é de 1,3 g/kg em arábica e 1,7 g/kg em robusta. Já a quantidade de ácidos clorogênicos nos grãos de cafés crus varia de 4,0 a 9,0%, em base seca, para café arábica e de 6,0 a 12,3 % para café robusta,

dependendo da técnica utilizada para a determinação dos mesmos (CLIFFORD, 1985; BALZER, 2001). Os ácidos clorogênicos, considerados compostos fenólicos, são responsáveis por grande parte da atividade antioxidante, tanto do café *in natura*, como da bebida, apresentando potencial atividade antibacteriana, antiviral e anti-hipertensiva (SMITH, 1985). Os compostos fenólicos constituem uma das principais classes de antioxidantes naturais. Eles são largamente encontrados em frutos, legumes, grãos, sementes, folhas, raízes, etc. Esses constituintes são também capazes de retardar o envelhecimento (KAWACHI et al., 1996; SÄÄKSJÄRVI et al., 2008). Os compostos fenólicos têm se tornado de grande relevância, decorrentes dessa característica antioxidante. Uma das principais classes de compostos fenólicos é a dos ácidos hidroxicinâmicos, dentre os quais destacam-se os ácidos cafeico, ferúlico e *p*-cumárico (FARAH et al., 2001; FARAH; DONANGELO, 2006).

Os compostos nitrogenados representam uma fração importante da composição do café. Dentro dessa classe de compostos, encontram-se a cafeína, a trigonelina, os aminoácidos e as proteínas. A cafeína é a substância do café associada a uma melhora no estado de alerta, na capacidade de aprendizado e resistência ao esforço físico. Seu teor em grãos de cafés crus varia fortemente com a espécie da planta e é da ordem de 2,2% para café robusta e 1,2% para café arábica, em base seca. Após ser transformada pelo organismo humano, ela pode contribuir para a atividade antioxidante da bebida (MACRAE, 1985; SMITH, 1985). Fatores ambientais e agriculturais não influenciam de modo significativo na quantidade de cafeína presente em grãos crus. A importância da trigonelina se dá, principalmente, pelos produtos de sua degradação durante a torra do café. Cafés arábica e robusta contêm 1,0% e 0,7% de trigonelina, em base seca, respectivamente. Assim como a cafeína, o teor de trigonelina varia com a espécie do café e com o método analítico utilizado na sua detecção. As proteínas presentes nos grãos de café encontram-se no citoplasma ou ligadas a polissacarídeos nas paredes celulares. Sua degradação durante a torração é responsável pelo aparecimento de voláteis que atribuirão aroma ao café. A idade da planta e o período de maturação dos frutos influem diretamente no teor de proteínas no grão. Não há evidências de que o teor de proteínas varie de acordo com a espécie de café. Em grãos crus, o teor de proteína bruta encontra-se entre 8,7 e 9,7%, em base seca (MACRAE, 1985). Aminoácidos livres também estão presentes em pequenas quantidades (0,15 a 0,25%, em base seca).

A quantidade de cinzas presentes nos grãos de cafés crus é da ordem de 4% em base seca, sendo o potássio, o mineral presente em maior quantidade (cerca de 40% do total) (CLARKE, 1985).

O teor de óleo do grão de café é de 15% para a espécie arábica e 10% para a robusta. Os lipídios de grãos de café estão presentes substancialmente no endosperma do fruto. O óleo de café não contém apenas triglicerídeos (75% em 100% de óleo de grãos crus de café arábica), mas também vários outros componentes, que são itens importantes e característicos do óleo.

Óleos e gorduras apresentam, como componentes, substâncias que podem ser classificadas em dois grupos: glicerídeos e não glicerídeos (que não contém a molécula de glicerol em sua estrutura). Óleos vegetais são geralmente constituídos em mais de 90% de glicerídeos, sendo a maior parte formada de triglicérides com pequenas frações de mono e diglicérides. Os não-glicerídeos de principal ocorrência nos óleos brutos são: fosfatídeos, ceras, esteróis, carotenoides, tocoferóis e outros componentes lipossolúveis (MORETTO; ALVES, 1998).

O óleo de café é composto, principalmente, por triacilglicerois em proporções similares às encontradas em óleos comestíveis tradicionais. Sua fração insaponificável é rica em diterpenos da família kaurane (cafestol, caveol e 16-O-metilcafestol), os quais têm recebido cada vez mais atenção, em razão de suas atividades biológicas diversificadas. Entre os esterois, também parte da matéria insaponificável, variados desmetil-, metil- e dimetilesterois já foram identificados (SPEER; KÖLLING-SPEER, 2006).

A importância do óleo de café se deve a seu elevado valor comercial. Comercialmente, existem dois principais tipos de óleo derivados dos grãos de café, sendo eles o óleo de café torrado e o óleo de café verde. O óleo de café torrado tem sido empregado na indústria de cosméticos, para a formulação de batons e produtos aromatizados, bem como na indústria de alimentos, como aromatizante e flavorizante. Contudo, o óleo de café torrado apresenta menor concentração dos diterpenos antioxidantes quando comparado ao café verde. Essa perda ocorre pelo processo de torragem e pode ser superior a 50%. As propriedades de ambos os óleos são semelhantes, diferenciando-se basicamente pelo aroma. O café verde tem um aroma parecido com as folhas verdes de café, enquanto o de café torrado é similar ao próprio café filtrado (FREIBERGER et al., 2015; OLIVEIRA et al., 2005). Já o óleo de café verde é um material bastante rico em matéria insaponificável, e os componentes presentes nessa fração, principalmente os esterois, possuem

propriedades cosméticas desejáveis como: retenção de umidade, penetração na pele, aderência e capacidade de bloquear a luz solar prejudicial à pele humana, o que garante sua utilização em cremes comerciais (ALVAREZ; RODRÍGUEZ, 2000; TURATTI, 2001).

No café torrado, o óleo é composto por ácidos graxos esterificados com glicerol (triacilgliceróis, 78%), por diterpenos (15%) e por uma pequena fração de esteres de esterol (KURZROCK; SPEER, 2001; BELITZ; GROSCH; SCHIEBERLE, 2009). O óleo de café torrado pode ser obtido por prensagem dos grãos torrados ou pela extração da borra de café solúvel (previamente seca), pelo solvente hexano, sua utilização é direcionada a produtos alimentícios, como recheios de balas ou realçador do sabor de café no próprio café solúvel. Pode ser utilizado também na fabricação de café gelado, café enlatado, cappuccinos, pudins, produtos que utilizam leite, produtos de confeitaria, sobremesas e sorvetes (TURATTI, 2001). Além disso, uma redução nos níveis de diterpenos no óleo de café torrado aumenta, significativamente, a sua estabilidade e do perfil sensorial, diminuindo o seu efeito de hipercolesterolemia (KÖLLING-SPEER; STROHSCHNEIDER; SPEER, 1999).

A composição do óleo do café, principalmente, o teor de ácidos graxos, é um diferenciador das variedades de café (MARTÍN et al., 2001). Assim, o ácido linoleico tem sido considerado um potencial marcador para distinguir entre amostras de café obtidas de variadas condições ambientais (ROMANO et al., 2014; FIGUEIREDO et al., 2015). Além das condições geobotânicas, o processo de torrefação pode influenciar o perfil lipídico do óleo de café (BUDRYN et al., 2012). Estudos sobre a extração do óleo de grãos torrados relatam a presença de ácidos graxos saturados e insaturados, principalmente, do ácido esteárico, palmítico, oleico, linoleico, linolênico e araquidônico (KOBELNILK et al., 2014).

Do ponto de vista alimentício, todos esses componentes fazem do café uma bebida natural e saudável e se ingerido em doses moderadas, pode fazer muito bem para a saúde, além de prevenir doenças (SMITH, 1985).

2.2 Processo de extração de óleos vegetais

Os métodos tradicionais para a extração dos óleos a partir de sementes são a prensagem e a extração com solvente ou a combinação de ambos. Segundo Tandy (2000), na extração do óleo de

sementes com altos teores de óleo (maiores que 30%), utiliza-se, inicialmente, o processo de extração por prensagem para a redução do teor de óleo até cerca de 15%, sendo utilizada a extração por solvente para extrair o restante. Em sementes com menor teor de óleo como a soja, que possui cerca de 20% de óleo, utiliza-se somente a extração por solvente.

Além dos métodos usuais, há a extração aquosa enzimática (ABDULKARIM, et al., 2005; MARIANO; COURI; FREITAS, 2009; LATIF; DIOSADY; ANWAR, 2008; NASCIMENTO et al., 2008; RAVINDRAN et al., 2017; SOTO; CHAWY; ZUNIGA, 2007), utilização de fluidos pressurizados (líquidos comprimidos e fluidos supercríticos) (DEL VALLE et al., 2000; GETACHEW et al., 2018; PÉREZ et al., 2015; SANTOS et al., 2016) e a extração assistida por ultrassom (AL-DHABI et al., 2017; LI, 2002; SHALMASHI, 2009; TAKEUCHI et al., 2009), entre outros.

2.2.1 Extração por prensagem

Nas primeiras décadas do século XX, as prensas utilizadas para a extração de oleaginosas, embora extraíssem um óleo de boa qualidade, deixavam resíduos no material sólido, resíduos estes que implicavam na perda de óleo, afetando a qualidade da torta, que é também um dos produtos residuais do extrator (BOSS, 2000). Atualmente, existem diversos tipos de prensas utilizadas para essa finalidade, podendo ser contínuas ou hidráulicas.

A simplicidade da operação em uma prensa mecânica não exige mão de obra qualificada para seu manuseio; é um sistema facilmente adaptável aos mais variados tipos de oleaginosas, bastando, para isso, alguns simples ajustes mecânicos, e todo o processo de expulsão do óleo é contínuo, ocorrendo em um curto espaço de tempo. O fato de não se utilizar produtos químicos nesse processo, torna-o mais seguro, podendo ser instalado em pequenas propriedades rurais além de permitir o uso do subproduto da extração mecânica, torta rica em proteína, como adubo e ração animal (SINGH; BARGALE, 2000).

As sementes ou amêndoas que apresentam de 30 a 50% de óleo, podem ser submetidas à extração de óleo em prensas contínuas, chamadas de *expeller*, ou em prensas hidráulicas (processo descontínuo). Esse processo pode ser utilizado para amendoim, girassol, gergelim, canola ou colza,

mamona, babaçu, castanha-do-pará, amêndoas em geral, ou seja, para materiais que apresentem baixa umidade (abaixo de 10%) e presença de material fibroso (ANTONIASSI, 2016).

As prensas contínuas são dotadas de uma rosca ou parafuso sem fim que esmaga o material, liberando o óleo. As prensas hidráulicas (prensagem descontínua) apresentam um cilindro perfurado onde se desloca um êmbolo que faz pressão na matéria-prima. No mercado brasileiro, existem prensas contínuas de capacidade de processar 40 a 200 kg/hora de matéria-prima. Esses equipamentos apresentam diferentes eficiências de extração dependendo do material a ser extraído e de seu desenho ou características de construção. Nesse processo, ocorre muito atrito interno que eleva a temperatura do material e do óleo e assim, o termo "prensagem à frio" não se aplica ou é muito difícil de ser atingido nessas condições. Mesmo que não se aqueça a matéria-prima antes da prensagem, o calor gerado é suficiente para aumentar a temperatura do equipamento, da torta parcialmente desengordurada e do óleo (ANTONIASSI, 2016; BUOSI, 2013).

Na prensagem, a extração de óleo não é completa e a torta obtida pode apresentar um alto teor de óleo residual, o que não será adequado para algumas rações e poderá promover a rancificação do material, se armazenado por longo tempo. A eficiência de extração depende do equipamento, das condições do processo e da matéria-prima. Assim, a prensagem de materiais com baixo teor de óleo, pode não ser viável do ponto de vista econômico. Por outro lado, óleos com alto valor agregado, para uso em cosméticos, por exemplo, podem viabilizar o processo de extração de óleo por prensagem (ANTONIASSI, 2016).

Antes da extração, é necessário realizar um tratamento térmico para a inativação enzimática, para condicionar a umidade antes da extração e desnaturar proteína para liberar o óleo. Para algumas matérias-primas, a prensagem pode ser realizada diretamente, para isso pode-se contar com a experiência das empresas que fabricam esses equipamentos. Existem alguns equipamentos para esse processo de tratamento térmico e filtros prensa para a filtração de óleo que apresenta muitos sedimentos (ANTONIASSI, 2016).

2.2.2 Extração sólido-líquido

A operação de extração sólido-líquido implica na separação de um soluto de uma matriz sólida. Aguilera (2003) menciona que, do ponto de vista da engenharia, a extração sólido-líquido

é uma operação de transferência de massa multicomponente, multifásica e em estado não estacionário. Os processos envolvidos no mecanismo de extração encontram-se apresentados na Figura 4.

Figura 4 - Esquema dos processos envolvidos na extração sólido-líquido.

Fonte: Aguilera (2003).

Inicialmente, ocorre a entrada do solvente na matriz sólida, seguida da solubilização e/ou fracionamento de componentes. Ocorre, então, o transporte de soluto para o exterior da matriz sólida e a migração do soluto extraído desde a superfície externa do sólido para a o seio da solução. Por fim, o movimento do extrato (solvente mais soluto) em relação ao sólido, seguido da separação e descarga do extrato e sólidos (COELHO FILHO, 2015; RICHARDSON; HARKER; BACKHURST, 2002).

2.2.2.1 Extração por solventes

A operação de extração por solvente é considerada a mais importante dentro de todo o processo de obtenção de óleo e farelo (torta), e consiste em um processo de transporte de massa de uma fase para outra, envolvendo um conjunto de operações como lixiviação, difusão, diálise e lavagem, com o proposito de separar um ou mais componentes da mistura (BECKER, 1978; WILLIAMS, 2005).

A extração por solvente é uma operação unitária simples. Robiquet aplicou esse processo pela primeira vez, em 1835, para a extração de compostos de flores (HUI et al., 2007). A extração dos componentes contidos em uma matriz sólida dá-se pela dissolução dos mesmos em um solvente

líquido. Esse processo é conhecido como lixiviação ou também como extração sólido-líquida. A solução obtida chamada de micela (óleo + solvente) é removida do extrator e encaminhada para um evaporador onde ocorre a remoção do solvente. Após a remoção completa do solvente, obtém-se um extrato concentrado (TREYBAL, 1981).

O objetivo da etapa de extração é reduzir o teor de óleo no floco ao menor valor possível com o uso mínimo de solvente. Normalmente, os flocos entram no extrator com um teor entre 15 e 20% de óleo. O processo de extração é dito eficiente se o rafinado deixar o extrator com teor de óleo menor que 1% (WILLIAMS, 2005).

A extração por solvente é indicada para matérias-primas de baixa umidade, como sementes, e quando se deseja maior rendimento de extração de óleo e obtenção de um farelo desengordurado. Esse fator é importante para a produção de derivados proteicos e também para a produção de ração, a partir do farelo, considerando que o óleo promove a sua rancificação, além do fato de que alguns animais não toleram grandes quantidades de óleo em sua ração (ANTONIASSI; FREITAS, 2016).

Moretto e Alves (1998) citam que na extração de óleos vegetais realizada por solventes, as sementes são extraídas com solventes apolares com ponto de ebulição até 70°C. O aumento dessa temperatura pode ser responsável pela formação de ácidos graxos livres, em razão da quebra de ligações entre ácidos graxos e glicerol.

Os solventes apolares são os que têm maior afinidade com a fração lipídica. Os solventes convencionais mais utilizados são o éter de petróleo e a hexana; os alternativos são o etanol (menos tóxico ao meio ambiente e ao homem e ainda possui a vantagem de ter sido obtido a partir do processamento da cana-de-açúcar), isopropanol, cetonas (acetona, butanona, metiletilcetona), solventes halogenados (diclorometano, tricloroetileno), fluidos supercríticos (dióxido de carbono), água com ou sem adição de enzimas, misturas de clorofórmio e metanol, de hexano e isopropanol, entre outros (JOHNSON; LUSAS, 1983; ROSENTHAL; PYLE; NIRANJAN, 1996; KUK; HRON, 1998; CABRAL; MORIS, 2010).

Três etapas são principais no processo de extração: (1) a penetração do solvente na célula, (2) formação de miscela intracelular, e (3) difusão do material extraído ao meio externo (JOHNSON; LUSAS, 1983; SCHNEIDER, 1980).

A escolha de um solvente adequado em combinação com o ajuste dos parâmetros operacionais influencia os processos de transferência de massa (LI et al., 2014). Alguns fatores que

influenciam o processo de extração por solvente têm sido estudados, ao longo dos anos, como, por exemplo, o tamanho de partícula, temperatura, pressão, agitação, umidade da semente, relação entre a quantidade de semente e solvente, aplicação de ultrassom, entre outros (OLIVEIRA et al., 2013).

A temperatura é um parâmetro importante nos processos de extrações. Em geral, o aumento da temperatura faz com que a solubilidade do óleo no solvente aumente e a viscosidade da solução diminua o que facilita a transferência de massa do processo (AMARANTE et al., 2014). Além disso, o aumento da temperatura pode aumentar a energia cinética das moléculas de solvente, o que proporciona uma maior extração (JAVED et al., 2015). No entanto, o aumento excessivo da temperatura provoca a perda de qualidade do óleo extraído.

A razão mássica solvente-sólido é um dos parâmetros importantes na extração, em que uma diluição mais elevada (maior proporção de solvente em relação à semente) proporciona aumento no coeficiente de transferência de massa, produzindo maior extração de óleos (SETH et al., 2007). A agitação favorece o movimento convectivo no solvente, compensando a gradual diminuição do gradiente causada pelo aumento da concentração do soluto.

O teor de água presente nas sementes destinadas à extração é outro fator que influencia no rendimento final. A presença de água nas partículas afeta, negativamente, a cinética e o rendimento de extração de óleo extraído durante o processo de lavagem e de difusão, isso porque a água altera a polaridade da mistura (SANTOS et al., 2010).

O efeito do tamanho da partícula no rendimento de extrações está associado ao aumento dos danos celulares e área superficial com a diminuição do tamanho das partículas. Isso favorece a extração do óleo presente na semente, no entanto, partículas excessivamente pequenas podem causar problemas operacionais como difícil separação das partículas que, muitas vezes, permanecem em suspensão (PATRICELLI et al., 1979).

2.2.2.1.1 Tipos de solventes utilizados no processo de extração

O hexano é o solvente orgânico mais utilizado no processo de extração, por ser o mais seletivo, possuir estreita faixa de ebulição e ser imiscível com a água, o que evita misturas azeotrópicas (MORETTO; ALVES, 1998). Porém, alguns pontos negativos como sua

inflamabilidade, custo, potencial poluidor e risco à saúde, pois é considerado uma neurotoxina, justificam o estudo de alternativas ao seu uso.

Hui et al. (2007) relatam que, durante o século XIX diferentes solventes foram estudados, tais como o éter de petróleo ou diclorometano, ambos muito utilizados na extração de óleos voláteis.

O etanol pode ser considerado como uma alternativa ao processo de extração, além de ser produzido por meio de fontes renováveis. A comparação das propriedades químicas permite verificar que os riscos operacionais oferecidos pelo etanol são menores do que aqueles oferecidos pelo hexano, pois apresenta maiores temperaturas de inflamação (22°C) e toxicidade mais baixa (MERCK, 2006a; 2006b). Além disso, o fato do etanol ser obtido a partir da cana-de-açúcar coloca o Brasil em uma posição privilegiada na eliminação do uso de derivados de petróleo no processamento de oleaginosas. Além das vantagens de ser obtido de fontes renováveis e não ser tóxico, o etanol independe do mercado internacional do petróleo (CARVALHO, 2001).

Na área de alimentos, utilizam-se solventes orgânicos, a fim de que os resíduos encontrados nos produtos sejam reduzidos. De acordo com o *Food Chemicals Codex* (IMNA, 2006), pode-se utilizar determinados solventes como acetona, etanol e hexano em processos da indústria alimentícia, porém faz-se necessária a sua eliminação na etapa final.

Segundo Mogensen e Jacobsen (1982) e Treybal (1981) alguns fatores importantes devem ser analisados na seleção do solvente a ser utilizado para que o processo seja viável. Esses fatores incluem a seletividade (habilidade do solvente em extrair o soluto do material em questão), viscosidade (solventes com alta viscosidade reduzem a taxa de transferência de massa, o que influencia a velocidade de extração), ponto de ebulição (a fim de que sejam evitadas perdas do solvente, no processo de extração deve-se utilizar temperaturas inferiores ao ponto de ebulição do solvente), volatilidade (há uma maior facilidade na recuperação de solventes mais voláteis, o que diminui os custos do processo, toxidez (ponto essencial na escolha do solvente levando-se em consideração o risco para o operador, risco quando liberado ao meio ambiente e risco para o consumidor), densidade (com o objetivo de facilitar a separação das fases, as densidades das mesmas devem ser diferentes), inflamabilidade (aspecto relevante relacionado à segurança) e custo (deve-se selecionar o mais viável, não ignorando a sua efetividade).

A fim de avaliar a eficiência do etanol na extração do óleo de sementes de mamona comparando-a com a extração utilizando hexano, Anthonisen (2007) verificou que o etanol constitui uma alternativa na extração, pois o óleo da mamona é um óleo rico em ácido graxo ricinoleico, que é solúvel em álcool. Os valores encontrados para a eficiência de extração de sólidos solúveis, usando Soxhlet com etanol foi de 53,8% (m/m) e com hexano 45,5% (m/m). Drummond et al. (2006) também avaliaram o uso do etanol como solvente na extração do óleo da mamona. Esses autores relataram que a mistura óleo e etanol seguiria diretamente para a reação de transesterificação sem a evaporação do solvente, diminuindo os custos do processo e tornando o produto menos poluente.

2.2.2.1.2 A importância do etanol como potencial solvente extrator

O cultivo da cana-de-açúcar é a terceira maior atividade agrícola do Brasil em termos de área de produção e de valor bruto produzido, sendo as primeiras a soja e o milho. Atualmente, a cana-de-açúcar ocupa uma média de 8,77 milhões de hectares em todo o país, produzindo 646 milhões de toneladas, sendo que seu histórico de cultivo tem sido alterado em virtude da expansão do cultivo no Bioma Cerrado, deixando a tradição de cultivo na costa nordestina e fluminense (CONAB, 2018a; IBGE, 2017). O Centro-Sul brasileiro congrega as maiores unidades de processamento de cana do país. Essa região foi responsável pela produção de cerca de 26,1 bilhões de litros de etanol o equivalente a, aproximadamente, 94% da produção nacional (UNICA, 2018). Como pode se observar na Figura 3, a região Centro-Sul brasileira detém a grande maioria da produção de grãos de café e etanol, tornando tecnicamente factível o uso do etanol no processo de extração de óleos vegetais. Esse processo pode ser bastante atrativo economicamente no país (RODRIGUES, 2011). A adoção de tecnologia adequada pode possibilitar a substituição de solventes não sustentáveis, por solventes GRAS (*Generally Recognized as Safe*).

Estudos de utilização do etanol como solvente para a obtenção de óleos vegetais têm sido realizados desde o início do século 20 (HRON; KOLTUN; GRACI, 1982; JOHNSON; LUSAS, 1983). Os japoneses já utilizavam a extração industrial do óleo de soja com etanol na década de 30 (SATO; ITO, 1932), no entanto, não deram continuidade ao processo, em decorrência do elevado custo do solvente (RODRIGUES, 2011). Kaparthi e Chari (1959), Fonseca e Regitano-d’Arce

(1993) estudaram a extração do óleo da torta do amendoim e do amendoim, respectivamente; Freitas et al. (2007) determinaram o rendimento da extração do óleo de castanha do Pará; Franco et al. (2007b) determinaram a solubilidade e cinética de extração do óleo de *Rosa rubiginosa*; Brossard-Gonzalez et al. (2010) compararam a prensagem e a extração por solvente do óleo de pinhão-manso; Rodrigues (2011) comparou a extração sólido-líquida com diversos tipos de solvente. Grande parte desses trabalhos realizou a extração com etanol comparada a outros métodos de extração, bem como a outros solventes. Estudos sistematizados que abordam, não somente as variáveis operacionais, mas também a avaliação de possíveis mudanças de óleos vegetais, como um todo, estão sendo realizados.

O índice de retenção é uma possível variável que deveria ser estudada para permitir a correta avaliação da viabilidade de mudança de solvente. Este permite definir a quantidade de solução que fica retida ao material insolúvel após o processo de extração (TAKEUCHI et al., 2009).

Os compostos minoritários extraídos (ou não) também devem ser analisados durante o processo de extração. Compostos como antioxidantes e ácidos fenólicos devem ser levados em consideração. No caso do café, compostos como ácidos clorogênicos e cafeína, hidrossolúveis, podem ser extraídos e contidos na fase extrato. Regitano-d'Arce (1985, 1991) avaliou a influência da hidratação do solvente biorenovável etanol na extração do ácido clorogênico e observou que a extração desse composto fenólico aumentou com o aumento da hidratação do solvente.

Em elevadas temperaturas, os triacilgliceróies possuem alta solubilidade em etanol e baixa solubilidade à temperatura ambiente, possibilitando uma pré-separação por resfriamento da fase oleosa e solvente sem necessidade do processo de "destilação da miscela", ou seja, com gasto energético menor. Assim, este é outro aspecto que deve ser analisado no processo de extração com esse solvente alternativo (RODRIGUES, 2011).

2.2.3 Processos alternativos de extração: Ultrassom

Segundo Cheeke (2017), o termo ultrassom é definido para a faixa de frequência acima de 20 kHz até 1 GHz. Para valores acima de 1 GHz se convencionou denominar o regime hipersônico. O processo de extração utiliza a faixa de ultrassom onde é produzida a cavitação. O ultrassom, pode ser gerado por meio de transdutores, como o usado por Mason et al. (2005), onde o sistema é

baseado na utilização de um transdutor de chapa reforçada para gerar ultrassom, incorporando uma placa de titânio (350 mm de diâmetro) que permite intensificar a energia ultrassônica de foco. Níveis de pressão sonora (SPL) em torno de 165 dB (3 W cm^{-2}) foram registrados a uma distância de aproximadamente 330 mm do centro da placa, quando uma potência máxima de 150 W foi aplicada ao transdutor.

Liang (1993) menciona que ultrassom de alta intensidade produz uma variedade de efeitos, tais como pressão por radiação, fluxo, cavitação, e a instabilidade da interface. Esses efeitos podem influenciar os processos de transferência de massa, produzindo mudanças ao longo dos gradientes de concentração, assim como nos coeficientes de difusão, ou na camada limite.

Spigno, Tramelli e De Faveri (2007) indicam que a melhora no processo de extração é atribuída ao rompimento das paredes celulares, redução do tamanho das partículas e à melhoria na transferência de massa do conteúdo da célula ao solvente causada pelo colapso da bolha produzida por cavitação. Visando à possibilidade de aperfeiçoar o processo de transferência de massa, Wei et al. (2008) avaliaram o rendimento da extração de óleo de sementes de colza (*Brassica napus*) utilizando o ultrassom, obtendo os seguintes parâmetros otimizados: 500 W de energia de ultrassom, tempo de extração de 60 minutos, relação líquido-sólido de 1 L g^{-1}. O rendimento experimental de óleo foi de 38,5%. Li (2002) também observou que a aplicação do ultrassom promoveu maior rendimento de óleo quando aplicado na extração de grãos de soja, se comparado a métodos tradicionais e, até mesmo, que o processo realizado em micro-ondas.

Os efeitos produzidos pelo ultrassom em processos de transferência de massa são influenciados pela intensidade aplicada. Mulet et al. (2003) relatam que à baixa intensidade não se observa mudança significativa na velocidade de transferência de massa, existindo um limite de vaporização pelo efeito da cavitação acústica para níveis de pressão sonora em torno de 140 dB (100 W m^{-2}). O efeito do fluxo acústico se torna maior do que a influência da convecção natural. Alta intensidade produz vibrações acústicas mais altas e, portanto, forte cavitação, e maior microfluxo. Segundo Mulet et al. (2002), ultrassom de alta intensidade é aplicado em baixas frequências (20 - 300 kHz) para obter níveis de alta potência (10-1000 W m^{-2}).

2.3 Aproveitamento de resíduos da agroindústria

Ao longo dos últimos anos muitos esforços têm sido feitos para a valorização dos resíduos e coprodutos agroindustriais, através de processos que transformam esses rejeitos em novos produtos com alto valor agregado (MAKRIS et al., 2007). Entretanto, no Brasil, ainda existem poucas iniciativas para a utilização em grande escala da maior parte dos resíduos agroindustriais. Considerando os impactos negativos no meio ambiente, que tem contribuído para o aumento nos problemas de saúde ocupacional, associados à degradação do ar, água e vegetação, é imperativo que se concentre esforços para desenvolver soluções concretas para o uso de resíduos com potencial para contribuir na oferta de novos produtos de alto valor agregado.

Existem hoje alguns grupos de pesquisa dedicados a produzir conhecimento e tecnologia para aproveitamento de resíduos da agroindústria, por meio de metodologias mais limpas, gerando materiais com propriedades nutracêuticas, funcionais e bioativas (atividades antioxidante, antimicrobiana, antialergênica, antiaterogênica, anti-inflamatória, antitrombótica ou efeitos cardioprotetores ou vasodilatadoras) (CRUZ et al., 2011; GARCIA-GARCIA, 2017; MELLO, 2006; RORIZ, 2012; SÁ LEITÃO; SÁ LEITÃO, 2015). O resíduo da indústria de vinho e suco de uva, por exemplo, é rico em polifenois que apresentam potencial antioxidante, auxiliando os organismos vivos no combate aos radicais livres e ao stress oxidativo (SPIGNO; TRAMELLI; DE FAVERI, 2007). Usando etanol e metanol como solventes, Casazza et al. (2012) compararam a extração de compostos fenólicos da semente e casca de uva com 4 diferentes técnicas: extração em *shaker*, extração em micro-ondas, extração em ultrassom e extração em reator com controle de pressão (200 bar) e temperatura (350ºC). Os compostos fenólicos foram medidos quali e quantitativamente por métodos espectrofotométricos e cromatográficos. O extrato etanólico apresentou maior capacidade antioxidante de 14,6 ± 0,4 e 1,1 ± 0,3 µL/ µg DPPH para a semente e casca de uva, respectivamente.

Um dos resíduos de biomassa mais pesquisados atualmente no Brasil é o bagaço da cana de açúcar, gerado em grandes quantidades pelo setor sucroalcooleiro. Dantas Filho (2009), em seu estudo, mostrou que o bagaço é um produto viável, tanto técnica como economicamente, para gerar energia elétrica, ainda, concluiu que alguns custos envolvidos no processo tendem a cair com a curva de aprendizado e o aumento da escala de produção. Estudos recentes de viabilidade técnica e econômica constataram a potencialidade dessa biomassa lignocelulósica para a produção do etanol de segunda geração (BIAGGI, 2017; DRABER, 2013; SOBRINHO CHEMMÉS et al.,

2013; SOARES, 2016). Segundo Gnansounou, Dariat e Wyman (2005) o etanol de segunda geração será comercializado nas próximas décadas, a preços competitivos, como energia renovável para transporte e ressalta que o bagaço de cana, principal resíduo da produção de etanol convencional no Brasil, é a biomassa mais promissora. Esse fato coloca o país em uma posição de destaque na produção de bioetanol.

O etanol, além de apresentar a vantagem de ser um solvente obtido a partir de fontes renováveis e ser menos tóxico que os solventes derivados de fontes fósseis, já é produzido no Brasil em grande escala, com potencial para ampliar sua oferta nas próximas décadas. A extração do óleo de café com álcool etílico é mais recomendada dos pontos de vista ambiental e da saúde ocupacional, e poderá ser muito vantajosa, pois seu caráter mais polar que o hexano (solvente mais utilizado na indústria de óleos) permite carrear substâncias características do flavor, compostos fenólicos, que são os maiores responsáveis pelo potencial antioxidante do café, e diterpenos como caveol e cafestol, que são associados à proteção contra raios UV (CAVIN et al., 2002; GROLLIER; PLESSIS, 1998; WAGEMAKER et al., 2011).

3 CONSIDERAÇÕES GERAIS

Face à necessidade da substituição de hexano por solventes alternativos em processos de extrações e subsidiando-se no fato de que o Brasil se encontra entre um dos maiores produtores de etanol do mundo, esse solvente torna-se uma alternativa viável para realizar processos de extração sólido-líquida. As extrações realizadas utilizando etanol podem apresentar menores rendimentos de extrações de sólidos solúveis do que as extrações realizadas utilizando hexano, no entanto, o etanol proporciona o aumento no rendimento de extração de compostos fenólicos e antioxidantes, obtendo óleos com diferentes concentrações de compostos minoritários. A fim de aumentar a solubilidade do óleo neutro em etanol, aumentando, assim, o rendimento da extração, alguns artifícios termodinâmicos podem ser utilizados, como o aumento da temperatura de operação ou a adição de cossolvente ao solvente. Além disso, a extração sólido-líquida pode ser uma opção para elevar o rendimento da extração de óleo de café verde, uma vez que o café verde possui um teor de óleo menor do que 20%, e da torta obtida da prensagem dos grãos, a qual apresenta teor residual de óleo. Como sugestões para trabalhos futuros:

a) Ampliar a quantificação de compostos.

b) Analisar a solubilidade do óleo extraído nos diferentes solventes e temperaturas.

c) Realizar o estudo cinético das extrações dos compostos minoritários.

d) Estudar o equilíbrio líquido-líquido entre os óleos obtidos e os solventes.

e) Avaliar o efeito da adição de água ao etanol nas extrações.

REFERÊNCIAS

ABDULKARIM, S. M. et al. Some physico-chemical properties of Moringa oleifera seed oil extracted using solvent and aqueous enzymatic methods. **Food Chemistry**, London, v. 93, n. 2, p. 253-263, 2005.

AGUILERA, J. M. Solid-liquid extraction. In: TZIA, C.; LIADAKIS, G. (Ed.). **Extraction optimization in food engineering**. New York: CRC, 2003. p. 35-55.

AL-DHABI, N. A. et al. Development and validation of ultrasound-assisted solid-liquid extraction of phenolic compounds from waste spent coffee grounds.**Ultrasonics Sonochemistry**, Oxford, v. 34, p. 206-213, 2017.

AL-HAMAMRE, Z. et al. Oil extracted from spent coffee grounds as a renewable source for fatty acid methyl ester manufacturing. **Fuel**, London, v. 96, n. 1, p. 70-76, 2012.

ALVAREZ, A. M. R.; RODRÍGUEZ, M. L. G. Lipids in pharmaceutical and cosmetic preparations. **Grasas y Aceites**, Sevilla, v. 51, n. 1/2, p. 74-96, 2000.

AMARANTE, R. C. A. et al. Oil extraction from castor cake using ethanol: kinetics and thermodynamics. **Industrial & Engineering Chemistry Research**, Washington, v. 53, n. 16, p. 6824-6829, 2014.

ANTHONISEN, D. G. **Caracterização de genótipos de mamona:** marcadores RAPD, teor de óleos nas sementes por Soxhlet e RMN e rendimento da extração do óleo usando etanol. 2007. 73 f. Dissertação (Mestrado em Ciência e Tecnologia dos Grãos)-Universidade Federal de Pelotas, Pelotas, 2007.

ANTONIASSI, R. **Prensagem em pequena escala**. Brasília, DF: Ageitec, 2016. Disponível em: <http://www.agencia.cnptia.embrapa.br/gestor/tecnologia_de_alimentos/arvore/CONT000gc8yujq302wx5ok01dx9lcudlguwx.html>. Acesso em: 13 maio 2017.

ANTONIASSI, R.; FREITAS, S. C. **Processamento**. Brasília, DF: Ageitec, 2016. Disponível em: <http://www.agencia.cnptia.embrapa.br/gestor/tecnologia_de_alimentos/arvore/CONT000gc8yujq302wx5ok01dx9lcx1g7v3u.html >. Acesso em: 14 jul. 2018.

AQUINO, L. P. et al. Extraction of oil from pequi fruit (*Caryocar Brasiliense* Camb.) using several solvents and their mixtures. **Grasas y Aceites**, Sevilla, v. 62, n. 3, p. 245-252, jul./set. 2011.

ASSOCIAÇÃO BRASILEIRA DA INDÚSTRIA DE CAFÉ. **Indicadores da indústria de café**. Rio de Janeiro, 2017. Disponível em: <http://abic.com.br/estatisticas/indicadores-da-industria/indicadores-da-industria-de-cafe-2017/>. Acesso em: 10 dez. 2017.

BALZER, H. H. 1-B acids in coffee. In: CLARKE, R. J.; VITZTHUM, O. G. (Ed.). **Coffee:** recent developments. Berlin: Blackwell Science, 2001. p. 18-32.

BECKER, W. Solvent extraction of soybeans. **Journal of the American Oil Chemists' Society**, Chicago, v. 55, n. 11, p. 754-761, 1978.

BELITZ, H. D.; GROSCH, W.; SCHIEBERLE, P. **Food chemistry**. 4th ed. Heidelberg: Springer-Verlag, 2009. 1070 p.

BIAGGI, D. E. **Inovações e tendências tecnológicas na produção de etanol de segunda geração a partir da cana-de-açúcar pela rota hidrolítica enzimática:** um estudo de prospecção tecnológica. 2017. 115 f. Dissertação (Mestrado em Gestão e Tecnologia em Sistemas Produtivos)-Centro Estadual de Educação Tecnológica Paula Souza, São Paulo, 2017.

BOSS, E. A. **Análise do desempenho de plantas de extração de óleo convencionais e de processos supercríticos**. 2000. 105 f. Dissertação (Mestrado em Engenharia Química)-Faculdade de Engenharia Química, Universidade Estadual de Campinas, Campinas, 2000.

BRADBURY, A. G. W. Chemistry I: non-volatile compounds: carbohydrates. In: CLARKE, R. J.; VITZTHUM, O. G. (Ed.). **Coffee:** recent developments. Oxford: Blackwell Science, 2001. p. 1-17.

BRASIL. Ministério da Agricultura, Pecuária e Abastecimento. **Departamento do café, do MAPA divulga informe estatístico do café do mês de maio**. Brasília, DF: EMBRAPA, 2015a. Disponível em: <http://www.agricultura.gov.br/vegetal/culturas/cafe/saiba-mais>. Acesso em: 14 maio 2017.

BRASIL. Ministério da Agricultura, Pecuária e Abastecimento. **Produção de café deve chegar a 44,28 milhões de sacas neste ano**. Belo Horizonte: Sistema FAEMG, 2015b. Disponível em: <http://www.sistemafaemg.org.br/Noticia.aspx?Code=8611&Portal=1&PortalNews=1&ParentCode=139&ParentPath=None&ContentVersion=R>. Acesso em: 14 maio 2017.

BROSSARD-GONZÁLEZ, C. O. et al. Preliminary evaluation of anhydrous ethanol as a solvent in the oilseed extraction of *Jatropha curcas* L. **Grasas y Aceites**, Sevilla, v. 61, p. 295-302, 2010.

BUDRYN, G. et al. Influence of roasting conditions on fatty acids and oxidative changes of *Robusta* coffee oil. **European Journal of Lipid Science and Technology**, Berlin, v. 114, n. 9, p. 1052-1061, 2012.

BUOSI, G. M. **Extração do óleo de abacate (*Persea americana* Mill.) visando a produção de biodiesel e sua caracterização**. 2013. 37 f. Trabalho de Conclusão de Curso (Graduação em Tecnologia em Biocombustíveis)-Universidade Federal do Paraná, Palotina, 2013.

CABRAL, M. S.; MORIS, V. A. da S. Reaproveitamento da borra de café como medida de minimização da geração de resíduos. In: ENCONTRO NACIONAL DE ENGENHARIA DE PRODUÇÃO, 30., 2010, São Carlos. **Anais Eletrônicos...** São Paulo: UFSCAR, 2010. Disponível em: <http://www.abepro.org.br/biblioteca/enegep2010_TN_STP_121_788_17072.pdf>. Acesso em: 12 maio 2017.

CAETANO, N. S.; SILVA, V. F. M.; MATA, T. M. Valorization of coffee grounds for biodiesel production. **Chemical Engineering Transactions**, Milano, v. 26, p. 267-272, 2012.

CAFÉ. **Tipos de café Arábica e Robusta (Conilon)**. Blog do café, 2015. Disponível em: <https://blogdocafe.com/2015/06/25/tipos-de-cafe-arabica-e-robusta-conilon/>. Acesso em: 8 ago. 2018.

CARDOSO, A. P. S. **Café:** cultura e tecnologia primária. Lisboa: IICT, 1994.

CARVALHO, L. C. C. Álcool do Brasil: energia limpa e renovável. **Agroanalysis**, São Paulo, v. 21, n. 9, p. 18-27, set. 2001.

CASAZZA, A. A. et al. High-pressure high-temperature extraction of phenolic compounds from grape skins. **International Journal of Food Science and Technology**, Oxford, v. 47, n. 2, p. 399-405, 2012.

CAVIN, C. et al. Cafestol and kahweol, two coffee specific diterpenes with anticarcinogenic activity. **Food and Chemical Toxicology**, Oxford, v. 40, n. 8, p. 1155-1163, 2002.

CHEEKE, J. D. N. **Fundamentals and applications of ultrasonic waves**. 2nd ed. Boca Raton: CRC, 2017. 504 p.

CLARKE, R. J. Water and mineral contents. In: CLARKE, R. J.; MACRAE, R. (Ed.). **Coffee:** chemistry. Rotterdam: Springer Netherlands, 1985. p. 42-82.

CLIFFORD, M. N. Chlorogenic acids. In: CLARKE, R. J. (Ed.). **Coffee:** chemistry. Rotterdam: Springer Netherlands, 1985. p. 153-202.

COELHO FILHO, A. C. A. **Extração sólido-líquido a quente de lipídios de alimentos industrializados**. 2015. 30 f. Trabalho de Conclusão de Curso (Graduação em Engenharia Química)-Universidade Federal de Alfenas, Poços de Caldas, 2015.

COMPANHIA NACIONAL DE ABASTECIMENTO. **Acompanhamento da safra brasileira de café:** primeiro levantamento, janeiro 2018, v. 5, n. 1. Brasília, DF, 2018a. 73 p.

COMPANHIA NACIONAL DE ABASTECIMENTO. **Acompanhamento da safra brasileira de café:** segundo levantamento, junho 2017, v. 2, n. 2. Brasília, DF, 2017. 59 p.

COMPANHIA NACIONAL DE ABASTECIMENTO. **Acompanhamento da safra brasileira de cana de açúcar:** primeiro levantamento, maio 2018, v. 5, n. 1. Brasília, DF, 2018b. 66 p.

CRUZ, S. A. et al. Recycled polymers for food contact. **Polímeros**, São Carlos, v. 2, n. 2, p. 340-345, 2015.

DAGOSTIN, J. L. A.; CARPINÉ, D.; CORAZZA, M. L. Extraction of soybean oil using ethanol and mixtures with alkyl esters (biodiesel) as co-solvent: kinetics and thermodynamics. **Industrial Crops and Products**, London, v. 74, p. 69-75, 2015.

DANTAS FILHO, P. L. **Análise de custos na geração de energia com bagaço de cana-de-açúcar:** um estudo de caso em quatro usinas de São Paulo. 2009. 175 p. Dissertação (Mestrado em Energia)-Universidade de São Paulo, São Paulo, 2009.

DEL VALLE, J. M. et al. Comparision of conventional and supercritical CO_2-extracted rosehip oil. **Brazilian Journal of Chemical Engineering**, São Paulo, v. 17, n. 3, p. 335-348, 2000.

DIAS, R.; BENASSI, M. Discrimination between Arabica and Robusta coffees using hydrosoluble compounds: is the efficiency of the parameters dependent on the roast degree? **Beverages**, Basel, v. 1, p. 127-139, 2015.

DRABER, K. M. M. **Etanol de segunda geração já é realidade**. 2017. 116 f. Dissertação (Mestrado Profissional em Gestão e Tecnologia em Sistemas Produtivos)-Centro Estadual de Educação Tecnológica Paula Souza, São Paulo, 2017.

DRUMMOND, A. R. F. et al. Metanol e etanol como solventes na extração do óleo da mamona. In: CONGRESSO DA REDE BRASILEIRA DE TECNOLOGIA DO BIODIESEL, 1., 2006, Brasília. **Anais...** Brasília, DF: Abipti; MCT, 2006. 1 CD-ROM.

FARAH, A.; DONANGELO, C. M. Phenolic compounds in coffee. **Brazilian Journal of Plant Physiology**, Londrina, v. 18, n. 1, p. 23-36, 2006.

FARAH, A. et al. Compostos fenólicos em café torrado. In: SIMPÓSIO DE PESQUISA DOS CAFÉS DO BRASIL, 2., 2001, Vitória. **Anais Eletrônicos...** Vitória: EMBRAPA Café, 2001. p. 1485-1492.

FERNANDES, S. M. et al. Constituintes químicos e teor de extrato aquoso de cafés arábica (C*offea arabica* L.) e conilon (*Coffea canephora* Pierre) torrados. **Ciência e Agrotecnologia**, Lavras, v. 27, n. 5, p. 1076-1081, set./out. 2003.

FIGUEIREDO, L. P. et al. Fatty acid s and parameters of quality of specialty coffees produced in different Brazilian regions. **African Journal of Agricultural Research**, Nairobi, v. 10, n. 35, p. 3484-3493, 2015.

FONSECA, H.; REGITANO-D'ARCE, M. A. B. Aflatoxin removal of peanut meals with aqueous ethanol. **Scientia Agricola**, Piracicaba, v. 50, n. 1, p. 154-156, 1993.

FRANCO, D. et al. Ethanolic extraction of *Rosa rubiginosa* soluble substances:oil solubility equilibria and kinetic studies. **Journal of Food Engineering**, London, v. 79, n. 1, p. 150-157, 2007a.

FRANCO, D. et al. Processing of *Rosa rubiginosa*: extraction of oil and antioxidant substances. **Bioresource Technology**, Essex, v. 98, n. 18, p. 3506-3512, 2007b.

FREIBERG, E. B. et al. Encapsulation of roasted coffee oil in biocompatible nanoparticles. **LWT - Food Science and Technology**, Trivandrum, v. 64, n. 1, p. 381-389, 2015.

FREITAS, S. P. et al. Extraction and simultaneous separation of the Brazil nuts oil with ethanol. **Ciência e Tecnologia de Alimentos**, Campinas, v. 27, p. 14-17, 2007.

GANDHI, A. P. et al. Studies on alternative solvents for the extraction of oil soybean. **International Journal of Food Science and Technology**, Oxford, v. 38, n. 3, p. 369-375, Mar. 2003.

GARCIA-GARCIA, G. et al. A methodology for sustainable management of food waste. **Waste and Biomass Valorization**, New York, v. 8, n. 6, p. 2209-2227, 2017.

GETACHEW, A. T. et al. Concurrent extraction of oil from roasted coffee (*Coffea arabica*) and fucoxanthin from brown seaweed (*Saccharina japonica*) using supercritical carbon dioxide. **Journal of CO_2 Utilization**, New York, v. 25, p. 137-146, 2018.

GNANSOUNOU, E.; DARIAT, A.; WYMAN, C. E. Refining sweet sorghum to ethanol and sugar: economic trade-offs in the context of North China. **Bioresource Technology**, Essex, v. 96, n. 9, p. 985-1002, 2005.

GROLLIER, J.; PLESSIS, S. **Use of coffee bean as a sun filter**. U.S. Patent n. 4793990. US Patent and Trade Mark Office, 27 Dec. 1988.

HRON, R. J.; KOLTUN, S. P.; GRACI, A. V. Biorenewable solvents for vegetable oil extraction. **Journal of the American Oil Chemists' Society**, Chicago, v. 59, n. 9, p. 674A-684A, 1982.

HUI, Y. H. et al. (Ed.). **Handbook of food products manufacturing**. New Jersey: J. Wiley, 2007. 2308 p.

ILLY, E. The complexity of coffee. **Scientific American**, New York, v. 286, n. 6, p. 86-91, 2002.

INSTITUTE OF MEDICINE OF NATIONAL ACADEMIES. **Food chemicals codex**. 5th ed. Washington, 2006.

INSTITUTO BRASILEIRO DE GEOGRAFIA E ESTATÍSTICA. **Nova publicação do IBGE analisa a dinâmica territorial da cana-de-açúcar**. Rio de Janeiro: Agência IBGE Notícias, 2017. Disponível em: <https://agenciadenoticias.ibge.gov.br/agencia-noticias/2013-agencia-de-noticias/releases/19013-nova-publicacao-do-ibge-analisa-a-dinamica-territorial-da-cana-de-acucar.html>. Acesso em: 14 jul. 2018.

INTERNATIONAL COFFEE ORGANIZATION. **Total production by all exporting countries**. London, 2018. Disponível em: <http://www.ico.org/prices/po-production.pdf>. Acesso em: 14 jul. 2018.

JAVED, F. Recovery of rice bran oil using solid-liquid extraction technique. **Journal of Food Process Engineering**, Westport, v. 38, n. 4, p. 357-362, 2015.

JOHNSON, L. A.; LUSAS, E. W. Comparison of alternative solvents for oils extraction. **Journal of the American Oil Chemists' Society**, Chicago, v. 60, n. 2, p. 229-242, 1983.

KAPARTHI, R.; CHARI, K. S. Solubilities of vegetable oils in aqueous ethanol and ethanol-hexane mixtures. **Journal of the American Oil Chemists' Society**, Chicago, v. 36, n. 2, p. 45-92, a4-a28, 1959.

KAWACHI, I. et al. A prospective study of coffee drinking and suicide in women. **Archives of Internal Medicine**, Chicago, v. 156, n. 5, p. 521-525, 1996.

KEMPER, T. G. Oil extraction. In: SHAHIDI, F. (Ed.). **Bailey's industrial oil and fat products**. New York: J. Wiley, 2005. p. 57-98.

KOBELNILK, M. et al. Behavior of coffee oil (*Robusta*and *Arabica*species). **Journal of Thermal Analysis and Calorimetry**, Oxford, v. 115, n. 3, p. 2045-2052, 2014.

KOHLHEPP, G. Análise da situação da produção de etanol e biodiesel no Brasil. **Estudos Avançados**, São Paulo, v. 24, n. 68, p. 223-253, 2010.

KÖLLING-SPEER, I.; STROHSCHNEIDER, S.; SPEER, K. Determination of free diterpenes in green and roasted coffees. **Journal of Separation Science**, New York, v. 22, n. 1, p. 43-46, 1999.

KUK, M. S.; HRON, R. J. Cottonseed extraction with a new solvent system: isohexane and alcohol mixtures. **Journal of the American Oil Chemists' Society**, Chicago, v. 75, n. 8, p. 927-930, 1998.

KURZROCK, T.; SPEER, K. Diterpenes and diterpene esters in coffee. **Food Reviews International**, London, v. 17, n. 4, p. 433-450, 2001.

LATIF, S.; DIOSADY, L. L.; ANWAR, F. Enzyme-assisted aqueous extraction of oil and protein from canola (*Brassica napus* L.) seeds. **European Journal of Lipid Science and Technology**, Berlin, v. 110, n. 10, p. 887-892, 2008.

LI, H. **Ultrasound and microwave assisted extraction of soybean oil**. 2002. 68 p. Dissertation (Master in Biosystems Engineering Technology)-University of Tenessee, Knoxville, 2002.

LI, X. et al. Solvent-mediated crystal-to-crystal transformations from a cationic homometallic metal-organic framework to heterometallic frameworks. **CrystEngComm**, London, v. 16, n. 37, p. 8818-8824, 2014.

LIMA FILHO, T. et al. Physico-chemical characterization of espresso coffee beverage prepared from blends of arabica and conilon coffees. **Revista Ceres**, Viçosa, MG, v. 62, n. 4, p. 333-339, 2015.

MACRAE, R. Nitrogenous components. In: CLARKE, R. J.; MACRAE, R. (Ed.). **Coffee:** chemistry. Rotterdam: Springer Netherlands, 1985. p. 115-152.

MAKRIS, D. P. et al. Polyphenolic content and in vitro antioxidant characteristics of wine industry and other agri-food solid waste extracts. **Journal of Food Composition and Analysis**, San Diego, v. 20, p. 125-132, 2007.

MANI, S.; JAYA, S.; VADIVAMBAL, R. Optimization of solvent extraction of moringa (*Moringa Oleifera*) seed kernel oil using response surface methodology. **Food and Bioproducts Processing**, Rugby, v. 85, n. 4, p. 328-335, 2007.

MARIANO, R. G. D.; COURI, S.; FREITAS, S. P. Enzymatic technology to improve oil extraction from *Caryocar brasiliense* Camb. (pequi) pulp. **Revista Brasileira de Fruticultura**, Jaboticabal, v. 31, n. 3, p. 637-643, 2009.

MARTÍN, M. J. et al. Fatty acid profiles as discriminant parameters for coffee varieties differentiation. **Talanta**, London, v. 54, n. 2, p. 291-297, 2001.

MARTINS, C. M.; AQUINO, F. J. T. Study of chemical composition, antioxidant activity of coffee (*Coffee arabica*) and its straw from cerrado. In: SEMINÁRIO DE INICIAÇÃO CIENTÍFICA, 12., 2008, Uberlândia. **Anais Eletrônicos...** Uberlândia: Ed. UFU, 2010. Disponível em: <https://ssl4799.websiteseguro.com/swge5/seg/cd2008/PDF/IC2008-0005.PDF>. Acesso em: 12 maio 2017.

MASON, T. et al. Application of ultrasound. In: SUN, D. W. (Ed.). **Emerging technologies for food processing**. Cambridge: Elsevier Academic Press, 2005. p. 323-350.

MELLO, L. M. R. **Produção e comercialização de uvas e vinhos:** panorama 2005. Bento Gonçalves: EMBRAPA, 2006.

MERCK. **Ficha de informações de segurança de produtos químicos:** etanol absoluto. São Paulo, 2006a.

MERCK. **Ficha de informações de segurança de produtos químicos:** n-Hexano. São Paulo, 2006b.

MOGENSEN, O. E.; JACOBSEN, F. M. Positronium yields in liquids determined by lifetime and angular correlation measurements. **Chemical Physisc**, College Park, v. 73, n. 1/2, p. 223-234, 1982.

MORETTO, E.; ALVES, R. F. **Tecnologia de óleos e gorduras vegetais na indústria de alimentos**. São Paulo: Varela, 1998. 150 p.

MULET, A. et al. Equilibrium isotherms and isoteric heats of morel (*Morchela esculenta*). **Journal of Food Engineering**, Essex, v. 53, p. 75-81, 2002.

MULET, A. et al. New food drying technologies: use of ultrasound. **Food Science and Technology International**, London, v. 9, n. 3, p. 215-221, 2003.

MUSSATTO, S. I. et al. Production, composition, and application of coffee and its industrial residues. **Food and Bioprocess Technologies**, Chicago, v. 4, p. 661-672, 2011.

NASCIMENTO, R. J. S. et al. Fatty acids composition of açaí pulp oil obtained by enzymatic technology and hexane. **Revista Brasileira de Fruticultura**, Jaboticabal, v. 30, n. 2, p. 498-502, 2008.

NAVARRO, S. L. B.; RODRIGUES, C. E. C. Extração de óleo de macadâmia com isopropanol: rendimento, estabilidade oxidativa e avaliação da fração proteica. In: CONGRESSO BRASILEIRO DE CIÊNCIA E TECNOLOGIA DE ALIMENTOS, 25., 2016, Gramado. **Anais Eletrônicos...** Gramado: SBCTA, 2016. Disponível em: <http://www.ufrgs.br/sbctars-eventos/xxvcbcta/anais/files/108.pdf>. Acesso em: 1 ago. 2018.

NEVES, C. **A estória do café**. Rio de Janeiro: IBC, 1974. 52 p.

O'BRIEN, R. D.; FARR, W. E.; WAN, P. J. **Introduction to fats and oils technology**. 2nd ed. Illinois: American Oil Chemists' Society, 2000. 618 p.

OLIVEIRA, A. L. et al. Brazilian roasted coffee oil obtained by mechanical expelling: compositional analysis by GC-MS. **Food Science and Technology**, Campinas, v. 25, n. 4, p. 677-682, 2005.

OLIVEIRA, C. M.; GARAVAZO, B. R.; RODRIGUES, C. E. C. Liquid-liquid equilibria for systems composed of rice bran oil and alcohol-rich solvents: application to extraction and deacidification of oil. **Journal of Food Engineering**, London, v. 110, n. 3, p. 418-427, June 2012.

OLIVEIRA, R. C.; BARROS, S. T. D.; GIMENES, M. L. The extraction of passion fruit oil with green solvents. **Journal of Food Engineering**, London, v. 117, n. 4, p. 458-463, 2013.

PATRICELLI, A. et al. Fattori che influenzano lèstrazione del lipidi da semi decorticati di girasole. **Rivista Italiana Delle Sostanze Grasse**, Milano, v. 61, p. 136-142, 1979.

PÉREZ, C. et al. Supercritical fluid extraction of grape seeds: extract chemical composition, antioxidant activity and inhibition of nitrite production in LPS-stimulated Raw 264.7 cells. **Food & Function**, London, v. 6, n. 8, p. 2607-2613, 2015.

RABASCO ALVAREZ, A. M. et al. Lipids in pharmaceutical and cosmetic preparations. **Grasas y Aceites**, Sevilla, v. 51, n. 1/2, p. 74-96, 2000.

RAVINDRAN, R. et al. Two-step sequential pretreatment for the enhanced enzymatic hydrolysis of coffee spent waste. **Bioresource Technology**, Essex, v. 239, p. 276-284, 2017.

REGITANO-D'ARCE, M. A. B. **Ensaios de extração de óleo de girasol (*Helianthus annuus* L.) com álcool etílico**. 1985. 110 p. Dissertação (Mestrado em Agronomia)-Escola de Agricultura "Luiz de Queiroz", Universidade de São Paulo, Piracicaba, 1985.

REGITANO-D'ARCE, M. A. B. **Extração de óleo de girassol com etanol:** cinética, ácido clorogênico, fração insaponificável. 1991. 145 p. Tese (Doutorado em Agronomia)-Faculdade de Ciências Farmacêuticas, Universidade de São Paulo, Piracicaba, 1991.

RENEWABLE FUELS ASSOCIATION. **World fuel ethanol production**. Washington, 2017. Disponível em: <http://www.ethanolrfa.org/resources/industry/statistics/#1454098996479-8715d404-e546>. Acesso em: 14 jul. 2018.

RICHARDSON, J. F.; HARKER, J. H.; BACKHURST, J. R. **Chemical engineering:** particle technology and separation processes. 5th ed. Oxford: Butter Worth Heinemann, 2002.

RODRIGUES, C. E. C. **Utilização de solvente biorenovável nos processos de extração e desacidificação de óleos vegetais**. 2011. 172 f. Tese (Livre Docência em Equilíbrio de Fases e Processos de Separação na Indústria de Alimentos)-Faculadade de Zootecnica e Engenharia de Alimentos, Universidade de São Paulo, Pirassununga, 2011.

RODRIGUES, C. E. C.; ARACAVA, K. K.; ABREU, F. N. Thermodynamic and statistical analysis of soybean oil extraction process using renewable solvent. **International Journal of Food Science and Technology**, Oxford, v. 45, n. 11, p. 2407-2414, Nov. 2010.

RODRIGUES, J. A. R. Do engenho à biorrefinaria: a usina de açúcar como empreendimento industrial para a geração de produtos bioquímicos e biocombustíveis. **Química Nova**, São Paulo, v. 34, n. 7, p. 1242-1254, 2011.

ROMANO, R. et al. Identification markers based on fatty acid composition to differentiate between roasted *Arabica*and *Canephora* (*Robusta*) coffee varieties in mixtures. **Journal of Food Composition and Analysis**, San Diego, v. 35, n. 1, p. 1-9, 2014.

RONCHI, C. P. **A origem do café conilon**. Vitória: Café Point, 2009. Disponível em: <http://www.cetcaf.com.br/informacoes%20gerais/origem%20cafe%20conilon/origem_cafe_conilon.htm>. Acesso em: 14 maio 2017.

RORIZ, R. F. C. **Aproveitamento dos resíduos alimentícios obtidos das Centrais de Abastecimento do Estado de Goiás S/A para alimentação humana**. 2012. 158 f. Dissertação (Mestrado em Ciência e Tecnologia de Alimentos)-Universidade Federal de Goiás, Goiânia, 2012.

ROSENTHAL, A.; PYLE, D. L.; NIRANJAN, K. Aqueous and enzymatic processes for edible oil extraction. **Enzyme and Microbial Technology**, New York, v. 19, n. 6, p. 402-420, 1996.

SÄÄKSJÄRVI, K. et al. Prospective study of coffee consumption and risk of Parkinson's disease. **European Journal of Clinical Nutrition**, London, v. 62, n. 7, p. 908-915, 2008.

SÁLEITÃO, B. R. G.; SÁLEITÃO, C. S. Sustentabilidade e elaboração de novos produtos através do aproveitamento de resíduo alimentar. **Revista de Produção Acadêmico-Científica**, Manaus, v. 2, n. 2, p. 97-104, 2015.

SANTOS, D. M. **Desenvolvimento de método para obtenção de energia a partir da produção de biodiesel via extração de óleo de borra de pó de café em escala laboratorial**. 2010. 48 f. Dissertação (Mestrado em Ciências)-Universidade de São Paulo, São Paulo, 2010.

SANTOS, P. et al. Supercritical CO_2 extraction of cumbaru oil (*Dipteryx alata* Vogel) assisted by ultrasound: global yield, kinetics and fatty acid composition. **The Journal of Supercritical Fluids**, New York, v. 107, p. 75-83, 2016.

SATO, M.; ITO, C. **Method of extracting fatty oil**. US Patent n. 1892366. Minami Manshu Tetsudo Kabushiki Kaisha, 27 Dec. 1932.

SCHNEIDER, F. H. Zur extraktiven lipid-freisetzung aus pflanzlichen zellen. **European Journal of Lipid Science and Technology**, Berlin, v. 82, n. 1, p. 16-23, 1980.

SETH, S. et al. Oil extraction rates of soya bean using isopropyl alcohol as solvent. **Biosystems Engineering**, London, v. 97, n. 2, p. 209-217, 2007.

SHALMASHI, A. Ultrasound-assisted extraction of oil from tea seeds. **Journal of Food Lipids**,

New York, v. 16, n. 4, p. 465-474, 2009.

SILVA, L. L. **Análise fitoquímica e determinação das atividades antioxidantes e vasculogênica dos extratos aquosos padronizados de *Coffea arabica* e *Theobroma cacao*.** 2005. 141 f. Dissertação (Mestrado em Biotecnologia)-Centro de Ciências Biológicas, Universidade Federal de Santa Catarina, Florianópolis, 2005.

SMITH, A. W. Introduction. In: CLARKE, R. J.; MACRAE, R. (Ed.). **Coffee:** chemistry. Dordrecht: Springer Netherlands, 1985. p. 1-41.

SINGH, J.; BARGALE, P. C. Development of a small capacity double stage compression screw press for oil expression. **Journal of Food Engineering**, London, v. 43, n. 2, p. 75-82, 2000.

SOARES, G. A. **O avanço das tecnologias de segunda geração e seus impactos na indústria do etanol**. 2016. 177 f. Dissertação (Mestrado em Economia da Indústria e Tecnologia)-Instituto de Economia, Universidade Federal do Rio de Janeiro, Rio de Janeiro, 2016.

SOBRINHO CHEMMÉS, C. et al. Estudo de métodos físico-químicos no pré-tratamento de resíduos lignocelulósicos para produção de etanol de segunda geração. In: SEMINÁRIO ESTUDANTIL DE PRODUÇÃO ACADÊMICA, 7., 2013, Salvador. **Anais Eletrônicos...** Salvador: Ed. UNIFACS, 2013. Disponível em: <https://revistas.unifacs.br/index.php/sepa/article/view/2816/2044>. Acesso em: 29 set. 2018.

SOMNUK, K.; EAWLEX, P.; PRATEEPCHAIKUL, G. Optimization of coffee oil extraction from spent coffee grounds using four solvents and prototype-scale extraction using circulation process. **Agriculture and Natural Resources**, New York, v. 51, p. 181-189, 2017.

SOTO, C.; CHAMY, R.; ZUNIGA, M. E. Enzymatic hydrolysis and pressing conditions effect on borage oil extraction by cold pressing. **Food Chemistry**, London, v. 102, n. 3, p. 834-840, 2007.

SPEER, K.; KÖLLING-SPEER, I. The lipid fraction of coffee bean. **Brazilian Journal of Plant Physiology**, Londrina, v. 18, n. 1, p. 201-216, 2006.

SPIGNO, G.; TRAMELLI, L.; DE FAVERI, D. M. Effects of extraction time, temperature and solvent on concentration and antioxidant activity of grape marc phenolics. **Journal of Food Engineering**, London, v. 81, n. 1, p. 200-208, 2007.

TAKEUCHI, T. M. et al. Low pressure solvent extraction (solid-liquid extraction, microwave assisted, and ultrasound assisted) from condimentary plants. In: MEIRELES, M. (Ed.). **Extracting bioactive compounds for food products:** theory and applications. Boca Raton: CRC, 2009. p. 137-218.

TAUNAY, A. de E. **História do café no Brasil:** no Brasil Imperial 1822-1872**.** Rio de Janeiro: Departamento Nacional do Café, 1939. 448 p.

TIR, R.; DUTTA, P. C.; AHMED, B. H. A. Y. Effect of the extraction solvent polarity on the sesame seeds oil composition. **European Journal of Lipid Science and Technology**, Berlin, v. 114, n. 12, p. 1427-1438, Dec. 2012.

TREYBAL, R. E. **Mass-transfer operations**. 3rd ed. New York: McGraw Hill, 1981.

TRUGO, L. C.; MACRAE, R. An investigation of coffee roasting using high performance liquid chromatography. **Food Chemistry**, London, v. 19, p. 1-9, 1986.

TURATTI, J. M. Extração e caracterização de óleo de café. In: SIMPÓSIO DE PESQUISA DOS CAFÉS DO BRASIL, 2., 2001, Vitória. **Anais Eletrônicos...** Vitória, 2001. Disponível em: <http://www.sbicafe.ufv.br/bitstream/handle/123456789/1291/155585_Art212f.pdf?sequence=1>. Acesso em: 13 maio 2017.

UNIÃO DA INDÚSTRIA DE CANA-DE-AÇÚCAR. **Produção:** etanol total 2017/2018. São Paulo, 2018. Disponível em: <http://www.unicadata.com.br/historico-de-producao-e-moagem.php?idMn=31&tipoHistorico=2&acao=visualizar&idTabela=1983&produto=etanol_total&safraIni=2017%2F2018&safraFim=2017%2F2018&estado=RS%2CSC%2CPR%2CSP%2CRJ%2CMG%2CES%2CMS%2CMT%2CGO%2CDF>. Acesso em: 14 jul. 2018.

VARDON, D. R. et al. Complete utilization of spent coffee grounds to produce biodiesel, bio-oil, and biochar. **ACS Sustainable Chemistry and Engineering**, Washington, v. 1, n. 10, p. 1286-1294, 2013.

WAGEMAKER, T. A. L. et al. Sun protection factor, content and composition of lipid fraction of green coffee beans. **Industrial Crops and Products**, London, v. 33, n. 2, p. 469-473, 2011.

WEI, F. et al. Quantitative determination of oil content in small quantity of oilseed rape by ultrasound-assisted extraction combined with gas chromatography. **Ultrasonics Sonochemistry**, Oxford, v. 15, n. 6, p. 938-942, 2008.

WILLIAMS, M. Recovery of oils and fats from oilseeds and fatty materials. In: SHAHIDI, F. (Ed.). **Bailey's industrial oil and fat products**. New York: J. Wiley, 2005. v. 6, p. 2589-2678.

SEGUNDA PARTE – ARTIGOS

ARTIGO 1 - GREEN COFFEE AND ITS RESIDUAL BIOMASS: CHARACTERIZATION FOR ITS INDUSTRIAL APPROACH

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Abstract

The increase in the production of green coffee oil by pressing has generated as a residue the press cake or biomass, whose characterization allows the evaluation of its potential as a source of extracts rich in bioactive compounds. In the present study, the physical and chemical composition of green coffee beans and its defatted biomass, a by-product from the coffee oil mechanical expelling industry, were determined. For so, soluble solids, bulk density, potassium leaching, electrical conductivity, acidity, nutritional value, sugar, wall components, enzymatic activity, caffeine, chlorogenic acids, tannins and phenolic content were carried out. By comparing the composition of the green beans and biomass, it could be observed that many compounds (protein, fiber, ash) were not extracted by the mechanical pressing process, in which the oil is removed but with a very low yield (< 30%). There was a reduction in the enzymatic activity of PPO (21.16 U g^{-1}), POD (3.18 U g^{-1}), and PG (7.39 U); the enzyme PME (17281.9 U g^{-1}) showed an increase in its activity in the biomass; the lipase activity was not statistically altered. The results indicated the potential of the biomass to obtain products based on caffeine (0.9%), chlorogenic acids (11.66 mg L^{-1}), phenolic compounds (131.1 mg 100 g^{-1}), oil (6.27%) and others. The green coffee beans biomass is still a rich source of nutrients that could be exploited by the industry. Data reported in this work helped to improve the understanding on the chemical composition of green coffee biomass that could be helpful for economical utilization of these products. These data would also provide a basic reference for product standards and quality control when the production of the green coffee biomass products comes to pilot and industrial scales.

Keywords: *Coffea arabica*; chemical composition; defatted coffee cake.

1. Introduction

Coffee is one of the most valuable traded food commodity. After crude oil, it is ranked second among all commodities (Esquivel and Jiménez, 2012). In 2015/2016, coffee production reached 148 million bags worldwide, and consumption of 151.3 million bags (ICO, 2017). *Coffea canephora* (Robusta) and *Coffea arabica* (Arabica) are the two main species of coffee that are grown for consumption (Denoeud et al., 2014). In general, Arabica species has more favorable sensory characteristics and higher commercial value. Green beans of both species can be distinguished by color, shape, and size (Dias and Benassi, 2015). But the most discriminant descriptors of both varieties are free amino acids, caffeine and chlorogenic acids, with Robusta having the highest content of caffeine and chlorogenic acids, and Arabica with the greatest amino acids content (Martín et al., 1998) and lipid content (Romano et al., 2014).

Coffee beans have been widely consumed roasted as a beverage for centuries, however, lately, the green bean (before roasting) and the by-products of the coffee industry (i.e. press cake) which are in many cases not properly handled and, therefore, an environmental concern, are also a potential source of compounds with functional properties. Green coffee has a complex chemical composition of polysaccharides, monosaccharides, lipids, sterols, fatty acids, phenolic acids, polyphenols, alkaloids, proteins, free amino acids, vitamins, and minerals (Ozcan et al., 2014; Parras et al., 2007). They are rich in bioactive compounds, with a highlight on caffeine, trigonelline, chlorogenic acids, tocopherols (α, β, γ), and diterpenes (mainly kawheol and cafestol) (Esquivel and Jiménez, 2012; Şemen et al., 2017; Speer and Kölling-Speer, 2006). Although it is already well established that green coffee beans are sources of bioactive compounds, such as polyphenols, their composition may vary with a series of factors that includes species, variety, cultivation, region, weather conditions, ripeness, time of harvest, and storage conditions (Faniadis et al., 2010; Haffner et al., 2002).

Green coffee beans can also be used to obtain oil from the pressing process of the green beans. The green coffee oil has been gaining great interest among the pharmaceutical chain, due to its interesting composition related mainly to the bioactive compounds, for its properties at maintaining natural skin moisture (Ferrari et al., 2010), and as a sun protector due to the ultraviolet absorption property of the main fatty acid, linoleic acid (Wagemaker et al., 2011). The obtainment

of green coffee oil generates one more by-product, the cake, or so called 'biomass', since it might be a valuable source of nutrient and bioactive compounds.

In addition, caffeine, chlorogenic acids, and polyphenols present in these materials may confer a toxic nature to them. Therefore, they may represent a pollution hazard if discharged into the environment. Despite this negative characteristic and the large amounts of these materials that are generated, there are few studies focusing on their use in different and profitable applications (Mussatto et al., 2006). Green coffee press cake is a rich residue concerning bioactive compounds and nutrients, different from usual wastes, and it is slowly beginning to be exploited (Affonso et al., 2016; Castro et al., 2018). Nevertheless, it is possible to find mostly, studies on grains rather than residues.

Due to the heterogeneous nature of coffee waste, most of the authors investigating its possible valorization carried out a selective fractionation of the coffee waste to analyze and determine the content of specific components, such as lignin, cellulose (Caetano et al., 2012; Tsai et al., 2012); tannins and total polyphenols (Anesini et al., 2008; Zhang et al., 2010). Nevertheless, to our knowledge there is no report on the composition of the by-product from the coffee oil industry, neither of a complete and integrated chemical characterization of this type of green coffee beans waste. Aiming to evaluate the green coffee beans (*C. arabica*) quality and its relation to the by-product (biomass) obtained from the beans mechanical pressing to obtain oil (generated by an oil industry), the goal of this work was to physicochemically characterize these materials, making information available on the effect of the pressing process on the biomass characteristics, that could add value to the coffee industry, hence, contributing to their economical exploitation. As chemical composition is critical information for product quality and for exploration of new uses, data obtained in this work will be essential to assess the potential use of this waste material as a source of high-added value compounds suitable for different bio-based applications.

2. Experimental

2.1 Sampling

Green coffee beans harvested in the Southeast area of Brazil (Guaxupé, Minas Gerais), in the year 2017 (dry process) and its biomass obtained from the oil mechanical expelling (continuous screw press - 550 kg h^{-1}) (Ecirtec, MPE-500 AC, Bauru, Brazil), both of the same variety (Arabica) and from the same batch, were kindly supplied by COOXUPÉ (Guaxupé, Minas Gerais, Brazil). For analysis, seeds were frozen in liquid nitrogen and ground to a fine powder in a laboratory mill (IKA, A-11, Xangai, China); the biomass was homogenized in a blender (Waring, 14-509-7A, Torrington, US). The powders, from both seeds and biomass, were then selected to their granulometry through Tyler sieves in an eletromagnetic shaker (Bertel, VP-01, Caieiras, Brazil). Samples retained in mesh 80 (0.177 mm) (-20/+80) were used. This granulometry is suggested by the National Renewable Energy Laboratory (NREL/TP-510-42620) (Sluiter et al., 2008), which presents specialized methodologies for biomass analysis. Samples with homogeneous granulometry were dried in a vacuum oven (16.8 kPa) at 40 ± 1 °C (Tecnal, TE-395, Piracicaba, Brasil) (~ 20 days for green coffee beans; ~ 7 days for biomass). All analyzes were performed in triplicate and the results were given on a dry basis (d.b.), except for the physical analysis, the enzymatic activity and potassium leaching assays, which were performed with fresh samples.

2.2 Reagents and chemicals

Chemicals were of analytical grade and purchased from Sigma-Aldrich (Saint Louis, MO, USA).

2.3 Physical analyzes

2.3.1 Bulk density

Coffee beans were weighed with the aid of a density determination kit (GEHAKA, Kit Peso Hectolitro, São Paulo, Brazil), and the beans average bulk density was evaluated as the ratio between the weight of the beans and the volume occupied by them (125 cm^3). For measuring the bulk density of the biomass, a previously calibrated 50 mL measuring cylinder was filled with a known mass (g) of the samples (12 g). The measuring cylinder base was gently tapped on the

laboratory bench, severally, to a constant volume; bulk density (g cm^{-3}) was calculated (Akpapuimam and Markakis, 1981; Ige et al., 1984) using Equation 1.

$$\text{Bulk density} = \frac{\text{Weight of samples (g)}}{\text{Volume of sample after tapping } (cm^3)} \quad \textbf{(1)}$$

2.3.2 Electrical conductivity of green coffee beans

The readings were performed in a benchtop conductivity meter (BEL Engineering, W12D, Piracicaba, Brazil) after 5 h of soaking (50 mL) at 25 °C the previously weighed beans (n = 50). The measurement was carried out in the soak water after removing the beans (Baalbaki et al., 2009; Loeffler et al. 1988). The electrical conductivity (EC) was calculated according with Equation (2).

$$\text{EC} = \frac{\text{Reading } (\mu\text{S cm}^{-1})}{\text{Weight of 50 beans (g)}} \quad \textbf{(2)}$$

2.4 pH and titrable acidity

Green coffee or biomass pH was recorded by preparing extractives with powders of the materials (5 g) in 50 mL distilled water (Mazzafera, 1999). The extract was shaken for 1 h (Solab, SL 180/A, Piracicaba, Brazil) in room temperature. Titratable acidity of coffee bean and biomass was determined titrating the same extract above against sodium hydroxide solution (0.1 mol L^{-1}) using a pH meter (Tecnal, Tec-3MP, Piracicaba, Brazil) to control the acidity of the solution. The volume of sodium hydroxide required for neutralization (pH 8.2) was noted and titratable acidity was calculated (AOAC, 2016).

2.5 Total soluble solids

Total soluble solids of the powdered coffee or biomass sample was determined by refluxing the coffee powder (2 g) with hot water (200 mL) for 1 h and made up to 500 mL. An aliquot (50

mL) was filtrated and evaporated to dryness, followed by heating in a hot air oven at 105 ± 1 °C to get constant weights, and the amount of total soluble solids was calculated (AOAC, 2016).

2.6 Proximate composition

Moisture content of coffee beans was determined following the ISO 1446 protocol, which assesses the moisture of green coffee beans through the mass loss under 105 °C, and also with the aid of a grain moisture meter (GEHAKA, G600, São Paulo, Brazil) on the mode of ISO 6673 for coffee beans (ISO, 2001, 2003). The biomass moisture was assessed by the method proposed by AOAC (2016), as well as the lipid, protein, fiber, and ash content, of both beans and biomass. To convert the nitrogen content to protein percentage, a factor of 6.25 was used. Carbohydrates fraction was calculated by difference (Carbohydrates = 100 - %lipid - %protein - %fiber - %ash) (d.b.).

2.7 Total sugar

Total sugar content was determined through the Dreywoods Anthrone's reagent method reported by Morris (1948), later modified by Beck and Bibby (1961).

2.8 Lignin, cellulose, and hemicellulose

Lignin, cellulose, and hemicellulose content was determined from the Neutral Detergent Fiber (NDF) and Acid Detergent Fiber contents (ADF). NDF determination was conducted according with Van Soest and Wine (1968), while ADF was determined following Van Soest (1963). Lignin content was obtained through the protocol proposed by Van Soest and Wine (1967). Cellulose and hemicellulose contents were calculated according to the Equations 3 and 4, respectively.

$$\text{Cellulose}\ (\%) = \text{ADF} - \text{Lignin} \quad \textbf{(3)}$$

$$\text{Hemicellulose (\%)} = \text{NDF} - \text{ADF} \quad \textbf{(4)}$$

2.19 Enzymatic activity

2.9.1 Polyphenoloxidase (PPO) and Peroxidase (POD)

Extraction. For each sample, 1 g of green coffee or biomass powder was extracted (under agitation) at 4 °C for 15 min with 5 mL of 0.1 M sodium phosphate (Na-Pi) buffer (pH 6.0). The suspension was centrifuged (15 min/ 15000 x g) (GS-15R, Beckman, Buckinghamshire, England), the supernatant was recovered and filtrated with a paper filter Whatman n° 1. The extract was kept at 4 °C. The method used to determine PPO activity was adapted from that of Ponting and Joslyn (1948). PPO activity was determined in a reaction mixture containing 1 mL of the extract, 1 mL of glycine (H_2NCH_2COOH) (0.2 M), and 3 mL of L-3,4-dihydroxyphenylalanine (DOPA) (0.005 M). The tubes containing the mixtures were incubated under 60 °C in a water bath for 1 h, and homogenized in a vortex. The absorbance of the solution was measured at 420 nm. For each tube with the sample, a blank was prepared containing 1 mL of extract and 4 mL of distilled water. POD activity assessment was adapted from Fehrmann and Dimond (1967). POD activity was determined in a reaction mixture containing 1 mL of the extract and 2 mL of 0.2 M Na-Pi buffer (pH 5.0). The tubes containing the mixture were incubated at 30°C in a water bath for 5 min. To each tube was added 0.4 mL of H_2O_2 (0.08%), and 0.4 mL of an alcoholic solution of guaiacol (1%). The tubes were incubated once again for 15 min at 30°C. The absorbance of the solution was measured at 470 nm. For each tube with the sample, a corresponding blank was prepared containing 1 mL of extract, 2 mL of buffer solution, and 0.8 mL of distilled water**.** Specific enzyme activities were reported as arbitrary units (U g^{-1}). One unit of enzyme activity was defined as the amount of enzyme causing an increase or a respectively decrease of absorbance of 0.1 unit per min at the corresponding wavelength.

2.9.2 Pectin methylesterase (PME) and Polygalacturonase (PG)

Both enzyme extracts were prepared following Buescher and Furmanski (1978), with modifications. A sample of 5 g was extracted with 50 mL of NaCl (0.2 mol L^{-1}) and polyvinylpyrrolidone (PVP) 1% to avoid phenol oxidation or its elimination from the extract in an ultraturrax (Tecnal, TE-102, Piracicaba, Brazil) for 1 min in an ice bath, followed by centrifugation (10 min/ 13000 x g) (Beckman, CS-15R, Indianapolis, US) and filtration. The extract was completed to a volume of 50 mL with NaCl (0.2 mol L^{-1}).

In order to determine PME activity, the carboxyl can be titrated during the enzymatic hydrolysis with NaOH solution at constant temperature and pH. Activity of the enzyme was measured quantitatively by polygalacturonic acid produced from pectin. Each assay was carried out by titrating 20 mL of 1% (w/v) pectin solution prepared in 0.1 mol L^{-1} NaCl of pH 8 at 30 °C with 0.025 mol L^{-1} NaOH. By the addition of small volumes of NaOH, the pH was maintained at 8. Then 3 mL of the enzyme extract was added and the reaction (extraction) mixture was kept for 5 min, where the pH was kept at 8. The same procedure was carried without the extract (blank). One unit of enzyme activity was defined as the amount of enzyme required to release 1 μmol of acid from pectin per min at pH 8 at 30 °C (Buescher and Furmanski, 1978). PG activity was estimated according with Proctor and Miesle (1991). The high salt extracts of the heated homogenates were assayed for PG activity by measuring the production of reducing sugars from polygalacturonic acid following the method of Nelson (1944). One unit of enzyme activity was defined as the amount of enzyme required to release 1 nmol of reducing sugar per min under the conditions of analysis.

2.9.3 Lipase

Lipase (glycerol ester hydrolase) extract was prepared according with Hassanien and Mukherjee (1986), with modifications by Liaquat and Owusu Apenten (2000). The enzymatic activity was adapted from Theimer and Rosnitschek (1978) and determined as follows. Olive oil was used as substrate (5% w/v) and emulsified (ultraturrax) (Tecnal, TE-102, Piracicaba, Brazil) for 3 min with Arabic gum (10% w/v) in NaCl buffer 0.1 M (pH 7) at room temperature,

immediately before use. In a 125 mL Erlenmeyer, 18 mL of the emulsion and 2 g of the extract were added. This solution was than incubated under agitation for 15 min, 37 °C, and 150 rpm at a Dubnoff Orbital water bath (Novatecnica, NT 230, Piracicaba, Brazil). The reaction was then interrupted by adding 20 mL of an acetone/ethanol (1:1 v/v) solution. Samples were titrated with NaOH (0.04 M) until pH 11 and time was recorded. The blank was prepared by adding 18 mL of emulsion (without enzyme preparation) and after the incubation, 20 mL of acetone/ethanol solution, followed by titration. One enzymatic activity unit is defined as the amount of enzyme necessary to release 1 μmol of fatty acid per min, and can be determined as in Equation 5.

$$\text{Lipase activity } (\mathrm{U\ g^{-1}}) = \frac{(\mathrm{V}-\mathrm{Vb})\times \mathrm{M}\times 1000}{\mathrm{m}\times \mathrm{t}} \qquad \textbf{(5)}$$

Where, V = volume spent to titrate the sample (mL); Vb = volume spent to titrate the blank (mL); M = NaOH molarity (mol L^{-1}); m = weight of the sample (g); t = time (min).

2.10 Caffeine

Green coffee powder (5 g) was extracted under reflux in a water bath at 95 °C along with MgO (3 g) for 45 min with distilled water (100 mL) and filtered. After filtration, the volume was made up to 50 mL. The filtrate (50 mL) was extracted thrice with 25 mL chloroform (25 mL × 3) under vigorous agitation, and the combined chloroform extract which contained caffeine was desolventized. The obtained caffeine was dissolved in water and the absorbance of the known strength of the solution was measured at 275 nm (Varian, Cary 50 Probe UV-Vis, Lake Forest, USA). The caffeine content was calculated using a calibration curve prepared from a caffeine standard (AOAC, 2016).

2.11 Total Chlorogenic acid

Chlorogenic acid was estimated by UV spectrophotometry. Extracts were prepared by adding 100 mL of isopropanol 70% to 0.5 g of sample. This solution was kept in a water bath (50 °C) under reflux for 4 h and then filtered. The volume was made up to 100 mL with isopropanol

70%. For determination, to 1 mL of the extract was added 10 mL of sodium metaperiodate 0.25%, kept in a water bath (28 °C) for 10 min, and read on a spectrophotometer (Varian, Cary 50 Probe UV-Vis, Lake Forest, USA) at 418 nm. A calibration curve was built using chlorogenic acid and sodium metaperiodate 0.25% (AOAC, 2016).

2.12 Total phenolic compounds (TPC)

The yield of TPC was determined according to the Folin–Ciocalteu's method (Waterhouse, 2002). The extracts were obtained by the method described by Brand-Williams et al. (1995), using methanol and acetone as the extracting solvents.

2.13 Tannin content

Tannin content was assessed based on the methodology proposed by Price et al. (1978). The extraction was carried out using methanol 50% and the standard curve was build using tannic acid (TA). The results were expressed as mg TA per 100 g of sample (d.b.).

2.14 Potassium leaching

After measuring the electrical conductivity, the same soak water was used to measure the potassium leaching. Only that, a dilution was carried out, where to 1 mL of the soak water 29 mL of distilled water was added. The readings were performed in a flame photometer (Tecnal, B462, Piracicaba, Brazil). The potassium leached by the water was calculated by multiplying the reading obtained in the flame photometer (mg potassium L^{-1}) by the volume of distilled water (mL) (dilution), and divided by the mass of the sample (g), the results were expressed as mg of potassium per kg^{-1} of fresh green beans (Equation 6).

$$\text{PL (mg kg}^{-1}) = \frac{\text{Reading } \times \text{Dilution}}{\text{Weight of 50 beans (g)}} \quad \textbf{(6)}$$

2.15 Statistical analysis

In this study, all the analyzes were carried out in triplicates. Data did comply with the parametrical requisites, thus, they were analyzed through an analysis of variance (ANOVA), and the Tukey's test ($p < 0.05$) was employed to compare the means of both materials (green coffee beans and biomass). All statistical analyzes were carried out using the software RSTUDIO version 3.2.0 (R Core Team, 2015).

3. Results and discussion

The bulk density (g cm^{-3}) was evaluated for both whole beans and biomass which presented similar ($p > 0.05$) values (0.61 ± 0.01 and 0.60 ± 0.01, respectively). Kornman (2016) classified low density coffee beans as lower than 0.64 g cm^{-3}, which is the case of the samples analyzed in this study, indicating that a greater amount of beans may be needed in order to fulfill a determined weight prior to transporting them. Concerning the biomass, there is no report on that measurement. Parodi (2017) reported the density of three different originated beans, from Thailand, Ethiopia, and El Salvador, all with a high density average of 0.66, 0.68, and 0.7 g cm^{-3}. While there are plenty of exceptions, density often varies from place to place in predictable ways. East African coffees, particularly those from Ethiopia and Kenya, tend to register very high on the density spectrum, as do coffees from Colombia. Conversely, coffees from Sumatra and Brazil tend to generally present low density by comparison (Kornman, 2016), as observed in this study.

pH and total acidity are relevant parameters to grade coffee beans. These parameters are shown in Table 1, and there were significant differences ($p < 0.05$) between beans and biomass. In this work, pH of green coffee beans was in the range reported in the literature (4.92 – 6.11) (Jeszka-Skowron et al., 2016; Ramalakshmi et al., 2007; Vasconcelos et al., 2007; Wintgens, 2008). And titrable acidity was lower than that reported by few authors, from 210 mL NaOH 100 g^{-1} (Ramalakshmi et al., 2007) to 277 mL NaOH g^{-1} (Vasconcelos et al., 2007). Higher titratable acidity in coffee could be attributed to fruit fermentation during the drying process. According to studies, acidity should increase as coffee quality decreases (Franca et al., 2005; Mazzafera, 1999).

The titratable acidity content in coffee beans may vary according to the fermentation levels that occur in the grains and also to the different maturation stages of the grains, and may also serve as a support to assist in the evaluation of the coffee beverage quality. Defects are responsible for

the increase of coffee acidity, especially black and burnt coffee. The sugars present in the mucilage, when in the presence of microorganisms or under anaerobiosis, are fermented producing alcohol, which is deployed in acetic, lactic, propionic and butyric acid (Navia et al., 2011). In this work, the acidity of the biomass was higher than of the beans, which could be associated to enzymatic activity, once they have not been totally inactivated during the pressing process (Table 1).

Table 1. Chemical characteristics of green coffee beans and its biomass

Attributes	Green coffee beans	Biomass
pH	5.74 ± 0.02 [a]	5.6 ± 0.02 [b]
Titrable acidity (mL NaOH 0.1 mol L^{-1} 100 g^{-1})	145.0 ± 0.06 [b]	281.3 ± 0.35 [a]
Soluble solids (%)	32.29 ± 3.83 [a]	33.45 ± 1.94 [a]
Moisture (%)	11.0 ± 0.06 [a]	4.35 ± 0.42 [b]
Total lipid (%)	8.61 ± 0.46 [a]	6.27 ± 0.22 [b]
Protein (%)	14.87 ± 0.55 [a]	15.86 ± 0.63 [a]
Fiber (%)	11.62 ± 1.30 [a]	12.46 ± 1.02 [a]
Ash (%)	1.75 ± 0.16 [b]	2.28 ± 0.02 [a]
Carbohydrates (%)	63.15	63.13
Energetic value (kcal 100 g^{-1})	389.57	372.39
Total sugar (%)	13.05 ± 0.01 [a]	14.43 ± 0.01 [a]
Lignin (%)	5.23 ± 0.34 [a]	4.02 ± 0.36 [b]
Cellulose (%)	18.88 ± 0.62 [b]	20.54 ± 0.39 [a]
Hemicellulose (%)	26.72 ± 2.19 [a]	25.61 ± 0.54 [a]
Polyphenoloxidase (U g^{-1}) (w.b.)	111.52 ± 0.0 [a]	21.16 ± 1.21 [b]
Peroxidase (U g^{-1}) (w.b.)	10.66 ± 0.01 [a]	3.18 ± 0.004 [b]
Pectin methylesterase (U g^{-1}) (w.b.)	11363.89±1136.4 [b]	17281.9±1646.4 [a]
Polygalacturonase (U) (w.b.)	12.21 ± 0.20 [a]	7.39 ± 0.11 [b]
Lipase (U g^{-1}) (w.b.)	41.12 ± 12.18 [a]	53.73 ± 1.42 [a]
Caffeine (g 100 g^{-1})	0.83 ± 0.12 [a]	0.9 ± 0.04 [a]
Chlorogenic acids (mg L^{-1})	11.05 ± 0.71 [a]	11.66 ± 0.50 [a]
TPC (mg 100 g^{-1})	101.6 ± 0.18 [b]	131.1 ± 0.17 [a]
Tannins (mg TA 100 g^{-1})	15307.18±1048.9 [a]	22485.30±937.1 [a]
Potassium leaching (g mL^{-1}) (w.b.)	368.58 ± 21.03	-

Values are Mean ± SD, (n=3). TA: Tannic acid.
Comparison between the samples was performed by one-way ANOVA followed by Tukey's multiple comparison test.
Values followed by different lower case letters in a row indicate statistically significant difference between the same parameters ($p < 0.05$).

The soluble solids content is used as the index of total sugars in fruits, indicating the degree of maturity. They are composed of water-soluble compounds, such as sugars, acids, vitamin C, and some pectin (Tian et al, 2007). Green coffee beans presented similar ($p > 0.05$) content of soluble solids compared to the biomass in this study (Table 1), indicating that during the oil obtaining

process only the oil was expelled from the vegetable matrix. Which is an interesting feature, once the biomass could also be destined to the soluble solids extraction industry, once it could be roasted and processed such as the coffee beans. In addition, the fact that it does have less oil content could be a reason to add value to this material. Total soluble solids average of Indian Arabica coffee is of 31.34% (d.b.) (Ramalakshmi et al., 2007), which is similar to the results obtained in this work for Brazilian Arabica, and within the range reported by Merritt and Proctor (1959) for green beans from different countries (30.4 – 34.4% d.b.).

Data in Table 1 show the observed proximate chemical composition (major components) of the Brazilian green coffee and biomass samples investigated. Moisture, lipid, and ash content presented significant difference ($p < 0.05$) for both materials, the other components were not influenced by the pressing process. By analyzing the samples, it was found that the moisture level of the beans in the present study was higher than the levels reported in the literature (5.5 to 7.6%) by Nogaim et al. (2013) who evaluated 70 samples of green coffee from Yemen (*C. arabica*). There is no report on the biomass moisture content of green coffee beans, which is much lower than that of the coffee beans once it went through a pressing process, which may remove the moisture due to the elevated temperature (~ 90 °C) reached along the process, as proved by Willems et al. (2008) for sesame press cake, where the ratio water/solids reduced from 0.07 to 0.05 after the pressing process (100 °C). Moisture is an important attribute and indicator of quality. A high moisture content of the beans is a loss of material and leads to physical and sensorial defects (Leroy et al., 2006). Beans could be easily attacked by fungi, allowing the accumulation of ochratoxin A to prohibited levels (in Europe, according to the Comission Regulation nº 123, 2005, higher than 5 mg kg^{-1}) and other mycotoxins, undergoing as well a marked color change, becoming darker (Coste and Cambrony, 1993). If the beans are too wet (above 12.5 % moisture w.b.), they will mold easily during storage. If the beans are too dry (below 8 % moisture), they will lose flavor. The International Coffee Council recommends that coffee should not be exported when outside of these limits as assessed by the ISO 6673 method (ICC, 2002, 2004; ISO, 2003). According with Kemper (2005), in case seeds are going to go through an oil obtaining process, they must have a low moisture content in order to prevent deterioration during storage and also to ensure that downstream unit operations are efficient. Specialty Coffee Association of America (SCAA, 2009) specifies that the ideal moisture of the crude grain or green coffee bean must be of 11.5%, but a variance from

10 to 12% is acceptable. Green coffee beans are within the limits allowed. According to the study carried out by Vasconcelos et al. (2007) moisture levels for green coffee were within the range reported in many literatures for good quality coffee (8.5 – 13%).

The protein content was similar ($p > 0.05$) for both beans and biomass, once the mechanical pressing, neither the warming did not influence the content of this nutrient. Farah (2012) affirmed that the total nitrogenous compounds (excluding caffeine and trigonelline) account for 9 to 16% of the green coffee chemical composition. Protein levels for green coffee were in the range reported in the literature for healthy coffee beans. Nogaim et al. (2013) reported protein content of 70 samples of Yemeni green coffee beans in the range of 7 to 16%. However, coffee is not a good nutritional source of protein because it lacks essential amino acids (Farah, 2012).

Lipids are present on the surface and in the interior matrix of green coffee beans**,** and their content depends on several factors, particularly, species and variety (Speer and Kölling-Speer, 2006). In this study, lipid content was relatively low for both beans and biomass. But it is relevant to observe that the pressing process employed to obtain the green oil was not efficient in removing the oil, once it was able to remove only about 27% of the oil present in the beans. Raw materials with low oil content (< 30%) should be directly processed by solvent extraction, as the low yield of their expression does not justify the costs generated by this method (Arișanu and Rus, 2013). Hence, this remaining oil in the biomass could have great and valuable exploitation by the industry. Behr and Gomes (2010) stated that many important catalytic functionalization (i.e. heterogeneous and homogeneous catalysis, like additions, reductions, oxidations and metathesis reactions) of fatty compounds and glycerol may result in new attractive products. Those products have emerging properties, so they could find a rapid introduction into the chemical market. In this study, the lipid content of green coffee beans was fairly lower than the range found in the literature of 9 – 15.36% (d.b.) (Geromel et al., 2006; Mazzafera, 1999; Mazzafera et al., 1998; Oliveira et al., 2006; Speer and Kölling-Speer, 2001).

Once the fiber content during the pressing process is not removed, it indicates that the biomass resulting from the process still is a by-product that could be exploited once it contains a relevant level of crude fiber.

Minerals are important constituents of human diet as they serve as cofactors for many physiological and metabolic processes (Ogunyinka et al., 2017). Mineral content (ash) in green

coffee and biomass were statistically different, indicating that all the mineral content remains in the grain structure after the oil removal. The ash content observed in this study presented great difference, being much lower, amongst the results found in the literature (4.8 – 6%) (Nogaim et al., 2013; Oliveira et al., 2006; Purohit and Rajyalakshmi, 2011).

The polysaccharides which make up about 50% of the green bean's dry weight, consist of three major types: mannans or galactomannans, arabinogalactan-proteins and cellulose, contributing to the tasteless flavor of green coffee (Coffee Chemistry, 2015). In addition, there are small amounts of pectic polysaccharides (Redgwell et al., 2002), and recently, xyloglucan was also shown to be present (Oosterveld et al., 2003). The results for total carbohydrates in green coffee beans and biomass are consistent with the literature, once green Arabica coffee consists of about 48 to 60% of polysaccharides (Arya and Rao, 2007; Fischer et al., 2001; Murkovic and Derler, 2006; Nogaim et al., 2013; Redgwell and Fischer, 2006). Once again, the by-product (biomass) which is consistently not being prospected by the industry, shows to be a valuable source of carbohydrates that could further be reinserted into the food chain.

In this study, sugar content was found to be similar ($p > 0.05$) for both beans and biomass (Table 1). For coffee cultivated under the shadow or full sun, Vaast et al. (2006) found sucrose levels lower (8.4%) than the average of this study. Rogers et al. (1999) reported sucrose levels from 5 to 12% for green coffee beans, also stated that sucrose represents, essentially, 100% of the total free sugars in mature grains. Low sucrose levels in immature beans are associated with bean maturation, whereas in the case of black and sour beans, low sucrose levels are due to fermentation (Vasconcelos et al., 2007). Vaast et al. (2006) affirmed that high sucrose content indicates incomplete maturation of the beans at the harvesting.

The cell wall constituents studied (lignin, cellulose, and hemicellulose) for green coffee beans and its biomass are presented in Table 1. Cell wall polysaccharides constitute half the dry weight of the native coffee bean (Campos-Vega et al., 2015; Fischer et al., 2001), what can be assured by the results obtained in this study (~ 50.8%); leading to affirm that green coffee bean is a rich source of polysaccharides. Lignin is a constituent of the cell walls of almost all dry land plant cell walls. It is the second most abundant natural polymer in the world, surpassed only by cellulose. Among the polymers found in plant cell walls, lignin is the only one that is not composed of carbohydrate (sugar) monomers (NSERC, 2010). Lignin and cellulose work together to provide a

structural function in plants and in green coffee beans they accounted for 24.11% of the cell wall constituents studied. The fibrous component, cellulose, is the primary load-bearing element while the matrix, lignin, provides stiffness and rigidity. Beyond the structural function, lignin plays several other important biological roles in plants. Because it is much less hydrophilic than cellulose and hemicellulose, it prevents the absorption of water by these polysaccharides in plant cell walls and allows the efficient transport of water in the vascular tissues. Lignin also forms an effective barrier against attack by insects and fungi (NSERC, 2010). The structure of lignocellulose is such that covalently cross-linked lignin and hemicellulose sheath a crystalline cellulose core resulting in a strong, recalcitrant network. Consequently, intensive treatment(s) of biomass is required to overcome the covalent linkages between the lignin and hemicellulose (Ravindran and Jaiswal, 2016). Disrupting lignocellulose in this way improves hydrolysis of hemicellulose and cellulose into industrially significant monosaccharides (Scully et al., 2016). According to Belitz et al. (2009), in Arabica green coffee, cellulose and hemicellulose are the main components of coffee silverskin, which is composed by 1 to 3% of lignin; 41 to 43% of cellulose; 5 to 10% of hemicellulose. During growth and development of the coffee bean cell wall there is a progressive change in both the relative content of the different polysaccharide types and their structural features. At the earliest stages of growth, cellulose and arabinogalactan appear to be the primary products of cell wall synthesis with the former the predominant polysaccharide (Redgwell and Fischer, 2006). During the middle stages of growth, cellulose synthesis appears to cease and there is a progressive increase in mannan/hemicellulose synthesis relative to the other wall polysaccharides as the grain approaches maturity (Redgwell and Fischer, 2006). It is possible to assume that green beans in study were in an ideal stage of maturation, once the highest polysaccharide observed was the hemicellulose. Polysaccharides and other large molecules have effects on the quality of coffee, being important in the retention of volatile compounds and the viscosity of coffee, that is, they give body to it (Fischer et al., 2001). On the other hand, the knowledge of the constitution of the cell structure makes it easier to select enzymes in order to enzymatically extract oil if that is the case. Green coffee bean and its biomass are mainly constituted by cellulose and hemicellulose; thus the enzymatic complexes involving cellulases and hemicellulases should be appropriate to carry out this operation.

Coffee beans contain a large range of enzymes, as all plant material. Some of these were investigated in this study, polyphenoloxidase (PPO) (EC 1.14.18.1), peroxidase (POD) (EC 1.11.1.7), pectinmethylesterase (PME) (EC 3.1.1.11), poligalacturonase (PG) (EC 3.2.1.15), and lipase (EC 3.1.1.3), which has not been closely studied in coffee. Enzymatic activity of green coffee beans and biomass are presented in Table 1.

PPO is a bifunctional copper-containing enzyme, also known as catechol oxidase, catecholase, diphenol oxidase, o-diphenolase, phenolase, cresolase, or tyrosinase. It triggers enzymatic browning by oxidizing phenols, usually impairing the appearance and the sensory and nutritional properties of raw material and food. In general, PPO and its substrates occupy different cellular compartments (pulp, outer layers of the bean, and in its central region) and interact upon membrane disruption. Hydroxylation of monophenols (cresolase activity) is an extra function shared by many PPO (Carmona et al., 1979; Pomerantz, 1966). Oxygen is required as a second substrate to achieve either cresolase or catecholase activity. PPO also seems to be involved in plant resistance against diseases (Ray and Hammerschmidt, 1998). In coffee (*C. arabica* L.) PPO was identified and characterized in leaves and endosperm and its activity was higher in early developmental stages of both leaves and endosperm of fruits (Mazzafera and Robinson, 2000). PPO activity of green coffee beans in this study was about 5 times higher than of the biomass. This indicates that the pressing process used to remove the fat content from green coffee beans might have degraded this enzyme, which could be due to the elevated temperatures (~70/80 °C) achieved during the process. According to several studies the optimum temperature for PPO may vary according with the material it is being extracted from; the temperature range may vary from 18 to 70 °C (Mizobutsi et al., 2010; Oktay et al., 1995; Serradell et al., 2000; Yue-Ming et al., 1997). Montavon and Bortlik (2004) found PPO enzymatic activity for mature green beans (color green – second stage of maturation) of Robusta coffee of 5.5 U mg^{-1} protein and concluded that PPO activities strongly decrease along maturation from green mature to over-ripe. Montavon et al. (2007) evaluated the enzymatic activity for a variety of green coffee and obtained the highest values for Arabica (~ 140 U mg). One other reason for low PPO activity may be due to the fact that a significant fraction of PPO was inactivated by POD- and PPO-produced quinones, which in turn inhibit PPO (Forsyth, 1964). Some decrease in PPO may be partially ascribed to increasing levels of storage proteins along maturation. Mature subclasses should then be less prone to oxidation upon

membrane disruption, a prerequisite for PPO to meet its substrate. For the reasons discussed above, the use of PPO activities as quality indicators as proposed by De Amorin and Silva (1968) can be misleading. Action of PPO requires the disruption of substrate and PPO-specific compartmentalization. It supposes harsh conditions in the field and/or harsh postharvest treatments. As mentioned by Mazzafera and Robinson (2000), low quality may be attributed to membrane damage rather than to PPO per se. Because PPO activity is launched upon damaging of the beans, enzymatic browning and low *in vitro* activities should be considered as a marker for inadequate treatment of the crop during harvest, processing, and storage. In healthy beans, however, PPO appears to play only a secondary role with respect to quality and redox status (Montavon and Bortlik, 2004).

Green beans at the earliest stage of maturation have the lowest POD activity and the highest PPO activity according with the study of Montavon and Bortlik (2004) with Robusta coffee. Which can relate to the results obtained in this work, whereas the beans were harvested in the mature stage. Again, the enzymatic activity of POD was lower for the biomass, which could be related to the temperature raising, even though POD is considered to be a highly heat resistant enzyme, in diverse vegetable tissues, over 70 – 80 °C its activity begins to decrease (Mizobutsi et al., 2010; Rodrigo et al., 1996). Montavon and Bortlik (2004) found the highest enzymatic activity for POD for Robusta green coffee at over-ripe stage of maturation (~ 90 U mg^{-1} protein). One should, however, keep in mind that specific assay conditions may also contribute to variations of specific activities. POD is also an heme-containing enzyme of major interest. It is known to oxidize various phenols and enediols substrates coupled to the consumption of H_2O_2 by a complex peroxidatic reaction cycle. POD may, however, convey multiple and opposite functions involving consumption and production of radical oxygen scavenger. As such, it plays an essential role in cell wall stiffening and cell wall loosening (Chen and Schopfer, 1999; Fry, 1998; Müse et al., 1997), two important processes for plant growth (Montavon and Bortlik, 2004).

Several enzymes have a role in the degradation of pectic substances. Among them, the most important and most studied in fruits are the hrydrolases PME and PG, however, the reports on these enzymes for green coffee are rare. PME and PG activities for green coffee and biomass are shown on Table 1. PME catalyzes the removal of the methyl groups from the polygalacturonic acid chain, leaving an increased number of free carboxyl groups that can then bind cations and cross-link the

pectin chains. The action of PME also makes the pectin susceptible to further degradation by PG because this enzyme acts only on segments of the pectin chain that have been demethylated by PME. PG cleaves the polygalacturonic acid backbone of the pectin and reduces the average length of the pectin chains (Anthon et al., 2002). Pectin degradation is usually accompanied by increased activity of cell wall hydrolases, such as PME and PG. The lower enzymatic level of PG on biomass could be due to temperature inactivation during the mechanical pressing, which is in the range of 25 – 60 °C for this enzyme (Anthon et al., 2002), depending on the matrix; the same authors also reported that the PME is much more heat resistant than PG, which could be another reason for the elevated activity of this enzyme in the biomass. The literature reports on PME activity during fruit maturation are controversial. PME activity may decrease, remain constant or increase during maturation, depending on the fruit and method of enzyme extraction. The changes are complicated by the presence of isoforms or enzyme inhibitors (Mohd Ali et al., 2004). Although the knowledge on these enzymes behavior in green coffee is scarce, both enzymes work actively together on fruits maturation, even during storage (Hobson et al., 1987), what makes the assessment of these enzymes activity so relevant.

The lipase enzyme belongs to the serine hydrolase family that includes several esterase enzymes. Lipases catalyze the hydrolysis of ester bonds at the interface between an aqueous and non-aqueous phase: such a feature distinguishes them from esterase (Panzanaro et al., 2010). Lipase activity found for both green beans and biomass were similar. To our knowledge there is no information on the lipase activity of green coffee. Panzanaro et al. (2010) investigated the lipase activity of olive fruits (*Olea europaea* L.) (cultivar Ogliarola) and observed an optimal activity of 5.2 nmol oleic acid h^{-1} mg^{-1} of total proteins for stage spotted II of ripening, ideal temperature of about 35 °C, and optimal pH of 5.5. It is possible to assume that besides analytical conditions, also the material being studied may add to activity variations, once the coffee beans were submitted to temperature > 35 °C (during defatting) and did not suffer inhibition concerning the lipase activity. Similar activities were observed for this enzyme in both beans and biomass indicating that once the biomass is intended to be further exploited or added to other processes the lipase activity should be taken into consideration once it may reduce the quality of the product due to its relevant oil content (Table 1).

Caffeine is a methylxanthine with bitter characteristics; however, it is responsible for no more than 10% of the perceived bitterness of the coffee beverage (Barone and Roberts, 1996). Caffeine content for green coffee beans and biomass is available in Table 1, which is in agreement with the literature value of 0.9% to 1.4% (Belitz et al., 2009; Franca et al., 2005; Wintgens, 2008). For the Robusta variety these values may vary between 1.7% and 4.0% (w/w) (Belitz et al., 2009). It was possible to confirm caffeine thermal stability, once the biomass presented the same content as the beans, also, it can be inferred that caffeine extraction did not occur along with the extraction of coffee oil by pressing, and that the biomass is a potential product for obtaining caffeine. This is another feature of this by-product that could make it possible to be applied in the food supplement, and pharmaceutical chain. Mazzafera et al. (1991) observed that Robusta coffee beans contained more caffeine than Arabica beans, and no differences in caffeine content were encountered among the defective beans, also higher caffeine content was reported in the endosperm of immature fruits and in the whole immature fruit.

CGA content for green beans and biomass are shown in Table 1. Although CGA are mostly found in the coffee seeds, they have also been found in the leaves and in the coffee pulp (Clifford and Ramirez-Martinez, 1991), and have also shown to tolerate in some extent temperature rise, once they were found also in the biomass in the same amount as in the seeds. CGA are a group of phenolic compounds representing 5 to 12% of the dry mass of raw coffee which are formed by esterification of quinic acid by a cinnamic acid (Farah and Donangelo, 2006; Nogaim et al., 2013). The main sub-groups are caffeoylquinic acids (CQA), dicaffeoylquinic (di-CQA) and feruloylquinic (FQA) (Farah and Donangelo, 2006). Meanwhile, assessment of caffeine, and CGA levels in green coffee is very important for the coffee industry, since they have a large effect on the final quality of the coffee products. Caffeine has been related to the pharmacological effects of coffee, and CGA has been associated with flavor formation and aroma production during coffee roasting (Nehlig, 2016).

In this work, CGA content in the coffee beans and biomass was found to be in this upper limit (11%). Studies performed in Denmark, the United States, Mediterranean countries, Japan, and Brazil have reported that coffee is the most important contributor to antioxidants intake in their diets due to the CGA content (Farah, 2012). CGA is one of the key components in coffee responsible for determining the beverage quality as well as its antioxidant activity and in turn for

health benefits. According to Farah and Donangelo (2006), CGA have strong antioxidant activity, showing hypoglycemic, antiviral, hepatic and immune protection activities in humans. Ramalakshmi et al. (2007), CGA content was found to be marginally higher in defective green coffee beans (6.83% to 8.80%) compared to graded green coffee beans (6.35 to 8.32%), both of which presented the lower limit about 50% lower than the values found in this study for green beans and biomass (Table 1). CGA content of Arabica coffee was found to be in the range of 3.61% to 6.14% d.b. (Martinez et al., 2013; Wintgens, 2008). CGA from biomass of coffee beans potential could be utilized as natural antioxidants and may help to add the benefits to the coffee growers/manufacturers. Hence, another relevant point concerning the CGA content in the green coffee biomass is that it is not is not extracted along with the oil and remains mostly in the biomass which leads to the conclusion, as well as for the caffeine content, that it is a potential source of these compounds.

TPC content found for green coffee beans and biomass is available in Table 1. TPC content in biomass was about 30% higher than that of the beans. What makes of the biomass, once more, a valuable input to the industry, and indicates that these compounds are not extracted along with the oil. The results also lead to the fact that the pressing process made these compounds more available for extraction, due to the cell rupture. Priftis et al. (2015) found lower content of TPC (5.2%) in green coffee aqueous extract. However, Siva et al. (2016) found higher content of these compounds in green coffee beans extracted with isopropanol (30.65%), and methanol (16.26%), indicating that, besides the cultivars, environmental condition, climate, and post harvesting manners, the extraction method of these compounds also have great influence on the results available. Extraction solvent plays an important factor that affects the antioxidant capacity and total phenolic content in food materials (Siva et al., 2016), as reported by Teh et al. (2014) who evaluated the effect of solvents on the recovery of TPC from hemp, flax, and canola (733.33, 774.33, and 2104.67 mg GAE/100 g fresh weight, respectively) seed cakes and came to a conclusion that a mixture of acetone, methanol, and water (7:7:6 v/v/v) was the best solvent alternative to yield these compounds. Gutiérrez et al. (2010) found 72.75 mg GAE 100 g^{-1} of extract for flaxseed cake TPC aqueous ethanolic (50%) extract and Ho et al. (2007) obtained TPC of 2080 mg GAE 100 g^{-1} of flaxseed cake extracted with pressurized low polarity water. Both authors reported that the cake presented higher content of TPC than the original material, as it was observed in this study,

corroborating the fact that the expelling process may allow TPC to be more available for further extraction. These studies confirm that the solvent type is paramount on the selectivity of TPC once green coffee beans are known as a great source of them and still presented lower content than the other materials aforementioned. The activity of the phenolic compounds in general has been extensively studied in their pure form to control nephritis lupica in humans (Oomah, 2001) showing the importance of these compounds for human health (Gutiérrez et al., 2010). Further, PC are directly related to the antioxidant activity of materials, the latter are extremely crucial in coffee beans to minimize the release of reactive oxygen species (ROS). This would enable the usage of these extracts to be used as ingredients in pharmaceutical industry or as food supplement as CGA (phenolic compounds) implies positive impact on weight loss (Siva et al., 2016).

In this work only phenolic and tannins were assayed (Table 1). The results revealed the presence of high antinutrient (tannins) value in both green coffee beans and biomass. The antinutrient was not removed after the defatting process by expelling, it is interesting to note that there were two times more tannin content in the biomass than it was found in the beans. The cellular damage caused to the beans by the pressing process may have had facilitated the extraction of these compounds. The values detected are not at a safe level that poses no danger in diets. It is believed that fermentation reduces antinutritional factors; therefore, the processing method may account for the result obtained. Xu and Hanna (2011) reported an average value of tannin content thirty times lower for hazelnut biomass (753 mg TA 100 g^{-1}), and Savolainen (1992) also found lower values than in this study for green coffee beans (660 mg TA 100 g^{-1}). As most phenolics and tannins are water soluble, soaking and thermal processing, such as cooking, toasting and extrusion, effectively reduces their levels (Enujiugha, 2003; Mukhopadhyay et al., 2007). It is worthy of note that although phenolics and tannins have a negative effect on utilizations of energy and nutrients, they currently are all considered to be health-promoting factors at low concentrations (Siddhuraju et al., 2002).

The electrical conductivity and potassium leaching tests have been consistently used as efficient quality markers because they present greater sensitivity in the detection of degradation occurring in the cellular membranes of the grains during inappropriate handling in the pre and post-harvest phases. In order to evaluate seeds quality, these tests based on the membrane integrity loss were developed. Lower vigor seeds release greater amount of electrolytes in a solution, resulting

in a high electrical conductivity value (Bedford, 1974) or in high concentration of certain ions, such as the potassium (Martinez et al., 2013). Amorim (1978) discussed that several conditions, including mechanical injury, affect the structure of cell membranes in coffee beans. When cell membranes collapse, they release cell contents, including enzymes such as polyphenol oxidase, proteases and lipases, altering the chemical composition of raw beans. Thus, potassium leaching and electrical conductivity, which is proportional to the amount of leached potassium, can be used to evaluate the collapse of membranes and consequently reduction in coffee quality. In this work, after 5 hours of soaking 50 beans in 50 mL water, beans showed potassium leaching and electrical conductivity content are in agreement with the literature for healthy cherry coffee beans (118. 31 μ Scm^{-1} g^{-1} and 33.34 ppm) (Borém et al., 2008).

4. Conclusion

By comparing the composition of the green beans and biomass, it could be observed that many compounds are not extracted in the mechanical pressing process, in which the oil is extracted but with a very low yield. The results indicate the potential of the biomass to obtain products based on caffeine, chlorogenic acids, phenolic compounds, oil and others. Also, there is the need to be attentive to the inactivation of the enzymes present in the material during processing, once they can cause degradation of compounds of interest if not inactivated. It can be concluded that the green coffee samples, as well as its biomass samples under this investigation showed a good grade on quality in most chemical composition parameters and these results are the beginning step for all interested researchers to do many scientific studies on coffee which is national crops in Brazil. The presence of phenolic compounds and chlorogenic acids in reasonable quantities makes them suitable as a source of natural antioxidant compounds. Besides to adding value to these unused materials, finding alternative forms to use them would be useful to decrease their impact to the environment. Among the studies up till now performed with these coffee industry wastes, most of them were focused on the use of spent coffee grounds. However, the chemical composition data suggest that the cake can be of value as raw material for other processes.

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REFERENCES

Akpapuimam, M. A. and Markakis, P. 1981. Physicochemical and nutritional aspects of Cowpea flour. Journal of Food Science 46(3): 972–973.

Amorim, H. V., 1978. Biochemical and histochemical aspects of green coffee beans related to deterioration of quality. Escola Superior de Agricultura Luiz de Queiroz (USP).

Anesini, C., Ferraro, G. E. and Filip, R. 2008. Total polyphenol content and antioxidant capacity of commercially available tea (*Camellia sinensis*) in Argentina. Journal of Agricultural and Food Chemistry 56(19): 9225–9229.

Anthon, G. E., Sekine, Y., Watanabe, N. and Barrett, D. M. 2002. Thermal inactivation of pectin methylesterase, polygalacturonase, and peroxidase in tomato juice. Journal of Agricultural and Food Chemistry 50(21): 6153–6159.

Association of the Official Analytical Chemists (AOAC). 2016. Official methods of analysis of the Association of Official Analytical Chemists International, 20th ed. Gaithersburg: AOAC.

Arișanu, A. O., Rus, F. 2013. The influence of process parameters on mechanical expression of sunflower oilseeds. Proceedind of the 5th International Conference on Computational Mechanics and Virtual Engineering, p. 341–346. Brașov, Romania.

Arya, M. and Rao, L. J. M. 2007. An impression of coffee carbohydrates. Critical Reviews in Food Science and Nutrition 47(1): 51–67.

Baalbaki, R., Elias, S., Filho, J. M. and McDonald, M. B. 2009. Seed vigor testing handbook. Washington: Association of Official Seed Analysis.

Barone, J. J. and Roberts, H. R. 1996. Caffeine consumption. Food Chemistry and Toxicology 34(1): 119–129.

Beck, D. J. and Bibby, B. G. 1961. A Modified anthrone colorimetric technique for use in investigations related to the cariogenicity of foodstuffs. Journal of Dental Research 40: 161–170.

Bedford, L. V. 1974. Conductivity tests in commercial and hand harvested seed of pea cultivars and their relation to field establishment. Seed Science and Technology 2: 323–335.

Behr, A. and Gomes, J. P. 2010. The refinement of renewable resources: New important derivatives of fatty acids and glycerol. European Journal of Lipid Science and Technology 112(1): 31–50.

Belitz, H.-D., Grosch, W. and Schieberle, P. 2009. Food chemistry. 4th ed. Berlin: Springer.

Bitter, T. and Muir, H. M. 1962. A modified uronic acid carbazole reaction. Analytical Biochemistry 4(4): 330–334.

Borém, F. M., Marques, E. R. and Alves, E. 2008. Ultrastructural analysis of drying damage in parchment Arabica coffee endosperm cells. Biosystems Engineering 99(1): 62–66.

Brand-Williams, W., Cuvelier, M. E. and Berset, C. 1995. Use of a free radical method to evaluate antioxidant activity. LWT - Food Science and Technology 28(1): 25–30.

Buescher, R. W. and Furmanski, R. J. 1978. Role of pectinesterase and polygalacturonase in the formation of woolliness in peaches. Journal of Food Science 43(1): 264–266.

Caetano, N. S., Silva, V. F. M. and Mata, T. M. 2012. Valorization of coffee grounds for biodiesel production. Chemical Engineering Transactions 26: 267–272.

Campos-Vega, R., Loarca-Piña, G., Vergara-Castañeda, H. and Oomah, B. D. 2015. Spent coffee grounds: A review on current research and future prospects. Trends in Food Science & Technology 45(1): 24-36.

Carmona, F. G., Pedreño, E., Galindo, J. D. and Cánovas, F. G. 1979. A new spectrophotometric method for the determination of cresolase activity of epidermis tyrosinase. Analytical Biochemistry 95(2): 433–435.

Castro, A. C. C. M., Oda, F. B., Almeida-Cincotto, M. G. J., Davanço, M. G., Chiari-Andréo, B. G., Cicarelli, R. M. B., Peccinini, R. G., Zocolo, G. J., Ribeiro, P. R. V., Corrêa, M. A., Isaac, V. L. B. and Santos, A. G. 2018. Green coffee seed residue: A sustainable source of antioxidant compounds. Food Chemistry 246: 48–57.

Chen, S. X. and Schopfer, P. 1999. Hydroxyl-radical production in physiological reactions. A novel function of peroxidase. European Journal of Biochemistry 260(3): 726–735.

Clifford, M. N. and Ramirez-Martinez, J. R. 1991. Tannins in wet-processed coffee beans and coffee pulp. Food Chemistry 40(2): 191–200.

Coffee Chemistry. 2015. Carbohydrates in coffee. Retrieved on September 4, 2018 from Coffee Chemistry Website: https://www.coffeechemistry.com/chemistry/carbohydrates/carbohydrates-in-coffee

Comission Regulation (EC), 2005. Official Journal of the European Union. EUR-Lex.

Coste, R. and Cambrony, H. 1993. Coffee: The plant and the product. Wageningen: Macmillan and CTA.

Denoeud, F., Carretero-Paulet, L., Dereeper, A., Droc, G., Guyot, R., Pietrella, M., Zheng, C., Alberti, A., Anthony, F., Aprea, G., Aury, J. -M., Bento, P., Bernard, M., Bocs, S., Campa,

C., Cenci, A., Combes, M. -C., Crouzillat, D., Da Silva, C., Daddiego, L., De Bellis, F., Dussert, S., Garsmeur, O., Gayraud, T., Guignon, V., Jahn, K., Jamilloux, V., Joet, T., Labadie, K., Lan, T., Leclercq, J., Lepelley, M., Leroy, T., Li, L.-T., Librado, P., Lopez, L., Munoz, A., Noel, B., Pallavicini, A., Perrotta, G., Poncet, V., Pot, D., Priyono, Rigoreau, M., Rouard, M., Rozas, J., Tranchant-Dubreuil, C., VanBuren, R., Zhang, Q., Andrade, A. C., Argout, X., Bertrand, B., de Kochko, A., Graziosi, G., Henry, R. J., Jayarama, Ming, R., Nagai, C., Rounsley, S., Sankoff, D., Giuliano, G., Albert, V. A., Wincker, P. and Lashermes, P. 2014. The coffee genome provides insight into the convergent evolution of caffeine biosynthesis. Science 345(6201): 1181–1184.

Dias, R. and Benassi, M. 2015. Discrimination between Arabica and Robusta coffees using hydrosoluble compounds: Is the efficiency of the parameters dependent on the roast degree? Beverages 1(3): 127–139.

European Coffee Federation (ECF). European Coffee Report 2016/17. Retrieved on September 1, 2018 from ECF Website: https://www.ecf-coffee.org/publications/european-coffee-report.

Enujiugha, V. N. 2003. Chemical and functional characteristics of Conophor nut. Pakistan Journal of Nutrition 2(3): 335–338.

Esquivel, P. and Jiménez, V. M. 2012. Functional properties of coffee and coffee by-products. Food Research International 46(2): 488–495.

Faniadis, D., Drogoudi, P. D. and Vasilakakis, M. 2010. Effects of cultivar, orchard elevation, and storage on fruit quality characters of sweet cherry (*Prunus avium* L.). Science Horticulturae. 125(3): 301–304.

Farah, A. 2012. Coffee constituents. In Chu, Y. -F. (Ed). Coffee: Emerging Health Effects and Disease Prevention, p. 21–55. Oxford: John Wiley & Sons, Inc. and The Institute of Food Technologists.

Farah, A. and Donangelo, C. M. 2006. Phenolic compounds in coffee. Brazilian Journal of Plant Physiology 18(1): 23–36.

Fehrmann, H. and Dimond, A. E. 1967. Peroxidase activity and *Phytophthora* resistance in different organs of the potato plant. Phytopathology 57: 69–72.

Ferrari, M., Ravera, F., De Angelis, E., Liverani, F. S. and Navarini, L. 2010. Interfacial properties of coffee oils. Colloids and Surfaces A: Physicochemical and Engineering Aspects 365(1-3) 79–82.

Fischer, M., Reimann, S., Trovato, V. and Redgwell, R. J. 2001. Polysaccharides of green Arabica and Robusta coffee beans. Carbohydrate Research 330(1): 93–101.

Forsyth, W. G. C. 1964. Physiological aspects of curing plant products. Annual Review of Plant

Physiology 15: 443–450.

Franca, A. S., Oliveira, L. S., Mendonça, J. C. F. and Silva, X. A. 2005. Physical and chemical attributes of defective crude and roasted coffee beans. Food Chemistry 90(1-2): 89–94.

Fry, S. C. 1998. Oxidative scission of plant cell wall polysaccharides by ascorbate-induced hydroxyl radicals. Biochemical Journal 332(Pt 2): 507–15.

Geromel, C., Ferreira, L. P., Guerreiro, S. M. C., Cavalari, A. A., Pot, D., Pereira, L. F. P., Leroy, T., Vieira, L. G. E., Mazzafera, P. and Marraccini, P. 2006. Biochemical and genomic analysis of sucrose metabolism during coffee (*Coffea arabica*) fruit development. Journal of Experimental Botany 57(12): 3243–3258.

Gutiérrez, C., Rubilar, M., Jara, C., Verdugo, M., Sineiro, J. and Shene, C. 2010. Flaxseed and flaxseed cake as a source of compounds for food industry. Journal of Soil Science and Plant Nutrition 10(4): 454–463.

Haffner, K., Rosenfeld, H. J., Skrede, G. and Wang, L. 2002. Quality of red raspberry *Rubus ideais* L. cultivars after storage in controlled and normal atmospheres. Postharvest Biology and Technology 24(3): 279–289.

Hassanien, F. R. and Mukherjee, K. D. 1986. Isolation of lipase from germinating oilseeds for biotechnological processes. Journal of the American Oil Chemists' Society 63(7): 893–897.

Ho, C. H. L., Cacace, J. E. and Mazza, G. 2007. Extraction of lignans, proteins and carbohydrates from flaxseed meal with pressurized low polarity water. LWT - Food Science and Technology 40(9): 1637–1647.

Hobson, G. E., Seymour, G. B., Harding, S. E., Taylor, A. J. and Tucker, G. A. 1987. Polyuronide solubilization during ripening of normal and mutant tomato fruit. Phytochemistry 26(7): 1871–1875.

International Coffee Council (ICC). 2004. Improving Quality. Retrieved on September 1, 2018 from ICO Website: http://www.ico.org/improving_quality.asp

International Coffee Council (ICC). 2002. Coffee quality-improvement programme - Implementation, Resolution No. 407. Retrieved on September 1, 2018 from ICO Website: http://www.ico.org/show_document.asp?id=133

International Coffee Organization (ICO). 2017. The current state of the global coffee trade. Retrieved on September 1, 2018 from ICO Website: http://www.ico.org/monthly_coffee_trade_stats.asp (accessed 7.15.17).

Ige, M. M., Ogunsua, A. O. and Oke, O. L. 1984. Functional properties of the proteins of some Nigerian oilseeds: Conophor seeds and three varieties of melon seeds. Journal of Agricultural

and Food Chemistry 32(4): 822–825.

International Organization for Standardization (ISO). 2003. ISO 6673:2003 - Green coffee - Determination of loss in mass at 105 degrees C.

International Organization for Standardization (ISO). 2001. ISO 1446:2001 - Green coffee — Determination of water content — Basic reference method.

Jeszka-Skowron, M., Sentkowska, A., Pyrzyńska, K. and De Peña, M. P. 2016. Chlorogenic acids, caffeine content and antioxidant properties of green coffee extracts: influence of green coffee bean preparation. European Food Research and Technology 242(8): 1403–1409.

Kemper, T. G. 2005. Oil extraction. In Shahidi, F. (Ed). Bailey's Industrial Oil and Fat Products, p. 57–98. New Jersey: John Wiley & Sons.

Kornman, C. 2016. Green Coffee Analytics Part IV – Density. Retrieved on 4 September, 2018 from Royal Coffee Website: https://royalcoffee.com/green-coffee-analytics-part-iv-density/

Leroy, T., Ribeyre, F., Bertrand, B., Charmetant, P., Dufour, M., Montagnon, C., Marraccini, P. and Pot, D. 2006. Genetics of coffee quality. Brazilian Journal of Plant Physiology 18(1): 229–242.

Liaquat, M. and Owusu Apenten, R. K. 2000. Synthesis of low molecular weight flavor esters using plant seedling lipases in organic media. Journal of Food Science 65(2): 295–299.

Loeffler, T. M., Tekrony, D. M. and Egli, D. B. 1988. The bulk conductivity test as an indicator of soybean seed quality. Journal of Seed Technology 12(1): 37–53.

Martinez, H. E. P., Poltronieri, Y., Farah, A. and Perrone, D. 2013. Zinc supplementation, production and quality of coffee beans. Revista Ceres 60(2): 293–299.

Martín, M. J., Pablos, F. and González, A. G. 1998. Discrimination between arabica and robusta green coffee varieties according to their chemical composition. Talanta 46(2): 1259–1264.

Mazzafera, P. 1999. Chemical composition of defective coffee beans. Food Chemistry 64(4): 547–554.

Mazzafera, P., Crozier, A. and Magalhães, A. C. 1991. Caffeine metabolism in *Coffea arábica* and other species of coffee. Phytochemistry 30(12): 3913–3916.

Mazzafera, P. and Robinson, S. P. 2000. Characterization of polyphenol oxidase in coffee. Phytochemistry 55(4): 285–96.

Mazzafera, P., Soave, D., Zullo, M. A. T. and Guerreiro Filho, O. 1998. Oil content of green beans from some coffee species. Bragantia 57(1): 45–48.

McCready, R. M. and McComb, E. A.,1952. Extraction and determination of total pectic materials in fruits. Analytical Chemistry 24(12): 1586–1588.

Merritt, M. C. and Proctor, B. E. 1959. Effect of temperature during the roasting cycle on selected components of different types of whole bean coffee. Journal of Food Science 24(6): 672–680.

Mizobutsi, G. P., Finger, F. L., Ribeiro, R. A., Puschmann, R., Neves, L. L. de M. and Mota, W. F. 2010. Effect of pH and temperature on peroxidase and polyphenoloxidase activities of litchi pericarp. Scientia Agricola 67(2): 213–217.

Mohd Ali, Z., Chin, L.-H. and Lazan, H. 2004. A comparative study on wall degrading enzymes, pectin modifications and softening during ripening of selected tropical fruits. Plant Science 167(2): 317–327.

Montavon, P. and Bortlik, K. 2004. Evolution of Robusta green coffee redox enzymatic activities with maturation. Journal of Agricultural and Food Chemistry 52(11): 3590–3594.

Montavon, P., Kukic, K. R. and Bortlik, K. 2007. A simple method to measure effective catalase activities: Optimization, validation, and application in green coffee. Analytical Biochemistry 360(2): 207–215.

Morris, D. L. 1948. Quantitative determination of carbohydrates with Dreywood's Anthrone reagent. Science 107(2775): 254–255.

Mukhopadhyay, N., Sarkar, S. and Bandyopadhyay, S. 2007. Effect of extrusion cooking on anti-nutritional factor tannin in linseed (*Linum usitatissimum*) meal. International Journal of Food Science and Nutrition 58(8): 588–594.

Murkovic, M. and Derler, K. 2006. Analysis of amino acids and carbohydrates in green coffee. Journal of Biochemistry and Biophysical Methods 69(1-2): 25–32.

Müse, G., Schindler, T., Bergfeld, R., Ruel, K., Jacquet, G., Lapierre, C., Speth, V. and Schopfer, P. 1997. Structure and distribution of lignin in primary and secondary cell walls of maize coleoptiles analyzed by chemical and immunological probes. Planta 201(2): 146–159.

Mussatto, S. I., Dragone, G. and Roberto, I. C. 2006. Brewers' spent grain: generation, characteristics and potential applications. Journal of Cereal Sciences 43(1): 1–14.

Navia, D., Velazco, R. and Hoyos, J. 2011. Production and evaluation of ethanol from coffee processing by-products. Vitae 18(3): 287–294.

Nehlig, A. 2016 Effects of coffee/caffeine on brain health and disease: What should i tell my patients? Practical Neurology 16(2): 89-95.

Nelson, N. A. 1944. A photometric adaptation of Somogyi method for the determination of glucose.

Journal of Biological Chemistry 153: 375–380.

Nogaim, Q. A., Al-Duais, M., Al-Warafi, A., Al-Erianee, H. and Al-Sayadi, M. 2013. The chemical composition of Yemeni green coffee. Journal of Food Chemistry and Nutrition 1(2): 42–48.

Natural Sciences and Engineering Research Council of Canada (NSERC) 2010. What is Lignin? Retrieved on September 1, 2018 from Lignoworks Website: http://www.icfar.ca/lignoworks/content/what-lignin.html.

Ogunyinka, B. I., Oyinloye, B. E., Osunsanmi, F. O., Kappo, A. P. and Opoku, A. R. 2017. Comparative study on proximate, functional, mineral, and antinutrient composition of fermented, defatted, and protein isolate of *Parkia biglobosa* seed. Food Science & Nutrition 5(1): 139–147.

Oktay, M., Küfreviolu, I., Kocaçalişkan, I. and Şaklrolu, H. 1995. Polyphenoloxidase from Amasya apple. Journal of Food Science 60(3): 494–496.

Oliveira, L. S., Franca, A. S., Mendonça, J. C. F. and Barros-Júnior, M. C. 2006. Proximate composition and fatty acids profile of green and roasted defective coffee beans. LWT - Food Science and Technology 39(3): 235–239.

Oomah, B. D. 2001. Flaxseed as a functional food source. Journal of the Science of Food and Agriculture 81: 889–894.

Oosterveld, A., Harmsen, J. S., Voragen, A. G. J. and Schols, H. A. 2003. Extraction and characterization of polysaccharides from green and roasted *Coffea arabica* beans. Carbohydrate Polymers 52(3) 285–296.

Ozcan, T., Akpinar-Bayizit, A., Yilmaz-Ersan, L. and Delikanli, B. 2014. Phenolics in human health. International Journal of Chemical Engineering and Applications 5(5): 393–396.

Panzanaro, S., Nutricati, E., Miceli, A. and De Bellis, L. 2010. Biochemical characterization of a lipase from olive fruit (*Olea europaea* L.). Plant Physiology and Biochemistry 48(9): 741–745.

Parodi, D., 2017. Analysis: Green coffee bean density. Mill47 Coffee. URL https://mill47.coffee/blogs/story-mill/green-bean-density (accessed 4.11.18).

Parras, P., Martínez-Tomé, M., Jiménez, A. M. and Murcia, M. A. 2007. Antioxidant capacity of coffees of several origins brewed following three different procedures. Food Chemistry 102(3): 582–592.

Pomerantz, S. H. 1966. The tyrosine hydroxylase activity of mammalian tyrosinase. The Journal of Biological Chemistry 241(1):161–168.

Ponting, J. D. and Joslyn, M. A. 1948. Ascorbic acid oxidation and browning in apple tissue extracts. Archives of Biochemistry 19(1): 47–63.

Price, M. L., Van Scoyoc, S. and Butler, L. G. 1978. A critical evaluation of the vanillin reaction as an assay for tannin in sorghum grain. Journal of Agricultural and Food Chemistry 26(5): 1214–1218.

Priftis, A., Stagos, D., Konstantinopoulos, K., Tsitsimpikou, C., Spandidos, D. A., Tsatsakis, A. M., Tzatzarakis, M. N. and Kouretas, D. 2015. Comparison of antioxidant activity between green and roasted coffee beans using molecular methods. Molecular Medicine Reports 12(5): 7292-7302.

Proctor, A. and Miesle, T. J. 1991. Polygalacturonase and pectinmethylesterase activities in developing highbush blueberries. Hortscience 26(5): 579–581.

Purohit, C. and Rajyalakshmi, P. 2011. Quality of products containing defatted groundnut cake flour. Journal of Food Science and Technology 48(1): 26–35.

R Core Team, 2015. R: A language and environment for statistical computing.

Ramalakshmi, K., Kubra, I. R. and Rao, L. J. M. 2007. Physicochemical characteristics of green coffee: Comparison of graded and defective beans. Journal of Food Science 72(5): S333–S337.

Ravindran, R. and Jaiswal, A. K. 2016. A comprehensive review on pre-treatment strategy for lignocellulosic food industry waste: Challenges and opportunities. Bioresource Technology 199: 92–102.

Ray, H. and Hammerschmidt, R. 1998. Responses of potato tuber to infection by *Fusarium sambucinum*. Physiological and Molecular Plant Pathology 53(2): 81–92.

Redgwell, R. and Fischer, M. 2006. Coffee carbohydrates. Brazilian Journal of Plant Physiology 18(1): 165-174.

Redgwell, R. J., Trovato, V., Curti, D. and Fischer, M. 2002. Effect of roasting on degradation and structural features of polysaccharides in Arabica coffee beans. Carbohydrate Research 337(5): 421–431.

Rodrigo, C., Rodrigo, M., Alavarruiz, A. and Frígola, A. 1996. Thermal inactivation at high temperatures and regeneration of green asparagus peroxidase. Journal of Food Protection 59(9): 1065–1071.

Rogers, W. J., Michaux, S., Bastin, M. and Bucheli, P. 1999. Changes to the content of sugars, sugar alcohols, myo-inositol, carboxylic acids and inorganic anions in developing grains from different varieties of Robusta (*Coffea canephora*) and Arabica (*Coffea arabica*) coffees. Plant

Science 149(2): 115–123.

Romano, R., Santini, A., Le Grottaglie, L., Manzo, N., Visconti, A. and Ritieni, A. 2014. Identification markers based on fatty acid composition to differentiate between roasted Arabica and Canephora (Robusta) coffee varieties in mixtures. Journal of Food Composition and Analysis 35(1): 1-9.

Savolainen, H. 1992. Tannin content of tea and coffee. Journal of Applied Toxicology 12(3): 191–2.

Specialty Coffee Association of America (SCAA). 2009. Grading Green Coffee. Retrieved on September 1, 2018 from SCAA Website: https://www.scaa.org/PDF/resources/grading-green-coffee.pdf.

Scully, D., Jaiswal, A. and Abu-Ghannam, N. 2016. An Investigation into spent coffee waste as a renewable source of bioactive compounds and industrially important sugars. Bioengineering 3(4): 1-13.

Şemen, S., Mercan, S., Yayla, M. and Açıkkol, M. 2017. Elemental composition of green coffee and its contribution to dietary intake. Food Chemistry 215: 92–100.

Serradell, M. de los A., Rozenfeld, P. A., Martínez, G. A., Civello, P. M., Chaves, A. R. and Anón, M. C. 2000. Polyphenoloxidase activity from strawberry fruit (*Fragaria ananassa*, Duch., cv Selva): Characterisation and partial purification. Journal of the Science of Food and Agriculture 80(9): 1421–1427.

Siddhuraju, P., Becker, K. and Makkar, H. P. S. 2002. Chemical composition, protein fractionation, essential amino acid potential and antimetabolic constituents of an unconventional legume, Gila bean (*Entada phaseoloides* Merrill) seed kernel. Journal of the Science of Food and Agriculture 82(2): 192–202.

Siva, R., Rajikin, N., Abdul Haiyee, Z. and Ismail, W. I. W. 2016. Assessment of antioxidant activity and total phenolic content from green coffee *Robusta sp.* beans. Malaysian Journal of Analytical ScienceS 20(5): 1059–1065.

Sluiter, A., Hames, B., Hyman, D., Payne, C., Ruiz, R., Scarlata, C., Sluiter, J., Templeton, D. and Wolfe, J. 2008. Determination of total solids in biomass and total dissolved solids in liquid process samples. Golden: National Renewable Energy Laboratory.

Speer, K. and Kölling-Speer, I. 2006. The lipid fraction of the coffee bean. Brazilian Journal of Plant Physiology 18(1): 201–216.

Speer, K. and Kölling-Speer, I. 2001. Lipids. In Clarke, R. J., Vitzthum, O. G. (Eds), p. 33–46. Coffee: Recent Developments. Oxford: Blackwell Science.

Teh, S. -S., Bekhit, A. and Birch, J. 2014. Antioxidative polyphenols from defatted oilseed cakes: Effect of solvents. Antioxidants 3(1): 67–80.

Theimer, R. R. and Rosnitschek, I. 1978. Development and intracellular localization of lipase activity in rapessed (*Brassica naus* L.) cotyledons. Planta 139(3): 249–256.

Tian, H., Ying, Y., Lu, H., Fu, X. and Yu, H. 2007. Measurement of soluble solids content in watermelon by Vis/NIR diffuse transmittance technique. Journal of Zhejiang University Science B 8(2): 105–110.

Tsai, W. T., Liu, S. C. and Hsieh, C. H. 2012. Preparation and fuel properties of biochars from the pyrolysis of exhausted coffee residue. Journal of Analytical and Applied Pyrolysis 93: 63–67.

Vaast, P., Bertrand, B., Perriot, J. -J., Guyot, B. and Génard, M. 2006. Fruit thinning and shade improve bean characteristics and beverage quality of coffee (*Coffea arabica* L.) under optimal conditions. Journal of the Science of Food and Agriculture 86(2): 197–204.

Van Soest, P. J. 1963. Use of detergents in the analysis of fibrous feeds. II. A rapid method for the determination of fiber and lignin. Journal of the Association of Official Agricultural Chemists 46(5): 830–835.

Van Soest, P. J. and Wine, R. H. 1968. Determination of lignin and cellulose in acid-detergen fiber with permanganate. Journal of the Association of Official Agricultural Chemists 51(4): 780–785.

Van Soest, P. J. and Wine, R. H. 1967. Use of detergents in the analysis of fibrous feeds. IV. Determination of plant cell-wall constituents. Journal of the Association of Official Agricultural Chemists 50(1): 50–55.

Vasconcelos, A. L. S., Franca, A. S., Glória, M. B. A. and Mendonça, J. C. F. 2007. A comparative study of chemical attributes and levels of amines in defective green and roasted coffee beans. Food Chemistry 101(1): 26–32.

Wagemaker, T. A. L., Carvalho, C. R. L., Maia, N. B., Baggio, S. R. and Guerreiro Filho, O. 2011. Sun protection factor, content and composition of lipid fraction of green coffee beans. Industrial Crops and Products 33(2): 469–473.

Waterhouse, A. L. 2002. Wine phenolics. Annals of the New York Academy of Sciences 957: 21–36.

Willems, P., Kuipers, N. J. M. and De Haan, A. B. 2008. Hydraulic pressing of oilseeds: Experimental determination and modeling of yield and pressing rates. Journal of Food Engineering 89(1): 8–16.

Wintgens, J. N. 2008. Coffee: Growing, Processing, Sustainable Production. Weinheim: Wiley-

VCH Verlag GmbH.

Xu, Y. and Hanna, M. A. 2011. Nutritional and anti-nutritional compositions of defatted Nebraska hybrid hazelnut meal. International Journal of Food Science and Technology 46: 2022–2029.

Yue-Ming, J., Zauberman, G. and Fuchs, Y. 1997. Partial purification and some properties of polyphenol oxidase extracted from litchi fruit pericarp. Postharvest Biology and Technology 10(3): 221–228.

Zhang, L. -L., Lin, Y. -M., Zhou, H. -C., Wei, S. -D. and Chen, J. -H. 2010. Condensed tannins from mangrove species Kandelia candel and Rhizophora mangle and their antioxidant activity. Molecules 15: 420–431.

ARTIGO 2 - AN INVESTIGATION INTO GREEN COFFEE PRESS CAKE AS A RENEWABLE SOURCE OF BIOACTIVE COMPOUNDS AN INDUSTRIALLY IMPORTANT INPUT: EFFECT OF SOLVENTS

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ABSTRACT

The residual biomass of coffee, obtained after the oil extraction from coffee beans, called coffee beans residual press cake, has been attracted interest as a source of compounds with antioxidant activity. This study investigated the effects of ethanolic and methanol-acetone extracts of green coffee beans (GCB) and its residual press cake (GCC) on the phenolic composition and antioxidant activity. The antioxidant activity was assayed through five different methods (total phenolic compounds, •DPPH, ABTS, FRAP, and β-carotene bleaching assay), and the phenolic profile of the samples through High Performance Liquid Cromatography. GCB and GCC enclosed chlorogenic (55.16 and 64.96%, respectively) and caffeic (25.07 and 44.37%, respectively) acids as the major components, and the cake presented higher antioxidant activity than the actual green bean. There was an interaction between the type of material and the type of solvent used in the extraction process, where, the antioxidant activity was higher when the GCC was extracted with the solvent mixture methanol-acetone. This study on the evaluation of the effect of solvent on the bioactive compounds from GCB and its by-product (GCC) showed that the latter can be a source of new value-added products, such as phenolic antioxidant adjunct for food or pharmaceutic processing.

Key-words: Antioxidant activity; phenolic compounds; waste; ethanol; solid-liquid extraction; coffee.

1. Introduction

Society has shown increasing interest in natural antioxidants, since they are safer than the synthetic ones, also these compounds have shown therapeutic (prevention of chronic diseases) and nutritive potentials (Reddy Palvai *et al.*, 2014). These compounds can be intended to many applications in the food industry, among them it could be mentioned the lipid oxidation and browning prevention, as functional ingredients, antimicrobials, flavoring, colorants, texturizer additives, and also, as food additives (Ayala-Zavala *et al.*, 2011). Hence, the studies on the bioactive compounds from agroindustrial waste have been increasingly growing and gaining attention (Moure *et al.*, 2001; Derakhshan *et al.*, 2018), once they might be valuable inputs to re-enter the industrial cycle.

Food residues can carry many high-value recoverable substances. Depending on the existence of appropriate technology, this waste can be transformed into added value products (Laufenberg *et al.*, 2003). One of the by-products from the coffee industry is the cake or meal. The defatted cake is the residue produced by the mechanical pressing of green coffee beans for oil expelling. So far, most coffee producers are dumping large quantities of this by-product in the environment, what might cause serious problems further on. Incorporating the biomass into animal feed could be an alternative (Franca and Oliveira, 2009; Galanakis, 2017; Martinez-Saez *et al.*, 2017). However, this practice can be narrowed due to the anti-nutritional activity of phenolic compounds, such as tannins, present in the residue. So far, most researches into coffee by-products have emphasized on spent coffee grounds which are by-products from the industrial-scale brewing of coffee beans (Scully *et al.*, 2016). Relatively few studies have investigated green coffee beans by-products generated through conventional pressing of green coffee beans by the oil industry.

It is of common knowledge that green coffee beans are a great source of bioactive compounds, such as antioxidants that work against free radicals in the human organism (Affonso *et al.*, 2016); however, there are scarce studies evaluating the potential of its press cake also as a source of these compounds which are of special interest to the food and pharmaceutic industries nowadays. Any molecule capable of remaining with one or more unpaired electrons in its last electron layer is called free radical, which is highly unstable and reactive. They are produced under physiological conditions and participate in a variety of normal cellular functions; if they are found

to be in excess in cells, their interaction can cause damage to proteins, lipids and DNA (Phaniendra *et al.*, 2015; Priftis *et al.*, 2015). An organism can obtain antioxidants through diet, besides from endogenous mechanisms; some of the most relevant antioxidants are the phenolic substances, which can be found specially in plant foods. Phenolic compounds are derived from the plant's secondary metabolism that operate in various cellular functions. When foods of plant origin are consumed, the absorbed polyphenols may draw a range of relevant bioactivities that have beneficial effects on health. These compounds can also be encountered in the coffee fruit, which is one of the most popular fruits consumed as a beverage all over the world. For a long time, caffeine was the only coffee compound to be investigated due to its believed positive effects on human health; nonetheless, there are other compounds that contribute and play an important role to its valuable properties, such as antioxidants. These are attributable specially to its polyphenolic content, with the most profuse phenolic substance being chlorogenic acids (CGA) (Pandey and Rizvi, 2009; Quideau *et al.*, 2011; Murthy and Naidu, 2012; Landete, 2013; Priftis *et al.*, 2015).

There are many *in vitro* techniques for evaluating the antioxidant activity of materials, and according with Moure et al. (2001) it must be measured trough different tests for different mechanisms, once each technique might evaluate a different type of antioxidant, also the matrix been analyzed has great influence on the responses. According with these authors, most of the methods are based on the capacity to scavenge distinct free radicals, but UV-absorption and chelation ability can also be accountable for the antioxidant capacity evaluation in systems. The most frequently used methods are the tests which measures the scavenging activity with different radical, such as ABTS•+ (radical cation of 2,2'-azinobis (3-ethylbenzothiozoline-6-sulphonate) and •DPPH (α,α-diphenil-beta-picrylhydrazyl radical), methods for determining oxygen reactive species, methods using metallic cations as catalysts during the oxidation assay (Ferric Reduction Antioxidative Power), beta-carotene oxidation in linoleic acid emulsion (Moure *et al.*, 2001), among others.

The use of coffee waste as an attainable source of bioactive compounds, particularly polyphenols, is up-and-coming, however, to date, it has been scarcely explored from a technological point of view (Esquivel and Jiménez, 2012). Therefore, the present study investigated the green coffee press cake as a feasible input for the generation of industrially important bioactive compounds, once it is known that green coffee beans are antioxidant-rich and most bioactive

compounds are hydrophilic compounds (Santos *et al.*, 2016; Oliveira *et al.*, 2018a) it is believed that these compounds remain in the cake after the oil extraction, making this residue a valued source of bioactive substances. Two solvent-types of extract were carried out, using ethanol and methanol-acetone. The antioxidant activity was assessed through five different assays (•DPPH, ABTS•+, FRAP, beta-carotene bleaching assay, and total phenolic), the phenolic profile was also assessed, and the results were compared to the ones obtained for green coffee beans.

2. Materials and methods

2.1 Material

Green coffee beans (GCB) (harvested in 2016) and cake (GCC), both from the same batch, were supplied by a local industrial coffee producer (Cooxupé, Guaxupé, Minas Gerais, Brazil) able to confirm the botanical origin (*Coffeea arabica*) of the coffee, as well as the general type of postharvest processing (dry process). The GCB were manually sorted to remove defected beans. Both beans and cake were milled in order to obtain homogeneous samples. The materials were ground in an analytical knife mill (IKA, A11, Wilmington, US), only that, GCB were dried in liquid nitrogen previously to milling. The final mesh size of the powders was 0.177 mm (NREL, 2008). The sieved powder was dried at 40 °C (~ 20 days for GCB; ~ 7 days for GCC) in a vacuum oven (absolute pressure = 16.8 kPa) (Tecnal, TE-395, Piracicaba, Brazil) and immediately submitted to analyzes.

2.2 Extracts

In order to perform the analyzes, extracts of both GCB and GCC were prepared following the procedures proposed by Larrauri *et al.* (1997). Two types of extracts were prepared, an ethanolic (99%) and a methanol (50%) – acetone (70%) (1:1 v/v) extract. Sequential extractions were performed. Shortly, 40 mL of methanol (50%) was added to 1.5 g sample. The solution remained at room temperature in the dark for 1 h and then submitted to centrifugation (15,000 rpm/15 min), the supernatant was filtered, and to the remaining solute in the centrifuge tube it was

added another 40 mL of acetone (70%), and the previous steps were repeated. After filtration, this supernatant was added to the methanolic extract (previously prepared), and the volume was made up to 100 mL with distilled water. For the ethanolic extract all the steps above mentioned were followed, only that instead of methanol and acetone, absolute ethanol was used in both extractions.

2.3 Total Polyphenol Content (TPC)

The yield of total polyphenols was determined according to the Folin–Ciocalteu's method (Waterhouse, 2002). The results were expressed as mg of gallic acid equivalent (GAE) per 100 g of dry sample.

2.4 •DPPH Radical Scavenging Activity

The DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical-scavenging capacity was estimated using the method of Blois (1958), later modified by Brand-Williams *et al.* (1995). An aliquot of 3.9 mL of the solution of •DPPH (60 μM) in methanol was added to 0.1 mL of the extract (three different dilutions were prepared for each sample). The extracts were allowed to react with DPPH radical solution for 1 h in the dark (time required to reach the steady state). The absorbance of the reaction mixture was measured at 515 nm. A calibration curve was prepared (0 – 60 μM DPPH 60 μM) using methanol as the solvent. The results were expressed as EC_{50} (Efficient Concentration) (g/g DPPH), which is the amount of sample necessary to decrease the •DPPH concentration by 50%.

2.5 ABTS•+ Radical Scavenging Method

The determination of the antioxidant activity by the ABTS•+ method was adapted from Re *et al.*, (1999). This method is based on the ability of different components to scavenge the ABTS•+ compared to a standard antioxidant (Trolox) in a dose-response curve. A solution of ABTS•+ was prepared using the reaction mixture of 5 mL of aqueous solution of ABTS (2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt) at concentration of 7 mM and 88 μL of

140 mM $K_2S_2O_8$. The final working solution of ABTS•+ was obtained after 16 h at room temperature in the dark. This solution was then diluted with absolute ethanol to reach the absorbance of 0.70 ± 0.02 at 734 nm. In order to spectrophotometrically measure the samples, an aliquot of 1 mL of the radical solution and 10 µL of sample were mixed. Three different aqueous dilutions of each sample were prepared. Calibration curve was built using Trolox at the concentrations of 100, 250, 500, 1000, 1500, and 2000 µM in ethanol, each added to 1 mL of diluted ABTS•+ (10 min in the dark). For the control 1 mL of diluted ABTS•+ was added to 10 µL of ethanol. The absorbance was acquired at 734 nm after 10 min using a spectrophotometer Cary 50 Probe UV-Vis (Varian, Lake Forest, USA). From the different dilutions of the extracts, a regression was obtained by plotting the absorbance on the y-axis and the dilution (mg/L) on the x-axis. To calculate the antioxidant activity, the absorbance equivalent to 1,000 µM of the trolox standard (obtained from the standard curve regression equation) (Nenadis *et al.*, 2004) was substituted in the regression equation obtained for each sample. The value obtained for the term x (angular coefficient) corresponds to the dilution of the sample (mg/L) equivalent to 1,000 µM trolox. The final result was given in µmol Trolox/g.

2.6 Ferric Reduction Antioxidative Power (FRAP)

The FRAP methodology was adapted from Benzie and Strain (1996). The FRAP reagent contained 2.5 mL of a 10 mM TPTZ solution in 40 mM HCl, 2.5 mL of 20 mM $FeCl_3$, and 25 mL of 0.1 M acetate buffer (pH 3.6), which was freshly prepared. Three different dilutions were prepared for each sample. An aliquot of 100 µL of extract, 300 µL of distilled water, and 3 mL of FRAP reagent were transferred into a test tube. The obtained solutions were homogenized and incubated at 37 ºC/30 min. The absorbance was measured at 594 nm against a reagent blank (FRAP reagent). Calibration curve was prepared using working aqueous solutions of $FeSO_4.7H_2O$ in the range of 250, 500, 1000, 1500 to 2000 µM each added to 3 mL of FRAP reagent and following the homogenization and incubation steps of the samples. From the three different dilutions of the extracts, a regression was obtained by plotting the absorbance (nm) on the y-axis and the dilution (mg/L) on the x-axis. To calculate the antioxidant activity, the absorbance equivalent to 1,000 µM of the standard (obtained from the standard curve regression equation) was substituted in the

regression equation obtained for each sample. The value obtained for the term x (angular coefficient) corresponds to the dilution of the sample (mg/L) equivalent to 1,000 µM ferrous sulfate. The final result was given in µM ferrous sulfate/g.

2.7 β-Carotene Bleaching Assay (BCBA)

Antioxidant activity by β-carotene/linoleic acid was determined according with the methodology proposed by Marco (1968), with modification. An aliquot (50 µL) of the β-carotene-chloroform solution (20 mg/mL) was added to a flask (500 mL) containing 40 µL of linoleic acid and 530 µL of Tween 40, then 1 mL of chloroform was added and mixed, which was further evaporated. Then, oxygenated distilled water was added (to the flask) in order to obtain an absorbance of 0.65 ± 0.05 units at 470 nm (control solution). An aliquot (0.4 mL) of the antioxidant extract was added to 5 mL of the control solution (CS) and the absorbance was immediately read at 470 nm (Abs_i); also the absorbance of the pure CS (Abs_i). After the first reading, the tubes containing both the samples and the CS were then incubated in a water bath at 40 °C/2 h. The measurements were performed once again after the water bath (Abs_f) using a spectrophotometer Cary 50 Probe UV-Vis (Varian, Lake Forest, USA). The oxygenated water was used as the blank. The antioxidant activity was calculated as the protection (%) relative to the control (Equations 2, 3, and 4).

$$\text{Reduction (nm)} = Abs_i - Abs_f \tag{2}$$

$$Oxidation\ (\%) = \left(\frac{Reduction\ of\ the\ sample}{Reduction\ of\ the\ CS}\right) \times 100 \tag{3}$$

$$Protection\ (\%) = 100 - Oxidation \tag{4}$$

2.8 Phenolic profile

Samples of extracts (20 µL), previously filtered through a micromembrane (0.45 µm), were separated by reverse phase chromatography at 15°C (Shimadzu, GCMA-QP2010 Plus, Tokyo, Japan), using a Nova-Pak C_{18} column (5 µm x 250 mm x 4.6 mm). DAD (Photodiode Array Detector) was established from 210 to 360 nm. The analysis parameters were set according with (Oliveira *et al.*, 2018). Quantitative determination was performed using the method of external standards with commercial standards (gallic acid, catechin, chlorogenic acid, caffeic acid, vanillin, ferulic acid, *p*-coumaric acid, *o*-coumaric acid, *m*-coumaric acid, resveratrol, and *trans*-cinnamic acid) (Supelco, Pennsylvania, USA). Calibration curves were obtained by injection of these standard solutions under the same conditions of the analyzed compounds.

2.9 Statistical analysis

A completely randomized design was used to plan the study and an analysis of variance (Anova) was carried out in order to evaluate the interaction among solvent (ethanol and methanol-acetone) and material types (GCB and GCC). The Scott Knott's test was used in order to observe the difference among the means, once this test is robust concerning the violation of data normality. RStudio software version 3.2.0 was used (R Core Team, 2015). A p-value < 0.05 was considered to indicate a statistically significant difference. In addition, a Principal Component Analysis (PCA) in order to examine the most representative form of the data from linear combinations of the original variables; the TPC, •DPPH, ABTS•+, FRAP, and BCBA assays were analyzed through PCA, also a correlation analysis using the Pearson's method was performed.

3. Results and discussion

3.1 Antioxidant activity, total phenolic compounds and phenolic profile

The results showed that different solvents had significant effects on the antioxidant compounds yield from GCB and GCC. The analysis of variance showed that the interaction between the type of solvent used to extract the target compounds and the type of material was significant at $p < 0.05$ for most of the response variables, such as for TPC ($p = 0.00074$; CV =

1.61%), •DPPH (p = 0.0003; CV = 6.56%), ABTS•+ (p = 0.0000002; CV = 8.68%), and BCBA (p = 0.000001; CV = 2.92%) indicating that the polarity of the solvent and the vegetable matrix are relevant input variables for the extraction process of bioactives.

The total phenols in GCB and GCC samples were estimated by using the Folin-Ciocalteu's colorimetric method. The content of all flavonoids, caffeic acid derivatives, and tannins are assayed by this method (Waterman and Mole, 1994). Results indicated that, overall, the cake contained the highest concentration of total phenols in either ethanolic or methanol-acetone extract than the respective green beans (Table 1).

Table 1. Antioxidant activity of green coffee beans (GCB) and its press cake (GCC).

Material	Extractor	TPC (mg GAE/100 g)	• DPPH (EC_{50}) (g/g DPPH)	ABTS•+ (µmol Trolox/g)	FRAP (µmol Ferrous sulphate/g)	BCBA (%Protection)
GCB	Ethanol	2059.84 ± 56.0^{b2}	39.40 ± 2.96^{a1}	1068.19 ± 3.29^{a2}	2500.47±165.29	77.74 ± 0.0^{a1}
	Methanol+Acetone	10156.62 ± 18.32^{B1}	16.92 ± 0.02^{A2}	25423.96 ± 93.1^{A1}	8913.24±385.69	71.78 ± 3.44^{A2}
GCC	Ethanol	3486.51 ± 223.26^{a2}	$28.94\pm0.01^{b\mathit{1}}$	1552.55 ± 76.81^{a2}	4308.73±236.95	47.84 ± 1.33^{b2}
	Methanol+Acetone	$13108.29\pm16.75^{A\mathit{1}}$	18.48 ± 1.68^{A2}	$23832.12\pm59.86^{A\mathit{1}}$	11076.88±531.53	$71.01\pm4.14^{A\mathit{1}}$

Data is expressed as mean ± standart deviation (n = 3).
Means in the same column followed by different lower case letters $^{(a\text{-}b)}$ (unfolding of the variable material into the ethanol solvent level) or by different upper case letters $^{(A\text{-}B)}$ (unfolding of the variable material into the methanol+acetone solvent level) are significantly different by the Skott Knott's test ($p < 0.05$).
Means in the same column followed by different arabic numbers $^{(1\text{-}2)}$ (unfolding of the variable solvent into the GCB level) or by different arabic numbers in Italic $^{(\mathit{1}\text{-}2)}$ (unfolding of the variable solvent into the GCC level) are significantly different by the Skott Knott's test ($p < 0.05$).

Different solvent types were found to have expressive influence on the TPC of both samples analyzed. Phytochemicals have distinct polarities, thus, they can be extracted by different solvents, which can determine difference in type, composition, and antioxidant activity of these compounds (Dehkharghanian *et al.*, 2010). The TPC of methanol-acetone extract was found to be about five times higher than the ethanolic extract, and the content in the GCC extract was approximately 30% greater than in the GCB extract. As for the ethanolic extract, the GCC presented about 70% more of TPC content than the GCB. In general, the content of TPC in regular GCB (d.b.) reported in the literature ranges from 4 to 8.4% for *Coffea arabica*, and from 7 to 14.4% for *Coffea canephora*, with some hybrids presenting intermediate levels (De Maria, Trugo, Moreira, & Werneck, 1994; Farah, Paulis, Trugo, & Martin, 2005; Ky et al., 2001; Rodríguez-Carpena, Morcuende, Andrade, Kylli, & Estévez, 2011).

Widyawati *et al.* (2014) reported that ethanol extracted sterol, flavonoid, phenolic, and alkaloid from *Pluchea indicia* more efficiently. Previous researches reported that alcoholic

solvents, such as methanol and ethanol, are able to solubilize phenolics with molecular weights ranging from low to medium and medium polarity (Lin *et al.*, 2009), anthocyanin, terpenoid, saponin, tannin, xantoxilin, quacinoid, lacton, flavone, phenone, and polyphenol (Cowan, 1999), aglycon flavonoid (Dehkharghanian *et al.*, 2010), and polar compounds, such as sugar, amino acid, and glycoside compounds (Houghton and Raman, 1998).

In their studies on the use of spent coffee grounds, Zuorro and Lavecchia (2012) proposed the extraction of phenolic compounds using ethanol/water solution. The percentage of ethanol in the solvent influenced the efficiency of the extraction of phenolic compounds obtained in the final extract, thus demonstrating the possibility of optimization of the extraction of bioactive compounds from this agroindustrial co-product.

Effectivity of methanol along with acetone in extracting phytochemical compounds from GCC was supported by total phenol and antioxidant assays. TPC extracted with the methanol and acetone mixture were efficient to donating atomic hydrogen to molybdenum ion in Folin-Ciocalteau phenol's reagent resulting in phenoxyl radicals stabilized by resonance or delocalization (Wong *et al.*, 2006). According with these authors, effectiveness of TPC depend on the type, number, structure, and position of hydroxyl group of the benzene ring. On the other hand, Siva *et al.* (2016) reported lower yield when a methanol/water (60:40) mixture was used to extract antioxidants from GCB compared to an alcoholic solvent (isopropanol/water).

TPC from coffee residues (coffee pulp, husk, silver skin, and spent coffee) were extracted using a mixture of isopropanol and water by Murthy and Naidu (2012). The by-products contained about 1 – 1.5% (w/w GA) total phenolic compounds with the highest yield for pulp, followed by silver skin (1.32%) and cherry husk (1.22%) when pretreated with viscozyme. Thus, phenolic-rich extracts can be obtained from coffee waste using an environmentally friendly and simple solvent-extraction procedure, as well as it was reported by Affonso et al. (2016), who obtained higher phenolic concentration for the GCC aqueous extract (3539 mg GAE/ 100 g), which was slightly higher than the result found in this study when ethanol was used as an ecofriendly solvent. TPC of GCB and GCC could be related with the antioxidant activity; Nakiboglu *et al.* (2007) reported that the ability of phytochemicals to donate hydrogen atom/electron could be essential to measure antioxidant capacity. Thus, it is possible that the prior extraction of the apolar fraction (oil) from the GCB allows obtaining a higher yield of phenolic compounds in the extract of the residual press

cake, once the polar fraction extracted during the mechanical pressing may not be yielded with phenolic compounds, also, the pressing may help the extraction of these compounds by breaking the beans cell wall making them available for extraction. Further, in the temperature conditions where the extraction processes were carried out (room temperature), where the solubility of the solvents are limited, the higher oil content in the GCB may have interfered the extraction of other compounds. After the harvest and along the coffee bean processing, some phenolic compounds can be isomerized, hydrolyzed, or degraded into low molecular weight compounds (Affonso *et al.*, 2016).

The antioxidant activity of each sample was evaluated using the •DPPH, ABTS•+, FRAP, and BCBA assays (Table 1). There is a variety of *in vitro* methods to assess the antioxidant activity of foods, plant origin preparations, and other substances; where most of them are based on the single electron (SET) and hydrogen atom (HAT) transfer reactions (Pokorná *et al.*, 2015). Their response depends on a range factors, such as pH, lipophilic and/or hydrophilic compounds, among others. Hence, in spite of the principle of each group of the antioxidant assay methods is similar, it is indicated to employ more than one technique in order to evaluate the antioxidant capacity of a matrix, especially when such phytochemicaly complex matrices as coffee beans products are being studied (Pokorná *et al.*, 2015). According to the assays, the GCC showed an increased antioxidant activity compared with the respective GCB (Table 1).

The 1,1-diphenyl-2-picrylhydrazyl (DPPH) test determines the antioxidant potential of phenolic compounds isolated or present in food and other biological samples. It is based on the ability of the stable free radical, •DPPH, to react with H^+ donor compounds, which may disrupt oxidative chain reactions (Brand-Williams *et al.*, 1995). For •DPPH analysis, once again the methanol-acetone extracts showed higher antioxidant activity by presenting the lowest EC_{50} values, however there was no significant difference between the two types of materials. Ramalakshmi *et al.* (2009) evaluated the antioxidant and anticarcinogenic activity of methanolic green coffee extracts of low-grade green coffee and spent coffee, which were found to have significant potential to scavenge the •DPPH (92 and 86.9%, respectively, for Arabica variety), and also anti-tumor activity. From the characterization of aqueous and alcoholic extracts of coffee grounds, Panusa *et al.* (2013) concluded that this residue, generated in large quantities throughout the world, is a source

of natural antioxidants; the analytical determinations showed the presence of phenolic compounds, chlorogenic acids, and flavonoids.

The ABTS•+ method allows the assessment of the antioxidant activity of the range of carotenoids, phenolics, and some plasma antioxidants (Re *et al.*, 1999). The differences between the •DPPH and ABTS•+ assays may be concerned to the varying reactivity of these radicals to the components of each coffee sample. In this work, the methanol-acetone extract presented the highest antioxidant activity when evaluated through the ABTS•+ assay, and there was no significant difference for either of the materials when extracted with ethanol or methanol-acetone (Table 1). Nenadis *et al*. (2004) observed that the substances containing at least one hydroxyl group in the aromatic ring were potentially active towards ABTS•+. Furthermore, the relative activity differences among the compounds, in comparison to those observed in the •DPPH assay, were rather suppressed. They also reported that an increase in the number of hydroxyl groups in the aromatic ring did not necessarily lead to an increase of the ABTS•+ assay values.

The FRAP assay identifies antioxidants that are able of a SET; on the other hand, the •DPPH assay allows the measuring SET and HAT antioxidants (Prior *et al.*, 2005), which makes it difficult to obtain a good agreement between the techniques. This assay showed no interaction of solvent and material types. The analysis of variance did not result significant for the interaction ($p = 0.51$), therefore, only the single effect of each variable was evaluated.

Concerning the FRAP assay, which, as mentioned above, measures antioxidants capable only of a SET, the trend observed in this work was followed, where the methanol-acetone extracts presented higher antioxidant activity (9995.06 μmol Fe(II)/ g) than the ethanolic extracts (3404.6 μmol Fe(II)/ g), as well as GCC (8369.62 μmol Fe(II)/ g) compared to GCB (6348.13 μmol Fe(II)/ g). Although, they are not dependent on each other, once there was no significative interaction between them. Martinez et al. (2013) reported 36.27 μM Fe(II)/ L in the FRAP assay for a methanolic extract obtained from Arabica green beans. The antioxidant phenolic compounds from spent coffee grounds were extracted by Mussatto *et al.* (2011) with 60% methanol (40 mL/ g solvent/ solid ratio, 90 min) producing a phenolic extract with 16 mg GAE/ g and with 0.10 mM Fe(II)/ g, simultaneously.

In order to enclose the most relevant current *in vitro* assays applied to evaluate the antioxidant activity of vegetable matrices, the BCBA was included in this study, once it is an assay

that is able to identify antioxidants mostly in hydrophobic environments. The BCBA clearly showed that the ethanolic extracts presented higher activity for GCB rather than GCC, reflecting the ability of this solvent to extract non polar compounds better than the methanol-acetone extracts, once the GCB are likely to have greater content of oil than GCC.

Also, there was no significative difference ($p < 0.05$) among the types of materials studied when methanol-acetone was used, demonstrating, in this case, that the antioxidant compounds (mainly hydrophilic) remained in the meal residue. This result may be an indicative of that the lipophilic antioxidant compounds may have been extracted along with the oil during the mechanical process, and these compounds are preferably extracted with ethanol.

Prieto and Vázquez (2014) also tested two different methods in order to assess the antioxidant activity of hydrophilic (Crocin bleaching assay) and lipophilic (BCBA) antioxidants from GCB extracts and observed that all coffee beans samples (Arabica and Robusta) encouraged the antioxidant capacity in both lipophilic and hydrophilic environments, however, the hydrophilic environments exhibited the highest activities.

As it can be seen, the extraction solvent plays an important role in the process as it affects the antioxidant capacity and TPC in food matrices.

An HPLC system was used to separate, identify, and quantify phenolic compounds in the GCB and GCC extracts. In this work, the GCC presented higher amount of phenolic compounds than the actual GCB, as it was previously shown in Table 1 and now, in Table 2.

Table 2. Phenolic profile of green coffee beans (GCB) and its press cake (GCC) (mg/100 g d.b.)

Phenolic compound	GCB	GCC
Gallic acid	0.013	0.050
Catechin	1.356	3.284
Chlorogenic acid	55.156	64.958
Caffeic acid	25.073	44.375
Vanillin	0.591	0.943
p-Coumaric	-	0.004
Ferulic acid	0.009	0.021
m-Coumaric	-	0.002
o-Coumaric	1.326	2.177
Resveratrol	0.165	0.316
Trans-cinnamic acid	0.082	0.163

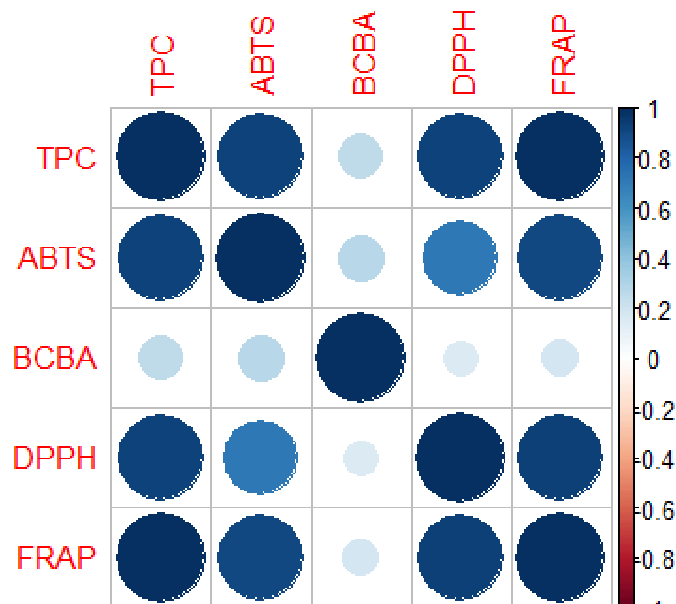
Showing once again, that the phenolic compounds are not being extracted along with the oil and that this waste may be a valuable industrial input. Moreover, the methanol-acetone extracts exhibited the highest antioxidant activity for most of the assays, thus, the phenolic profile was only assessed for this type of extract. The major phenolic compounds identified in both materials were chlorogenic and caffeic acids, followed by catechin and *o*-coumaric acid. Also, the acids *p*-coumaric and *m*-coumaric were found only in the GCC, in very small contents. Esquivel and Jiménez (2012) also reported chlorogenic acid as the major phenolic in Arabica green beans.

The antioxidant activity of GCB and GCC extracts was contributed by the phytochemical compounds content. This research shows that extracts rich in phenolic compounds can be obtained from the GCC with a solvent of simple handling, environmentally safe and from a renewable source, such as ethanol.

3.2 Correlation analysis and Principal Component Analysis

A Pearson's correlation matrix involving all response variables (•DPPH, ABTS•+, FRAP, BCBA, and TPC) ($p < 0.01$) is shown in Figure 1.

Figure 1. Pearson's correlation matrix involving all response variables (•DPPH, ABTS•+, FRAP, BCBA, and TPC) ($p < 0.01$).



Specifically, the correlation coefficient ($p < 0.01$) was 0.92 between TPC and •DPPH, 0.92 between TPC and ABTS•+, 0.99 between TPC and FRAP, 0.71 between ABTS•+ and •DPPH, 0.9 between ABTS•+ and FRAP, and 0.93 between •DPPH and FRAP. The coefficient between the other variable (BCBA) was not significant, neither at 1 nor at 5% of significance. The correlations between the TPC and both the free radical scavenging assays, •DPPH and ABTS•+, and also FRAP, indicated that there was an association between the total amount of polyphenols and the antioxidant activity, that is, higher amounts of polyphenols led to enhanced antioxidant potency. This trend was also observed by Priftis *et al*. (2015) who investigated the antioxidant activity of green and roasted coffee beans extracts. In addition, the correlation between •DPPH, ABTS•+, and FRAP suggests that the same compounds of the extracts are likely responsible for the scavenging of both free radicals and for the reducing power of the ferric ion.

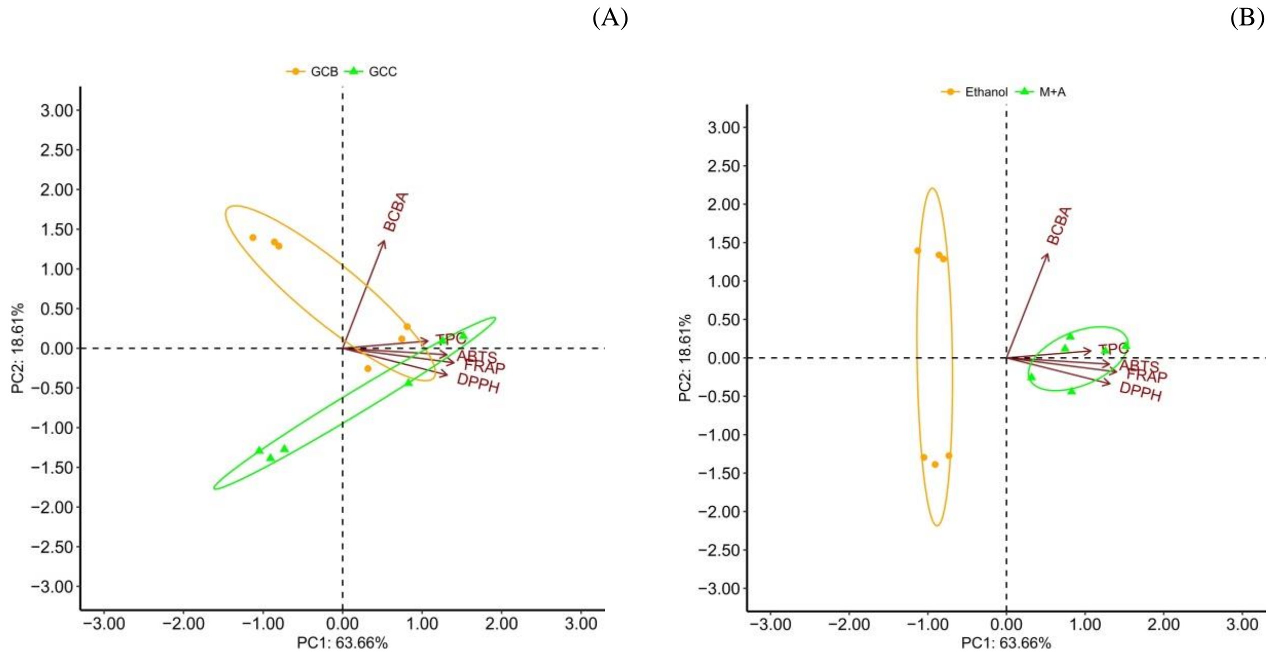
PCA has been applied to evaluate differences between GCB and GCC antioxidant activity in each type of solvent. Figure 2 shows two main principal components (PCs) characterizing the antioxidant capacities (TPC, •DPPH, ABTS•+, FRAP, and BCBA) of the extracts obtained from the GCB and GCC (Figure 2A) with different solvents (Figure 2B) with a cumulative explained total variance of 82.27% for both of them.

The first principal component (PC1) had the highest eigenvalue of 4.66, and accounted for 63.66% of the variability in the data set. The second PC (PC2) had eigenvalue of 0.25 and accounted for 18.61% of the variance in the data. For both the material and solvent type, the remaining three generated PCs yielded progressively smaller eigenvalues and did not explain significant variability in the data (< 3% total). PCA performed on the complete data set of samples confirmed that GCC formed a separate cluster in the PC1 versus PC2 plot (82.27% of total system variability).

Thus, it can explain that GCC contains the highest content of antioxidants extracted by all of the assays, except for BCBA. Simultaneously, solvent type influenced on the differences in PCA, and this was confirmed in a separate cluster. Moreover, it was noted that the results from both •DPPH and ABTS•+ assays did correlate with the TPC and FRAP analyzes (Figure 1). Indeed, it was found positive correlation for these analyzes as shown in Figure 2.

Figure 2. Principal Component Analysis (PCA) evaluating the response variables for the material (A) and solvents (B). GCB: Green coffee beans; GCC: Green coffee cake.

(A) (B)



4. Conclusion

The extraction solvent played an important role in the antioxidant extraction process by affecting the antioxidant capacity and total phenolic content in the vegetable matrices analyzed, GCB and GCC. The solvent system methanol-acetone was found to be the most effective to recovering hydrophilic antioxidant components from the materials, and GCC exhibited higher antioxidant capacity compared to GCB. When the BCBA assay was carried out, the solvent ethanol seemed to be a better option to extract antioxidants from GCB. It was found positive correlation for •DPPH, ABTS•+, TPC, and FRAP analyzes, where the highest correlations were found for TPC - FRAP, ABTS - DPPH, and TPC - FRAP. The antioxidant activity of GCB and GCC extracts was contributed by the phytochemical compounds content. This research shows that extract rich in phenolic compounds can be obtained from the GCC with a solvent of simple handling, environmentally safe and from a renewable source. Also, GCC is a valuable source of bioactive compounds that should be taken into consideration, once this industrial by-product has not been given the enough attention so far. This study highlighted GCC, an underutilized source of industrial

waste, as an up-and-coming source of industrially important extractable bioactives, while also demonstrating the importance of selecting appropriate solvents and assays in order to extract bioactives.

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REFERENCES

Affonso, R.C.L., Voytena, A.P.L., Fanan, S., Pitz, H., Coelho, D.S., Horstmann, A.L., Pereira, A., Uarrota, V.G., Hillmann, M.C., Varela, L.A.C., Ribeiro-do-Valle, R.M. & Maraschin, M. (2016). Phytochemical composition, antioxidant activity, and the effect of the aqueous extract of coffee (*Coffea arabica* L.) bean residual press cake on the skin wound healing. *Oxidative Medicine and Cellular Longevity*, **2016**, 1–10.

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

Benzie, I.F.F. & Strain, J.J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of "Antioxidant Power": The FRAP assay. *Analytical Biochemistry*, **239**, 70–76.

Blois, M.S. (1958). Antioxidant determinations by the use of a stable free radical. *Nature*, **181**, 1199–1200.

Brand-Williams, W., Cuvelier, M.E. & Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *LWT - Food Science and Technology*, **28**, 25–30.

Cowan, M.M. (1999). Plant products as antimicrobial agents. *Clinical Microbiology Reviews*, **12**, 564–82.

Dehkharghanian, M., Adenier, H. & Vijayalakshmi, M.A. (2010). Study of flavonoids in aqueous spinach extract using positive electrospray ionisation tandem quadrupole mass spectrometry. *Food Chemistry*, **121**, 863–870.

Derakhshan, Z., Ferrante, M., Tadi, M., Ansari, F., Heydari, A., Hosseini, M.S., Conti, G.O. & Sadrabad, E.K. (2018). Antioxidant activity and total phenolic content of ethanolic extract of pomegranate peels, juice and seeds. *Food and Chemical Toxicology*, **114**, 108–111.

Esquivel, P. & Jiménez, V.M. (2012). Functional properties of coffee and coffee by-products. *Food Research International*, **46**, 488–495.

Farah, A., Paulis, T. de, Trugo, L.C. & Martin, P.R. (2005). Effect of roasting on the formation of chlorogenic acid lactones in coffee. *Journal of Agricultural and Food Chemistry*, **53**, 1505–1513.

Franca, A.S. & Oliveira, L.S. (2009). Coffee processing solid wastes: Current uses and future perspectives. In: *Agricultural wastes* (edited by G. Ashworth & P. Azevedo). New York: Nova Science Publishers.

Galanakis, C.M. (2017). *Handbook of coffee processing by-products: Sustainable applications*. 1st edn. London: Academic Press.

Houghton, P.J. & Raman, A. (1998). *Laboratory Handbook for the Fractionation of Natural Extracts*. *Laboratory Handbook for the Fractionation of Natural Extracts*. Boston, MA: Springer US.

Ky, C.-L., Louarn, J., Dussert, S., Guyot, B., Hamon, S. & Noirot, M. (2001). Caffeine, trigonelline, chlorogenic acids and sucrose diversity in wild *Coffea arabica* L. and *C. canephora* P. accessions. *Food Chemistry*, **75**, 223–230.

Landete, J.M. (2013). Dietary Intake of Natural Antioxidants: Vitamins and Polyphenols. *Critical Reviews in Food Science and Nutrition*, **53**, 706–721.

Larrauri, J.A., Rupérez, P. & Saura-Calixto, F. (1997). Effect of drying temperature on the stability of polyphenols and antioxidant activity of red grape pomace peels. *Journal of Agricultural and Food Chemistry*, **45**, 1390–1393.

Laufenberg, G., Kunz, B. & Nystroem, M. (2003). Transformation of vegetable waste into value added products: (A) the upgrading concept; (B) practical implementations. *Bioresource Technology*.

Lin, H.-Y., Kuo, Y.-H., Lin, Y.-L. & Chiang, W. (2009). Antioxidative effect and active components from leaves of lotus (*Nelumbo nucifera*). *Journal of Agricultural and Food Chemistry*, **57**, 6623–6629.

Marco, G.J. (1968). A rapid method for evaluation of antioxidants. *Journal of the American Oil Chemists? Society*, **45**, 594–598.

Mareček, V., Mikyška, A., Hampel, D., Čejka, P., Neuwirthová, J., Malachová, A. & Cerkal, R. (2017). ABTS and DPPH methods as a tool for studying antioxidant capacity of spring barley and malt. *Journal of Cereal Science*, **73**, 40–45.

Maria, C.A.B. De, Trugo, L.C., Moreira, R.F.A. & Werneck, C.C. (1994). Composition of green coffee fractions and their contribution to the volatile profile formed during roasting. *Food Chemistry*, **50**, 141–145.

Martinez-Saez, N., García, A.T., Pérez, I.D., Rebollo-Hernanz, M., Mesías, M., Morales, F.J., Martín-Cabrejas, M.A. & Castillo, M.D. del. (2017). Use of spent coffee grounds as food ingredient in bakery products. *Food Chemistry*, **216**, 114–122.

Martinez, H.E.P., Poltronieri, Y., Farah, A. & Perrone, D. (2013). Zinc supplementation, production and quality of coffee beans. *Revista Ceres*, **60**, 293–299.

Moure, A., Cruz, J.M., Franco, D., Domínguez, J.M., Sineiro, J., Domínguez, H., José Núñez, M.

& Parajó, J.C. (2001). Natural antioxidants from residual sources. *Food Chemistry*, **72**, 145–171.

Murthy, P.S. & Naidu, M.M. (2012). Recovery of phenolic antioxidants and functional compounds from coffee industry by-products. *Food and Bioprocess Technology*, **5**, 897–903.

Mussatto, S.I., Machado, E.M.S., Martins, S. & Teixeira, J.A. (2011). Production, composition, and application of coffee and its industrial residues. *Food and Bioprocess Technology*, **4**, 661–672.

Nakiboglu, M., Urek, R.O., Kayali, H.A. & Tarhan, L. (2007). Antioxidant capacities of endemic *Sideritis sipylea* and *Origanum sipyleum* from Turkey. *Food Chemistry*, **104**, 630–635.

Nenadis, N., Wang, L.-F., Tsimidou, M. & Zhang, H.-Y. (2004). Estimation of scavenging activity of phenolic compounds using the ABTS $^{•+}$ assay. *Journal of Agricultural and Food Chemistry*, **52**, 4669–4674.

Oliveira, É.R., Caliari, M., Soares Júnior, M.S., Oliveira, A.R., Duarte, R.C.M. & Vilas Boas, E.V.B. (2018). Assessment of chemical and sensory quality of sugarcane alcoholic fermented beverage. *Journal of Food Science and Technology*, **55**, 1–10.

Pandey, K.B. & Rizvi, S.I. (2009). Plant polyphenols as dietary antioxidants in human health and disease. *Oxidative Medicine and Cellular Longevity*, **2**, 270–8.

Panusa, A., Zuorro, A., Lavecchia, R., Marrosu, G. & Petrucci, R. (2013). Recovery of natural antioxidants from spent coffee grounds. *Journal of Agricultural and Food Chemistry*, **61**, 4162–4168.

Phaniendra, A., Jestadi, D.B. & Periyasamy, L. (2015). Free radicals: Properties, sources, targets, and their implication in various diseases. *Indian Journal of Clinical Biochemistry*, **30**, 11–26.

Pokorná, J., Venskutonis, P.R., Kraujalyte, V., Kraujalis, P., Dvořák, P., Tremlová, B., Kopřiva, V. & Ošťádalová, M. (2015). Comparison of different methods of antioxidant activity evaluation of green and roast *C. arabica* and *C. robusta* coffee beans. *Acta Alimentaria*, **44**, 454–460.

Prieto, M.A. & Vázquez, J.A. (2014). In vitro determination of the lipophilic and hydrophilic antioxidant capacity of unroasted coffee bean extracts and their synergistic and antagonistic effects. *Food Research International*, **62**, 1183–1196.

Priftis, A., Stagos, D., Konstantinopoulos, K., Tsitsimpikou, C., Spandidos, D.A., Tsatsakis, A.M., Tzatzarakis, M.N. & Kouretas, D. (2015). Comparison of antioxidant activity between green and roasted coffee beans using molecular methods. *Molecular Medicine Reports*, **12**.

Prior, R.L., Wu, X. & Schaich, K. (2005). Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. *Journal of Agricultural and Food Chemistry*, **53**, 4290–4302.

Quideau, S., Deffieux, D., Douat-Casassus, C. & Pouységu, L. (2011). Plant polyphenols: Chemical properties, biological activities, and synthesis. *Angewandte Chemie International Edition*, **50**, 586–621.

R Core Team. (2015). R: A language and environment for statistical computing.

Ramalakshmi, K., Rao, L.J.M., Takano-Ishikawa, Y. & Goto, M. (2009). Bioactivities of low-grade green coffee and spent coffee in different in vitro model systems. *Food Chemistry*, **115**, 79–85.

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M. & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine*, **26**, 1231–1237.

Reddy Palvai, V., Mahalingu, S., Urooj, A. & Cristina Breschi, M. (2014). *Abrus precatorius* leaves: Antioxidant activity in food and biological systems, pH, and temperature stability. *International Journal of Medicinal Chemistry*, 1–7.

Rodríguez-Carpena, J.-G., Morcuende, D., Andrade, M.-J., Kylli, P. & Estévez, M. (2011). Avocado (*Persea americana* Mill.) phenolics, in vitro antioxidant and antimicrobial activities, and inhibition of lipid and protein oxidation in porcine patties. *Journal of Agricultural and Food Chemistry*, **59**, 5625–5635.

Santos, M.C.P. & Gonçalves, É.C.B.A. (2016). Effect of different extracting solvents on antioxidant activity and phenolic compounds of a fruit and vegetable residue flour. *Scientia agropecuaria*, **7**, 07-14.

Santos, P., Aguiar, A.C., Viganó, J., Boeing, J.S., Visentainer, J. V & Martínez, J. (2016). Supercritical CO2 extraction of cumbaru oil (Dipteryx alata Vogel) assisted by ultrasound: Global yield, kinetics and fatty acid composition. *The Journal of Supercritical Fluids*, **107**, 75–83.

Scully, D., Jaiswal, A. & Abu-Ghannam, N. (2016). An Investigation into spent coffee waste as a renewable source of bioactive compounds and industrially important sugars. *Bioengineering*, **3**, 33.

Siva, R., Rajikin, N., Abdul Haiyee, Z. & Iryani Wan Ismail, W. (2016). Assessment of antioxidant activity and total phenolic content from green coffee robusta sp. beans. *Malaysian Journal of Analytical Sciences*, **20**, 1059–1065.

Tono, T., Fujita, S. & Kawabe, M. (1989). Determination of chlorogenic acid in coffee samples

by difference spectral method and DEAEToyopearl column chromatography. *Nippon Shokuhin Kogyo Gakkaishi*, **36**, 587–591.

Waterhouse, A.L. (2002). Wine phenolics. *Annals of the New York Academy of Science*, **957**, 21–36.

Waterman, P.G. & Mole, S. (1994). *Analysis of phenolic plant metabolites*. Oxford: Blackwell Scientific Publications.

Widyawati, P.S., Dwi, T., Budianta, W., Kusuma, F.A. & Wijaya, E.L. (2014). Difference of solvent polarity to phytochemical content and antioxidant activity of *Pluchea indicia* Less leaves extracts. *International Journal of Pharmacognosy and Phytochemical Research*, **6**, 850–855.

Wolfe, K., Wu, X. & Liu, R.H. (2003). Antioxidant activity of apple peels. *Journal of Agricultural and Food Chemistry*, **51**, 609–614.

Wong, S.P., Leong, L.P. & Koh, J.H.W. (2006). Antioxidant activities of aqueous extracts of selected plants. *Food Chemistry*, **99**, 775–783.

Zuorro, A. & Lavecchia, R. (2012). Spent coffee grounds as a valuable source of phenolic compounds and bioenergy. *Journal of Cleaner Production*, **34**, 49–56.

ARTIGO 3 - POTENTIAL OF ALTERNATIVE SOLVENTS TO EXTRACT BIOLOGICALLY ACTIVE COMPOUNDS FROM GREEN COFFEE BEANS AND ITS RESIDUE FROM THE OIL INDUSTRY

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ABSTRACT

Food waste causes great impact on society and the environment. Residues disposal is observed throughout the production chain, which establishes a need to use both food as a whole and the possibility of maintaining habitual consumption with the use of residues for new food products or in another industrial branch. In this perspective, this study aimed to evaluate the role of selected traditional organic solvents the extraction of total soluble solids and bioactives from green coffee beans and its press meal, as an alternative to make use of this waste generated by the oil industry. Six solvents with different polarities were used (acetone, ethanol, ethyl acetate, hexane, isopropanol, and petroelum ether) in order to evaluate the yield of soluble solids and bioactive activity (Folin-Ciocalteau's method, DPPH, ABTS, FRAP, and β-carotene bleaching assay - BCBA) of green coffee beans and its industrial press meal. The results showed that the tested solvents played an important role in the extraction of total solids and antioxidant capacity from the matrices analyzed. Ethanol was found to be the optimal extraction solvent for extractable solids from both green coffee beans and its press meal, as well as for phenolic and antioxidant compounds. Only that, for BCBA analysis, ethyl acetate yielded the best results for the antioxidant activity of green beans and meal. Therefore, ethanol is recommended for the extraction of soluble solids, phenolic, and antioxidant compounds from green coffee beans and its press meal for further isolation and utilization.

Keywords: biorenewable solvents; green coffee; press cake; ethanol; solid-liquid extraction; bioactives.

1. INTRODUCTION

Extraction solvent plays an important role that affects the antioxidant capacity and total phenolic content in food materials (Siva et al., 2016). Solid–liquid extraction method of phenolic compounds with different solvents from vegetable sources are the most commonly used for isolating these compounds (Alothman et al., 2009; Cottica et al., 2011). Crude phenolic extracts contain complex mixtures of some classes of phenols, which are selectively soluble in the different solvents. In this sense, solvent polarity plays a key role in increasing phenol solubility (Naczk and Shahidi, 2006). The recovery of phenolic compounds is dependent on the solvent used in their extraction and its polarity (Alothman et al., 2009).

Naczk and Shahidi (2006) identified a group of factors that influence the quantification of phenolics in plant materials. The chemical nature of the phenolic compounds, the extraction method employed and the assay method were some of those factors. Compounds with antioxidant activity can preserve flavor and color, avoid vitamin destruction in foods and more importantly protect living systems from oxidative damage (Moure et al., 2001). Several scientific studies have proved the capacity of antioxidants to protect cells from free radical damage and to preserve severe diseases (Gaulejac et al., 1999; Moure et al., 2001). Antioxidants are found in fruits such as prunes (Donovan et al., 1998), berries (Abuja et al., 1998; Heinonen et al., 1998a; Heinonen et al., 1998b; Kähkönen et al., 2001; Kim et al., 2013), and coffee (Esquivel and Jiménez, 2012; Gawlik-Dziki et al., 2014; Jaiswal et al., 2012; Kamiyama et al., 2015; Mills et al., 2013; Rodrigues et al., 2015; Smrke et al., 2013; van der Werf et al., 2014). They are also available in various forms such as phenolics, flavonoids, coumarins, xanthons, lignans, tannins, curcumanoids, tocopherol, lycopene and β-carotene (Mohdaly et al., 2010).

Antioxidants are extremely crucial in coffee beans to minimize the release of reactive oxygen species (ROS). If the production of ROS exceeds the capacity of antioxidant systems, damage to sub-cellular compartments occurs, leading to cell destruction (Davey et al., 2000). Hence it is important to maintain the antioxidant and total phenolic pool so that the integrity of green coffee beans is not compromised. The green beans are bioactively rich, being chlorogenic acids, caffeine, diterpenes (mainly kawheol and cafestol), trigonelline, and tocopherols (α, β, γ) the most relevant compounds (Esquivel and Jiménez, 2012; Şemen et al., 2017; Speer and Kölling-

Speer, 2006). This would enable the usage of the beans extracts, which have been widely prospected for their antioxidant potential, to be used as ingredients in pharmaceutical industry or as food supplement (i.e. chlorogenic acids imply positive impact on slimming and weight loss) (Siva et al., 2016).

Coffee is one of the world's largest fruit crops with a global production of around 158.93 millions of 60 kg bags in 2017 (ICO, 2018). This also generates an enormous amount of waste, including coffee pulp, husk, silver skin, and spent coffee. Coffee waste has a heavy environmental impact due to the high content of phenols that considerably increase chemical and biochemical oxygen demands (Spigno et al., 2007). Among these by-products from the coffee industry there is the press coffee biomass generated from the oil mechanical expelling process. This by-product is still containing a substantial content of oil and also presents a great content of bioactive compounds, once the pressing process is not efficient to remove completely the oil present in the beans; also, the bioactive compounds, which are hydrophilic compounds, remaining mainly in the biomass (Oliveira et al., 2018). Thus, this is an economically valuable industrial output that can be exploited and might have further application interests. In order to exploit this by-product, the solid-liquid extraction may be an interesting proposal.

Solvent extraction has been widely adapted for economical and practical concerns. The matter is washed usually with hexane. Thereafter, the hexane is separated from oil by evaporation (Serrato, 1981). Hexane has been widely used for oil extraction because of easy oil recovery, narrow boiling point (63 – 69 °C) and excellent solubilizing ability (Liu and Mamidipally, 2005). However, hexane is released into the environment and reacts with the pollutants to form ozone and photochemicals (Hanmoungjai et al., 2000), and it is proved to affect the neural system when inhaled by humans because of its solubility in neural lipids. Toxicity has been observed in piglets fed with defatted meal containing residual hexane which was left over after the process (EPA, 2005). Therefore, health perspective, safety and environment concerns have triggered to look for a substitute to n-hexane without compromising the yield of oil. Hence, green solvents coupled with technology are a viable alternative for oil extraction (Jeevan Kumar et al., 2017). Green solvents are aimed to develop an environment friendly process with simultaneous reduction of pollutants (Anastas and Warner, 1998; Wan et al., 1995) for oil extraction.

Green solvents are derived either from naturally or agricultural residues or petroleum sources, which have good solubilizing properties like conventional solvents. Recent advances on green approaches have great impetus in oil industry because of green solvents (Jeevan Kumar et al., 2017). Hence, green solvents, such as ethanol, acetone, ethyl acetate, and isopropanol have huge potential to replace n-hexane without any compromise in oil recovery from the process, as previously reported by a range of authors (Castro et al., 2018; Mazzafera et al., 1998; Pellegrini et al., 2003; Somnuk et al., 2017; Tsukui et al., 2014). The Food and Drug Administration (FDA) guidance for industry classifies the latter solvents as in class 3, which are considered as the less toxic and harmful for human health (FDA, 2017). It is considered that amounts of these residual solvents of 50 mg per day or less would be acceptable without justification. Higher amounts may also be acceptable provided they are realistic in relation to manufacturing capability and good manufacturing practice (FDA, 2017).

Therefore, the proposal of this work was to evaluate the technical viability of conventional solid-liquid extraction (Soxhlet) of soluble solids and bioactives from green coffee beans and its press meal using renewable, consumer-safe and environmentally friendly solvents, seeking the most noble use of residues that have compounds of economic interest.

This work is the first attempt to identify the best green solvents in the conventional extraction of soluble solids and antioxidant compounds from green coffee beans cultivated in Southeastern Brazil and the respective meal obtained from the coffee oil expelling industry. For this, various antioxidant capacity assays were performed, ABTS, DPPH, BCBA (β-carotene bleaching assay), FRAP, and TPC (Total Phenolic Compounds). Also, principal component analysis (PCA) has been carried out to study the influence of the extraction solvent and time on the antioxidant compounds of the extracts. This research serves as a good basis for other researchers to investigate green coffee waste antioxidants and soluble solids in future research.

2. MATERIALS AND METHODS

2.1. Chemicals

All chemicals and solvents were of analytical grade and purchased from Sigma-Aldrich (St. Louis,

US).

2.2. Raw material

Green coffee beans (11% moisture d.b.) and meal (4.35% moisture d.b.) were kindly donated by a local industry (Cooxupé) located in Guaxupé in the South region of Minas Gerais (Brazil). Both materials were imediately submitted to freezing (-18 °C) until further processing. At the convenience, beans (under nitrogen atmosphere) and meal were milled (IKA, A11, Wilmington, US) in order to obtain homogeneous samples with a mesh size of 0.177 mm (NREL, 2008). Each powder was then dried (40 °C) in a vacuum oven (16.8 kPa) (Tecnal, TE-395, Piracicaba, Brasil), and imediately submitted to the extraction process. The proximal composition of the materials was carried out by Oliveira et al. (2018a) (Table 1).

Table 1 Chemical composition of green coffee beans and its press meal

Component (%)	Green Coffe Beans	Green Coffee Press Meal
Total lipid	8.61 ± 0.46	6.27 ± 0.22
Protein	14.87 ± 0.55	15.86 ± 0.63
Fiber	11.62 ± 1.30	12.46 ± 1.02
Ash	1.75 ± 0.16	2.28 ± 0.02
Carbohydrates	63.15	63.13

2.3. Solid-liquid extraction (SLE) of soluble solids

Green beans and meal were extracted using a solid–liquid ratio (SLR) of 1:5 (w/w) and four different types of environmentaly safe organic solvents (acetone, ethanol, ethil acetate, isopropanol), and also hexane and petroleum ether were used in order to compare the results. The extractions were carried out in a Soxhlet type apparatus (ISB, OXY-901, Bom Princípio, Brasil) during two periods of extraction (3 h and 5 h). The first period (3 h) was chosen as it is indicated by AOAC (2016), and the period of 5 h was observed to be about enough time to completely extract the soluble solids. The temperature used was according to the boiling point of each solvent (Table 2). Samples of ~ 2 g were weighed and placed in a cellulose cartridge, which was appropriately sealed and placed into the Soxhlet apparatus. After each period of extraction, the extract was

submitted to evaporation in order to recover the solvent, and forwarded to a drying process in an oven (100 ºC/ 2 h) to eliminate all the remaining solvent, and make it possible to calculate the yield recovery of soluble solids/oil by gravimetry (Equation 1).

$$Yield\ (\%)(d.b.) = \frac{Reboiler\ with\ sample\ (g) - Reboiler\ without\ sample\ (g)}{Sample\ mass\ (g)} \times 100 \quad (1)$$

Table 2 Boiling Point of solvents of different polarity and dieletric constant and temperature used in the solid-liquid extraction (SLE)

Solvent	Boiling Point (ºC)*	SLE Temperature (ºC)	Polarity Index**	Dieletric Constant (ε, °C) ***
Acetone	56.3	60	5.4	21.01, 20
Ethanol	78.3	80	5.2	24.6, 20
Ethyl Acetate	77.1	80	4.3	6, 25
Hexane	68.7	70	0.0	1.89, 20
Isopropanol	82.3	85	4.3	18.3, 25
Petroleum Ether	60	60	0.0	-

***(BrandTech Scientific, 2018)
**Snyder Polarity Index (Snyder, 1974)
* (Furniss et al., 2007; Lide, 2006)

2.4. Extracts for antioxidant activity

The extracts for antioxidant activity were obtained as described in section 2.3. Only that, after the adequate period of extraction, the solvent was not recovered and the extract was fully collected and kept frozen until further analyzes. The volume of the extracts was duly noted in order to be used in the calculation of the antioxidant activities.

2.5. Determination of the antioxidant activity

The antioxidant activity of each extract was assessed through five different assays.

2.4.1. Total polyphenols (TP)

The content of total polyphenols (TP) in the extracts was determined through a colorimetric method using the Folin–Ciocalteau's assay based on Waterhouse (2002), as described by Oliveira et al. (2018b). A calibration curve of gallic acid (ranging from 50 to 400 mg/mL) was prepared,

and the results determined by the linear regression of the curve were expressed as mg of gallic acid equivalent (GAE) per g of dry weight of raw material (d.b.). In this method, an aliquot (0.5 mL) of a suitable extract sample was added to 0.25 mL of Folin–Ciocalteu's reagent (10%). The solution was mixed and 0.2 mL of sodium carbonate (Na_2CO_3) (4%) solution was added to it. The final mixture was shaken thoroughly and then incubated for 120 min in the dark at room temperature. The absorbance was measured at 750 nm using a UV–Vis spectrophotometer (Mettler Toledo, Fast Track, Barueri, São Paulo).

2.4.2. Free radical scavenging activity

The free radical scavenging activity of the extracts was assessed through the ABTS (2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)) and DDPH (2,2-difenil-1-picrilhidrazil) tests. The determination of the antioxidant activity by the ABTS•+ method was carried out based on Mareček et al. (2017), as described by Oliveira et al. (2018b). The DPPH free radical-scavenging capacity was estimated using the method of Blois (1958), later modified by Brand-Williams, Cuvelier, & Berset (1995), and the results were presented in terms of EC_{50} (half maximal effective concentration), which represents the concentration of a compound where 50% of its maximal effect is observed.

2.4.4. Ferric Reducing Antioxidant Power (FRAP)

The FRAP methodology was adapted from Benzie & Strain (1996) and carried out as described by Oliveira et al. (2018b).

2.4.5. β-Carotene Bleaching Assay (BCBA)

Antioxidant activity by β-carotene/linoleic acid assay was determined according with the methodology proposed by Marco (1968).

3. Phenolic Profile

The phenolic profile of the soluble solids obtained through the ethanolic extraction was identified through High Performance Liquid Chromatography. The parameters of the analysis are described in the work of Oliveira et al. (2018c). Standards from Supelco (Pennsylvania, USA) were used: gallic acid, catechin, chlorogenic acid, caffeic acid, vanillin, ferulic acid, *p*-coumaric acid, *o*-coumaric acid, *m*-coumaric acid, resveratrol, and *trans*-cinnamic acid.

2.5. Statistical analysis

Data (performed in triplicates) were statistically analyzed through the analysis of variance (Anova) in order to evaluate the interaction amongst the type of material, type of solvent, and time of extraction, followed by the Scott Knott's text, once this a very robust text concerning the violation of the data normality. A Principal Component Analysis (PCA) was also performed in order to reveal which factor exerted the greatest influence on the response variables studied. All the analyzes were carried out using a significance of 5% with the aid of RStudio software version 3.2.0 (R Core Team, 2015).

3. RESULTS AND DISCUSSION

3.1 Effect of extraction solvents on total soluble solids

The main constituents of vegetable oils are the triacylglycerols, which are esters derived from glycerol and (saturated or unsaturated) aliphatic fatty acids. The main solvent industrially known to extract oil from oilseeds is the hexana, which is a mixture of isomers from hexane with aliphatic structures. Concerning the non-polar solvents, hexane was selected because of its industrial application for oil extraction, and petroleum ether due to its large usage in the lab routine. Due to the potential health hazard of these solvents, great efforts have been made into researching alternatives, with low toxicity and that could generate high quality outputs (Tir et al., 2012). The most promising biorenewable solvents to extract oil are the polar ones, which are able to break up

the cell wall of the vegetable matrix, and thus, allow the complete extraction of its content (Tir et al., 2012).

The apolar solvents present high selectivity to the oil, and their yields can therefore be directly related to the oil content extracted by the pressing process. It was observed in this study (Table 3) that the oil content present in the meal obtained from the mechanical pressing process was close (slightly higher) to the oil content of the green coffee beans (Oliveira et al., 2018a), indicating the very low yield of the mechanical pressing process and the non-extractability through this operation for materials with low oil content, such as coffee.

Table 3 Soluble solids/oil yield (%) (d.b.) of green coffee beans (C) and its press meal (M) extracted with different solvents and periods

Material	Acetone	Ethanol	Ethyl Acetate	Hexane	Isopropanol	Petroleum Ether
	3 h					
C	$8.31{\pm}0.38^{c,\alpha,B}$	$11.78{\pm}1.19^{a,\alpha,A}$	$6.44{\pm}0.14^{d,\alpha,A}$	$8.85{\pm}0.41^{c,\alpha,A}$	$10.23{\pm}0.96^{b,\alpha,A}$	$7.67{\pm}0.8^{c,\alpha,A}$
M	$8.67{\pm}0.22^{a,\alpha,A}$	$9.92{\pm}0.91^{a,\beta,B}$	$6.70{\pm}0.46^{b,\alpha,A}$	$6.65{\pm}0.08^{b,\alpha,B}$	$9.22{\pm}0.27^{a,\alpha,A}$	$6.98{\pm}0.26^{b,\alpha,B}$
	5 h					
C	$8.47{\pm}0.29^{d,\alpha,A}$	$14.16{\pm}0.35^{a,\alpha,A}$	$9.77{\pm}0.37^{c,\alpha,A}$	$8.31{\pm}0.31^{d,\alpha,A}$	$11.21{\pm}0.80^{b,\alpha,A}$	$7.94{\pm}0.05^{d,\alpha,A}$
M	$8.99{\pm}1.0^{b,\alpha,A}$	$13.34{\pm}1.65^{a,\alpha,B}$	$7.62{\pm}0.47^{c,\alpha,B}$	$6.75{\pm}0.07^{c,\alpha,B}$	$10.15{\pm}0.77^{b,\alpha,B}$	$7.15{\pm}0.13^{c,\alpha,A}$

Results are expressed as mean ± standard deviation (n = 3). $^{a–d}$ Means in the same row (for each extraction time) followed by different lowercase letters are significantly different ($P < 0.05$) (unfolding of the solvent type into the time and material type). $^{A–B}$ Means in the same column (for each extraction time) followed by different uppercase letters are significantly different ($P < 0.05$) (unfolding of the material type into the time and solvent type). $^{\alpha,\beta}$ Means in the same column (for both extraction times) followed by different greek letters are significantly different ($P < 0.05$) (unfolding of the extraction time into the material and solvent types). Results are according to the Scott Knott's test.

The solvent used for extraction is selected according to the aim of the process, the nature of the components being extracted, the properties of the matrix, the availability of reagents and equipment, the cost and safety concerns (Yu et al., 2002). The results showed that different solvents had significant effect on the extractable solids yield from green coffee beans and its press meal. The interaction amongst type of solvent, type of material, and period of extraction was significant at $p < 0.05$, indicating that the polarity of solvent, vegetable matrix, and the length of the process are relevant variables when proposing this type of process. In advance, while unfolding the extraction time into the material and solvent types, it was observed that for either the beans or the meal, the time did not show significance among either of the solvents.

Further, it was posssible to observe that the polar solvents (Table 2) were able to recover higher soluble solids content, which may be due to their ability to solubilize other components from the matrix, besides the oil (Nagendra Prasad et al., 2011). According to the latter, the alcoholic solvents, because of their greater polarity, are able to extract larger amounts of compounds that are

not acylglycerols, in other words, the oil extracted with these solvents presents a greater amount of phospholipids and unsaponifiable material in their composition. Also, according with Suchinina et al. (2011), the extract yield increases with increasing solvent polarity. The authors observed that several factors including steric effects of the solvent molecule probably had an effect on the extraction yield of *Euphrasia brevipila* if the polarity was increased to greater than 1.8 (dipole moment). The solid-liquid extraction studies carried out with ethanol present, in general, higher extraction of sugars, phospholipids, pigments and waxes besides obtaining a meal of better sensorial and nutritional quality in relation to that obtained with hexane (Beckel et al., 1948; Fonseca and Regitano-d'Arce, 1993; Johnson and Lusas, 1983; Regitano-d'Arce et al., 1994).

Absolute ethanol, which is the most polar solvent among the ones being investigated, was the most efficient solvent at extracting soluble solids from both materials studied. Its performance showed a slight dependence on the extraction time. Isopropanol was the second most efficient solvent at recovering soluble solids from both beans and meal, except for the meal submitted to 3 h extraction.

The soluble solids yield of coffee beans showed the greatest difference between the two times of extraction when extracted with ethyl acetate and isopropanol, showing that the time is a relevant parameter when working with these solvents. Which was not the case for the other solvents, such as hexane, and petroleum ether, once there was only a slight difference in the yield of both beans and meal, concerning the extraction time, meaning that, 3 h should be enough to complete the process and exhaust the soluble solids from the vegetable matrix. In addition, hexane and petroleum ether are the least polar solvents, showing therefore, the lowest yields, which is within the yield range (7.5 to 9.5%) reported by Tsukui et al. (2014), who evaluated the Soxhlet extraction of 13 different Brazilian green Arabica coffee beans using petroleum ether as the extractor. Dibert et al. (1989) reported a mean value of 8.3% of Robusta green coffee oil extracted with hexane for 16 h in a Soxhlet apparatus, confirming that the longer extraction time is not necessary in order to deplete the oil from the matrix. The apolarity of these solvents allow the extraction of only apolar substances, such as fat, wax, etc., once these are solvents that present selectivity to the lipidic matrix solely, justifying the lower yields obtained with them.

The solubility of phenolic compounds, which are derived from the pentose phosphate, shikimate, and phenylpropanoid pathways in plants, the dominant group of phytochemicals or

secondary metabolites, is strongly governed by the type of solvent used, the degree of polymerization of phenolic compounds, as well as by the interaction of phenolic with other food constituents and the formation of insoluble complex (Djeridane et al., 2006; Randhir et al., 2004). Results of SLE of phenolic compounds with different solvents are reported in Table 4.

Table 4 Total phenolic content (mg GAE/ 100 g) (d.b.) of green coffee beans (C) and its press meal (M) extracted with different solvents and periods

Material	3 h					
	Acetone	Ethanol	Ethyl Acetate	Hexane	Isopropanol	Petroleum Ether
C	$1737.81 \pm 28.24^{b,\alpha,A}$	$4048.34 \pm 177.05^{a,\alpha,B}$	$313.84 \pm 51.58^{d,\alpha,A}$	$785.42 \pm 32.54^{c,\alpha,A}$	$1477.21 \pm 79.03^{b,\alpha,A}$	$246.92 \pm 16.24^{d,\alpha,B}$
M	$1359.48 \pm 98.56^{b,\alpha,B}$	$6799.41 \pm 75.95^{a,\alpha,A}$	$245.64 \pm 32.05^{d,\alpha,B}$	$179.29 \pm 3.18^{d,\alpha,B}$	$1684.9 \pm 70.72^{b,\alpha,A}$	$448.18 \pm 18.56^{c,\alpha,A}$
	5 h					
C	$101.24 \pm 8.32^{d,\beta,B}$	$2281.58 \pm 116.16^{a,\beta,B}$	$167.44 \pm 7.96^{c,\beta,A}$	$90.15 \pm 6.25^{d,\beta,A}$	$1338.67 \pm 20.15^{b,\alpha,B}$	$131.28 \pm 15.31^{c,\beta,A}$
M	$1048.33 \pm 8.03^{c,\beta,A}$	$2403.77 \pm 115.15^{a,\beta,A}$	$81.92 \pm 1.97^{d,\beta,B}$	$48.84 \pm 7.7^{e,\beta,B}$	$1650.35 \pm 27.85^{b,\alpha,A}$	$8.26 \pm 0.0^{f,\beta,B}$

Results are expressed as mean ± standard deviation (n = 3). [a–d] Means in the same row (for each extraction time) followed by different lowercase letters are significantly different ($P < 0.05$) (unfolding of the solvent type into the time and material type). [A–B] Means in the same column (for each time) followed by different uppercase letters are significantly different ($P < 0.05$) (unfolding of the material type into the time and solvent type). [α,β] Means in the same column (for both extraction times) followed by different greek letters are significantly different ($P < 0.05$) (unfolding of the extraction time into the material and solvent types). Results are according to the Scott Knott's test.

The results showed that the extraction solvents and time had a signifficant impact on the extraction yields of TPC from both materials. Ethanol, isopropanol, and acetone presented the highest extraction yields of TPC, respectively, as expected, once these are within most polar solvents (Table 4). These results are in agreement with previous studies, which also reported the performance of different solvents on the extraction of phenolic compounds (Chavan et al., 2013; Dailey and Vuong, 2015; Sulaiman et al., 2013). The results could also be related to the extraction temperature used during the process, once, for each solvent, a different temperature was applied, however this parameter was not considered as a variable in the experimental design. Concerning the ethanolic extract, it seems that the pressing process favored the extraction of phenolic compounds. On the other hand, the exposure to elevated temperatures led to their degradation. However, it is important to highlight that in spite of the solvents performance, the content of these compounds may vary for each different matrix. The recovery of TPC was also time dependent, mostly for the treatments where apolar solvents were employed. However, the longer extraction period did not favor the recovery of these compounds, which could be explained by their instability at elevated temperatures. The results from the isopropanol extract showed to be the least affected by the length of extraction. These findings further confirm that the extraction solvents play an important role in the extraction of phenolic compounds from the samples. Zieliński and Kozłowska

(2000) reported that 80% acetone was the most efficient solvent mixture for phenolic extraction from cereal seeds. Similarly, Zhou and Yu (2004) reported that 50% acetone wheat extracts contained the highest TPC, whereas ethanol was the least effective solvent, showing that the type of matrix is a relevant factor that may affect the recovery of these compounds. El-Ghorab et al. (2010) reported that the highest TPC was found in the cumin methanolic extract (35.5 mg GAE/g d.b.), whereas the hexane extract showed the lowest one (10.6 mg GAE/g d.b.), as it was found in this study for the meal. This trend is also supported by a previous study that showed that hexane yielded the lowest total phenolic content and antioxidant capacity in seed cakes, from evening primrose, burdock, sesame, and woad (Peschel et al., 2007). They found that hexane is only suitable to extract phosphatides, lipid and other fat soluble components such as tocopherols, tocotrienols and carotenoids but it is too weak to extract hydrophilic phenolic compounds due to the low polarity of hexane. Hence, there is a contrast for the types and polarity of solvent for extracting a specific component in the seed cakes. In order to increase the extraction of total phenolic content in the seed cakes, the polarity of hexane needs to be increased by mixing with other polar solvents such as methanol, acetone and ethanol.

The dielectric constant of a solvent is a measure of how well the solvent can insulate opposite charges from one another (Bruice, 2007). The greater the value of the dielectric constant of a solvent, the smaller the interaction between ions of opposite charge dissolved in that solvent. Cheok et al. (2012) reported that the TPC of mangosteen hull powder was directly proportional to the dielectric constants of the organic solvents studied by them. Acetone, ethanol, and isopropanol which have the highest dielectric constant among the organic solvents used, are very good at insulating the charges of phenolic compounds of green coffee beans and meal, and as a result, yielded the highest TPC. The interaction between a solvent and a molecule or an ion dissolved in that solvent is called solvation (Bruice, 2007). Hence, the solvation power of the selected solvents on the TPC of green coffee and its press meal can be listed as ethanol > isopropanol > acetone > ethyl acetate > hexane > petroleum ether. Even though the dielectric constants of acetone, isopropanol, and ethanol are similar, the TPC obtained from acetone and isopropanol extracts were significantly lower ($p < 0.05$). This could be due to the longer alkyl groups in isopropanol compared to those of ethanol, which implies that the solvation power of a solvent is not solely dependent on the dielectric constant, but also possibly is influenced by the chemical structure of an organic

solvent (Bruice, 2007).

Previous studies have shown the potential recovery of polyphenols in agrowastes, such as rice hulls (Ramarathnam et al., 1989), buckwheat hulls (Watanabe et al., 1997), pistachio hulls (Goli et al., 2005), and citrus peels, where polyphenol recovery was higher in the peels compared to the edible portion (Gorinstein et al., 2001). For seed cakes, most polyphenols were not successfully extracted into the oil after cold-pressing (Żuk et al., 2011), as showed in this study for the green coffee press meal. Several solvent systems have been used to extract polyphenols from plant materials. For example, methanol:acetone:water (7:7:6, v/v/v) was used to extract phenolic compounds from de-oiled mesocarp of palm fruit (Neo et al., 2008). Methanol, ethanol, acetone, hexane, diethyl ether and petroleum ether were used to extract phenolic compounds from potato peel, sugar beet pulp and sesame cake (Mohdaly et al., 2010). In addition, methanol 80% was used to extract phenolic compounds from flax seeds cake (Żuk et al., 2011). Suchinina et al. (2011) reported that the number of phenolic acids in the extract increased with increasing solvent polarity. Thus, petroleum ether extracted one; ethylacetate, two; and trichloromethane, butanol, ethanol, and water, three compounds. The TPC percentage by mass of aqueous green coffee beans extract varied from 3.2 to 5.2% (Priftis et al., 2015). The polyphenolic percentages obtained in the present study are in agreement with those presented in the relevant literature, despite the fact that, depending on the variety, large variations have been detected (Dziki et al., 2015; Gawlik-Dziki et al., 2014; Mills et al., 2013; Rodrigues et al., 2015).

The solvent nature is the most controversial parameter that influences antioxidant capacities (Naczk and Shahidi, 2004; Turkmen et al., 2006). There are many assays to evaluate the antioxidant activity of plant materials. For example, DPPH free radical-scavenging assay, ferric reducing/antioxidant power (FRAP) assay, β-carotene bleaching assay (BCBA) (Chen et al., 2012), and ABTS free radical-scavenging assay.

Concerning the DPPH free radical-scavenging assay, ethanol and isopropanol were more efficient to extract antioxidant compounds from both materials at both extraction periods (Table 5), since they presented the lowest EC_{50}, which may be due to the polarity of these solvents (Table 2); petroleum ether (3 h) and hexane (5 h) were the least efficient. Ethanol was able to extract higher amounts of antioxidants from the meal over a longer extraction period. The coffee samples yielded higher antioxidant activity when extracted using ethanol, ethyl acetate, and hexane (3 h).

While, hexane extracted more antioxidants from the meal at the extraction period of 3 h. Indicating that 3 h are enough to carry the process under these conditions. The antioxidant activity did not show significative difference for acetone extracts of both material types and periods of extraction. The material type influenced the extractions carried for 3 h, when ethanol, hexane, isopropanol and petroleum ether were employed. Yet, after 5 h of extraction, the type of material influenced all treatments, where the highest activity was found for coffee extracted with acetone, hexane, and ether; and for the meal extracted with ethyl acetate and hexane, indicating the thermal stability of the compounds capable of scavenging the DPPH free radical.

Table 5 DPPH free radical-scavenging assay (EC_{50}) (g/g DPPH) (d.b.) of green coffee beans (C) and its press meal (M) extracted with different solvents and periods

Material	3 h					
	Acetone	Etanol	Ethyl Acetate	Hexane	Isopropanol	Petroleum Ether
C	$364 \pm 19.14^{b,\alpha,A}$	$39.18 \pm 0.26^{d,\beta,B}$	$481.98 \pm 30.24^{a,\beta,A}$	$348.29 \pm 39.47^{b,\beta,B}$	$274.33 \pm 14.54^{c,\alpha,A}$	$450.64 \pm 26.69^{a,\alpha,B}$
M	$369.07 \pm 0.11^{c,\alpha,A}$	$62.60 \pm 0.35^{e,\alpha,A}$	$329.48 \pm 19.20^{c,\alpha,A}$	$507.02 \pm 1.18^{b,\beta,A}$	$155.87 \pm 4.61^{d,\alpha,B}$	$1564.88 \pm 90.16^{a,\alpha,A}$
	5 h					
C	$274.45 \pm 11.56^{c,\alpha,B}$	$56.45 \pm 3.17^{d,\alpha,A}$	$643.86 \pm 20.19^{a,\alpha,A}$	$512.04 \pm 24.65^{b,\alpha,B}$	$78.02 \pm 3.47^{d,\beta,A}$	$454.22 \pm 12.57^{b,\alpha,B}$
M	$328.45 \pm 21.91^{c,\alpha,A}$	$18.40 \pm 1.5^{e,\beta,B}$	$140.86 \pm 34.81^{d,\beta,B}$	$1710.84 \pm 28.62^{a,\alpha,A}$	$57.28 \pm 5.43^{e,\beta,B}$	$974.67 \pm 19.18^{b,\beta,A}$

Results are expressed as mean ± standard deviation (n = 3). $^{a–e}$ Means in the same row (for each extraction time) followed by different lowercase letters are significantly different ($P < 0.05$) (unfolding of the solvent type into the time and material type). $^{A–B}$ Means in the same column (for each time) followed by different uppercase letters are significantly different ($P < 0.05$) (unfolding of the material type into the time and solvent type). $^{\alpha,\beta}$ Means in the same column (for both extraction times) followed by different greek letters are significantly different ($P < 0.05$) (unfolding of the extraction time into the material and solvent types). Results are according to the Scott Knott's test.

Significant differences ($p < 0.05$) of % DPPH• inhibition between extracts using different solvents were found by Teh et al. (2014). They also observed that extracts with hexane presented the lowest antioxidant activity. This trend was previously reported by few authors who studied the hexane extracts of potato peel, sugar beet pulp, sesame cake (Mohdaly et al., 2010), and medicinal plants (Djeridane et al., 2006). Hence, this showed that high-polarity solvents (ethanol and acetone) were more effective to extract antioxidant compounds that exhibited more efficient radical scavenging property compared to low-polarity solvents (Teh et al., 2014). According with Zhou and Yu (2004), antioxidant compounds of various polarities could exist in seed cakes, in general.

The impact of two parameters (temperature and duration) on the ABTS radical scavenging capacity of antioxidant compounds was observed (Table 6). There was not a trend, but a diversity of behavior of the extracts along the extraction period.

Table 6 ABTS free radical-scavenging assay (μmol trolox/g) (d.b.) of green coffee beans (C) and its press meal (M) extracted with different solvents and periods

Material	3 h					
	Acetone	Etanol	Ethyl Acetate	Hexane	Isopropanol	Petroleum Ether
C	$1049.64 \pm 36.13^{b,\beta,A}$	$3528.64 \pm 25.76^{a,\alpha,A}$	$1313.71 \pm 48.69^{b,\alpha,B}$	$1322.21 \pm 31.33^{b,\alpha,A}$	$1309.11 \pm 68.05^{b,\alpha,B}$	$836.95 \pm 35.86^{b,\alpha,A}$
M	$1002.26 \pm 2.76^{b,\beta,A}$	$3427.82 \pm 173.88^{a,\beta,A}$	$1867.61 \pm 32.56^{b,\alpha,A}$	$705.58 \pm 15.92^{b,\alpha,B}$	$2134.74 \pm 108.02^{b,\alpha,A}$	$796.39 \pm 15.74^{b,\alpha,A}$
	5 h					
C	$1965.58 \pm 43.15^{b,\alpha,A}$	$2922.21 \pm 49.68^{a,\beta,B}$	$790.38 \pm 9.25^{b,\beta,B}$	$701.1 \pm 23.56^{b,\beta,A}$	$1142.86 \pm 34.78^{b,\alpha,B}$	$431.86 \pm 24.61^{b,\beta,A}$
M	$1477.9 \pm 34.83^{c,\alpha,B}$	$8457.48 \pm 163.3^{a,\alpha,A}$	$1559.26 \pm 5.02^{c,\alpha,A}$	$325.55 \pm 9.4^{c,\beta,B}$	$2012.92 \pm 34.79^{b,\alpha,A}$	$465.07 \pm 28.47^{c,\beta,A}$

Results are expressed as mean ± standard deviation (n = 3). $^{a–d}$ Means in the same row (for each extraction time) followed by different lowercase letters are significantly different ($P < 0.05$) (unfolding of the solvent type into the time and material type). $^{A–B}$ Means in the same column (for each time) followed by different uppercase letters are significantly different ($P < 0.05$) (unfolding of the material type into the time and solvent type). $^{\alpha,\beta}$ Means in the same column (for both extraction times) followed by different greek letters are significantly different ($P < 0.05$) (unfolding of the extraction time into the material and solvent types). Results are according to the Scott Knott's test.

Ethanol was once again the most efficient solvent on extracting antioxidant compounds, from both materials after 3 h and 5 h of extraction. For the beans, the longer extraction time had low influence on extracting the antioxidants, except when acetone was used as the solvent. For the meal, the longer time of extraction allowed a 2-fold higher recovery when ethanol was used in the process, probably because the extracted compounds are less soluble compounds, also more difficult to be extracted, so their extraction may be easier when other compounds had already been extracted. While ethyl acetate did not show significative difference between extraction periods for the meal.

Ethyl acetate and isopropanol were more efficient to extract antioxidants from the meal after 3 h, while hexane performed better with coffee samples. There was no difference between the extraction power of ethanol and acetone regarding the material types after 3 h of extraction. On the other hand, after 5 h of process, acetone, hexane, and petroleum ether were more effective to yield antioxidants from coffee samples. While, ethanol, ethyl acetate, and isopropanol extracted better the antioxidants from the meal. Regarding the solvent petroleum ether, there was no effect of the material type after 5 h.

Isopropanol did not show difference between the extraction periods and material types. The lowest yields were observed for the least polar solvents (hexane = petroleum ether < ethyl acetate) (Table 2), as it was observed for the DPPH and TPC assays.

The ABTS assay was also carried out and investigated by van der Werf et al. (2014) for green coffee. Phenolic/antioxidant coffee extracts by Accelerated Solvent Extraction (ASE) of green coffee, using methanol, were analyzed by an ABTS decolorization assay. This study revealed a general decrease of radical scavenging capacity related to native phenolic compounds. They reported 4043 ± 133 μmol Trolox/L in green coffee and observed that when the coffee bean is submitted to elevated temperatures (235 °C), the antioxidant activity is prone to decrease over the

time. It is well known that elevated temperatures greatly affect the chemical composition of the coffee beans (Kamiyama et al., 2015; Smrke et al., 2013), new compounds may be formed, which exhibit antioxidant activity, whereas other ingredients, such as chlorogenic acids may be broken down (Esquivel and Jiménez, 2012; Jaiswal et al., 2012). However, as it was presented in this work, it may vary according with the parameters involved in the process, such as type of solvent, vegetable matrix, temperature, time, and type of extraction.

As it can be observed in Table 7, the FRAP assay was the least selective for assessing green coffee and its press meal antioxidant activity.

Table 7 Ferric Reducing Antioxidant Power (FRAP) (μmol Fe (II)/g) (d.b.) of green coffee beans (C) and its press meal (M) extracted with different solvents and periods

Material	3 h					
	Acetone	Etanol	Ethyl Acetate	Hexane	Isopropanol	Petroleum Ether
C	2740.7±293.51$^{b,\alpha,A}$	5019.91±248.35$^{a,\alpha,B}$	179.44±15.40$^{d,\alpha,A}$	1209.44±174.35$^{c,\alpha,A}$	1672.04±28.4$^{c,\alpha,B}$	29.94±0.01$^{e,\alpha,A}$
M	1150.12±23.97$^{c,\alpha,B}$	9622.34±362.21$^{a,\alpha,A}$	61.99±0.43$^{d,\alpha,B}$	0.0±0.0$^{d,\alpha,B}$	2744.15±29.22$^{b,\alpha,A}$	0.0±0.0$^{d,\alpha,A}$
	5 h					
C	1119.92±44.36$^{b,\beta,A}$	2449.58±19.4$^{a,\beta,A}$	140.14±12.28$^{c,\alpha,A}$	14.75±5$^{d,\beta,A}$	993.7±9.13$^{b,\beta,B}$	23.69±5.38$^{d,\alpha,A}$
M	1018.20±44.26$^{c,\alpha,A}$	2563.75±51.82$^{a,\beta,A}$	24±0.82$^{d,\beta,B}$	0.0±0.0$^{d,\alpha,A}$	1652.87±137.88$^{b,\beta,A}$	0.0±0.0$^{d,\alpha,A}$

Results are expressed as mean ± standard deviation (n = 3). $^{a–d}$ Means in the same row (for each extraction time) followed by different lowercase letters are significantly different (P < 0.05) (unfolding of the solvent type into the time and material type). $^{A–B}$ Means in the same column (for each time) followed by different uppercase letters are significantly different (P < 0.05) (unfolding of the material type into the time and solvent type). $^{\alpha,\beta}$ Means in the same column (for both extraction times) followed by different greek letters are significantly different (P < 0.05) (unfolding of the extraction time into the material and solvent types). Results are according to the Scott Knott's test.

Ethanol was the solvent that better extracted antioxidant compounds from the matrices, that were allowed to be detected through the FRAP assay. Acetone and isopropanol were also able to extract antioxidant compounds detected by this assay, however, they were less effective than ethanol. For both materials, it was found that 3 h were enough in order to extract antioxidants assessed by the FRAP assay, indicating that these compounds may be sensitive to the temperature.

The apolar solvents are not indicated to extract components to be detected by the FRAP assay, which leads to a conclusion that only polar solvents are indicated to extract antioxidant compounds to be assessed through FRAP, also DPPH, and ABTS, assays. In addition, time was a relevant parameter to this analysis, once after 3 h of extraction, the content of antioxidants decreased greatly, indicating these are thermosensitive compounds. The meal presented once again great content, similar to coffee beans, of antioxidant compounds. FRAP assay results showed a similar trend to the DPPH• assay comparing the solvents used on the extraction process in the work of Teh et al. (2014). The results showed that extracts with methanol-acetone-water exhibited the

highest reducing power, 3.51 ± 0.04, 1.48 ± 0.00, 8.78 ± 0.07 μmol Fe (II)/g fresh weight for hemp, flax and canola seed cakes, respectively. Again, extracts with hexane exhibited the lowest reducing power with the aqueous polar solvents proving superior to the pure polar solvents (Teh et al., 2014). In this study, the only relationship observed between DPPH and FRAP assays was that the longer period of extraction did affect the antioxidant power of the extracts.

Concerning the β-carotene bleaching assay, the oxidation of linoleic acid generates peroxyl-free radicals due to the abstraction of hydrogen atom from dialytic methylene groups of linoleic acid (Kumaran and Karunakaran, 2006). The free radical then will oxidize the highly unsaturated β-carotene. The presence of antioxidants in the extract will minimize the β-carotene oxidation by hydroperoxides. These will be later neutralized by the antioxidants from the extracts. Thus, the degradation rate of β-carotene depends on the antioxidant activity of the extracts. The results for BCBA of green coffee beans and its press meal are available in Table 8.

Table 8 β-carotene Bleaching Assay (BCBA) (% protection) (d.b.) of green coffee beans (C) and its press meal (M) extracted with different solvents and periods

Material	Acetone	Etanol	Ethyl Acetate	Hexane	Isopropanol	Petroleum Ether
			3 h			
C	$55.75 \pm 8.31^{b,\alpha,A}$	$46.07 \pm 1.05^{b,\alpha,A}$	$80.31 \pm 0.93^{a,\alpha,A}$	$52.84 \pm 7.43^{b,\alpha,A}$	$48.64 \pm 8.43^{b,\alpha,A}$	$71.19 \pm 2.95^{a,\alpha,A}$
M	$48.92 \pm 0.0^{b,\alpha,B}$	$15.66 \pm 3.19^{c,\alpha,B}$	$83.48 \pm 7.62^{a,\alpha,A}$	$8.18 \pm 0.0^{c,\alpha,B}$	$22.01 \pm 1.99^{b,\beta,B}$	$24.81 \pm 1.77^{b,\beta,B}$
			5 h			
C	$44.06 \pm 3.49^{b,\alpha,A}$	$57.35 \pm 3.44^{b,\alpha,A}$	$98.32 \pm 1.39^{a,\alpha,A}$	$64.67 \pm 1.7^{b,\alpha,A}$	$62.19 \pm 0.30^{b,\alpha,A}$	$72.16 \pm 1.46^{b,\alpha,A}$
M	$33.27 \pm 3.57^{c,\beta,A}$	$28.73 \pm 3.57^{c,\alpha,B}$	$92.8 \pm 0.46^{a,\alpha,A}$	$7.62 \pm 1.00^{d,\alpha,B}$	$47.16 \pm 0.40^{b,\alpha,B}$	$62.3 \pm 1.17^{b,\alpha,A}$

Results are expressed as mean ± standard deviation (n = 3). $^{a–d}$ Means in the same row (for each extraction time) followed by different lowercase letters are significantly different ($P < 0.05$) (unfolding of the solvent type into the time and material type). $^{A–B}$ Means in the same column (for each time) followed by different uppercase letters are significantly different ($P < 0.05$) (unfolding of the material type into the time and solvent type). $^{\alpha,\beta}$ Means in the same column (for both extraction times) followed by different greek letters are significantly different ($P < 0.05$) (unfolding of the extraction time into the material and solvent types). Results are according to the Scott Knott's test.

This was the only assay that showed, in general, greater results for the coffee samples, which was probably due to the apolar nature of this assay, once the meal contains less amount of apolar substances after the mechanical pressing process. Interestingly, polar (acetone, ethanol, and isopropanol) and non-polar (hexane and petroleum ether) solvents showed similar results for coffee beans, with ethyl acetate being the solvent that better extracted the antioxidants to be assessed through BCBA, for both beans and meal. Antioxidants yield was greater for coffee beans when petroleum ether, and isopropanol were used, respectively, after 3 h. Considering the unfolding of the extraction time into the material and solvent types, the other treatments were not significantly different. While for the meal, after the same period of extraction, only acetone was effective; the

other treatments were not significant ($p < 0.05$). Most of the solvents were able to extract more antioxidants from the beans rather than the meal, except for ethyl acetate which did not show significative difference between materials after this extraction period. After the longer extraction period (5 h), there was difference between the material types when extracted with ethanol, hexane, and isopropanol, where the beans antioxidant activity was higher. Moreover, the significant variations in antioxidant potential according to the solvents used is essentially due to the difference in polarity, and thus, different extractability of the antioxidant compounds (Djeridane et al., 2006; Maisuthisakul et al., 2007). Variations in phenolic composition could be explained by several factors, whether genetic or related to the geographic origin, plant organ, harvest time, extraction method, type of cultivar and storage conditions (Duda-Chodak et al., 2010; McGhie et al., 2005; Tsao et al., 2005). The season and even sunlight duration are also known to influence the plant metabolism since some compounds may be accumulated at a particular period to respond to environmental changes (von Koenen, 2001). Recent interest in food phenolics has increased owing to their roles as antioxidants and scavengers of free radicals and their implication in the prevention of many pathologies, such as cardiovascular diseases (Ursini et al., 2009), and certain types of cancer (Hudson et al., 2000). Quantitative analysis of phenolic compounds using the spectrophotometrical method indicated that beans contained less compounds than the meal (Table 3). However, contents obtained by HPLC (Table 9) were significantly higher than those obtained by the spectrophotometrical method.

Table 9 Phenolic profile of green coffee beans and its press meal oils extracted with ethanol (mg/ 100 g)

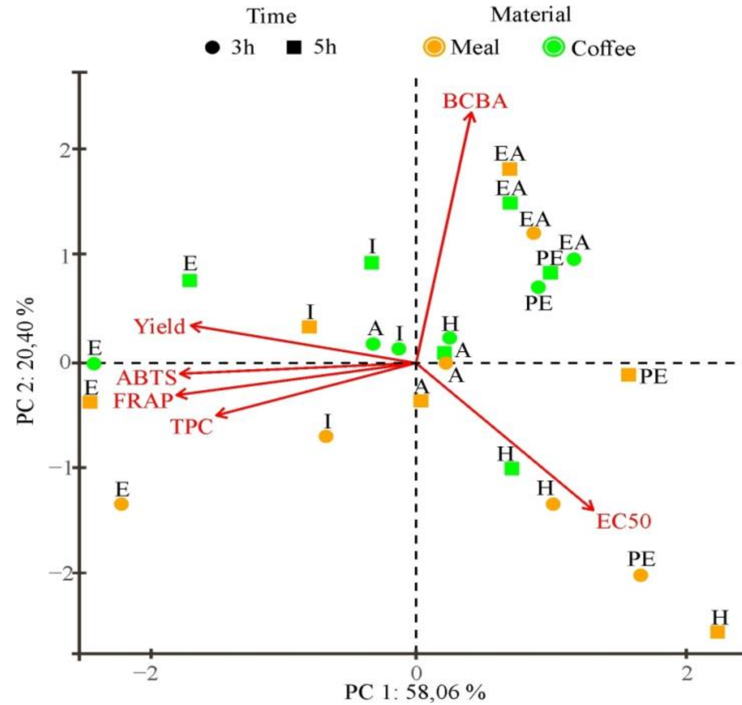
Phenolic compound	Green coffee oil	Oil from green coffee press meal
Gallic acid	0.012	0.006
Catechin	0.175	-
Chlorogenic acid	17.524	0.237
Caffeic acid	21.956	4.579
Vanillin	0.293	0.006
p-Coumaric	0.003	-
Ferulic acid	0.005	-
m-Coumaric	0.001	-
o-Coumaric	0.859	0.004
Resveratrol	0.116	0.009
Trans-cinnamic acid	0.058	-

This was predictable due to the low selectivity of Folin–Ciocalteu's reagent, as it reacts positively with different phenolic and non-phenolic substances (Mariod et al., 2017), also, it was possible to identify only few (eleven) acids, compared to the whole profile present in these materials.

Principal Component Analysis

The results from the experiments together with the independent parameters were analyzed using PCA. A PCA facilitates the discovery of a pattern in multivariated data, a pattern that can be difficult to identify with a univariate analytical approach. A loading plot describes the contribution of each variable to each principal component (PC), and also affords a view of the variable similarities. Based on the PCA (Figure 1), PC1 and PC2 together explained 78.46% of the relation among the independent variables and the recovery yield and antioxidant data. Considering data from the PCA, it can be concluded from the loading plot that most of the variation can be ascribed to the polarity of the extractor solvent and type of material. Coffee beans and meal soluble solids yield and antioxidant activities assessed through ABTS, FRAP, and Folin-Ciocalteu's method (TPC) presented a good correlation with the coffee beans ethanolic extract, while the meal extracted with ethanol for 5 h exhibited the highest correlation with these variables. According with the literature, ethanol is able to extract medium polarity phenolic compounds with different ranges of molecular weight (Lin et al., 2009), anthocyanins and terpenoid (Cowan, 1999), and polar compounds, such as sugar, amino acid, and glycoside compounds (Houghton and Raman, 1998). This shows that the methodologies above correlated are able to identify these compounds. The three methodologies are able to measure the antioxidant activity of phenolic compounds; in addition, ABTS reacts mainly with carotenoids and plasma antioxidants (Re et al., 1999), and FRAP measures antioxidants directly from the sample, and does not react only with thiols (Halvorsen et al., 2002; Payne et al., 2013). As observed earlier in this study, petroleum ether and hexane, non-polar solvents, exhibited the highest EC_{50} correlation, once the higher this value is, the lower is the antioxidant activity of the material, showing their inability to extract antioxidants through the DPPH assay. Further, the differences between the DPPH and $ABTS^{\bullet+}$ assays may be ascribed to the varying reactivity of these 2 radicals to the components of each coffee sample.

Fig. 1 Principal Component Analysis showing the relationship among the response variables (yield, DPPH, ABTS, FRAP, BCBA) and type of material (green coffee beans and press meal), type of solvent (A: acetone, E: ethanol, EA: ethyl acetate, H: hexane, I: isopropanol, and PE: petroleum ether), and extraction period (3 h and 5 h)



4. CONCLUSION

The extraction solvent played an important role in the antioxidant extraction process by affecting the antioxidant capacity and total phenolic content in the green coffee beans and its press meal; also in the total soluble solids yield. Ethanol was found to be the optimal extraction solvent for extractable solids from both green coffee beans and its press meal, as well as for phenolic and antioxidant compounds. Only that for BCBA analysis, ethyl acetate yielded the best results for the antioxidant activity of green beans and meal. Therefore, ethanol is recommended for extraction of soluble solids, phenolic, and antioxidant compounds from green coffee beans and its press meal for further isolation and utilization. This research showed that extracts rich in phenolic compounds can be obtained from the green beans, and mainly from its press meal with a solvent of simple handling, environmentally safe and from a renewable source. Also, the green coffee bean press meal is a valuable resource of bioactive compounds that should be taken into consideration, once

this industrial by-product has not been given the enough attention so far. This study highlighted the press meal, an underutilized source of industrial waste, as a promising source of industrially important extractable bioactives, while also revealing the importance of selecting appropriate solvents while extracting bioactives and the proper assay to do so. A deeper analysis and more in-depth studies of GCC are required.

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REFERENCES

Abuja, P.M., Murkovic, M., Pfannhauser, W., 1998. Antioxidant and prooxidant activities of elderberry (*Sambucus nigra*) extract in low-density lipoprotein oxidation. J. Agric. Food Chem. 46, 4091–4096. doi:10.1021/JF980296G

Alothman, M., Bhat, R., Karim, A.A., 2009. Antioxidant capacity and phenolic content of selected tropical fruits from Malaysia, extracted with different solvents. Food Chem. 115, 785–788. doi:10.1016/J.FOODCHEM.2008.12.005

Anastas, P.T., Warner, J.C., 1998. Green chemistry: Theory and practice. Oxford University Press, New York.

AOAC, Association of Official Analytical Chemists, 2016. Official methods of analysis of the Association of Official Analytical Chemists International, 20th ed. AOAC, Gaithersburg.

Beckel, A.C., Belter, P.A., Smith, A.K., 1948. Solvent effects on the products of soybean oil extraction. J. Am. Oil Chem. Soc. 25, 7–9. doi:10.1007/BF02553630

Benzie, I.F.F., Strain, J.J., 1996. The ferric reducing ability of plasma (FRAP) as a measure of “Antioxidant Power”: The FRAP assay. Anal. Biochem. 239, 70–76. doi:10.1006/abio.1996.0292

Blois, M.S., 1958. Antioxidant determinations by the use of a stable free radical. Nature 181, 1199–1200. doi:10.1038/1811199a0

Brand-Williams, W., Cuvelier, M.E., Berset, C., 1995. Use of a free radical method to evaluate antioxidant activity. LWT - Food Sci. Technol. 28, 25–30. doi:10.1016/S0023-6438(95)80008-5

BrandTech Scientific, 2018. Solvent Boiling Points Chart [WWW Document]. URL https://www.brandtech.com/solventboilingpointschart/ (accessed 6.25.18).

Bruice, P.Y., 2007. Organic chemistry, 5 th. ed. Pearson International Edition, New Jersey.

Castro, A.C.C.M., Oda, F.B., Almeida-Cincotto, M.G.J., Davanço, M.G., Chiari-Andréo, B.G., Cicarelli, R.M.B., Peccinini, R.G., Zocolo, G.J., Ribeiro, P.R.V., Corrêa, M.A., Isaac, V.L.B., Santos, A.G., 2018. Green coffee seed residue: A sustainable source of antioxidant compounds. Food Chem. 246, 48–57. doi:10.1016/J.FOODCHEM.2017.10.153

Chavan, J.J., Gaikwad, N.B., Kshirsagar, P.R., Dixit, G.B., 2013. Total phenolics, flavonoids and antioxidant properties of three *Ceropegia* species from Western Ghats of India. South African J. Bot. 88, 273–277. doi:10.1016/J.SAJB.2013.08.007

Chen, T., He, J., Zhang, J., Li, X., Zhang, H., Hao, J., Li, L., 2012. The isolation and identification of two compounds with predominant radical scavenging activity in hempseed (seed of *Cannabis sativa* L.). Food Chem. 134, 1030–1037. doi:10.1016/J.FOODCHEM.2012.03.009

Cheok, C.Y., Chin, N.L., Yusof, Y.A., Law, C.L., 2012. Extraction of total phenolic content from *Garcinia mangostana* Linn. hull. I. effects of solvents and UV–Vis spectrophotometer absorbance method. Food Bioprocess Technol. 5, 2928–2933. doi:10.1007/s11947-011-0627-2

Cottica, S.M., Sawaya, A.C.H.F., Eberlin, M.N., Franco, S.L., Zeoula, L.M., Visentainer, J. V, 2011. Antioxidant activity and composition of propolis obtained by different methods of extraction. J. Braz. Chem. Soc. 22, 929–935. doi:10.1590/S0103-50532011000500016

Cowan, M.M., 1999. Plant products as antimicrobial agents. Clin. Microbiol. Rev. 12, 564–82.

Dailey, A., Vuong, Q., 2015. Optimization of aqueous extraction conditions for recovery of phenolic content and antioxidant properties from macadamia (*Macadamia tetraphylla*) skin waste. Antioxidants 4, 699–718. doi:10.3390/antiox4040699

Davey, M.W., Montagu, M. Van, Inzé, D., Sanmartin, M., Kanellis, A., Smirnoff, N., Benzie, I.J., Strain, J.J., Favell, D., Fletcher, J., 2000. Plant L-ascorbic acid: Chemistry, function, metabolism, bioavailability and effects of processing. J. Sci. Food Agric. 80, 825–860. doi:10.1002/(SICI)1097-0010(20000515)80:7<825::AID-JSFA598>3.0.CO;2-6

Dibert, K., Cros, E., Andrieu, J., 1989. Solvent extraction of oil and chlorogenic acid from green coffee part I: Equilibrium data. J. Food Eng. 10, 1–11. doi:10.1016/0260-8774(89)90017-4

Djeridane, A., Yousfi, M., Nadjemi, B., Boutassouna, D., Stocker, P., Vidal, N., 2006. Antioxidant activity of some algerian medicinal plants extracts containing phenolic compounds. Food Chem. 97, 654–660. doi:10.1016/J.FOODCHEM.2005.04.028

Donovan, J.L., Meyer, A.S., Waterhouse, A.L., 1998. Phenolic composition and antioxidant activity of prunes and prune juice (*Prunus domestica*). J. Agric. Food Chem. 46, 1247–1252. doi:10.1021/JF970831X

Duda-Chodak, A., Tarko, T., Satora, P., Sroka, P., Tuszyński, T., 2010. The profile of polyphenols and antioxidant properties of selected apple cultivars grown in Poland. J. Fruit Ornam. Plant Res. 18, 39–50.

Dziki, D., Gawlik-Dziki, U., Pecio, Ł., Różyło, R., Świeca, M., Krzykowski, A., Rudy, S., 2015. Ground green coffee beans as a functional food supplement – Preliminary study. LWT - Food Sci. Technol. 63, 691–699. doi:10.1016/j.lwt.2015.03.076

El-Ghorab, A.H., Nauman, M., Anjum, F.M., Hussain, S., Nadeem, M., 2010. A comparative

study on chemical composition and antioxidant activity of ginger (*Zingiber officinale*) and cumin (*Cuminum cyminum*). J. Agric. Food Chem. 58, 8231–8237. doi:10.1021/jf101202x

EPA, U.S., 2005. IRIS Toxicological Review and Summary Documents for N-Hexane (EPA/635/R-03/012).

Esquivel, P., Jiménez, V.M., 2012. Functional properties of coffee and coffee by-products. Food Res. Int. 46, 488–495. doi:10.1016/J.FOODRES.2011.05.028

Fonseca, H., Regitano-d'Arce, M.A.B., 1993. Aflatoxin removal of peanut meals with aqueous ethanol. Sci. Agric. 50, 154–156. doi:10.1590/S0103-90161993000100021

Furniss, B.S., Hannaford, A.J., Smith, P.W.G., Tatchell, A.R., 2007. Vogel's texbook of practical organic chemistry, 5th ed. Pearson Education, London.

Gaulejac, N.S.-C. de, Provost, C., Vivas, N., 1999. Comparative study of polyphenol scavenging activities assessed by different methods. J. Agric. Food Chem. 47, 425–431. doi:10.1021/JF980700B

Gawlik-Dziki, U., Świeca, M., Dziki, D., Kowalska, I., Pecio, Ł., Durak, A., Sęczyk, Ł., 2014. Lipoxygenase inhibitors and antioxidants from green coffee—mechanism of action in the light of potential bioaccessibility. Food Res. Int. 61, 48–55. doi:10.1016/J.FOODRES.2014.05.002

Goli, A.H., Barzegar, M., Sahari, M.A., 2005. Antioxidant activity and total phenolic compounds of pistachio (*Pistachia vera*) hull extracts. Food Chem. 92, 521–525. doi:10.1016/J.FOODCHEM.2004.08.020

Gorinstein, S., Martín-Belloso, O., Park, Y.-S., Haruenkit, R., Lojek, A., Ĉíž, M., Caspi, A., Libman, I., Trakhtenberg, S., 2001. Comparison of some biochemical characteristics of different citrus fruits. Food Chem. 74, 309–315. doi:10.1016/S0308-8146(01)00157-1

Halvorsen, B.L., Holte, K., Myhrstad, M.C.W., Barikmo, I., Hvattum, E., Remberg, S.F., Wold, A.-B., Haffner, K., Baugerød, H., Andersen, L.F., Moskaug, Ø., Jacobs, D.R., Blomhoff, R., 2002. A systematic screening of total antioxidants in dietary plants. J. Nutr. 132, 461–471. doi:10.1093/jn/132.3.461

Hanmoungjai, P., Pyle, L., Niranjan, K., 2000. Extraction of rice bran oil using aqueous media. J. Chem. Technol. Biotechnol. 75, 348–352. doi:10.1002/(SICI)1097-4660(200005)75:5<348::AID-JCTB233>3.0.CO;2-P

Heinonen, I.M., Lehtonen, P.J., Hopia, A.I., 1998. Antioxidant activity of berry and fruit wines and liquors. J. Agric. Food Chem. 46, 25–31.

Heinonen, I.M., Meyer, A.S., Frankel, E.N., 1998. Antioxidant activity of berry phenolics on

human low-density lipoprotein and liposome oxidation. J. Agruicultural Food Chem. 46, 4107–4112. doi:10.1021/JF980181C

Houghton, P.J., Raman, A., 1998. Laboratory Handbook for the Fractionation of Natural Extracts, Laboratory Handbook for the Fractionation of Natural Extracts. Springer US, Boston, MA. doi:10.1007/978-1-4615-5809-5_1

Hudson, E.A., Dinh, P.A., Kokubun, T., Simmonds, M.S., Gescher, A., 2000. Characterization of potentially chemopreventive phenols in extracts of brown rice that inhibit the growth of human breast and colon cancer cells. Cancer Epidemiol. Biomarkers Prev. 9, 1163–70.

ICO, International Coffee Organization, 2018. Worldwide production of coffee. Int. Coffee Organ. URL http://www.ico.org/prices/po-production.pdf (accessed 3.21.18).

Jaiswal, R., Matei, M.F., Golon, A., Witt, M., Kuhnert, N., 2012. Understanding the fate of chlorogenic acids in coffee roasting using mass spectrometry based targeted and non-targeted analytical strategies. Food Funct. 3, 976. doi:10.1039/c2fo10260a

Jeevan Kumar, S.P., Prasad, S.R., Banerjee, R., Agarwal, D.K., Kulkarni, K.S., Ramesh, K. V, 2017. Green solvents and technologies for oil extraction from oilseeds. Chem. Cent. J. 11, 9. doi:10.1186/s13065-017-0238-8

Johnson, L.A., Lusas, E.W., 1983. Comparison of alternative solvents for oils extraction. J. Am. Oil Chem. Soc. 60, 229–242. doi:10.1007/BF02543490

Jouki, M., Khazaei, N., 1995. Compare of extraction of phenolic compounds from Pistacia atlantica in different solvents. Adv. Biomed. Res. 361–365.

Kähkönen, M.P., Hopia, A.I., Heinonen, M., 2001. Berry phenolics and their antioxidant activity. J. Agric. Food Chem. 49, 4076–4082. doi:10.1021/jf010152t

Kamiyama, M., Moon, J.-K., Jang, H.W., Shibamoto, T., 2015. Role of degradation products of chlorogenic acid in the antioxidant activity of roasted coffee. J. Agric. Food Chem. 63, 1996–2005. doi:10.1021/jf5060563

Kim, J.G., Kim, H.L., Kim, S.J., Park, K.-S., 2013. Fruit quality, anthocyanin and total phenolic contents, and antioxidant activities of 45 blueberry cultivars grown in Suwon, Korea. J. Zhejiang Univ. - Sci. B 14, 793–799. doi:10.1631/jzus.B1300012

Kumaran, A., Karunakaran, R.J., 2006. Nitric oxide radical scavenging active components from *Phyllanthus emblica* L. Plant Foods Hum. Nutr. 61, 1–5. doi:10.1007/s11130-006-0001-0

Lide, D.R., 2006. CRC handbook of chemistry and physics, 87th ed. CRC Press, Boca Raton.

Lin, H.-Y., Kuo, Y.-H., Lin, Y.-L., Chiang, W., 2009. Antioxidative effect and active

components from leaves of lotus (*Nelumbo nucifera*). J. Agric. Food Chem. 57, 6623–6629. doi:10.1021/jf900950z

Liu, S.X., Mamidipally, P.K., 2005. Quality comparison of rice bran oil extracted with d-Limonene and hexane. Cereal Chem. J. 82, 209–215. doi:10.1094/CC-82-0209

Maisuthisakul, P., Suttajit, M., Pongsawatmanit, R., 2007. Assessment of phenolic content and free radical-scavenging capacity of some Thai indigenous plants. Food Chem. 100, 1409–1418. doi:10.1016/J.FOODCHEM.2005.11.032

Marco, G.J., 1968. A rapid method for evaluation of antioxidants. J. Am. Oil Chem. Soc. 45, 594–598. doi:10.1007/BF02668958

Mareček, V., Mikyška, A., Hampel, D., Čejka, P., Neuwirthová, J., Malachová, A., Cerkal, R., 2017. ABTS and DPPH methods as a tool for studying antioxidant capacity of spring barley and malt. J. Cereal Sci. 73, 40–45. doi:10.1016/j.jcs.2016.11.004

Mariod, A.A., Mirghani, M.E.S., Hussein, I., 2017. *Vangueria madagascariensis* (Rubiaceae) as new oil source, in: Mariod, A.A., Mirghani, M.E.S., Hussein, I. (Eds.), Unconventional Oilseeds and Oil Sources. Academic Press, London, pp. 167–180.

Mazzafera, P., Soave, D., Zullo, M.A.T., Guerreiro Filho, O., 1998. Oil content of green beans from some coffee species. Bragantia 57, 45–48. doi:10.1590/S0006-87051998000100006

McGhie, T.K., Hunt, M., Barnett, L.E., 2005. Cultivar and growing region determine the antioxidant polyphenolic concentration and composition of apples grown in New Zealand. J. Agric. Food Chem. 53, 3065–3070. doi:10.1021/jf047832r

Mills, C.E., Oruna-Concha, M.J., Mottram, D.S., Gibson, G.R., Spencer, J.P.E., 2013. The effect of processing on chlorogenic acid content of commercially available coffee. Food Chem. 141, 3335–3340. doi:10.1016/J.FOODCHEM.2013.06.014

Mohdaly, A.A., Sarhan, M.A., Smetanska, I., Mahmoud, A., 2010. Antioxidant properties of various solvent extracts of potato peel, sugar beet pulp and sesame cake. J. Sci. Food Agric. 90, 218–226. doi:10.1002/jsfa.3796

Moure, A., Cruz, J.M., Franco, D., Domínguez, J.M., Sineiro, J., Domínguez, H., José Núñez, M., Parajó, J.C., 2001. Natural antioxidants from residual sources. Food Chem. 72, 145–171. doi:10.1016/S0308-8146(00)00223-5

Naczk, M., Shahidi, F., 2006. Phenolics in cereals, fruits and vegetables: Occurrence, extraction and analysis. J. Pharm. Biomed. Anal. 41, 1523–1542. doi:https://doi.org/10.1016/j.jpba.2006.04.002

Naczk, M., Shahidi, F., 2004. Extraction and analysis of phenolics in food. J. Chromatogr. A

1054, 95–111.

Nagendra Prasad, M.N., Sanjay, K.R., Shravya Khatokar, M., Vismaya, M.N., Nanjunda Swamy, S., 2011. Health benefits of rice bran - A review. J. Nutr. Food Sci. 1, 1–7.

Neo, Y.P., Ariffin, A., Tan, C.P., Tan, Y.A., 2008. Determination of oil palm fruit phenolic compounds and their antioxidant activities using spectrophotometric methods. Int. J. Food Sci. Technol. 43, 1832–1837. doi:10.1111/j.1365-2621.2008.01717.x

Oliveira, É.R., Carvalho, G.R., Santos, P.R., Queiroz, F., 2018a. Green coffee and its residual biomass: Characterization for its industrial approach. Int. Food Res. J.

Oliveira, É.R., Cirillo, M.Â., Silva, R.F., Santos, P.R., Queiroz, F., 2018b. An investigation into green coffee press cake as a renewable source of bioactive compounds an industrially important input: Effect of solvents. Int. J. Food Sci. Technol.

Oliveira, É.R., Caliari, M., Soares Júnior, M.S., Oliveira, A.R., Duarte, R.C.M., Vilas Boas, E.V.B., 2018c. Assessment of chemical and sensory quality of sugarcane alcoholic fermented beverage. J. Food Sci. Tec. 55, 1-10. 10.1007/s13197-017-2792-4

Optiz, S.E.W., Smrke, S., Goodman, B.A., Yeretzian, C., 2014. Methodology for the measurement of antioxidant capacity of coffee: A validated platform composed of three complementary antioxidant assays, in: Preedy, V.R. (Ed.), Processing and impact on antioxidants in beverages. Academic Press, Oxford, UK, pp. 253–264.

Payne, A.C., Mazzer, A., Clarkson, G.J.J., Taylor, G., 2013. Antioxidant assays - consistent findings from FRAP and ORAC reveal a negative impact of organic cultivation on antioxidant potential in spinach but not watercress or rocket leaves. Food Sci. Nutr. 1, 439–444. doi:10.1002/fsn3.71

Pellegrini, N., Serafini, M., Colombi, B., Del Rio, D., Salvatore, S., Bianchi, M., Brighenti, F., 2003. Total antioxidant capacity of plant foods, beverages and oils consumed in Italy assessed by three different in vitro assays. 133, 2812–9.

Peschel, W., Dieckmann, W., Sonnenschein, M., Plescher, A., 2007. High antioxidant potential of pressing residues from evening primrose in comparison to other oilseed cakes and plant antioxidants. Ind. Crops Prod. 25, 44–54. doi:10.1016/J.INDCROP.2006.07.002

Priftis, A., Stagos, D., Konstantinopoulos, K., Tsitsimpikou, C., Spandidos, D.A., Tsatsakis, A.M., Tzatzarakis, M.N., Kouretas, D., 2015. Comparison of antioxidant activity between green and roasted coffee beans using molecular methods. Mol. Med. Rep. 12. doi:10.3892/mmr.2015.4377

R Core Team, 2015. R: A language and environment for statistical computing.

Ramarathnam, N., Osawa, T., Namiki, M., Kawakishi, S., 1989. Chemical studies on novel rice hull antioxidants. 2. Identification of isovitexin, a C-glycosyl flavonoid. J. Agric. Food Chem. 37, 316–319. doi:10.1021/jf00086a009

Randhir, R., Lin, Y.-T., Shetty, K., 2004. Phenolics, their antioxidant and antimicrobial activity in dark germinated fenugreek sprouts in response to peptide and phytochemical elicitors. Asia Pac. J. Clin. Nutr. 13, 295–307.

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., Rice-Evans, C., 1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic. Biol. Med. 26, 1231–1237. doi:10.1016/S0891-5849(98)00315-3

Regitano-d'Arce, M.A., Assis, R. de P., Lima, U. de A., 1994. Functional properties of sunflower seed meal obtained by ethanol extraction. Arch. Latinoam. Nutr. 44, 29–32.

Rodrigues, N.P., Salva, T. de J.G., Bragagnolo, N., 2015. Influence of coffee genotype on bioactive compounds and the in vitro capacity to scavenge reactive oxygen and nitrogen species. J. Agric. Food Chem. 63, 4815–4826. doi:10.1021/acs.jafc.5b00530

Şemen, S., Mercan, S., Yayla, M., Açıkkol, M., 2017. Elemental composition of green coffee and its contribution to dietary intake. Food Chem. 215, 92–100. doi:10.1016/j.foodchem.2016.07.176

Serrato, A.G., 1981. Extraction of oil from soybeans. J. Am. Oil Chem. Soc. 58, 157–159. doi:10.1007/BF02582327

Siva, R., Rajikin, N., Abdul Haiyee, Z., Iryani Wan Ismail, W., 2016. Assessment of antioxidant activity and total phenolic content from green coffee robusta sp. beans. Malaysian J. Anal. Sci. 20, 1059–1065. doi:10.17576/mjas-2016-2005-10

Smrke, S., Opitz, S.E.W., Vovk, I., Yeretzian, C., 2013. How does roasting affect the antioxidants of a coffee brew? Exploring the antioxidant capacity of coffee via on-line antioxidant assays coupled with size exclusion chromatography. Food Funct. 4, 1082. doi:10.1039/c3fo30377b

Snyder, L.R., 1974. Classification of the solvent properties of common liquids. J. Chromatogr. A 92, 223–230. doi:10.1016/S0021-9673(00)85732-5

Somnuk, K., Eawlex, P., Prateepchaikul, G., 2017. Optimization of coffee oil extraction from spent coffee grounds using four solvents and prototype-scale extraction using circulation process. Agric. Nat. Resour. 51, 181–189. doi:10.1016/j.anres.2017.01.003

Speer, K., Kölling-Speer, I., 2006. The lipid fraction of the coffee bean. Braz. J. Plant Physiol 18, 201–216.

Spigno, G., Tramelli, L., De Faveri, D.M., 2007. Effects of extraction time, temperature and solvent on concentration and antioxidant activity of grape marc phenolics. J. Food Eng. 81, 200–208. doi:10.1016/j.jfoodeng.2006.10.021

Suchinina, T. V., Shestakova, T.S., Petrichenko, V.M., Novikova, V. V., 2011. Solvent polarity effect on the composition of biologically active substances, UV spectral characteristics, and antibacterial activity of Euphrasia brevipila herb extracts. Pharm. Chem. J. 44, 683–686. doi:10.1007/s11094-011-0542-x

Sulaiman, S., Abdul Aziz, A.R., Aroua, M.K., 2013. Optimization and modeling of extraction of solid coconut waste oil. J. Food Eng. 114, 228–234. doi:10.1016/J.JFOODENG.2012.08.025

Teh, S.-S., Bekhit, A., Birch, J., 2014. Antioxidative polyphenols from defatted oilseed cakes: Effect of solvents. Antioxidants 3, 67–80. doi:10.3390/antiox3010067

Tir, R., Dutta, P.C., Badjah-Hadj-Ahmed, A.Y., 2012. Effect of the extraction solvent polarity on the sesame seeds oil composition. Eur. J. Lipid Sci. Technol. 114, 1427–1438. doi:10.1002/ejlt.201200129

Tsao, R., Yang, R., Xie, S., Sockovie, E., Khanizadeh, S., 2005. Which polyphenolic compounds contribute to the total antioxidant activities of apple? J. Agric. Food Chem. 53, 4989–4995. doi:10.1021/jf048289h

Tsukui, A., Santos Júnior, H.M., Oigman, S.S., de Souza, R.O.M.A., Bizzo, H.R., Rezende, C.M., 2014. Microwave-assisted extraction of green coffee oil and quantification of diterpenes by HPLC. Food Chem. 164, 266–271. doi:10.1016/j.foodchem.2014.05.039

Turkmen, N., Sari, F., Velioglu, Y.S., 2006. Effects of extraction solvents on concentration and antioxidant activity of black and black mate tea polyphenols determined by ferrous tartrate and Folin–Ciocalteu methods. Food Chem. 99, 835–841. doi:10.1016/J.FOODCHEM.2005.08.034

Ursini, F., Tubaro, F., Rong, J., Sevanian, A., 2009. Optimization of nutrition: Polyphenols and vascular protection. Nutr. Rev. 57, 241–249. doi:10.1111/j.1753-4887.1999.tb06951.x

van der Werf, R., Marcic, C., Khalil, A., Sigrist, S., Marchioni, E., 2014. ABTS radical scavenging capacity in green and roasted coffee extracts. LWT - Food Sci. Technol. 58, 77–85. doi:10.1016/J.LWT.2014.02.053

von Koenen, E., 2001. Medicinal, poisonous, and edible plants in Namibia. Klauss Hess Publishers, Göttingen.

Wan, P.J., Pakarinen, D.R., Hron, R.J., Richard, O.L., Conkerton, E.J., 1995. Alternative hydrocarbon solvents for cottonseed extraction. J. Am. Oil Chem. Soc. 72, 653–659. doi:10.1007/BF02635650

Watanabe, M., Ohshita, Y., Tojiro, T., 1997. Antioxidant compounds from Buckwheat (*Fagopyrum esculentum* Möench) hulls. J. Agric. Food Chem. 45, 1039–1044. doi:10.1021/JF9605557

Waterhouse, A.L., 2002. Wine phenolics. Ann. New York Acad. Sci. 957, 21–36.

Yu, L., Haley, S., Perret, J., Harris, M., 2002. Antioxidant properties of hard winter wheat extracts. Food Chem. 78, 457–461. doi:10.1016/S0308-8146(02)00156-5

Zhou, K., Yu, L., 2004. Effects of extraction solvent on wheat bran antioxidant activity estimation. LWT - Food Sci. Technol. 37, 717–721. doi:10.1016/J.LWT.2004.02.008

Zieliński, H., Kozłowska, H., 2000. Antioxidant activity and total phenolics in selected cereal grains and their different morphological fractions. J. Agric. Food Chem. 48, 2008–16.

Żuk, M., Dymińska, L., Kulma, A., Boba, A., Prescha, A., Szopa, J., Mączka, M., Zając, A., Szołtysek, K., Hanuza, J., 2011. IR and Raman studies of oil and seedcake extracts from natural and genetically modified flax seeds. Spectrochim. Acta Part A Mol. Biomol. Spectrosc. 78, 1080–1089. doi:10.1016/j.saa.2010.12.054

ARTIGO 4 - GREEN COFFEE PRESS CAKE: BIORENEWABLE SOLVENT EXTRACTION AND THERMODINAMIC PARAMETERS

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ABSTRACT

The soluble solids recovery, the retention index, and thermodynamic parameters of green coffee press cake and beans extraction using green solvents were investigated. The extraction parameters investigated were temperature (35 to 55 °C), type of material (coffee beans and press cake), and type of solvent (ethanol, acetone, and ethyl acetate). The extractions were carried out in finit bath at a fixed solvent to solute mass ratio (5:1) (w/w). The recovery of soluble solids from the materials was obtained through mass transfer and the system mass balance. It was observed that higher temperatures affected positively the extraction yields. At 55 °C, the extraction yields were not improved when using ethanol as the solvent. The thermodynamic analyzes showed that both ΔH and ΔS were positive for this process, while negative values of ΔG were found, that means the process evaluated could be characterized as endothermic, irreversible, and occurs spontaneously.

Keywords: *Coffea arabica*; alternative solvents; thermodynamic; solid-liquid extraction; food waste.

INTRODUCTION

Recently, another operation has been introduced to the coffee industry, the oil expression, which generates a great amount of residues. The green coffee oil expression is carried out through a screw press, and it is believed that this operation is not efficient enough to remove the oil from the matrix. And besides leaving a residual oil in the meal, a great amount of bioactives may also be found in this residue, once most of the phenolic compounds are of hydrophilic nature. According with Affonso et al. [1], the coffee beans press cake is a residual biomass from the coffee beans oil extraction process, claimed to be rich in bioactive compounds of interest for human health, food industry, and cosmetics. There is considerable emphasis on the recovery of plant biomass originating from the food industry in order to target it to other industries [2], adding value and reducing eventual environmental damage. In this sense, the large amount of beans produced in Brazil gives rise to certain amounts of biomass with an important potential for the development of other products such as energetic beverages and cosmetics.

An option to optimize the mechanical extraction process could be alying this method with the solvent extraction, aiming at the exhaustion of the compounds of interest remaining in the residue, or even use the the solvent extraction as the only step. The extraction process aims at providing a maximum yield of substances of the highest quality. Yet, literature about the most effective methods and the solvents to extract soluble compounds is abundant but to some extent contradictory [3]. Considering the structure of the matrices and their physicochemical properties, it would be impossible to propose a universal extraction protocol. Different solvent systems have been used for the extraction of solids from plant material, the extraction yield is influenced by the solvent nature and the extraction method. According to Akowuah et al. [4], depending on the solvent used for extracting compounds, extracts obtained from the same material may vary widely with respect to their concentration and composition. Type of solvent, temperature, solid–liquid ratio, particle size and agitation are important parameters for extraction of solids from food or plant matrices.

There are several studies on the extraction behavior of different plant materials such as coffee bean [5], soy bean [6], neem seed [7], aniseed [8], avocado [9], pongam seeds [10], pequi and murici seeds [11], and walnuts [12]. These studies were conducted in aqueous form or solvent extraction

including the parameters mentioned. It is generally reported that the extraction rate increases with increasing in temperature and liquid/solid ratio, and decreasing the particle size, however these parameter shall be extensively studied, once high temperatures may favor the extraction yield of solids and desfavor the recovery of phenolic and atioxidants [13]. Earlier works reported extraction efficiency in different coffee species by using different extraction methods, such as, conventional [14–16], pressurized liquid [5,17], ultrasonic assisted, [18], supercritical CO_2 [19,20], and microwave extraction [21]. However, there is no reported study on organic solvent extraction behavior of total soluble solids from green coffee beans and its press cake in order to state the efficiency of the mechanical oil extraction. Therefore, the present study was carried out in order to determine the recovery yield of total soluble solids from green coffee beans and its press cake extracted by organic ecofriendly solvents, including ethanol, at different temperatures, and also to study and establish useful and relevant thermodynamic parameters of the process.

MATERIAL AND METHODS

Sampling

The samples of Arabica green coffee beans were harvested in the cherry state in the region of Guaxupé (Minas Gerais) located in the Southest Region of Brazil. Both beans and its press meal were kindly donated by Cooxupé (Guaxupé, Brazil). Green coffee press cake samples were obtained after mechanically screw pressing the beans for the extraction of the oil fraction (100 kg h^{-1}; Ecirtec, MPE-500 AC, Bauru, Brazil). After extraction, the cake was packed in polyethylene bags, with silica pouches, and frozen for further analyzes. Prior being submitted to the solid-liquid extraction, both materials were ground (IKA, A11, Wilmington, US) and sieved (0.177 mm) (Bertel, VP-01, Caieiras, Brazil) (NREL, 2008). Each powder was then dried (40 °C) in a vacuum oven (16.8 kPa) (Tecnal, TE-395, Piracicaba, Brazil) (approximately 20 days for green coffee beans and 7 days for the cake), and further used to the extraction process. The physical and chemical characterization of the materials is available at Oliveira et al.[22].

Solvent Extraction

The solvent extraction of soluble solids from green coffee and its press cake was carried out in batches using ethanol (boiling point = 78 °C, 99% purity), acetone (boiling point = 56 °C, 99% purity) and ethyl acetate (boiling point = 78 °C, 99% purity), at constant solid-liquid ratio (1:5 w/w). Each experiment was carried out thrice in different days. The samples were placed in Falcon tubes (50 mL) and added with the solvents in the convenient proportion. The tubes were then placed in a stirring Dubnoff Orbital water bath (220 rpm; Novatecnica, NT 230, Piracicaba, Brazil) at constant temperature (according to each experiment) (Table 1) for 4 h (period determined in preliminary tests as enough to reach the equilibrium). A mild temperature range was chosen with the purpose of not degrading possible bioactive compounds present in the extracts to be analyzed in further studies.

Table 1. Planning of the solid-liquid extraction of soluble solids from green coffee beans and its press cake.

Experiment	Ethanol	Acetone	Ethyl Acetate	Temperature (°C)
1	-	+	-	35
2	-	-	+	35
3	+	-	-	35
4	-	+	-	39
5	-	-	+	39
6	+	-	-	39
7	-	+	-	45
8	-	-	+	45
9	+	-	-	45
10	-	+	-	50.9
11	-	-	+	50.9
12	+	-	-	50.9
13	-	+	-	55
14	-	-	+	55
15	+	-	-	55

(-): Absence of solvent; (+): Presence of solvent.

After the extraction time had elapsed, aliquots of the supernatant phase (extract) (~ 2 mL) were removed with the aid of pre-weighed microsyringes. The sample withdrawn with the aid of the syringe was placed in a Petri dish, also pre-weighed. Ethanol was used to wash the syringe (after the extract phase disposal) in order to remove any solids that may have become adhered to the syringe wall. The Petri dish containing the extract phase + ethanol was oven dried at 100 °C for at least 2 h in order to evaporate the solvent. The raffinate phase was subjected to centrifugation

(3500 rpm/ 1 min) (Excelsa II – 206 BL, FANEM, São Paulo, Brazil) after the removal of the extract phase, then the remaining liquid was discarded and a sample of the raffinate phase was withdrawn and immediately weighed into a Petri dish, which was subjected to drying (100 °C/ 2 h). Further, the samples without the solvent were cooled down in desiccators and weighed.

Determination of Soluble Solids Mass Transfer

For the extraction experiments performed, the extract (EP) and the raffinate (RP) phases were determined in terms of the mass fractions of the components. The mass fraction of EP was determined using the data obtained from the samples taken with the microsyringe. The withdrawn EP sample mass was calculated using the mass difference of the syringe with extract and the empty syringe. The soluble solids mass in the EP sample was calculated from the mass difference of the Petri dish containing the extract sample after the solvent evaporation and the empty Petri dish. The mass fraction of soluble solids ($w_{1,EP}$) and the mass fraction of the solvent ($w_{2,EP}$) in the EP, both insoluble solids free ($w_{3,EP}$= 0), were calculated according to equations 1 and 2, respectively.

$$w_{1,EP} = \frac{m_f - m_i}{m_{EP}} \quad (1)$$

$$w_{2,EP} = 1 - w_{1,EP} \quad (2)$$

Where, m_f and m_i are the final and initial weight of the Petri dish, respectively, and m_{EP} is the mass of the EP sample withdrawn.

The mass fraction of the solvent in RP ($w_{2,RP}$) was calculated by the difference of weights found before and after drying (Equation 3).

$$w_{2,RP} = \frac{m_f - m_i}{m_{RP}} \quad (3)$$

Where, m_f and m_i are the final and initial weight of the Petri dish, respectively, and m_{RP} is the mass of the raffinate phase sample withdrawn.

The mass of the system (solvent and material) (m_{system}), and the mass fraction of each component in the system are known variables. The remaining variables - mass of EP (m_{EP}), mass of RP (m_{RP}), soluble solids mass fraction in RP ($w_{1,RP}$), and insoluble solids mass fraction in RP ($w_{3,RP}$) - were determined through a global mass balance (Equation 4). And the mass balance for each component of the system (*i*) is presented in Equation 5.

$$m_{system} = m_{EP} + m_{RP} = m_2 + m_s \quad (4)$$

Where, m_{system} is the mass of the global system, m_{EP} is the mass of the extract phase withdrwan with the syringe, m_{RP} is the mass of the raffinate phase collected, m_2 is the solvent mass used in the extraction process, and m_s is the mass of sample used in the experiment.

$$w_{\mathrm{i},system} m_{system} = w_{\mathrm{i},EP} m_{EP} + w_{\mathrm{i},RP} m_{RP} \quad (5)$$

Where, *i* = 1 (soluble solids) or 2 (solvent) or 3 (insoluble solids).

The mass fraction of the componente *i* (w_i) is defined by Equation 6, where m_i is the mass of a given component in the EP or RP (m_P).

$$w_i = \frac{m_i}{m_P} \quad (6)$$

The soluble solids mass transfer (Γ_1) in the extraction process was calculated through Equation (7) (Rodrigues et al., 2010), where m_s is the mass of samples (beans and cake) used in the extraction process, and $w_{1,s}$ is the mass fraction of soluble solids in the seeds before the extraction process (Soxhlet extraction using ethanol as the solvent).

$$\Gamma_1 \text{ (\%)} = 100 \ x \left(\frac{w_{1,EP} m_{EP}}{w_{1,s} m_s}\right) \tag{7}$$

The retention index (RI) corresponds to the mass of adhered solution per soluble solids mass, and was calculated using Equation (8):

$$\text{RI} = \frac{w_{1,RP} m_{RP} + w_{2,RP} m_{RP}}{w_{3,RP} m_{RP}} = \frac{m_{1,RP} + m_{2,RP}}{m_{3,RP}} \tag{8}$$

Thermodynamic Study

Enthalpy (ΔH) (kJ/mol), entropy (ΔS) (J/(mol K)), and Gibbs free energy (ΔG) (kJ/mol) variations are important thermodynamic parameters in order to study the spontaneity of the extraction process involving different systems. Thermodynamic parameters of the soluble solids solid-liquid extraction process from green coffee beans and its press cake were assessed for each solvent using Equations 9 – 11. The ΔH and ΔS from the press cake and green coffee beans extraction were calculated by a linear fit of the Van't Hoff equation (Equation 10). Where, K_e is the distribution coefficient for the solid-liquid system, $m_{1,EP}$ (kg) is the soluble solids mass in the EP, $m_{1,RP}$ (kg) is the soluble solids mass in the RP, R (8.3145 kJ/(kmol K)) is the universal constant of gases, and T (K) is the temperature.

$$K_e = \frac{m_{1,\text{EP}}}{m_{1,\text{RP}}} \tag{9}$$

$$\ln K_e = -\frac{\Delta H}{RT} + \frac{\Delta S}{R} \tag{10}$$

$$\Delta \text{G} = \Delta \text{H} - \text{T} \cdot \Delta \text{S} \tag{11}$$

Statistical Analysis

A completely randomized design (CRD) was applied in order to plan a factorial experiment

with three replicates. Type of material (M), temperature (T), and type of solvent (S) were the independent variables studied as a factorial 2x3x5 (MxTxS) (Equation 12). The ANOVA was carried out with the effect of each variable and their interactions were evaluated. Scott Knotts's test was further applied to compare the means of the response variables (yield or RI) or their interactions, when siginificative ($p < 0.05$). All the analyzes were performed using the software RStudio version 3.2.0 (R Core Team, 2015).

$$Y_{ijk} = \mu + \alpha_i + \beta_j + \partial_k + (\alpha\beta)_{ij} + (\alpha\,\partial)_{ik} + (\partial\beta)_{kj} + (\alpha\beta\,\partial)_{ijk} + e_{ijkz} \quad (12)$$

Where, Y_{ijkz} is the observed value for the response variable in study respective to the z-th repetition of the combination of the i, j, k levels of the factors M, S, T, respectively; μ is the average of all experimental units of the response variable; α_i, β_j, ∂_k are the effects of the i, j, k levels of each factor M, S, T in the observed value Y_{ijk} , respectively; $(\alpha\beta)_{ij}$, $(\alpha\,\partial)_{ik}$, $(\partial\beta)_{kj}$, $(\alpha\beta\,\partial)_{ijk}$ are the effects of the interactions of the factors in study; e_{ijk} is the error associated with the observed value Y_{ijk}; $i = 2, j = 3, k = 5$.

RESULTS AND DISCUSSION

Soluble solids recovery (Γ_1) and Retention index (RI)

Factorial assays allow for savings in time and resources, but mainly, they enable broader conclusions about the factors including the study of the interaction among them and greater precision for the estimates of the main effects of the factors, also, the degree of freedom associated with the residue is high when compared to the simple experiments of the same factors, which contributes to decrease the residual variance, increasing the precision of the experiment. The results of the ANOVA, considering the sources of variation, such as material (M), solvent (S), and temperature (T), employed in the extraction process, are presented in Table 2.

Table 2. ANOVA table (p < 0.05) for the soluble solids mass transfer (Γ_1) and retention index (RI) of the solid-liquid extraction process of green coffee beans and its press cake using different solvents and temperatures.

Source	Γ_1				RI				$P_r > F$	
	GL	SQ	QM	F_c	GL	SQ	QM	F_c	Γ	RI
Material (M)	1	2.52	2.52	531.03	1	6.34	6.34	19.36	< 0.01*	< 0.01*
Solvent (S)	2	1.42	0.71	150.38	2	0.14	0.07	1763.4	< 0.01*	< 0.01*
Temperature (T)	4	5.49	1.37	289.48	4	0.08	0.02	< 0.01	< 0.01*	< 0.01*
M*S	2	0.18	0.1	19.32	2	0.24	0.12	32.77	< 0.01*	< 0.01*
S*T	8	0.57	0.07	15.14	8	0.26	0.03	8.98	< 0.01*	< 0.01*
M*T	4	0.27	0.07	14.04	4	0.26	0.07	18.4	< 0.01*	< 0.01*
M*S*T	8	0.32	0.04	8.39	8	0.13	0.02	< 0.01	< 0.01*	< 0.01*
Residue	60	0.28	0.005		60	0.22	0.004			
Total	89	11.07	0.12		89	7.67	0.09			

*Sigificant at p < 0.05.

In this part of the analysis, ANOVA initially compares the model (all known sources of variation) randomly, and then compares each of the effects of the model (Table 2). In the ANOVA, the most important point to be evaluated is the chance of an effect being due to randomization ($P_r > F$). In Table 2, the probabilities were very close to zero, that is, there was a chance near 0% of the effect of a given source of variation being due to randomization. Considering the significance level of 5%, which was used in this study, in order to assess the significance of a given effect it is sufficient to verify that the value found for Pr > F is less than 0.05.

Considering the response variable Γ_1, the ANOVA came out significant ($p < 0.05$) for all effects (main effects and interactions). In this case, only the ternary interaction (M*S*T) was analyzed. This case occurs when the nule hypothesis for the interaction between the factors is rejected. This result implies that the effects of the factors act in a dependent way. In this case, the comparisons between the levels of one factor take into account the level of the other factor, since the significant result for the interaction indicates that the effect of one factor depends on the level of the other factor. Since the results of each effect were dependent on the other two effects, Scott Knott's test was only performed for the significative ternary interaction, which was analyzed through the unfolding of the interactions in each combination of levels (Table 3).

Considering the solvent effect on each level of temperature and material (Table 3), it was possible to observe that the Γ_1 (%) for green coffee beans was higher when acetone was used as the extractor solvent in all temperatures analyzed. The solvent ethyl acetate was only more efficient than ethanol to extract soluble solids at 55°C. At 39, 45, and 50.9 °C there was no significative difference among these two solvents performance, showing that either one could be used in order

to achieve a similar result, also, that their polarity difference did not influence the extraction recovery of soluble solids; however, it could influence the extract composition, which was not evaluated. In case of opting for one rather than the other, the solvent ethanol might be a good choice, once it is highly available (what lowers its cost) in many countries, such as Brazil, also due to its non-polluting and favorable to handling features. A wide range of literature has brought to interest that, in general, ethanol allows a better quality meal recovery and results in a greater extraction of sugars, free fatty acids, pigments, waxes, and peroxides [24–27].

Table 3. Soluble solids mass transfer ($\%\Gamma_1$) (d.b.) of the solid-liquid extraction process of green coffee beans and its cake using different solvents and temperatures.

Solvent	Coffee				
	35 °C	39 °C	45 °C	50.9 °C	55 °C
Acetone	$42.96\pm1.07^{a,D,\alpha}$	$52.28\pm0.98^{a,C,\alpha}$	$54.12\pm6.72^{a,C,\alpha}$	$82.46\pm7.47^{a,B,\alpha}$	$90.04\pm3.7^{a,A,\alpha}$
Ethyl Acetate	$23.07\pm0.0^{c,E,\alpha}$	$37.86\pm2.46^{b,D,\alpha}$	$43.7\pm1.81^{b,C,\alpha}$	$60.0\pm2.06^{b,B,\alpha}$	$65.68\pm5.41^{b,A,\alpha}$
Ethanol	$34.58\pm3.39^{b,C,\alpha}$	$36.79\pm2.64^{b,C,\alpha}$	$41.62\pm2.19^{b,B,\alpha}$	$63.39\pm1.78^{b,A,\alpha}$	$42.18\pm0.85^{c,B,\alpha}$
	Cake				
Acetone	$27.92\pm0.89^{a,C,\beta}$	$37.56\pm1.94^{a,B,\beta}$	$38.28\pm3.58^{a,B,\beta}$	$39.29\pm0.2^{b,B,\beta}$	$56.66\pm2.77^{a,A,\beta}$
Ethyl Acetate	$22.68\pm3.88^{a,D,\alpha}$	$31.84\pm2.02^{b,C,\beta}$	$34.26\pm3.13^{a,B,\beta}$	$35.27\pm2.46^{b,B,\beta}$	$47.42\pm3.66^{b,A,\beta}$
Ethanol	$24.63\pm1.67^{a,B,\beta}$	$26.56\pm1.81^{c,B,\beta}$	$29.64\pm0.89^{b,B,\beta}$	$44.33\pm1.39^{a,A,\beta}$	$46.54\pm2.21^{b,A,\alpha}$

Mean ± standard deviation (n = 3).
Different lower case letters in the same column, for each solvent level, indicate significant difference among the treatments by the Scott Knott's test ($p < 0.05$) (Solvent unfolding within each temperature and material levels).
Different upper case letters on the same line, for each temperature level, indicate a significant difference among the treatments by the Scott Knott's test ($p < 0.05$) (Temperature unfolding within each material and solvent levels).
Different Greek letters in the same column, for each material level, indicate a significant difference between the treatments by the Scott Knott's test ($p < 0.05$) (Material unfolding within each temperature and solvent levels).

From these results, another fact that could be stated is that inspite of acetone being only slightly less polar than ethanol, it showed far better performance in the extraction process, which could be explained by the composition of the vegetable matrix analyzed.

The solvent effect on the extraction of soluble solids from the press cake showed a different behavior from that presented for the beans. In this case, acetone performed better only at 39 and 55 °C, and at 45 °C it showed the same efficiency as ethyl acetate. Ethanol was the greatest solvent in recovering the soluble solids from the cake at 50.9 °C; showing that at higher temperatures ethanol should be used, and at lower temperatures, acetone should be the best choice.

Concerning the temperature effect on each level of solvent and material (Table 2), overall, the greatest Γ_1 (%) for green coffee beans was obtained at the two highest temperatures studied. Acetone presented the best results, showing that the temperature may exert great influence on extraction processes, as observed also for the ethyl acetate results, where the higher yields were

obtained at the highest temperatures. The elevation of temperature from 35 to 50.9 °C promoted an increase of 91% and 83% on the results obtained with acetone and ethanol, respectively. On the other hand, acetone performed similarly at 39 and 45 °C, meaning that the lowest temperature could be used in order to save energetic costs in the process. This energetic cost saving could be also observed when ethanol was applied at 35 and 39 °C, where no difference in the results were observed.

The temperature effect on the extraction of soluble solids from the press cake presented results as expected, once the most reasonable recovery yields were obtained at 55 °C. Ethyl acetate results were similar at 45 and 50.9 °C, indicating that the process could be carried out at 45 °C without harming the process results. The same trend was observed for acetone and ethanol results, where in the case of acetone, it is suggested to perform the process at 39 °C rather than at 45 °C or 50.9 °C; and concerning the ethanol results, it is cost saving to carry the process at 35 °C, rather than at 39 °C or 45 °C.

As expected, the temperature effect on the recovery yield was greater for the green coffee beans which present higher content of extractable solids compared to its press cake. This fact could be also conffirmed when analyzing the effect of the materials on each level of solvent and temperature (Table 3), where the Γ_1 (%) was significantly higher for the beans rather than for the cake at all temperatures and most of the solvents (excluding ethanol, that showed similar results for both materials at 55 °C, and ethyl acetate, at 35 °C). When a low solvent to solute ratio is used, as in this study (5:1 w/w), the extraction may be limited by the saturation of the solids that are soluble in the solvent, and, generally, by raising the temperature up to a point, there is an increase of the solids solubility in the solvent and consequent increase in the yield of the extraction process. The solubility of the oil or extractable solids in the solvent may be related to the temperature, matrix composition, and also to the type of extraction process, being an important criteria for the process, since with an increase in temperature the solubility increases and, consequently, there is an improvement in the extraction efficiency [28–30].

Another interesting behavior observed was the lowering of the recovery of soluble solids from green beans when the temperature was raised from 50.98 to 55 °C and the extraction was carried out with ethanol, which could be explained, according with Dibert et al. [31], by the fact that for a given mass ratio, when there is no adsorption of the solute to the solid matrix, the extraction

yields at equilibrium do not depend on the temperature of the system. These authors studied the equilibrium data of the green coffee oil extraction using hexane (20 h; 30, 40, and 50°C), and also reported this trend when raising the temperature from 40 to 50 °C at the same mass ratio applied in this study. They also observed that the equilibrium concentration yield decreased with increasing solid-liquid mass ratio.

The retention index (RI) is an important extraction parameter to be analyzed, once it measures the adhered solution to the inert. This variable substantially impacts the extractors design, as long as it influences in the number of stages of the process and also in the solvent recovering stage – higher the RI the higher is the operational cost [23]. The RI is directly related to the solution viscosity, particle size, and to the physicochemical afinity between the solution and the oily matrix [32]. Concerning this response variable (RI), the ANOVA (Table 2) was significant to the triple interaction (M*S*T), as well as it was for the Γ_1. Taking into account only the interaction amongst material, solvent, and temperature, since the linear factors should not be discussed if the interactions were significant, the results for RI are presented in Table 4, where it is possible to evaluate the behavior of the RI for the unfolding of each independent variable analyzed into the other variables.

It was observed that for all solvents and temperatures there was a significant difference among the results of the adhered solution mainly to the green coffee beans, with the highest values obtained for this material. Although both materials had a similar composition, the heat treatment and the pressing of the material influenced the retention of the solution, which is interesting, once it indicates that with application of pre-treatments there is an improvement in the efficiency of the process.

The higher RI was observed when ethyl acetate was employed as the solvent, at most temperature levels, except at 35 °C, in which the solvent ethanol presented higher RI. Concerning the press cake, only at 35 °C there was a significant difference amongst the solvents; the solvent ethanol presented higher RI at 35 °C. On the other hand, the other solvents showed that they are not distinguishable to this variable.

Table 4. Retention index (RI) of the solid-liquid extraction process of green coffee beans and its press cake.

Temperature (°C)	RI		
	Acetone	Ethyl Acetate	Ethanol
		Green Coffee	
35	$1.12 \pm 0.02^{b,A,\beta}$	$1.20 \pm 0.24^{b,A,\beta}$	$1.36 \pm 0.05^{a,A,\alpha}$
39	$1.03 \pm 0.08^{b,A,\beta}$	$1.31 \pm 0.01^{a,A,\beta}$	$1.10 \pm 0.07^{b,A,\delta}$
45	$1.38 \pm 0.26^{a,A,\alpha}$	$1.39 \pm 0.18^{a,A,\alpha}$	$1.26 \pm 0.01^{b,A,\beta}$
50.9	$1.05 \pm 0.02^{b,A,\beta}$	$1.42 \pm 0.16^{a,A,\alpha}$	$1.04 \pm 0.06^{b,A,\delta}$
55	$1.01 \pm 0.03^{c,A,\beta}$	$1.33 \pm 0.03^{a,A,\beta}$	$1.19 \pm 0.01^{b,A,\gamma}$
		Press Cake	
35	$0.70 \pm 0.06^{b,B,\alpha}$	$0.65 \pm 0.02^{b,B,\beta}$	$0.83 \pm 0.03^{a,B,\alpha}$
39	$0.70 \pm 0.01^{a,B,\alpha}$	$0.60 \pm 0.04^{a,B,\beta}$	$0.66 \pm 0.0^{a,B,\gamma}$
45	$0.60 \pm 0.03^{a,B,\beta}$	$0.63 \pm 0.01^{a,B,\beta}$	$0.59 \pm 0.0^{a,B,\gamma}$
50.9	$0.75 \pm 0.01^{a,B,\alpha}$	$0.75 \pm 0.01^{a,B,\alpha}$	$0.77 \pm 0.02^{a,B,\beta}$
55	$0.64 \pm 0.01^{a,B,\beta}$	$0.65 \pm 0.03^{a,B,\beta}$	$0.70 \pm 0.02^{a,B,\beta}$

Mean ± standard deviation (n = 3).
Different lower case letters in the same row, for each solvent level, indicate significant difference among the treatments by the Scott Knott's test ($p < 0.05$) (Solvent unfolding within each temperature and material levels).
Different upper case letters in the same column, for each material level, indicate a significant difference among the treatments by the Scott Knott's test ($p < 0.05$) (Material unfolding within each temperature and solvent levels).
Different Greek letters in the same column, for each temperature level, indicate a significant difference between the treatments by the Scott Knott's test ($p < 0.05$) (Temperature unfolding within each material and solvent levels).

By unfolding the interaction of each solvent level within each material and temperature levels of the beans extraction, acetone and ethyl acetate showed the best results in the extreme (the lowest and the highest) temperature levels; ethanol, however, followed the expected trend, where the lowest content of adhered solution to the raffinate was verified at the highest temperatures. This same behavior of the solvent ethanol was observed for the press cake; acetone showed the lowest values of RI at 45 and 55 °C.

This leads us to conclude that the higher temperature empolyed in the extraction process (55 °C) was more efficient to extract the soluble solids from the green coffee materials, since it presented the lowest RI, however, it is important to state that the RI is not the only parameter affecting the yield of the process. Although, it was expected that the lowest temperatures would present greater RI values, as occurred in the case of ethanol for both the press cake and coffee beans, since at low temperatures the lowest yields were obtained. The increase in temperature provides a decrease in the viscosity of the solution [33], allowing the decrease of the amount of adhered solution in the inert matrix. This is due to the fact that RI is an important variable due to its direct impact on the solvent content used in the extraction process, amount of extract obtained, losses of solution in the raffinate stream [32], and recovering losses.

According to Rittner [26], polar alcohols have high affinity for the solid matrix, tending to become more adhered to it, and consequently increase the RI. However, this could not be corroborated to our results, once the greater RI values were obtained to the solvent ethyl acetate, which was the least polar solvent studied.

Araújo et al. [11] reported the dependence of the RI on the temperature and solvent type for the soluble solids extraction process of pequi and murici seeds. When using ethanol as an extractor, the authors observed that the increase in temperature promoted an increase in RI, whereas when isopropanol was used, the RI reduced when the temperature was raised from 35 to 45 °C; as the concentration of ethanol in the solvent increased, the value of the RI response variable occurred.

Thermodynamic study

In order to perform the thermodynamic study of the soluble solids extraction from green coffee beans and its press cake with different alternative solvents, the distribution coefficients (K_e) were experimentally determined (Table 5). The thermodynamic parameters (ΔH, ΔS, and ΔG) estimated for the extraction of soluble solids were obtained from the values of K_e. ΔH and ΔS values were obtained from the linear and angular coefficients of the linear regressions of the experimental data through equation (10) ($R^2 > 90\%$). ΔG values were obtained using equation (11). The values of the thermodynamic parameters are shown in Table 5. In the thermodynamic study, the enthalpy (ΔH) and entropy (ΔS) variation values were positive. The Gibbs energy variation (ΔG) presented negative values. The positive ΔH values indicate the endothermic nature of the process, which requires energy to occur. The positive values of the ΔS indicate an increase in the degree of molecular disorder during the process for the solids-oil-solvent system, and also the irreversibility of the same. This effect was expected because the oil transferred from a solid phase (beans or cake) to a liquid one (solvent) [33].The extraction process involves the mixing of at least two different substances, which leads to the increase of their disorder [34]. The same trend was also observed by other authors for the extraction of oil from several plant matrices, regardless of the type of solvent used [33,35–38]. Concerning the ΔS, positive for all treatments, a trend was observed, the values were lower to the extraction of soluble solids for green coffee beans using ethyl acetate. The process is controlled by the ΔS, being favored by the temperature increase, where this trend

significantly influenced the recovery of soluble solids; and for all conditions makes the process more spontaneous, with low ΔG values [11].

Table 5. Thermodynamic parameters of the solid-liquid extraction of soluble solids from green coffee beans and its press cake using alternative solvents.

Solvent	Temperature (°C)	Green coffee beans				Press cake			
		K_e	ΔH (kJ/ mol)	ΔS (J/ mol/ K)	ΔG (kJ/ mol)	K_e	ΔH (kJ/ mol)	ΔS (J/ mol/ K)	ΔG (kJ/ mol)
Acetone	35	0.69±0.11	93994.12	301.17	-22466.61	0.39±.02	26535.39	79.21	-6127.72
	39.02	1.1±0.04			-22612.22	0.57±0.05			-6165.53
	45	1.34±0.038			-22828.83	0.62±0.09			-6222.5
	50.98	4.42±0.19			-23045.44	0.73±0.15			-6279.47
	55	6.32±0.06			-23191.05	0.78±0.06			-6317.77
Ethyl acetate	35	0.83±0.0	53831.71	169.01	-12738.28	0.41±0.07	25587.48	75.49	-5875.28
	39.02	0.61±0.06			-12819.99	0.47±0.04			-5911.78
	45	0.78±0.06			-12941.55	0.52±0.07			-5966.07
	50.98	1.5±0.13			-13063.11	0.62±0.06			-6020.37
	55	1.99±0.6			-13144.82	0.8±0.13			-6056.87
Ethanol	35	0.49±0.09	38846.59	120.18	-9126.08	0.33±0.03	44853.69	135.47	-10415.39
	39.02	0.61±0.06			-9184.18	0.36±0.03			-10480.89
	45	0.71±0.06			-9270.61	0.42±0.02			-10578.32
	50.98	1.57±0.29			-9357.05	0.8±0.04			-10675.76
	55	0.94±0.37			-9415.15	0.87±0.08			-10741.26

According with Cooney et al. [39], the most effective ethanolic solvents in oil extraction are those with low water content, once they cause greater variations of ΔS and, consequently, result in negative ΔG. Thus, the moisture content in ethanol is not favorable in the extraction of oils. In this way, the drying of the raw material is an important factor in the yield of the process, once the moisture of the raw material migrates to the extract phase, decreasing the solubilizing power of ethanol [40]. Also, in processes where ΔH > 0 under conditions of higher temperatures, ΔS becomes dominant and, consequently, the oil dissolution process becomes spontaneous.

For green coffee beans and its press cake, in the temperature range of operation, the lowest values of ΔG were obtained in the extraction with acetone and ethyl acetate, respectively; what may explain the good yields obtained with these solvents. These results could be also related to the fact that acetone may show a better solubility to the oil.

In the literature, it is reported the thermodynamic parameters for several systems using ethanol as solvent, generally comparing with the results obtained with hexane, and other alternative solvents considered less toxic. Liauw et al. [36] studied the process of neem oil extraction with ethanol, using the same solvent:seed mass ratio and temperature range as in this study, although, different particle sizes were applied. They obtained ΔH in the range of 75 – 115 kJ/mol, and ΔS

values from 263 to 392 J/mol/K). ΔH and ΔS results for hexane were lower than those obtained with ethanol, and it was a system where ethanol extraction exhibited lower values of ΔG than the hexane extraction process. Meziane and Kadi (2008) studied the process of olive oil extraction (oil content of 11.07%) with ethanol (96%), and temperature range of 20 – 50 °C. The authors observed ΔH values of 12.91 kJ/mol, ΔS of 59.33 J/mol/K, and ΔG < 0. Similar parameters were obtained by Amarante et al. [33] in the process of extracting castor oil (oil content of 14.78%) using ethanol, and temperature range of 20 – 55 °C (ΔH 12.27 kJ/mol, ΔS 57.41 J/mol/K, ΔG < 0). Sulaiman et al. [38] while studying the oil extraction process from coconut residues using hexane and petroleum ether obtained positive values for ΔH and ΔS, and negative results for ΔG.

Evaluating all parameters analyzed (extraction yield, RI, and thermodynamic parameters), it was possible to observe that acetone and ethanol were more efficient in the extraction of soluble solids from green coffee beans and its press cake. Showing that the applying of alternative solvents is possible, which allow the achievement of satisfactory yields, which can be improved with further studies. The negative value for ΔG for the soluble solids extraction from green coffee beans and its press cake showed that the process was feasible and spontaneous and that the extraction increased with increasing the temperature as ΔG became more negative. The value of the thermodynamic parameters indicated that the extraction was endothermic and the process was irreversible.

CONCLUSION

The experimental results showed that the conditions during the extraction process had a significant influence on the extractability of total soluble solids from green coffee beans and from its press cake. The highest efficiency of soluble solids compounds extraction at the temperature of 55 °C and 4 h of the extraction was achieved with the solvents acetone and ethanol.

The solution retained in the raffinate phase from green coffee beans extraction process was greater than for the green coffee press cake. On the other hand, the soluble solids extraction yield was higher for the beans concerning each solvent used. The increase in temperature favored the extraction yield. For all operational levels the ΔH and ΔS were positive, and the ΔG was negative, being the process, independent of the solvent, endothermic and spontaneous, favored by the temperature increase. The thermodynamic study showed that the soluble solids extraction from

green coffee beans and from its press cake is a process that requires more energy to be carried out, therefore the study of parameters such as temperature and type of solvent is very important in order to optimize such processes.

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REFERENCES

(1) Affonso, R. C. L.; Voytena, A. P. L.; Fanan, S.; Pitz, H.; Coelho, D. S.; Horstmann, A. L.; Pereira, A.; Uarrota, V. G.; Hillmann, M. C.; Varela, L. A. C.; et al. Phytochemical Composition, Antioxidant Activity, and the Effect of the Aqueous Extract of Coffee (*Coffea Arabica* L.) Bean Residual Press Cake on the Skin Wound Healing. *Oxid. Med. Cell. Longev.* **2016**, *2016*, 1923754.

(2) Laufenberg, G.; Kunz, B.; Nystroem, M. Transformation of Vegetable Waste into Value Added Products: (A) the Upgrading Concept; (B) Practical Implementations. *Bioresource Technology*. Elsevier April 1, 2003, pp 167–198.

(3) Contini, M.; Baccelloni, S.; Massantini, R.; Anelli, G. Extraction of Natural Antioxidants from Hazelnut (*Corylus Avellana* L.) Shell and Skin Wastes by Long Maceration at Room Temperature. *Food Chem.* **2008**, *110* (3), 659–669.

(4) Akowuah, G. A.; Zhari, I.; Norhayati, I.; Sadikun, A. Radical Scavenging Activity of Methanol Leaf Extracts of *Orthosiphon Stamineus*. *Pharm. Biol.* **2005**, *42* (8), 629–635.

(5) Oliveira, N. A. de; Cornelio-Santiago, H. P.; Fukumasu, H.; Oliveira, A. L. de. Green Coffee Extracts Rich in Diterpenes – Process Optimization of Pressurized Liquid Extraction Using Ethanol as Solvent. *J. Food Eng.* **2018**, *224*, 148–155.

(6) Cheng, M.-H.; Sekhon, J. J. K.; Rosentrater, K. A.; Wang, T.; Jung, S.; Johnson, L. A. Environmental Impact Assessment of Soybean Oil Production: Extruding-Expelling Process, Hexane Extraction and Aqueous Extraction. *Food Bioprod. Process.* **2018**, *108*, 58–68.

(7) Adewoye, T. L.; Ogunleye, O. O. Optimization of Neem Seed Oil Extraction Process Using Response Surface Methodology. *J. Nat. Sci. Res.* **2012**, *2* (6), 66–75.

(8) Scopel, R.; da Silva, C. F.; Lucas, A. M.; Garcez, J. J.; do Espirito Santo, A. T.; Almeida, R. N.; Cassel, E.; Vargas, R. M. F. Fluid Phase Equilibria and Mass Transfer Studies Applied to Supercritical Fluid Extraction of Illicium Verum Volatile Oil. *Fluid Phase Equilib.* **2016**, *417*, 203–211.

(9) Krumreich, F. D.; Borges, C. D.; Mendonça, C. R. B.; Jansen-Alves, C.; Zambiazi, R. C. Bioactive Compounds and Quality Parameters of Avocado Oil Obtained by Different Processes. *Food Chem.* **2018**, *257*, 376–381.

(10) Kumar, R. C.; Benal, M. M.; Prasad, B. D.; Krupashankara, M. S.; Kulkarni, R. S.; Siddaligaswamy, N. H. Microwave Assisted Extraction of Oil from Pongamia Pinnata Seeds. *Mater. Today Proc.* **2018**, *5* (1), 2960–2964.

(11) Araújo, A. M. A.; Oliveira, É. R.; Carvalho, G. R.; Menezes, E. G. T.; Dias, B. O.; Queiroz, F. Solvent Effect on the Extraction of Soluble Solids from Murici and Pequi Seeds. *J. Food Process Eng.* **2018**.

(12) Chen, J.; Yu, X.; Geng, Q.; Li, M. Combination of Span 20 and PH-Assisted Walnut Oil Extraction during Aqueous Extraction Process. *LWT* **2018**, *91*, 477–483.

(13) Torun, M.; Dincer, C.; Topuz, A.; Sahin-Nadeem, H.; Ozdemir, F. Aqueous Extraction Kinetics of Soluble Solids, Phenolics and Flavonoids from Sage (*Salvia Fruticosa* Miller) Leaves. *J. Food Sci. Technol.* **2015**, *52* (5), 2797–2805.

(14) Bravo, J.; Monente, C.; Juániz, I.; De Peña, M. P.; Cid, C. Influence of Extraction Process on Antioxidant Capacity of Spent Coffee. *Food Res. Int.* **2013**, *50* (2), 610–616.

(15) Somnuk, K.; Eawlex, P.; Prateepchaikul, G. Optimization of Coffee Oil Extraction from Spent Coffee Grounds Using Four Solvents and Prototype-Scale Extraction Using Circulation Process. *Agric. Nat. Resour.* **2017**, *51*, 181–189.

(16) Najdanovic-Visak, V.; Lee, F. Y.-L.; Tavares, M. T.; Armstrong, A. Kinetics of Extraction and in Situ Transesterification of Oils from Spent Coffee Grounds. *J. Environ. Chem. Eng.* **2017**, *5* (3), 2611–2616.

(17) Belandria, V.; Aparecida de Oliveira, P. M.; Chartier, A.; Rabi, J. A.; de Oliveira, A. L.; Bostyn, S. Pressurized-Fluid Extraction of Cafestol and Kahweol Diterpenes from Green Coffee. *Innov. Food Sci. Emerg. Technol.* **2016**, *37*, 145–152.

(18) Al-Dhabi, N. A.; Ponmurugan, K.; Maran Jeganathan, P. Development and Validation of Ultrasound-Assisted Solid-Liquid Extraction of Phenolic Compounds from Waste Spent

Coffee Grounds. *Ultrason. Sonochem.* **2017**, *34*, 206–213.

(19) De Marco, I.; Riemma, S.; Iannone, R. Life Cycle Assessment of Supercritical CO2 Extraction of Caffeine from Coffee Beans. *J. Supercrit. Fluids* **2018**, *133*, 393–400.

(20) Getachew, A. T.; Saravana, P. S.; Cho, Y. J.; Woo, H. C.; Chun, B. S. Concurrent Extraction of Oil from Roasted Coffee (Coffea Arabica) and Fucoxanthin from Brown Seaweed (Saccharina Japonica) Using Supercritical Carbon Dioxide. *J. CO2 Util.* **2018**, *25*, 137–146.

(21) Tsukui, A.; Santos Júnior, H. M.; Oigman, S. S.; de Souza, R. O. M. A.; Bizzo, H. R.; Rezende, C. M. Microwave-Assisted Extraction of Green Coffee Oil and Quantification of Diterpenes by HPLC. *Food Chem.* **2014**, *164*, 266–271.

(22) Oliveira, É. R.; Carvalho, G. R.; Santos, P. R.; Queiroz, F. Green Coffee and Its Residual Biomass: Characterization for Its Industrial Approach. *Int. Food Res. J.* **2018**.

(23) Rodrigues, C. E. C.; Aracava, K. K.; Abreu, F. N. Thermodynamic and Statistical Analysis of Soybean Oil Extraction Process Using Renewable Solvent. *Int. J. Food Sci. Technol.* **2010**, *45* (11), 2407–2414.

(24) Beckel, A. C.; Belter, P. A.; Smith, A. K. Solvent Effects on the Products of Soybean Oil Extraction. *J. Am. Oil Chem. Soc.* **1948**, *25* (1), 7–9.

(25) Hron, R. J.; Koltun, S. P. An Aqueous Ethanol Extraction Process for Cottonseed Oil. *J. Am. Oil Chem. Soc.* **1984**, *61* (9), 1457–1460.

(26) Rittner, H. Extraction of Vegetable Oils with Ethyl Alcohol. *Oléagineux* **1992**, *47* (1), 39–42.

(27) Gandhi, A. P.; Joshi, K. C.; Jha, K.; Parihar, V. S.; Srivastav, D. C.; Raghunadh, P.; Kawalkar, J.; Jain, S. K.; Tripathi, R. N. Studies on Alternative Solvents for the Extraction of Oil-I Soybean. *Int. J. Food Sci. Technol.* **2003**, *38* (3), 369–375.

(28) Takeuchi, T. M.; Pereira, C. G.; Braga, M. E. M.; Maróstica Jr., M. R.; Leal, P. F.; Meireles, M. A. A. Low Pressure Solvent Extraction (Solid-Liquid Extraction, Microwave Assisted, and Ultrasound Assisted) from Condimentary Plants. In *Extracting bioactive compounds for food products: Theory and applications*; Meireles, M. A. A., Ed.; CRC Press: Boca Raton, 2009.

(29) Azmir, J.; Zaidul, I. S. M.; Rahman, M. M.; Sharif, K. M.; Mohamed, A.; Sahena, F.; Jahurul, M. H. A.; Ghafoor, K.; Norulaini, N. A. N.; Omar, A. K. M. Techniques for Extraction of Bioactive Compounds from Plant Materials: A Review. *J. Food Eng.* **2013**, *117* (4), 426–436.

(30) Varzakas, T.; Tzia, C. *Food Engineering Handbook: Food Process Engineering*; Varzakas, T., Tzia, C., Eds.; CRC Press: Boca Raton, 2015.

(31) Dibert, K.; Cros, E.; Andrieu, J. Solvent Extraction of Oil and Chlorogenic Acid from Green Coffee Part I: Equilibrium Data. *J. Food Eng.* **1989**, *10* (1), 1–11.

(32) Wlsniak, J.; Hillel, J.; Katz, O. Holdup and Extraction Characteristics of Jojoba Meal. *J. Am. Oil Chem. Soc.* **1987**, *64* (9), 1352–1354.

(33) Amarante, R. C. A.; Oliveira, P. M.; Schwantes, F. K.; Morón-Villarreyes, J. A. Oil Extraction from Castor Cake Using Ethanol: Kinetics and Thermodynamics. *Ind. Eng. Chem. Res.* **2014**, *53* (16), 6824–6829.

(34) Johnson, L. A.; Lusas, E. W. Comparison of Alternative Solvents for Oils Extraction. *J. Am. Oil Chem. Soc.* **1983**, *60* (2), 229–242.

(35) Kostić, M. D.; Joković, N. M.; Stamenković, O. S.; Rajković, K. M.; Milić, P. S.; Veljković, V. B. Optimization of Hempseed Oil Extraction by N-Hexane. *Ind. Crops Prod.* **2013**, *48*, 133–143.

(36) Liauw, M. Y.; Natan, F. A.; Widiyanti, P.; Ikasari, D.; Indraswati, N.; Soetaredjo, F. E. Extraction of Neem Oil (Azadirachta Indica A. Juss) Using n-Hexane and Ethanol: Studies of Oil Quality, Kinetic and Thermodynamic. *ARPN J. Eng. Appl. Sci.* **2008**, *3* (3), 49–54.

(37) Meziane, S.; Kadi, H. Kinetics and Thermodynamics of Oil Extraction from Olive Cake. *J. Am. Oil Chem. Soc.* **2008**, *85* (4), 391–396.

(38) Sulaiman, S.; Abdul Aziz, A. R.; Aroua, M. K. Optimization and Modeling of Extraction of Solid Coconut Waste Oil. *J. Food Eng.* **2013**, *114* (2), 228–234.

(39) Cooney, M.; Young, G.; Nagle, N. Extraction of Bio-oils from Microalgae. *Sep. Purif. Rev.* **2009**, *38* (4), 291–325.

(40) Aquino, L. P.; Ferrua, F. Q.; Borges, S. V.; Antoniassi, R.; Luiz, J.; Correa, G.; Cirillo, M. A. Influence of Pequi Drying (*Caryocar Brasiliense* Camb.) on the Quality of the Oil Extracted. *Food Sci. Technol.* **2009**, *29* (2), 354–357.

ARTIGO 5 – SOLID-LIQUID EXTRACTION OF GREEN COFFEE BEANS AND ITS PRESS CAKE USING BIO-RENEWABLE SOLVENTS AS AN ALTERNATIVE TO MECHANICAL PRESSING

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Abstract

The aim of this work was to optimize the performance of alternative solvents, such as ethanol, acetone, and ethyl acetate in different temperatures when used in the extraction process of green coffee beans (GB) and its press cake (PC) through an experimental design and response surface. The three independent variables involved were the mass fraction content of each cosolvent (acetone and ethyl acetate) in the solvent ethanol and the temperature; the response variables were the recovery yield and retention index (RI). It was found that polynomial models can be used to predict the transfer of soluble solids from GB and PC from experiments using ethanol and cosolvents. Concerning the GB, the increase in temperature showed a positive effect on the yield, as well as the acetone presence in the system. While for the PC it is also desirable to work with higher temperatures (> 45°C), and both acetone and ethyl acetate seemed to influence the process. The extraction process of both materials had the temperature significantly influencing the RI in the process, however, this response variable could not be described by an empirical model. The optimal recovery yield for the extraction of GB (65%) could be obtained when using 40% of acetone and 60% of ethanol at 55 °C; while for the press cake (58%), 40% of acetone, 20% of ethyl acetate, and 40% of ethanol at 45 °C. Further, the RI results showed that the soluble solids extraction process of the PC using ethanol and cosolvents is more feasible than for the GB, as it was 66% lower than the latter.

Keywords: green coffee, cosolvent, extraction, alternative solvents, ethanol; food residue.

1. Introduction

Mechanical pressing oil extraction can be a low cost operation depending on the matrix being processed, nonetheless, it yields much lower than other processes, such as the solvent extraction, which is based on the transfer of soluble components in the solvent from a material by chemical affinity (Fornasari et al., 2017); besides, the mechanical pressing is worthy only for material with higher oil content (> 20%). In order to extract oil from food matrices, different methods and their combination can be applied, such as the use of mechanical pressing followed by solvent extraction, microwave (Torres-León, Rojas, Serna-Cock, Belmares-Cerda, & Aguilar, 2017) or ultrasound (Perrier et al., 2017) assisted extractions.

It is attainable that alcohols were once in the past contemplated as promissing solvents to extract oils from food matrices, mainly seeds. However, due to the low fixed oil yield and economic factors, the employment of n-hexane prevailed (Lusas, Watkais, & Rhee, 1994). Hexane is a mixture of petroleum derivatives, it is inflammable, very volatile, and toxic (Graham Solomons & Fryhle, 1998; Wakelyn & Wan, 2005), it also can be emitted during the extraction process, making it compulsory its recovery, once it is an air polutant (Hanmoungjai, Pyle, & Niranjan, 2000; Wan, Pakarinen, Hron, Richard, & Conkerton, 1995), and has been identified as a neurotoxin (Lusas et al., 1994). Due to these disadvantages, alternative solvents to hexane, such as alcoholic ones, have regained attention from the industry and scientist communities. Environmental, health, and safety issues have promoted increased interest in these solvents. They are propably going to be gradually substituted by alternative solvents that are recognized as economically viable and environmentally safer (Baümler, Carrín, & Carelli, 2016; Li et al., 2014).

Many types of solvents have been proposed to replace hexane as the solvent to extract oil from vegetable sources (Bessa, Ferreira, Rodrigues, Batista, & Meirelles, 2017). The literature has shown the technical feasibility of using alcohols as extractants in order to substitute hexane (Baümler et al., 2016; Hron, Koltun, & Graci, 1982; Rittner, 1992; E. Rodrigues et al., 2011), including coffee beans (Bitencourt, Ferreira, Oliveira, Cabral, & Meirelles, 2018; Oliveira, Cornelio-Santiago, Fukumasu, & Oliveira, 2018).

The solvent ethanol has a range of advantages from an environmental point of view, since it is produced by biotechnological processes. In addition, it is higly available at low cost, especially

in Brazil, which is, along with the United States, the largest producer of this solvent worldwide, besides, it is less aggressive to the environment and it is generally recognized as safe (GRAS) (ICO, 2018; Rodrigues, Gonçalves, Batista, & Meirelles, 2007; Rodrigues, Alcázar-Alay, Petenate, & Meireles, 2014). The U.S. Food and Drug Administration (FDA) (FDA, CDER, & CBER, 2017) classifies ethanol as class 3, which does not include any solvent known to be a human health hazard at levels normally accepted in the pharmaceutical industry, meaning that ethanol is considered to be less toxic and poses lower risk to human health. According to the FDA guideline, 50 mg of ethanol per day are an acceptable level without justification. Furthermore, regarding fire hazard, ethanol is less flammable and less explosive than hexane (Johnson & Lusas, 1983; NFPA, 1991). On the other hand, there are drawbacks, it presents lower selectivity to the extractable compounds, has higher latent heat of vaporization compared to hexane. But according with Bessa et al. (2017) and with Hron & Koltun (1984) the latter could be worked around due to its partial miscibility with oils at room temperature, once it allows two liquid phases to be formed (an alcohol-rich and an oil-rich phase), which is particularly advantageous for solvent recovery, and oil deacidification via liquid–liquid extraction, if it is the case.

Other GRAS solvents can also be used in the extraction process of soluble solids and bioactives from vegetable matrices, such as acetone (Aquino, Borges, Queiroz, Antoniassi, & Cirillo, 2011; Javed, Ahmad, Rehman, Zafar, & Malik, 2015; Mani, Jaya, & Vadivambal, 2007; Oliveira, Barros, & Gimenes, 2013) and ethyl acetate (Ishida & Chapman, 2009; Strati & Oreopoulou, 2011; Villanueva Bermejo et al., 2013). These solvents could also be employed as cosolvents in order to improve the results of the process. The addition of polar cosolvents may be employed in order to improve the solubility of polar and high molecular weight substances, despite a possible decrease in selectivity (Brunner, 1994). The addition of cosolvents may enhance the physical interactions between solute and solvent molecules which, depending on the nature of the solute, can lead to chemical interactions such as hydrogen bonding, and a consequent increase of the overall solubility (Brunner, 1994; Galanakis, 2017), also, modification of the critical temperature of the mixed solvent when compared to pure solvent (Azevedo, Mazzafera, Mohamed, Melo, & Kieckbusch, 2008; Brunner, 1994; Kim & Johnston, 1987). The extraction of caffeine from coffee beans using moistened green coffee beans and water saturated supercritical CO_2 as a

solvent is an effective exemple of the use of a cosolvent (Lack & Seidlitz, 1993; Peker, Srinivasan, Smith, & McCoy, 1992).

In the light of this background, the employment of alternative solvents maybe an interesting alternative to be applied in the extraction of soluble solids from green coffee beans. In addition to its main purpose, to be consumed as a beverage, coffee beans play an important role in the world trade scenario due to their concentration of oil and bioactive compounds in the green beans (Al-Hamamre, Foerster, Hartmann, Kröger, & Kaltschmitt, 2012; Caetano, Silva, & Mata, 2012; Vardon et al., 2013), which in turn carries significant soluble solids indices (Oliveira, Carvalho, Santos, & Queiroz, 2018), being promising for application in the food and pharmaceutical industries.

Besides the great potential of the green coffee oil, such as cosmetics emollient, antioxidant properties, and quimoprotector (Rabasco Alvarez, González Rodríguez, & Rodríguez, 2000), the beans are not a source of oil (~15%) (Dias & Benassi, 2015; Mussatto, Machado, Martins, & Teixeira, 2011), which makes of them an improper input for the mechanical pressing process. According with Somnuk, Eawlex, & Prateepchaikul (2017), in spite of the mechanical action applied with the purpose of extracting the oil from the interior of the bean to the maximum, the organic residues after treatment (press cake), still have solids content retained, varying around 10 - 15% in relation to their weight, depending on the coffee variety (Abdullah & Bulent Koc, 2013; Al-Hamamre et al., 2012; Castro et al., 2018; Jenkins, Stageman, Fortune, & Chuck, 2014; Somnuk et al., 2017) Thus, the extraction process methods association (mechanical and solid-liquid processes) could be an interesting alternative to obtain better yields of extracts from green coffee beans. Therefore, the aim of this work was to determine the performance of alternative solvents, such as ethanol, acetone, and ethyl acetate, and cosolvents when used in the extraction process of green coffee beans and its press cake.

2. Materials and Methods

2.1 Materials

The samples of Arabica green coffee beans were harvested in the cherry state in the region of Guaxupé (Minas Gerais) located in Southest Region of Brazil. Both beans and its press meal were kindly donated by Cooxupé. Green coffee press cake samples were obtained after mechanically screw pressing the beans for the extraction of the oil fraction (100 kg h^{-1}; Ecirtec, MPE-500 AC, Bauru, Brazil). After extraction, the cake was packed in polyethylene bags, with silica pouches, and freezed for further analysis. Prior being submitted to the solid-liquid extraction, both materials were ground (IKA, A11, Wilmington, US) and sieved (> 0.84 and < 0.177 mm) with the aid of a sieve shaker (Bertel, VP-01, Caieiras, Brazil) (Sluiter et al., 2008). Each powder was then dried (40 °C) in a vacuum oven (16.8 kPa) (Tecnal, TE-395, Piracicaba, Brasil) (approximately 20 days for green coffee beans and 7 days for the cake), and further used to the extraction process. The physical and chemical characterization of the materials is available in Oliveira et al. (2018).

2.2 Solvent Extraction

The solvent extraction of soluble solids from green coffee (GC) and its press cake (PC) was carried out in batches using ethanol (99%) as the main solvent, and acetone (99%) and ethyl acetate (99%) as cosolvents, at the constant solid-liquid ratio of 1:5 (w/w). Extractions using only ethanol at 35, 39, 45, 50.9, and 55 °C were also carried out in order to compare the results.

The equilibrium studies were performed using a magnetically stirred batch system immersed in a temperature-controlled water bath. Solid and solvent were brought to the extraction temperature (35 to 55 °C) separately before each experiment. The samples were placed in Falcon tubes (50 mL) and added with the solvents at a fixed ratio. The tubes were then placed in the stirring Dubnoff Orbital water bath (Novatecnica, NT 230, Piracicaba, Brazil) (220 rpm) at constant temperature (according to each experiment) for 4 h (period determined in preliminary tests as enough to reach the equilibrium). After the extraction time had elapsed, aliquots of the supernatant phase (extract) (~2 mL) were removed with the aid of pre-weighed microseringes. The sample withdrawn with the aid of the syringe was placed in a Petri dish, also pre-weighed. Ethanol was used to wash the syringe in order to remove any solids that may have been adhered to syringe wall. The Petri dish containing the extract phase of the sample + ethanol was oven dried at 100 °C for at

least 2 h in order to evaporate the solvent. The raffinate phase was subjected to centrifugation (3500 rpm/ 1 min) (FANEM, Excelsa II – 206 BL, São Paulo, Brazil), then the remaining extract phase was discarded and a sample of the raffinate phase was withdrawn and immediately weighed into a Petri dish, which was subjected to drying (100 °C/ 2 h). Further, the samples without the solvent were cooled down in desiccators and weighed. The residual extractable material in the moisture-free material was determined as the difference between the maximum extractable material determined by Soxhlet using ethanol as the solvent, and the extracted material in the liquid phase.

2.3 Solids mass transfer (Recovery yield)

For the extraction experiments performed, the extract (EP) and the raffinate (RP) phases were determined in terms of the mass fractions of the components. The mass fraction of EP was determined using the data obtained from the samples taken with the microsyringe. The withdrawn EP sample mass was calculated using the mass difference of the syringe with extract and the empty syringe. The soluble solids mass in the EP sample was calculated from the mass difference of the Petri dish containing the dried extract sample and the empty Petri dish. The mass fraction of soluble solids ($w_{1,EP}$) and the mass fraction of the solvent ($w_{2,EP}$) in the EP, both insoluble solids free ($w_{3,EP} = 0$), were calculated according to equations 1 and 2, respectively.

$$w_{1,EP} = \frac{m_f - m_i}{m_{EP}} \quad (1)$$

$$w_{2,EP} = 1 - w_{1,EP} \quad (2)$$

Where, m_f and m_i are the final and initial weight of the Petri dish, respectively, and m_{EP} is the mass of the extract phase sample withdrawn.

The mass fraction of the solvent in RP ($w_{2,RP}$) was calculated by the difference of weights found before and after drying (Equation 3).

$$w_{2,RP} = \frac{m_f - m_i}{m_{RP}} \quad (3)$$

Where, m_f and m_i are the final and initial weight of the Petri dish, respectively, and m_{RP} is the mass of the raffinate phase sample withdrawn.

The mass of the system (solvent and material) (m_{system}), and the mass fraction of each component in the system are known variables. The remaining variables - mass of EP (m_{EP}), mass of RP (m_{RP}), soluble solids mass fraction in RP ($w_{1,RP}$), and insoluble solids mass fraction in RP ($w_{3,RP}$) - were determined through a global mass balance (Equation 4), and a mass balance for each component of the system (i) (Equation 5).

$$m_{system} = m_{EP} + m_{RP} = m_2 + m_s \quad (4)$$

Where, m_{system} is the mass of the global system, m_{EP} is the mass of the extract phase, m_{RP} is the mass of the raffinate phase, m_2 is the solvent mass used in the extraction process, and m_s is the mass of bean or cake used in the experiment.

$$w_{i,system} m_{system} = w_{i,EP} m_{RP} + w_{i,RP} m_{RP} \quad (5)$$

Where, i = 1 (soluble solids) or 2 (solvent) or 3 (insoluble solids).

The parameter w_i is defined by Equation 6, where mi is the mass of a given component in the EP or RP (m_P).

$$w_i = \frac{m_i}{m_P} \quad (6)$$

The soluble solids mass transfer in the extraction process was calculated through Equation (7) (Rodrigues et al., 2010), where m_s is the mass of samples (beans and cake) used in the

extraction process, and $w_{1,s}$ is the mass fraction of soluble solids in the bean or cake before the extraction process.

$$\Gamma_1 \, (\%) = 100\left(\frac{w_{1,EP} m_{EP}}{w_{1,s} m_s}\right) \quad (7)$$

The retention index (RI) corresponds to the mass of adhered solution per soluble solids mass, and can be calculated using Equation (8).

$$RI = \frac{w_{1,RP} m_{RP} + w_{2,RP} m_{RP}}{w_{3,RP} m_{RP}} = \frac{m_{1,RP} + m_{2,RP}}{m_{3,RP}} \quad (8)$$

2.4 Experimental Design and Central Composite Rotational Design (CCRD)

Effects of solvent extraction parameters including solvent-to-cosolvent ratio (ethanol, acetone, and ethyl acetate), and extraction temperature (35 to 55 °C) were investigated by applying a full factorial design (2^3) with two levels (+1 and -1), two axial points, and three repetitions at the central point (Table 1).

The proposed extraction process was modeled by means of CCRD and RSM (Response Surface Methodology), and statistically tested using the analysis of variance (ANOVA) through Statistica 8.0 software (StatSoft Inc., 2007).

The coded (x_i) and uncoded levels of independent variables are shown in Table 1, and 17 experimental runs in random order were required. The general form of the quadratic regression model was expressed as the following equation:

$$Y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_{11} x_1^2 + \beta_{22} x_2^2 + \beta_{33} x_3^2 + \beta_{12} x_1 x_2 + \beta_{13} x_1 x_3 + + \beta_{23} x_2 x_3 + \varepsilon_{123} \quad (9)$$

Where Y is the response function (Γ_1 or RI), x_i (i =1, 2, 3) is the studied variables, β_0 is the constant term, β_i is the linear coefficient, β_{ii} and β_{ij} are the quadratic and interactive terms, respectively, of the model. The effect of the mass fraction of acetone (x_1; $0 - 40\%$ w/w), and ethyl acetate (x_2; $0 - 40\%$ w/w) in the solvent ethanol, and of the extraction temperature (x_3; $35 - 55$ °C) were

studied. The optimization was carried out simultaneously for both response variables through the Desiribility function.

3. Results and Discussion

Table 1 shows the component values of the experimental design matrix and the responses regarding the extraction process of soluble solids (SS). The SS recovery (% Γ_1) from GB ranged from 36.73 to 61.62%, and for the PC the results were in the range of 22.62 to 56.75%. Further, an extraction with pure ethanol was carried out in order to compare the results (Table 2).

Table 1. Experimental design and response values of the extraction yield and rentention index (RI) of green coffee beans (GB) and its press cake (PC).

Run	x_1(%w/w)	x_2(%w/w)	x_3(°C)	GB		PC	
				Γ_1 (%)	RI	Γ_1 (%)	RI
1	-1 (8.04)	-1 (8.04)	-1 (39)	38.73±0.78	0.71±0.0	30.91±0.1	0.73±0.0
2	-1 (8.04)	1 (31.95)	1 (50.9)	42.29±0.79	0.7±0.06	47.7±1.48	0.7±0.03
3	1 (31.95)	-1 (8.04)	1 (50.9)	54.91±1.44	1.12±0.01	35.46±0.39	0.73±0.07
4	1 (31.95)	1 (31.95)	-1 (39)	40.56±0.4	0.36±0.43	26.12±0.33	0.75±0.05
5	0 (20)	0 (20)	0 (45)	39.96±1.35	1.34±0.04	24.61±0.3	0.82±0.06
6	-1 (8.04)	-1 (8.04)	1 (50.9)	55.9±1.27	1.16±0.01	35.02±2.92	0.75±0.15
7	-1 (8.04)	1 (31.95)	-1 (39)	41.36±0.79	1.28±0.05	27.93±1.57	0.68±0.05
8	1 (31.95)	-1 (8.04)	-1 (39)	36.73±1.03	1.26±0.03	46.32±.35	0.74±0.0
9	1 (31.95)	1 (31.95)	1 (50.9)	61.62±2.15	1.22±0.1	38.27±0.38	0.7±0.27
10	0 (20)	0 (20)	0 (45)	39.2±2.6	1.21±0.02	52.39±0.26	0.7±0.04
11	-1.67332 (0)	0 (20)	0 (45)	37.41±1.93	1.24±0.11	22.62±1.4	0.77±0.01
12	1.67332 (40)	0 (20)	0 (45)	45.44±0.48	1.38±0.02	56.07±2.08	0.71±0.02
13	0 (20)	-1.67332 (0)	0 (45)	43.63±1.62	1.17±0.04	31.18±2.62	0.74±0.01
14	0 (20)	1.67332 (40)	0 (45)	40.65±2.34	1.35±0.02	23.02±2.63	0.78±0.0
15	0 (20)	0 (20)	-1.67332 (35)	43.24±0.36	1.06±0.17	32.63±0.24	0.65±0.02
16	0 (20)	0 (20)	1.67332 (55)	45.89±4.08	0.7±0.0	45.25±1.62	0.52±0.01
17	0 (20)	0 (20)	0 (45)	47.0±1.32	1.2±0.03	56.75±0.59	0.72±0.02

Value outside the brackets represents coded levels.
x_1: Mass fraction of acetone in the solvent ethanol
x_2: Mass fraction of ethyl acetate in the solvent ethanol
x_3: Temperature (°C)

Table 2 shows the positive effect of the use of cosolvents on the extraction of soluble solids both from the beans and cake. All the results (Γ_1) regarding the GB extraction were lower than those obtained used the cosolvents at the same operation temperature, except at 55 °C, where the results were similar. A similar trend was observed for the PC, only that at 50,98 °C the Γ_1 was

greater when employing ethanol as the solvent. The use of cosolvents, not only favored the Γ_1, but also the retention index (RI), mainly obtained from the GB extraction.

Table 2. Extraction recovery yield (Γ_1) and retention index (RI) of the solid-liquid extraction of green coffee beans (GB) and its press cake (PC) with absolute ethanol as the solvent.

Temperature (°C)	GB		PC	
	Γ_1 (%)	RI	Γ_1 (%)	RI
35	24.63±1.67	1.36±0.05	34.58±3.39	0.83±0.03
39.02	26.56±1.81	1.1±0.07	36.79±2.64	0.66±0.0
45	29.64±0.89	1.26±0.01	41.62±2.19	0.59±0.0
50.98	44.33±1.39	1.04±0.06	63.39±1.78	0.77±0.02
55	46.54±2.21	1.19±0.01	42.18±0.85	0.7±0.02

The significance of the model was evaluated through the ANOVA as well as the variables and their individual and mutual effects. Table 3 shows the ANOVA results for Γ_1 from both materials. In order to present the significance of each variable, the p-values of each factor are shown in Table 3.

Table 3. ANOVA for the response surface complete quadratic model of soluble solids transfer (Γ_1) from green coffee beans (GB) ($R^2 = 0.74$) and its press cake (PC) ($R^2 = 0.75$).

Source	Sum of square		Degree of freedom		Mean square		p-value	
	GB	PC	GB	PC	GB	PC	GB	PC
x_1	123.35	539.62	1	1	123.35	539.62	0.0003*	0.009*
x_1^2	8.79	51.83	1	1	8.79	51.83	0.2609	0.3863
x_2	4.33	66.88	1	1	4.33	66.88	0.4261	0.3264
x_2^2	17.4	774.62	1	1	17.4	774.62	0.1196	0.0028*
x_3	560.91	315.28	1	1	560.91	315.28	0.0*	0.0414*
x_3^2	68.16	62.17	1	1	68.16	62.17	0.0045*	0.3436
x_1x_2	115.74	183.29	1	1	115.74	183.29	0.0005*	0.1118
x_1x_3	111.59	127.56	1	1	111.59	127.56	0.0006*	0.1803
x_2x_3	44.65	373.67	1	1	44.65	373.67	0.0172*	0.0279*
Lack of fit	454.21	629.07	5	5	90.84	125.81	0.0*	0.1402
Pure error	124.46	1252.34	19	19	6.55	65.91		
Total	1610.87	4263.19	33	33				

*Significant at $p \leq 0.05$.

The p-value is used as a tool to check the significance of each coefficient and the interaction strength between each independent variable. The corresponding variables will be more significant if the p value becomes smaller. Thus, the smaller the p-values, the more significant the corresponding coefficients are (Jang, Lee, Lee, Choi, & Kim, 2017; Muralidhar, Chirumamilla, Ramachandran, Marchant, & Nigam, 2001; Quanhong & Caili, 2005).

In the case of GB, the significant effect of the temperature (x_3) was positively higher than the effect of the interactions between variables. In the studied operational variables, the acetone mass fraction (x_1) was the second variable that showed the greatest positive influence on the Γ_1 of GB, followed by the interaction between variables x_1x_2, and x_1x_3, which the effects were very close in absolute values. Further, the variable x_3 showed significative effect for both linear and quadratic terms, indicating that the increase in temperature of the system enhanced the efficiency of the process. The interaction between the ethyl acetate mass fraction and the temperature (x_2x_3) was also significative, however with little effect on the results. Thus, the ethyl acetate employement is suggested, once it seems to increase the acetone extraction effect at low temperatures.

Concerning the PC, the mass fraction of ethyl acetate (x_2^2) showed the greatest negative influence on the Γ_1. The significance of the negative quadratic term indicates that there is a point of maximum for this variable, indicating that the concentration of this solvent initially favors the solubilization of soluble solids, reaching a maximum, and posterior addition leads to a reduction of their solubilization power. While the the intereraction between ethyl acetate mass fraction and the temperature (x_2x_3) showed the greatest positive effect on the results, followed by acetone mass fraction (x_1) and the temperature (x_3). A different behavior was observed by Oliveira et al. (2012) for the temperature, who studied the rice bran oil extraction with hydrated ethanol (60 – 90 °C; RSL 1:3.5 (w:w); 175 rpm), where only the variable temperature, with linear effect, was significative. The authors observed that the temperature rise positively influenced only the yield of low-hydrated solvents, indicating that the moisture content in the solvent suppressed the extraction yield. The temperature affects the solubility of the analytes in the solvent, because it is directly correlated to the solvent density as well as to the vapor pressure of the analytes (Turner, Whitehand, Nguyen, & McKeon, 2004).

In the case of a quadratic model, other parameters should be taken into consideration other than the R^2 in order to be able to assure the validity of the model, such as the F-value, which indicates the significance of each controlled factor on each tested model, the residual standard error, the independency and the normality of the residues, and also the analytical discerniment of the scientist should be taken into account. By means of the F test, the model (Eq. 10 and 11) for the Γ_1 obtained via solvent extraction was significant and, therefore, predictive for both materials (GB and PC), because F_c (calculated F) (7.5 and 3.38, respectively) was higher than F_t (reference F)

(2.5 and 2.3, respectively). These analyzes make it possible to validate empirical reduced coded models and build the response surfaces (Figure 1).

$$\Gamma_{GB} = 44.38 + 2.13x_1 + 0.63x_1^2 - 0.4x_2 + 0.88x_2^2 + 4.54x_3 + 1.75x_3^2 + 2.69x_1x_2 + 2.64x_1x_3 - 1.67x_2x_3 \quad (10)$$

$$\Gamma_{PC} = 37.19 + 4.45x_1 - 1.53x_1^2 - 1.57x_2 - 5.9x_2^2 + 3.4x_3 - 1.67x_3^2 - 3.4x_1x_2 - 2.8x_1x_3 + 4.83x_2x_3 \quad (11)$$

The application of RSM offers, based on parameter estimates, an empirical relationship between the response variable and the test variables under consideration (Jang et al., 2017). Response surface was used to represent the models at the central point of the fixed variable. Figures 1 A-E show the effect of the variables on the response (%Γ_1) for GB and PC solvent extraction. It is inferable that the rise in temperature (x_3) influenced the Γ_1 of both materials (Figures 1x and 1x), and the increase of the mass fraction of ethyl acetate (x_2) in the system influenced the Γ_1 of PC (Figure1E). Specifically, in the case of GB, by increasing the acetone concentration at elevated temperatures, and in the absence of ethyl acetate, it was observed greater Γ_1 (Figure 1B); while at lower temperatures and by raising the ethyl acetate content, it was observed an adverse effect (Figure 1A). In the case of the PC, an interesting behavior could be observed, as the increase in the acetone concentration favored the extraction at any temperature range (Figure 1E). These behavior explaining could involve the fact that in the beans sample there is a greater content of oil, and in the cake, due to the smaller content, other compounds could have been extracted, besides the influence of the type of material. In the beans it would be necessary to evaluate the behavior of the oil solubility in different mixtures of acetone and ethanol.

Figure 1A shows an interesting behavior of the soluble solids yield from the GB when varying the cosolvents concentrations (acetone and ethyl acetate), where the variable x_3 (temperature) was fixed at the central point (45 °C). By the plot, it was possible to observe that the extraction of soluble solids showed a tendency to increase in the extreme points of the experimental variables, indicating that ethanol itself could be an interesting option to extract the solids from this material. Eventhough the graphs were not shown for other temperature levels; this behavior was observed at all levels studied.

Figure 1B, where the variable x_2 (ethyl acetate mass fraction in ethanol) was fixed at the central point (20%), represents the behavior of the interaction among x_1 (acetone mass fraction in ethanol) and x_3 (temperature) in the extraction results of GB. The optimum yield could be obtained with the highest temperature and acetone mass fraction in ethanol. Again, this behavior was observed for all levels of the variable x_2, meaning that the use of acetone favors the extraction while using no acetate or using higher levels of this solvent.

Figure 1C shows the responce surface plot for GB, where the variable x_1 (acetone mass fraction in ethanol) was fixed at the central point (20%), which represents the behavior of the soluble solids yield with the variation of the ethyl acetate mass fraction (x_2) and along with the temperature (x_3). The region with the highest yield results was found to be at elevated temperatures and very little content of the cosolvent. This trend could be explained by the fact that elevated temperatures lower the ethyl acetate effect on the extraction.

Loyao, Villasica, Dela Peña, & Go (2018) studied the lipid extraction from spent coffee grounds using alternative solvents (ethanol, isopropanol, and ethyl acetate) and also observed that the least polar solvents did not yield the best results; ethanol yielded the greatest amount of extractable solids (23 g/100 g d.b.) corresponding to a recovery of 145%. The higher yields may be attributed to the co-extraction of polar components other than lipids. On the other hand, Efthymiopoulos et al. (2018) while carring the extraction of lipids from spent coffee grounds at elevated temperatures conditions observed higher yields for apolar solvents, what could be explained by the decreasing in the selectivity of the process due to the high extraction temperature achieved with solvents (ie. hexanol), resulting in the extraction of cell wall components and bound lipids (Campos-Vega, Loarca-Piña, Vergara-Castañeda, & Oomah, 2015; Zuorro & Lavecchia, 2012).

The behavior of the soluble solids yield of PC with acetone (x_1) and acethyl acetate (x_2) fixed at the central point (20%) is presented in Figures 1D and 1E, respectively. In Figure 1D its possible to observe a strong influence of the presence of ethyl acetate at high temperatures on the results. Besides, the greatest yield is atainable near the central point, once due to the point of maximum the high and low concentration regions show lower yield. What allows the conclusion that costs could be saved by using less amount of solvent and obtaining reasonable results. Figure

1E enables the latter, once it shows that the best extraction results could be achieved when employing high temperatures (> 45 °C) and content of acetone (> 20%).

The numerical optimization was used in order to obtain the optimum values for yield variable studied. However, it was not possible to optimize the response, once saddle points were obtained. Therefore, the numerical optimization was performed through the desirability function method (spline fit) in order to determine the optimum level of the process variable (Γ_1) for both materials (Figure 2). For GB, a temperature of 55 °C, 40% of acetone and no concentration of ethyl acetate were determined to be the optimal conditions for extraction. The maximum response was found as 65%, under these operating conditions. While for the PC, temperatures above the central point are indicated, where the optimum result was obtained at 45 °C. Concerning the cosolvents concentration, 40% of acetone and 20% of ethyl acetate were the proper proportion in order to achieve the best results. The maximum response was found as 58%, under these operating conditions. Both results were higher than those obtained by when using only ethanol as the extractor solvent, reassuring the efficiency of using cosolvents in extraction processes.

According to Amarante, Oliveira, Schwantes, & Morón-Villarreyes (2014), the temperature increase usually influences the extraction of soluble solids due to the increase of the solubility of the oil and the decrease of the viscosity of the solution, facilitating the mass transfer process. Javed et al. (2015) pointed out that further extraction may occur with increasing temperature as it may increase the kinetic energy of the solvent molecules. Although the raise of temperature promotes an increase of the solubility of the solute in the solvent, the final effect of this variable on the yield of the extract will also be influenced by its effect on the RI.

The RI has a decisive impact on the number of stages carried out to complete the extraction and on the desolventization step, and it depends on the viscosity of the extract solution, and mainly on the physicochemical affinity between the solvents, cosolvents and the solid matrix (Bessa et al., 2017). The design matrix and real values of the experiments to evaluate the effect of three variables including the mass fraction of acetone (x_1), ethyl acetate (x_2), and extraction temperature (x_3) on the RI are presented in Table 1, also, a control extraction was carried out with ethanol and the results are presented in Table 2.

The RI indicates the amount of solution retained in the RP, thus, the lower this index is, the most efficient is the extraction process. The highest RI values were obtained for GB, with an

average of 1.09; while the results for PC averaged 0.72, which is 66% lower than the former. Thus, the results indicate that GB are less feasable to the solvent recovery process, once the adhered solution in the fibers (insoluble solids) are higher, making it difficult to recover the solvent from the RP.

The highest RI for the GB extraction process was obtained for run 12 (1.38 kg adhered solution/ kg insoluble solids), which did not present the greatest Γ_1 (45.44%). The highest RI for PC was of 0.82 kg adhered solution/ kg insoluble solids (run 5), corresponding to a Γ_1 of 24.61% as expected, once this was one of the lowest yields obtained for this material. Concerning the control ethanolic extraction, it was observed that the RI was much higher for the GB extraction compared to the same temperatures at the experimental design where cosolvents were applied, which is accordance with the lower results obtained for the Γ_1 for this material extracted only with ethanol, which shows the importance of the use of cosolvents in order to extract solids from coffee beans (Tables 1 and 2). Regarding the PC, the Γ_1 was also lower at the control ethanolic extraction, however, the RI was lower than with the use of cosolvents, which could be explained by the fact that the RI is not the only parameter influencing the process output.

Through the ANOVA for the response variable RI (Table 4) it was observed that only one effect was significative ($p \leq 0.05$) for PC (x_3^2), and three effects for GB (x_3^2, x_1x_2, x_1x_3), showing the great influence the temperature has on this variable.

Table 4. ANOVA for the response surface complete quadratic model of soluble solids transfer (RI) from green coffee beans (GB) ($R^2 = 0.38$) and its press cake (PC) ($R^2 = 0.38$).

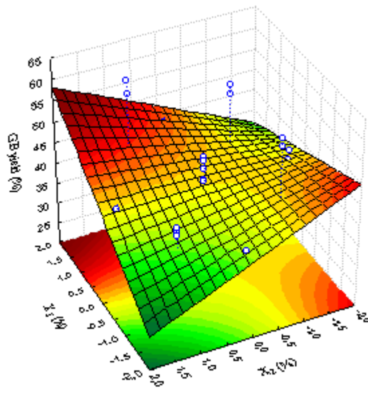
Source	Sum of square		Degree of freedom		Mean square		p-value	
	GB	PC	GB	PC	GB	PC	GB	PC
x_1	0.0173	0.0003	1	1	0.017	0.0003	0.2906	0.8235
x_1^2	0.0067	0.0005	1	1	0.007	0.0005	0.5069	0.7838
x_2	0.0222	0.0003	1	1	0.022	0.0003	0.2323	0.8328
x_2^2	0.0259	0.0032	1	1	0.026	0.0032	0.1985	0.4921
x_3	0.00003	0.0084	1	1	0.0	0.0084	0.963	0.274
x_3^2	0.6482	0.0572	1	1	0.65	0.0572	0.0*	0.0088*
x_1x_2	0.2155	0.0025	1	1	0.22	0.0025	0.001*	0.5467
x_1x_3	0.1854	0.0026	1	1	0.19	0.0026	0.002*	0.5381
x_2x_3	0.0001	0.0003	1	1	0.0	0.0003	0.9256	0.82
Lack of fit	1.5478	0.0244	5	5	0.31	0.0049	0.0	0.6081
Pure error	0.2778	0.1269	19	19	0.015	0.0067		
Total	2.9414	0.2454	33	33				

*Significant at $p \leq 0.05$.

Nonetheless, inspite of the significative effects, the correlation coefficient (R^2) was not significant (< 70%) for either of the models. Thus, it was not possible to stablish an empirical model for this response, consequently, no response surface could be plotted.

Based on the results, the solid-liquid extraction could be suggested as an alternative process to extract soluble solids from green coffee beans and its press cake, as the mechanical pressing process seems no to be removing a substantial content of oil, which is remaining in the cake, as observed in this study. Also, the solid-liquid extraction could be used as a second step to the mechanical pressing process, once there is still great content of soluble solids, such as the oil, in this material after the pressing.

(A)



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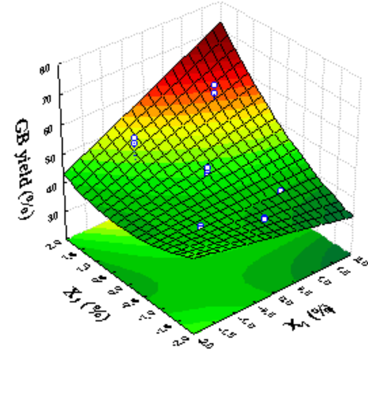
< 35
< 30

(B)



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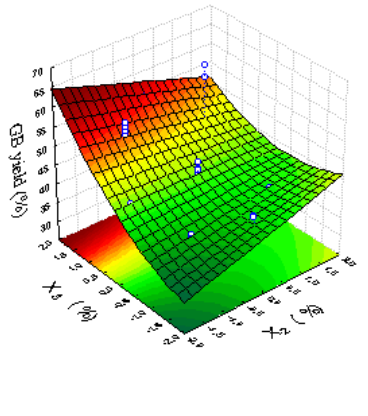
(C)



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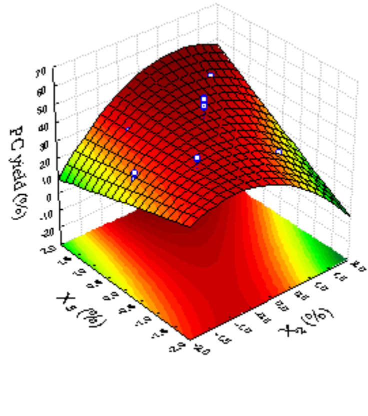
(D)



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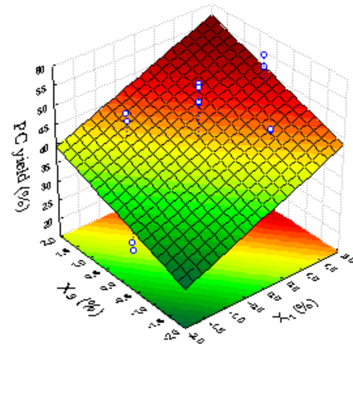
< 10
< 0

(E)



> 55
< 55
< 50
< 45
< 40
< 35
< 30

Fig. 1. Response surfaces generated by the second-order model that show the effects of the variables x_1 (acetone mass fraction in ethanol), x_2 (ethyl acetate mass fraction in ethanol), and x_3 (temperature) on the yield of the green coffee beans (GB) (A, B, C) and its press cake (PC) (D, E) obtained by solid-liquid extraction.

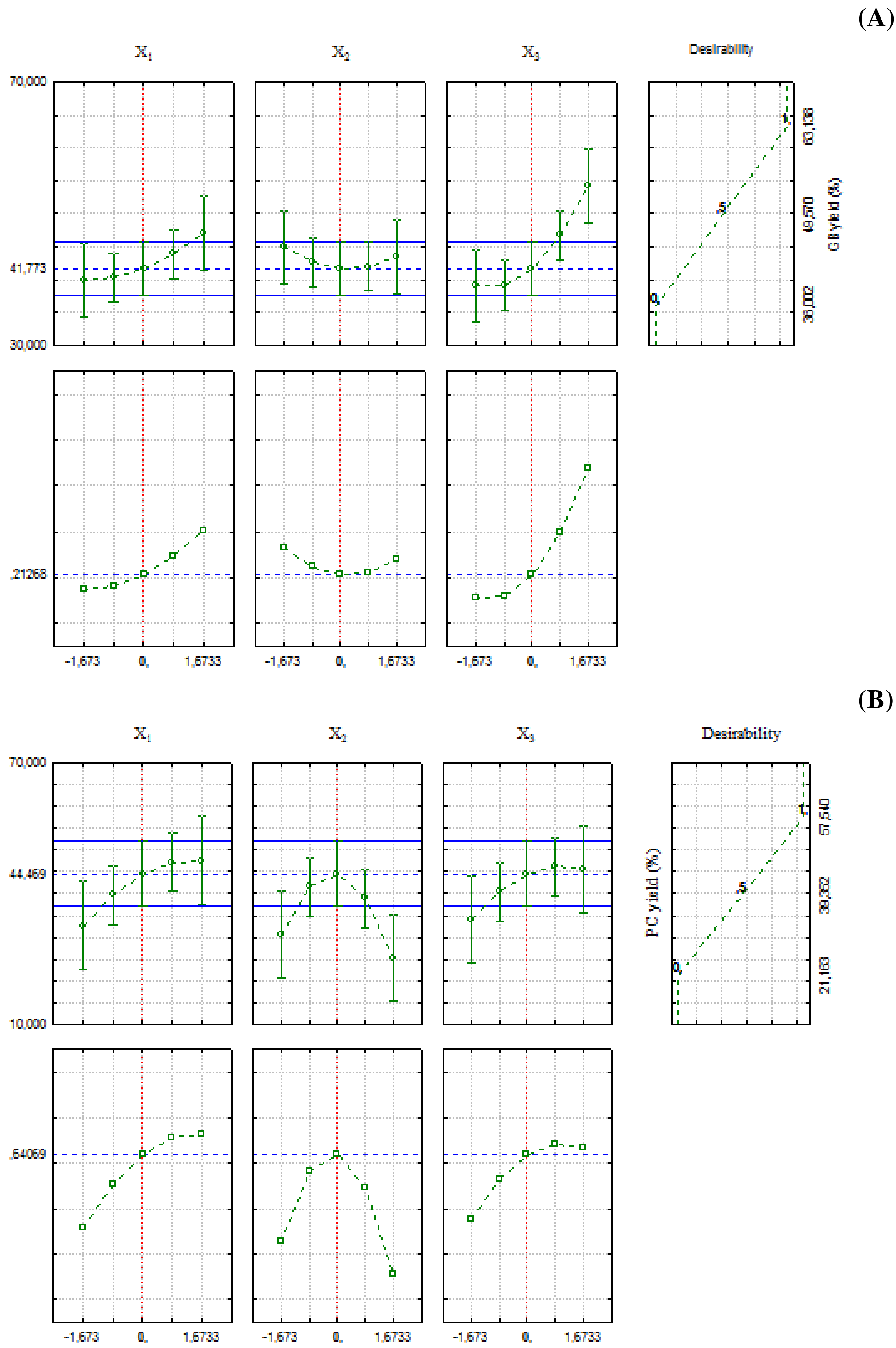


Fig. 2. Desirability profiles of the solid-liquid extraction yield of green coffee beans (GB) (A) and its press cake (PC) (B) (x_1: acetone mass fraction in ethanol, x_2: ethyl acetate mass fraction in ethanol, x_3: temperature).

4. Conclusion

According with the experimental design and response surface analysis, quadratic polynomial models can be used to predict the transfer of soluble solids from green coffee beans and its press cake from experiments using ethanol as the solvent, and acetone and ethyl acetate as cosolvents, carried out at equilibrium conditions. The three independent variables involved in the models were the mass fraction content of each cosolvent (acetone and ethyl acetate) in the solvent ethanol and temperature. Within the range of the operating conditions studied, a saddle point was obtained for both models, thus, the desirability function was used in order to obtain the optimal operation conditions. Concerning the green beans, the increase in temperature showed a positive effect on the yield, as well as the acetone presence in the system. While for the press cake it is also desirable to work with higher temperatures (> 45°C), and both acetone and ethyl acetate seemed to influence the process. The extraction process of both materials had the temperature significantly influencing the retention index in the process, however, this response variable could not be described by the empirical model. The optimal recovery yield for the extraction of green coffee beans (65%) could be obtained when using 40% of acetone and 60% of ethanol at 55 °C; while for the press cake (58%), 40% of acetone, 20% of ethyl acetate, and 40% of ethanol at 45 °C. Further, the retention index results showed that the soluble solids extraction process of the press cake using ethanol and cosolvents is more feasible than for the green coffee beans, as it was 66% lower than the latter. Thus, the solid-liquid extraction could be suggested as an alternative process to extract soluble solids from green coffee beans and its press cake, also, it could be used as a second step to the mechanical process, once there is still great content of solids in this material after the pressing.

Acknowledgments

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References

Abdullah, M., & Bulent Koc, A. (2013). Oil removal from waste coffee grounds using two-phase solvent extraction enhanced with ultrasonication. *Renewable Energy*, *50*, 965–970. http://doi.org/10.1016/j.renene.2012.08.073

Al-Hamamre, Z., Foerster, S., Hartmann, F., Kröger, M., & Kaltschmitt, M. (2012). Oil extracted from spent coffee grounds as a renewable source for fatty acid methyl ester manufacturing. *Fuel*, *96*(1), 70–76. http://doi.org/10.1016/j.fuel.2012.01.023

Amarante, R. C. A., Oliveira, P. M., Schwantes, F. K., & Morón-Villarreyes, J. A. (2014). Oil extraction from castor cake using ethanol: kinetics and thermodynamics. *Industrial & Engineering Chemistry Research*, *53*(16), 6824–6829. http://doi.org/10.1021/ie500508n

Aquino, L. P., Borges, S. V, Queiroz, F., Antoniassi, R., & Cirillo, M. A. (2011). Extracción de aceite del fruto pequi (*Caryocar brasiliense* Camb.) utilizando diversos disolventes y sus mezclas. *Grasas y Aceite*, *62*(3), 245–252. http://doi.org/10.3989/gya.091010

Azevedo, A. B. A. d., Mazzafera, P., Mohamed, R. S., Melo, S. A. B. V. de, & Kieckbusch, T. G. (2008). Extraction of caffeine, chlorogenic acids and lipids from green coffee beans using supercritical carbon dioxide and co-solvents. *Brazilian Journal of Chemical Engineering*, *25*(3), 543–552. http://doi.org/10.1590/S0104-66322008000300012

Baümler, E. R., Carrín, M. E., & Carelli, A. A. (2016). Extraction of sunflower oil using ethanol as solvent. *Journal of Food Engineering*, *178*, 190–197. http://doi.org/10.1016/j.jfoodeng.2016.01.020

Bessa, L. C. B. A., Ferreira, M. C., Rodrigues, C. E. C., Batista, E. A. C., & Meirelles, A. J. A. (2017). Simulation and process design of continuous countercurrent ethanolic extraction of rice bran oil. *Journal of Food Engineering*, *202*, 99–113. http://doi.org/10.1016/j.jfoodeng.2017.01.019

Bitencourt, R. G., Ferreira, N. J., Oliveira, A. L., Cabral, F. A., & Meirelles, A. J. A. (2018). High pressure phase equilibrium of the crude green coffee oil – CO2 – ethanol system and the oil bioactive compounds. *The Journal of Supercritical Fluids*, *133*, 49–57. http://doi.org/10.1016/J.SUPFLU.2017.09.017

Brunner, G. (1994). *Gas extraction: An introduction to fundamentals of supercritical fluids and the application to separation processes*. (G. Brunner, Ed.). Berlin: Steinkopff-Verlag Heidelberg.

Caetano, N. S., Silva, V. F. M., & Mata, T. M. (2012). Valorization of coffee grounds for biodiesel production. *Chemical Engineering Transactions*, *26*, 267–272.

Campos-Vega, R., Loarca-Piña, G., Vergara-Castañeda, H., & Oomah, B. D. (2015). Spent coffee

grounds: A review on current research and future prospects. *Trends in Food Science and Technology*.

Castro, A. C. C. M., Oda, F. B., Almeida-Cincotto, M. G. J., Davanço, M. G., Chiari-Andréo, B. G., Cicarelli, R. M. B., … Santos, A. G. (2018). Green coffee seed residue: A sustainable source of antioxidant compounds. *Food Chemistry*, *246*, 48–57. http://doi.org/10.1016/J.FOODCHEM.2017.10.153

Dias, R., & Benassi, M. (2015). Discrimination between Arabica and Robusta coffees using hydrosoluble compounds: Is the efficiency of the parameters dependent on the roast degree? *Beverages*, *1*(3), 127–139. http://doi.org/10.3390/beverages1030127

Efthymiopoulos, I., Hellier, P., Ladommatos, N., Russo-Profili, A., Eveleigh, A., Aliev, A., … Mills-Lamptey, B. (2018). Influence of solvent selection and extraction temperature on yield and composition of lipids extracted from spent coffee grounds. *Industrial Crops and Products*, *119*, 49–56. http://doi.org/10.1016/j.indcrop.2018.04.008

FDA, CDER, & CBER. (2017). Q3C — Tables and List Guidance for Industry. Silver Spring: FDA.

Fornasari, C. H., Secco, D., Santos, R. F., da Silva, T. R. B., Galant Lenz, N. B., Tokura, L. K., … Gurgacz, F. (2017). Efficiency of the use of solvents in vegetable oil extraction at oleaginous crops. *Renewable and Sustainable Energy Reviews*, *80*, 121–124. http://doi.org/10.1016/J.RSER.2017.05.123

Galanakis, C. M. (2017). *Handbook of coffee processing by-products: Sustainable applications*. (C. M. Galanakis, Ed.) (1st ed.). London: Academic Press.

Graham Solomons, T. W., & Fryhle, C. B. (1998). *Organic Chemistry*. (G. Solomons & C. Fryhle, Eds.) (7th ed.). New York: John Wiley & Sons.

Hanmoungjai, P., Pyle, L., & Niranjan, K. (2000). Extraction of rice bran oil using aqueous media. *Journal of Chemical Technology & Biotechnology*, *75*(5), 348–352. http://doi.org/10.1002/(SICI)1097-4660(200005)75:5<348::AID-JCTB233>3.0.CO;2-P

Hron, R. J., & Koltun, S. P. (1984). An aqueous ethanol extraction process for cottonseed oil. *Journal of the American Oil Chemists Society*, *61*(9), 1457–1460. http://doi.org/10.1007/BF02636363

Hron, R. J., Koltun, S. P., & Graci, A. V. (1982). Biorenewable solvents for vegetable oil extraction. *Journal of the American Oil Chemists' Society*, *59*(9), 674A–684A. http://doi.org/10.1007/BF02636034

ICO. (2018). Total production by all exporting countries. Retrieved July 15, 2018, from http://www.ico.org/historical/1990 onwards/PDF/1a-total-production.pdf

Ishida, B. K., & Chapman, M. H. (2009). Carotenoid extraction from plants using a novel, environmentally friendly solvent. *Journal of Agricultural and Food Chemistry*, *57*(3), 1051–1059. http://doi.org/10.1021/jf8026292

Jang, S., Lee, A. Y., Lee, A. R., Choi, G., & Kim, H. K. (2017). Optimization of ultrasound-assisted extraction of glycyrrhizic acid from licorice using response surface methodology. *Integrative Medicine Research*, *6*(4), 388–394. http://doi.org/10.1016/j.imr.2017.08.003

Javed, F., Ahmad, S. W., Rehman, A., Zafar, S., & Malik, S. R. (2015). Recovery of rice bran oil using solid-liquid extraction technique. *Journal of Food Process Engineering*, *38*(4), 357–362. http://doi.org/10.1111/jfpe.12166

Jenkins, R. W., Stageman, N. E., Fortune, C. M., & Chuck, C. J. (2014). Effect of the type of bean, processing, and geographical location on the biodiesel produced from waste coffee grounds. *Energy and Fuels*, *28*(2), 1166–1174. http://doi.org/10.1021/ef4022976

Johnson, L. A., & Lusas, E. W. (1983). Comparison of alternative solvents for oils extraction. *Journal of the American Oil Chemists' Society*, *60*(2), 229–242. http://doi.org/10.1007/BF02543490

Kim, S., & Johnston, K. P. (1987). Clustering in supercritical fluid mixtures. *AIChE Journal*, *33*(10), 1603–1611. http://doi.org/10.1002/aic.690331004

Lack, E., & Seidlitz, H. (1993). Commercial scale decaffeination of coffee and tea using supercritical CO2. In M. B. King & T. R. Bott (Eds.), *Extraction of natural products using near-critical solvents* (1st ed., p. 325). Heidelberg: Springer. http://doi.org/10.1007/978-94-011-2138-5

Li, Y., Sui, X., Qi, B., Zhang, Y., Feng, H., Zhang, Y., … Wang, T. (2014). Optimization of ethanol-ultrasound-assisted destabilization of a cream recovered from enzymatic extraction of soybean oil. *Journal of the American Oil Chemists' Society*, *91*(1), 159–168. http://doi.org/10.1007/s11746-013-2352-7

Loyao, A. S., Villasica, S. L. G., Dela Peña, P. L. L., & Go, A. W. (2018). Extraction of lipids from spent coffee grounds with non-polar renewable solvents as alternative. *Industrial Crops and Products*, *119*, 152–161. http://doi.org/10.1016/j.indcrop.2018.04.017

Lusas, E. W., Watkais, L. R., & Rhee, K. C. (1994). Separación de grasas y aceites por métodos no tradicionales. *Aceites y Grasas*, *14*, 87–99.

Mani, S., Jaya, S., & Vadivambal, R. (2007). Optimization of solvent extraction of moringa (*Moringa Oleifera*) seed kernel oil using response surface methodology. *Food and Bioproducts Processing*, *85*(4), 328–335. http://doi.org/10.1205/FBP07075

Muralidhar, R. V, Chirumamilla, R. R., Ramachandran, V. N., Marchant, R., & Nigam, P. (2001).

Racemic resolution of RS-baclofen using lipase from *Candida cylindracea*. *Mededelingen (Rijksuniversiteit Te Gent. Fakulteit van de Landbouwkundige En Toegepaste Biologische Wetenschappen)*, *66*(3a), 227–32.

Mussatto, S. I., Machado, E. M. S., Martins, S., & Teixeira, J. A. (2011). Production, composition, and application of coffee and its industrial residues. *Food and Bioprocess Technology*, *4*, 661–672. http://doi.org/10.1007/s11947-011-0565-z

NFPA. (1991). *Fire protection guide to hazardous materials*. (NFPA, Ed.) (10th ed.). Quincy.

Oliveira, N. A. de, Cornelio-Santiago, H. P., Fukumasu, H., & Oliveira, A. L. de. (2018). Green coffee extracts rich in diterpenes – Process optimization of pressurized liquid extraction using ethanol as solvent. *Journal of Food Engineering*, *224*, 148–155. http://doi.org/10.1016/J.JFOODENG.2017.12.021

Oliveira, É. R., Carvalho, G. R., Santos, P. R., & Queiroz, F. (2018). Green coffee and its residual biomass: Characterization for its industrial approach. *International Food Research Journal*.

Oliveira, R. C., Barros, S. T. D., & Gimenes, M. L. (2013). The extraction of passion fruit oil with green solvents. *Journal of Food Engineering*, *117*(4), 458–463. http://doi.org/10.1016/J.JFOODENG.2012.12.004

Oliveira, R., Oliveira, V., Aracava, K. K., & Rodrigues, C. E. da C. (2012). Effects of the extraction conditions on the yield and composition of rice bran oil extracted with ethanol—A response surface approach. *Food and Bioproducts Processing*, *90*(1), 22–31. http://doi.org/10.1016/j.fbp.2011.01.004

Peker, H., Srinivasan, M. P., Smith, J. M., & McCoy, B. J. (1992). Caffeine extraction rates from coffee beans with supercritical carbon dioxide. *AIChE Journal*, *38*(5), 761–770. http://doi.org/10.1002/aic.690380513

Perrier, L., Blondal, E., Ayala, A. P., Dearborn, D., Kenny, T., Lightfoot, D., … MacDonald, H. (2017). Research data management in academic institutions: A scoping review. *Plos One*, *12*(5), e0178261. http://doi.org/10.1371/journal.pone.0178261

Quanhong, L., & Caili, F. (2005). Application of response surface methodology for extraction optimization of germinant pumpkin seeds protein. *Food Chemistry*, *92*(4), 701–706. http://doi.org/10.1016/J.FOODCHEM.2004.08.042

Rabasco Alvarez, A. M., González Rodríguez, M. L., & Rodríguez, M. L. G. (2000). Lipids in pharmaceutical and cosmetic preparations. *Grasas y Aceites*, *51*(1–2), 74–96. http://doi.org/10.3989/gya.2000.v51.i1-2.409

Rittner, H. (1992). Extraction of vegetable oils with ethyl alcohol. *Oléagineux*, *47*(1), 39–42.

Rodrigues, C. E. C., Aracava, K. K., & Abreu, F. N. (2010). Thermodynamic and statistical analysis of soybean oil extraction process using renewable solvent. *International Journal of Food Science & Technology*, *45*(11), 2407–2414. http://doi.org/10.1111/j.1365-2621.2010.02417.x

Rodrigues, C. E. C., Gonçalves, C. B., Batista, E., & Meirelles, A. J. A. (2007). Deacidification of vegetable oils by solvent extraction. *Recent Patents on Engineering*, *1*(1), 95–102. http://doi.org/10.2174/187221207779814699

Rodrigues, E., Poerner, N., Rockenbach, I. I., Gonzaga, L. V, Mendes, C. R., & Fett, R. (2011). Phenolic compounds and antioxidant activity of blueberry cultivars grown in Brazil. *Food Science and Technology (Campinas)*, *31*(4), 911–917. http://doi.org/10.1590/S0101-20612011000400013

Rodrigues, L. M., Alcázar-Alay, S. C., Petenate, A. J., & Meireles, M. A. A. (2014). Bixin extraction from defatted annatto seeds. *Comptes Rendus Chimie*, *17*(3), 268–283. http://doi.org/10.1016/J.CRCI.2013.10.010

Sluiter, A., Hames, B., Hyman, D., Payne, C., Ruiz, R., Scarlata, C., … Wolfe, J. (2008). *Determination of total solids in biomass and total dissolved solids in liquid process samples*. *Laboratory Analytical Procedure (LAP)*. Golden.

Somnuk, K., Eawlex, P., & Prateepchaikul, G. (2017). Optimization of coffee oil extraction from spent coffee grounds using four solvents and prototype-scale extraction using circulation process. *Agriculture and Natural Resources*, *51*, 181–189. http://doi.org/10.1016/j.anres.2017.01.003

StatSoft Inc. (2007). STATISTICA (Data analysis software system) version 8.0. Tulsa (OK): StatSoft Inc.

Strati, I. F., & Oreopoulou, V. (2011). Effect of extraction parameters on the carotenoid recovery from tomato waste. *International Journal of Food Science & Technology*, *46*(1), 23–29. http://doi.org/10.1111/j.1365-2621.2010.02496.x

Torres-León, C., Rojas, R., Serna-Cock, L., Belmares-Cerda, R., & Aguilar, C. N. (2017). Extraction of antioxidants from mango seed kernel: Optimization assisted by microwave. *Food and Bioproducts Processing*, *105*, 188–196. http://doi.org/10.1016/j.fbp.2017.07.005

Turner, C., Whitehand, L. C., Nguyen, T., & McKeon, T. (2004). Optimization of a supercritical fluid extraction/reaction methodology for the analysis of castor oil using experimental design. *Journal of Agricultural and Food Chemistry*, *52*(1), 26–32. http://doi.org/10.1021/jf0347665

Vardon, D. R., Moser, B. R., Zheng, W., Witkin, K., Evangelista, R. L., Strathmann, T. J., … Sharma, B. K. (2013). Complete utilization of spent coffee grounds to produce biodiesel,

bio-oil, and biochar. *ACS Sustainable Chemistry and Engineering*, *1*(10), 1286–1294. http://doi.org/10.1021/sc400145w

Villanueva Bermejo, D., Luna, P., Manic, M. S., Najdanovic-Visak, V., Reglero, G., & Fornari, T. (2013). Extraction of caffeine from natural matter using a bio-renewable agrochemical solvent. *Food and Bioproducts Processing*, *91*, 303–309. http://doi.org/10.1016/j.fbp.2012.11.007

Wakelyn, P. J., & Wan, P. J. (2005). Solvent extraction to obtain edible oil products. In C. C. Akoh (Ed.), *Handbook of Functional Lipids* (1st ed., pp. 89–131). Boca Raton (US): CRC Press. http://doi.org/doi:10.1201/9781420039184.ch5\r10.1201/9781420039184.ch5

Wan, P. J., Pakarinen, D. R., Hron, R. J., Richard, O. L., & Conkerton, E. J. (1995). Alternative hydrocarbon solvents for cottonseed extraction. *Journal of the American Oil Chemists' Society*, *72*(6), 653–659. http://doi.org/10.1007/BF02635650

Zuorro, A., & Lavecchia, R. (2012). Spent coffee grounds as a valuable source of phenolic compounds and bioenergy. *Journal of Cleaner Production*, *34*, 49–56. http://doi.org/https://doi.org/10.1016/j.jclepro.2011.12.003

ARTIGO 6 – ULTRASOUND ASSISTED EXTRACTION OF GREEN COFFEE BEANS: ALTERNATIVE SOLVENTS

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Abstract

This study aimed to optimize the soluble solids solid-liquid ultrasound-assisted extraction (20 kHz, 400 W, 20% amplitude and a 3 mm microtip) using bio-renewable solvents from green coffee beans. The effect of the cosolvents (acetone and ethyl acetate) concentration (0 – 40%) in the solvent ethanol, and the extraction time (5 – 60 min) on the extraction yield and retention index were evaluated through a central composite rotational design and response surface. A desirability function was used in order to optimize the results of both response variables analyzed, where the optimal conditions were found to be 16.41 to 31.45% for acetone; 31.45 to 40% for ethyl acetate; 54.44 to 60 min for time. The results showed that the extraction time is a parameter with high influence on recovery of soluble solids and retention index of green coffee beans solid-liquid extraction process.

Keywords: *Coffea arabica*; Solid-liquid extraction; Ultrasound; alternative solvents.

1. Introduction

Coffee is one of the most popular and consumed fruits in the world. Brazil itself is, worldwide, one of the largest producers and consumers of this crop. Apart from its use as a beverage, the green coffee beans are becoming of interest due to its oil content and quality, mainly regarding its bioactivity.

Lipids are among the most important components of green coffee beans from a quantitative point of view. In the mature coffee endosperm, they are present in the form of oil bodies functioning as an energy reserve, mobilized during germination [1,2]. The two commercially relevant coffee species, *Coffea arabica* and *Coffea canephora*, contain from 7% to 17% of lipids (dry basis), the average lipid content of green Arabica being significantly higher (15%) than that of Robusta (10%) [3,4]. Other coffee species, such as *C. heterocalyx* and *C. salvatrix*, may contain lipids up to 20–30% [5]. In terms of chemical composition, the coffee lipid fraction, also known as coffee oil, is extremely complex; it is mainly constituted by triacylglycerols with a fatty acid profile similar to that of common edible vegetable oils. However, the relatively large unsaponifiable fraction (up to 18.5% w/w) [6,7] as well as the presence of antioxidants, caffeine and other phenolics, makes of the coffee oil an interesting product in order to be applied in the industry, such as pharmaceutical, cosmetics, and food.

The coffee oil is usually obtained by the mechanical pressing process, which may not be as feasible as other processes due to its low recovery of oil and antioxidants [8], such as the solid-liquid extraction. The lipidic fraction of vegetable matrices are usually extracted with conventional Soxhlet apparatus using petroleum-derived solvents such as n-hexane and petroleum ether [9–12]. Conventional solid-liquid extraction techniques as maceration and Soxhlet extraction methods consume time and use large amounts of solvents [13,14]. Nowadays, the development of the concept of green extraction, the environment friendly procedures are becoming of interest. The use of new technologies, as microwave and ultrasound offer several advantages like reducing time, cost, solvent consumption and power not to affect the stability of extracted compounds, simplifying processes and improving the extraction quantitatively and qualitatively [15–18]. The ultrasound technology can be employed in order to improve the solid-liquid extraction, being widely used to modify the food matrix physical properties, mainly due to the cavitation phenomena [19].

The application of ultrasound has been extensively studied for the extraction of oil compounds from vegetable matrices [20–24]. Besides working for oil extraction, ultrasound-assisted extraction can also, significantly, improve the yield of bioactive substances, achieving higher efficiencies and shorter reaction times at lower temperatures and lower process costs [21,25]. These studies demonstrate that the use of an ultrasound-assisted oil extraction offers advantages, including saving time, reducing solvent consumption and increasing oil recovery. However, most of the studies have being carried out using hexane as the solvent. Thus, the aim of this work was to study the effect of green solvents, using acetone and ethyl acetate as cosolvents to ethanol, and time on the extraction of soluble solids from an industrial waste proveniente from the coffee industry (green coffee press cake) assisted by an intensification technology, such as the ultrasound.

2. Material and methods

2.1 Materials

Green coffee beans (GCB) (*Coffea arabica*) samples were obtained from a local coffee and oil producing cooperative (Cooxupé, Guaxupé, Minas Gerais, Brazil). The GCB were packed in polyethylene bags in order to be transported and stored until the experiment was carried out. It was homogenized in a blender in orther to obtain a standardized granulometry of the powder by sieving through standard Tyler mesh (−20/+80 mesh) with the aid of a sieve shaker (A5911, Intertest Benelux, Netherlands) [26]. Further, it was vacuum dried (40 °C) (absolute pressure = 16.8 kPa) (TE-395, Tecnal, Piracicaba, Brazil) until the weight was stable, and kept in dark recipients in a desiccator at room temperature. Characterization of the material was carried out by Oliveira et al. (2018), where the crude oil content of the GCB was assessed by Sohxlet extraction using ethanol as the solvent, and an average of 14.16 g/100 g in dry basis (d.b.) was obtained. Analytical grade solvents and chemicals were used and obtained from Sigma Aldrich (Brazil). The solvents employed in the extraction process were ethanol (99% purity), acetone (99% purity), and ethyl acetate (99% purity).

2.2 Ultrasound-assisted extraction of soluble solids from GCB using green solvents

Ultrasonic extraction was carried out in a digital sonifier (Branson 450, Branson Ultrasonics Corporation, Danbury, USA) with a frequency of 20 kHz, power of 400 W, 20% amplitude, and a 3 mm microtip (109-067-683). To a sample of 3 g it was added 15 g of solvent ethanol and mixtures with acetone and/or ethyl acetate in a Falcon tube (50 mL) which was properly sealed, at the constant solid-liquid ratio of 1:5 (w/w). The ultrasonic generator probe was submerged into the suspension by an orifice made to the center of the tube's cap. The extractions were performed under different conditions of time (5 – 60 min) and concentration of acetone and/or ethyl acetate (0 - 40%) in ethanol. Ended the extraction time, the resulting suspension, extract phase (EP), was filtered using a paper filter (Whatmann nº1), and placed in a pre-weighed Petri dish. Ethanol was used to wash the recipient (with the EP) in order to remove any solids that may have been adhered to its wall. The Petri dish containing the EP + ethanol was oven dried at 60 °C for at least 2 h in order to evaporate the solvent. The raffinate phase (RP), which remained in the extraction tube after the removal of the EP, was subjected to centrifugation (3500 rpm/1 min) (Excelsa II – 206 BL, FANEM, São Paulo, Brazil), then the remaining liquid was discarded and a sample of the RP was withdrawn and immediately weighed into a Petri dish, which was also subjected to drying (60 °C/ 2 h). Further, the samples without the solvent were cooled down in desiccators and weighed. The residual extractable material in the dried material was determined as the difference between the maximum extractable material determined by Soxhlet using ethanol as the solvent, and the extracted material in the liquid phase.

2.3 Recovery yield determination through mass balance

For the extraction experiments performed, the extract (EP) and the raffinate (RP) phases were determined in terms of the mass fractions of the components. The mass fraction of EP was determined using the data obtained from the samples taken with the microsyringe. The withdrawn EP sample mass was calculated using the mass difference of the syringe with extract and the empty syringe. The soluble solids mass in the EP sample was calculated from the mass difference of the Petri dish containing the extract sample and the empty Petri dish. The mass fraction of soluble

solids ($w_{1,EP}$) and the mass fraction of the solvent ($w_{2,EP}$) in the EP, both insoluble solids free ($w_{3,EP} = 0$), were calculated according to equations 1 and 2, respectively.

$$w_{1,EP} = \frac{m_f - m_i}{m_{EP}} \quad (1)$$

$$w_{2,EP} = 1 - w_{1,EP} \quad (2)$$

Where, m_f and m_i are the final and initial weight of the Petri dish, respectively, and m_{EP} is the mass of the extract phase sample withdrawn.

The mass fraction of the RP was determined through a mass balance. The mass fraction of the solvent in RP ($w_{2,RP}$) was calculated by the difference of weights found before and after drying (Equation 3).

$$w_{2,RP} = \frac{m_f - m_i}{m_{RP}} \quad (3)$$

Where, m_f and m_i are the final and initial weight of the Petri dish, respectively, and m_{RP} is the mass of the raffinate phase sample withdrawn.

The mass of the system (solvent and material) (m_{system}), and the mass fraction of each component in the system are known variables. The remaining variables - mass of EP (m_{EP}), mass of RP (m_{RP}), soluble solids mass fraction in RP ($w_{1,RP}$), and insoluble solids mass fraction in RP ($w_{3,RP}$) - were determined through a global mass balance (Equation 4). And the mass balance for each component of the system (i) is presented in Equation 5.

$$m_{system} = m_{EP} + m_{RP} = m_2 + m_s \quad (4)$$

Where, m_{system} is the mass of the global system, m_{EP} is the mass of the extract phase withdrawn with the syringe, m_{RP} is the mass of the raffinate phase collected, m_2 is the solvent mass used in the extraction process, and m_s is the mass of sample used in the experiment.

$$w_{i,system} m_{system} = w_{i,EP} m_{EP} + w_{i,RP} m_{RP} \quad (5)$$

Where, i = 1 (soluble solids) or 2 (solvent) or 3 (insoluble solids).

The parameter w_i is defined by Equation 6, where m_i is the mass of a given component in the EP or RP (m_P).

$$w_i = \frac{m_i}{m_P} \quad (6)$$

The soluble solids mass transfer in the extraction process was calculated through Equation (7) (Rodrigues et al., 2010), where m_s is the mass of beans used in the extraction process, and $w_{1,s}$ is the mass fraction of soluble solids in the beans before the extraction process.

$$\Gamma_1\ (\%) = 100\left(\frac{w_{1,EP} m_{EP}}{w_{1,s} m_s}\right) \quad (7)$$

The retention index (RI) corresponds to the mass of adhered solution per soluble solids mass, and can be calculated using Equation (8):

$$RI = \frac{w_{1,RP} m_{RP} + w_{2,RP} m_{RP}}{w_{3,RP} m_{RP}} = \frac{m_{1,RP} + m_{2,RP}}{m_{3,RP}} \quad (8)$$

2.4 Experimental Design and statistical analysis

The effects of the parameters cosolvent-to-solvent ratio, and extraction time were investigated by applying a full factorial design (2^3) with two levels (+1 and -1), two axial points, and three repetitions at the central point, summing 17 experimental runs. The proposed extraction

process was modeled by means of Central Composite Rotational Design (CCRD) and Response Surface Methodology (RSM), and statistically tested using the analysis of variance (ANOVA) through the Statistica 8.0 software [28]. The coded (x_i) and uncoded levels of independent variables are shown in Table 1. The general form of the quadratic regression model was expressed as Equation 9.

$$Y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_{11} x_1^2 + \beta_{22} x_2^2 + \beta_{33} x_3^2 + \beta_{12} x_1 x_2 + \beta_{13} x_1 x_3 + +\beta_{23} x_2 x_3 \quad (9)$$

Where Y is the response function (Γ_1 or RI), x_i is the studied independent variables, β_0 is the constant term, β_i is the linear coefficient, β_{ii} and β_{ij} are the quadratic and interactive terms, respectively, of the model. The effect of the mass fraction of acetone (x_1; 0 - 40% w/w), and ethyl acetate (x_2; 0 - 40% w/w) in the solvent ethanol, and of the extraction time (x_3; 5 – 60 min) were studied. The optimal process condition was determined by numerical optimization technique. The developed second-order polynomial mathematical model was converted into a conical form and the Eigen value was calculated. Based on the Eigen values, the nature of the optimal condition (point of maximum, minimum or a saddle point response) was identified through the Statistica 8.0 software [28].

3. Results and discussion

CCRD with RSM was used to determine the optimal experimental conditions for the extraction of soluble solids (SS) from GCB. The purpose was to reach the most appropriate solvent system composition and time for the extraction of SS from the solid matrices. Taking into account the chemical nature of the analytes under study as well as the matrices involved, the RI was also considered as a response factor that might influence the process. Based on previous studies of this research group, acetone and ethyl acetate were selected as potential cosolvents to ethanol in order to evaluate the process [29–31]. The presence of several hydroxyl or carboxyl groups, allows more hydrogen bonds to be formed, thus increasing the stability of the extracts [32]. Moreover, the selected solvent system components are readily available, inexpensive and widely used.

Table 1 shows the component values of the experimental design matrix and the responses regarding the extraction process of soluble solids (SS) and retention index (RI).

Table 1. Experimental design and response values of the extraction yield and retention index (RI) of green coffee beans (GCB).

Run	x_1 (%w/w)	x_2 (%w/w)	x_3 (min)	Γ_1 (%)	RI
1	-1 (8.04)	-1 (8.04)	-1 (16.13)	115.04	0.42
2	-1 (8.04)	1 (31.95)	1 (48.87)	102.59	0.25
3	1 (31.95)	-1 (8.04)	1 (48.87)	103.76	0.58
4	1 (31.95)	1 (31.95)	-1 (16.13)	86.24	0.33
5	0 (20)	0 (20)	0 (32.5)	99.54	0.18
6	-1 (8.04)	-1 (8.04)	1 (48.87)	129.1	0.48
7	-1 (8.04)	1 (31.95)	-1 (16.13)	80.77	0.68
8	1 (31.95)	-1 (8.04)	-1 (16.13)	124.22	0.52
9	1 (31.95)	1 (31.95)	1 (48.87)	113.73	0.09
10	0 (20)	0 (20)	0 (32.5)	103.08	0.19
11	-1.67332 (0)	0 (20)	0 (32.5)	70.4	0.3
12	1.67332 (40)	0 (20)	0 (32.5)	81.87	0.49
13	0 (20)	-1.67332 (0)	0 (32.5)	101.3	0.52
14	0 (20)	1.67332 (40)	0 (32.5)	87.58	0.52
15	0 (20)	0 (20)	-1.67332 (5)	91.03	0.43
16	0 (20)	0 (20)	1.67332 (60)	145.56	0.37
17	0 (20)	0 (20)	0 (32.5)	107.58	0.19

Value outside the brackets represents coded levels.
x_1: Mass fraction of acetone in the solvent ethanol
x_2: Mass fraction of ethyl acetate in the solvent ethanol
x_3: Time (min)

The SS recovery (% Γ_1) from GCB ranged from 70.39 (9.97 g of soluble solids/ 100 g of dry beans) to 145.56% (21.25 g of soluble solids/ 100 g of dry beans), where the lowest and greatest yields were achieved at runs 11 (0% acetone, 20% ethyl acetate, 32.5 min) and 16 (20% acetone, 20% ethyl acetate, 60 min), respectively. The greatest recovery of 14.16 g/100 g surpassed the yield obtained by the traditional Soxhlet extraction with ethanol. Regarding the RI, which is a parameter that has a decisive impact on the number of stages carried out to complete the extraction, and on the desolventization step, that depends on the viscosity of the extract solution, and mainly on the physicochemical affinity between the solvents, cosolvents and the solid matrix [33], the aim is to achieve the lowest values in order to obtain an efficient process, once it indicates the amount of solution retained in the RP. Thus, the results were as expected, once the lowest value was obtained after a longer period of extraction (run 9), which corresponds to one of the greatest recovery yields obtained in the process.

The results were statistically tested through an ANOVA (Table 2), in order to stablish an empiric model to express the process.

Table 2. ANOVA and regression coefficients for the response surface complete quadratic model of soluble solids transfer (Γ_1) ($R^2 = 0.84$) and retention index (RI) ($R^2 = 0.8$) from green coffee beans (GCB).

Source	Sum of square		Degree of freedom		Mean square		p-value		Regression coefficients	
	Γ_1	RI	Γ_1	RI	Γ_1	RI	Γ_1	RI	Γ_1	RI
x_1	28.44	9^10^{-6}	1	1	28.44	9^10^{-6}	0.3171	0.6662	1.44	0.0
x_1^2	577.75	0.05	1	1	577.75	0.05	0.027*	0.0007*	-7.21	0.07
x_2	918.27	0.03	1	1	918.27	0.03	0.017*	0.0012*	-8.22	-0.05
x_2^2	4.94	0.13	1	1	4.94	0.13	0.6369	0.0002*	-0.67	0.11
x_3	1323.46	0.03	1	1	1323.46	0.03	0.012*	0.0012*	9.87	-0.05
x_3^2	686.46	0.05	1	1	686.46	0.05	0.023*	0.0007*	7.85	0.07
x_1x_2	134.18	0.06	1	1	134.18	0.06	0.1028	0.0006*	4.1	-0.09
x_1x_3	104.04	0.005	1	1	104.04	0.005	0.1272	0.007*	-3.61	0.03
x_2x_3	388.01	0.08	1	1	388.01	0.08	0.039*	0.0005*	6.9	-0.1
Lack of fit	1184.88	0.07	5	5	236.98	0.014	0.07	0.003*		
Pure error	32.54	7^10^{-5}	2	2	16.27	4^10^{-5}				
Total	5902.17	0.44	16	16						

*Significant at $p \leq 0.05$.

The ANOVA results showed that it is possible to plot the response surface for this experimental design. The resulting correlation coefficients for both Γ_1 ($R^2 = 0.8$) and RI ($R^2 = 0.84$) indicated that the experimental data were in satisfactory agreement with predicted responses for each model, or that, the samples variation of 80% and 84% are attributable to the independent variables, namely cosolvent type and ratio in the system and extraction time. The elevated R^2 values (> 70%) indicate the quality of the model regarding the high predictability of responses and experimental significance. The lack-of-fit (p-value) was calculated as 0.07 for Γ_1. The fitness of the model was evaluated through the lack of fit test ($p > 0.05$), indicating that this model accurately represents the experimental data [34–36]. The lack of-fit was significative for the RI response, which implies that a higher degree model should be used in order to represent the data, however, each of the observed values was compared with the predicted values calculated from the model, as depicted in Fig. 1, which shows the accordance of the majority of the values, also the adequate level of the correlation coefficient allows the usage of the parameters of this model. The predicted results, which correspond to the complete model (including the non-significant parameters), closely

matched the experimental results as the model explains 80% and 84% of the variability among samples, for Γ1 (%) and RI results, respectively.

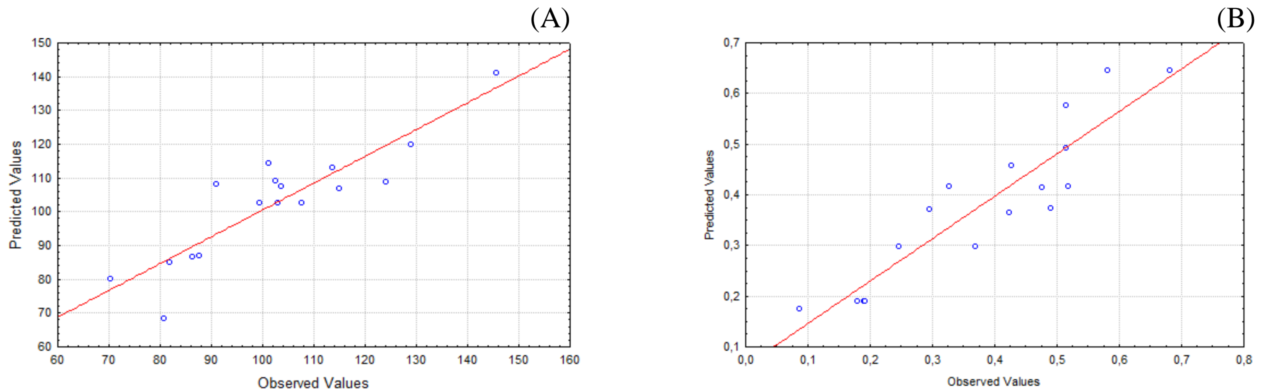

Fig. 1. Comparison between predicted and observed soluble solids yield (A) and retention index (B) results obtained from the green coffee beans ultrasound-assisted extraction.

The significance of the F value depends on the number of degrees of freedom in the model and is shown in the p-value column (95% confidence level). Thus, the effects lower than 0.05 in this column were considered significant. The significance of the corresponding variables would consist in the F value becoming greater and the p value becoming smaller. The F-test suggested that the model, for each of the response analyzed, had a high F-value (F = 3, F = 4), and a low p value (< 0.05), indicating that the models were highly significant, reassuring the decision to use the RI model. In the case of Γ1, the linear (x_2 , x_3), quadratic (x_1^2, x_3^2), and interaction terms (x_2 x_3) had high model F-values of 56.44, 81.34, 35.51, 42.19, and 23.85, respectively, and all p-values (p < 0.05) were low, indicating that these factors were significant and should be used in order to build the model. While, for the RI, only the linear effect of the variable x_1 (acetone mass fraction) was not significant, whereas the quadratic terms of the variables x_2 and x_3 , and the interaction between x_2 and x_3 and x_1 x_2 were the most significant. This significance of each term of the models correspondent to the Γ1 and RI (p ≤ 0.05) can be vizualized in the Pareto chart (Fig. 2).

The significance of the negative quadratic terms indicates that there is a point of maximum for the variable, as for the variables x_1 and x_2 for the rensponse Γ1. Thus the addition of acetone and ethyl acetate influenced the Γ1 from GCB. The quadratic term coefficient (x_1^2) (acetone) was negative and significant for GCB, indicating that the increasing of acetone content initially favors

the solubilization of soluble solids, reaching a maximum, and posterior addition leads to a reduction of their solubilization power.

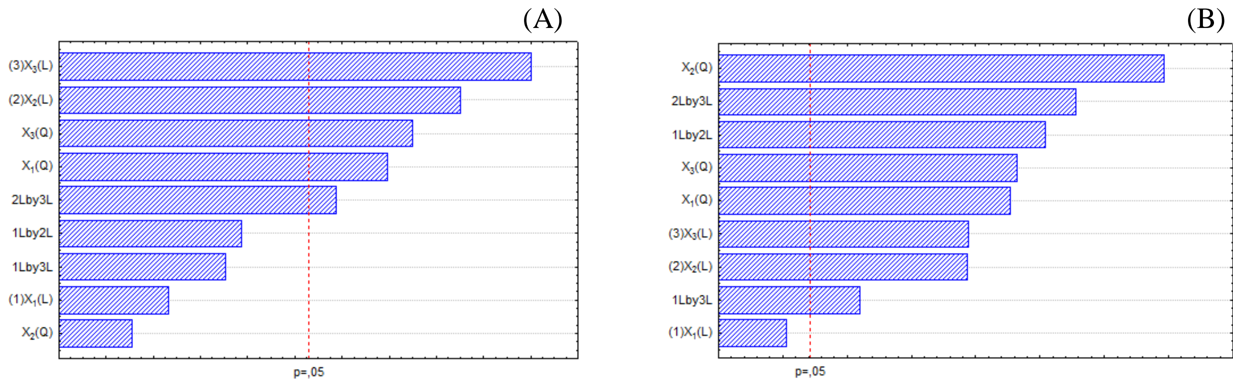

Fig. 2. Pareto charts representing the effects of the independent variables on the soluble solids yield (A) and retention index (B) obtained from the green coffee beans ultrasound-assisted extraction concerning the complete model.

A similar interpretation could be carried to the variable time (x_3^2) once the quadratic term was significant, however, positive, indicating that the time is effective in order to extract the soluble solids from coffee beans through ultrasonication. By extending the extraction time there is the rupture of the material which, initially leads to a lowering of the RI, and later, in longer periods there is a raise of the adhered solution.

The linear coefficient of the variable x_2 (ethyl acetate mass fraction in ethanol) for both models exhibited negative and significative effect on the extraction. The quadratic term (x_2^2) was only significative for RI, and also positive, indicating that there is a point of minimum related to it, that is, the process should be operated at this point, once the low RI indices are required in order to achieve higher recoveries.

The application of RSM yielded the following reduced regression equations, which is an empirical relationship between Γ_1 (Eq. 10) and RI (Eq. 11), and the tested significant variables in coded units.

$$\Gamma_{GCB} = 101.72 + 1.45x_1 - 7.2x_1^2 - 8.22x_2 - 0.66x_2^2 + 9.87x_3 + 7.8x_3^2 + 4.09x_1 x_2 - 3.6x_1 x_3 + 6.96x_2 \quad (10)$$

$$\mathrm{RI} = 0.19 + 0.0008x_1 + 0.07x_1^2 - 0.05x_2 + 0.11x_2^2 - 0.05x_3 + 0.07x_3^2 - 0.09x_1 x_2 + 0.03x_1 x_3 - 0.1x_2 x_3 \quad (11)$$

The numerical optimization was used in order to obtain the optimum values for each independent variable studied. However, it was possible to optimize only the response RI ($x_1 =$ 23.82; $x_2 =$ 28.6; $x_3 =$ 33.6; real values), once the optimization of the response Γ1 resulted in a saddlepoint for the variable x_2 (acetone mass fraction in ethanol) ($x_1 =$ -1.58; $x_2 =$ -4.89; $x_3 =$ 1.17; coded values). Therefore, in order to be able to discuss both variables results, the desirability function (spline fit) was used to obtain the simultaneous optimization of both response variables (Γ1 and RI) (Fig. 3).

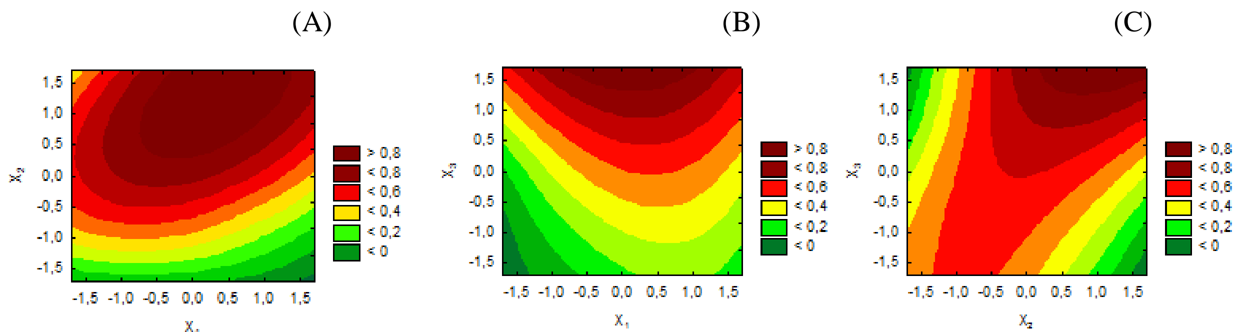

Fig. 4. Contour plots (desirability function) for the soluble solids extraction and retention index when optimizing the proportion of cosolvents acetone (x_1) vs. ethyl acetate (x_2) in the system (A), time (x_3) vs. mass fraction of acetone (B), and temperature vs. mass fraction of ethyl acetate (C), where the third variable was kept constant (optimal value).

As shown in Fig. 3 the desirability function increases when the time and the mass fraction of ethyl acetate decreases. The desirable experimental conditions for the extraction process were of 16.41 to 31.45 for x_1; 31.45 to 40 for x_2; 54.44 to 60 for x_3.

Fig. 3A shows the effect of both cosolvents proportion on the recovery of SS and RI from GCB. A quadratic effect of mass fraction of acetone in ethanol and a linear effect of ethyl acetate concentration on the response were observed. Fig. 3B shows the effect of the extraction time and acetone concentration; on the SS recovery, whereas the extraction time had both linear and quadratic effects. Fig. 3C depicts the influence of ethyl acetate concentration and time, where it can be seen a linear effect for the former, and a quadratic effect for the latter. Therefore, increasing the extraction time resulted in a higher SS yield.

It is inferable that the rise in the extraction time and ethyl acetate mass fraction influenced the Γ_1 (%) of GCB. For the time (x_3) there is a range where the yield is maximum, and the ethyl acetate (x_2), as a cosolvent, positively influenced the extraction, there is a range where the yield is minimum, thus, this cosolvent must be used out of this range of concentration (Figure 3C). Oliveira et al. (2018a) reported a different trend while extracting soluble solids from green coffee beans and its press cake using the same solvents at 35 to 55°C. The authors observed that the presence of ethyl acetate did not favor the process, while in this study the opposite was observed, this fact could be explained by the difference in the temperature applied in the process. Ethyl acetate probably is more efficient at higher temperatures, where it becomes less viscous, which is the case of this study where while applying the ultrasound, the temperature reached up to 70 °C.

Ultrasound can induce acoustic cavitation and rupture of plant cells [37]. When extraction time is extended, plant cells are completely collapsed by the effects of acoustic cavitation, and extraction yields increase. However, when plant cells rupture, insoluble substances and cytosol get suspended in the extraction liquid, thus resulting in the lower permeability of the solvent [38]. Moreover, specific constituents get readsorbed on the ruptured plant particles due to their relatively large specific surface areas, and this decreases the yields of the recovered compounds [39]. Thus, it is counter-productive to extend extraction duration once the maximum extraction yield has been achieved [40,41].

Bimakr et al. (2012) while optimizing the ultrasound extraction of soluble solids from winter melon (*Benincasa hispida*) seeds using ethanol as the solvent, observed an optimum extraction time of 35 min, they reported that at the beginning of the process washing (dissolution of soluble substances on surfaces of the material) happens with a fast increase, and later, the slowing of the process was observed by a low raise in the recovery, which is defined by a mass transfer of the solute from the material into the solvent by diffusion and osmotic processes [42]. Sun and Tomkinson (2002) observed that the extraction yield was increased up to 26 min of sonication time, and reported that longer extraction periods could cause structural alteration and disintegration due to the excess heating effect and overexposure to ultrasound treatment for longer extraction time which could diminish the extraction yield, and also long time exposure with low extraction yield is not a viable condition from the economical point of view.

Regarding the effect of the cosolvents employed in the solvent ethanol, as stated above, the addition of ethyl acetate showed to be more effective as a cosolvent rather than acetone in order to yield extractable solids from green coffee beans. Also, it was found that the extraction time was the most significant variable among the process variables studied. According with Suchinina et al. (2011), the extract yield increases with increasing solvent polarity, however, this was not observed in this study, once ethyl acetate is less polar than acetone and ethanol, with a polarity index of 4.3 [45]. This condition could be attributed to the positive effect of adding solvents of different polarities in order to attain higher extraction characteristics, or even to the fact that the coffee sample was constituted of more apolar solvents that could be better extracted by a less polar solvent, such as ethyl acetate. Also, the solvents properties in the cavitation effect, such as vapor pressure and viscosity, could be a reason to justify this trend. Elevated vapor pressures can lower the cavitation effect due to the bubbles' rupture before their growing.

4. Conclusion

In this study, response surface methodology with a central composite rotational design was applied to investigate the ultrasound-assisted extraction of soluble solids from green coffee beans. The experimental results showed that all three process variables, including acetone mass fraction in ethanol, ethyl acetate mass fraction in ethanol, and time, influenced the extraction of solubles solids. It was also studied the retention index as a response variable. It was found that the extraction time was the most significant variable among the process variables studied. An empirical quadratic polynomial correlation has been proposed to estimate the optimum operating condition of the process. The highest recovery yield (145.56% d.b.) was obtained when the extraction process was carried out at the central point for the cosolvents concentration and at 60 min, and the lowest RI at 31.95% of each cosolvent and at 48.87 min. The expected lowest RI was to match the highest soluble solids recovery; however, it was correspondent to one of the highest recoveries achieved. The results of the comparative study revealed that the solids recovery obtained from ultrasound-assisted extraction was higher than that obtained by the Soxhlet method. It could be suggested that ultrasound-assisted extraction is an effective and indeed feasible method for the extraction of soluble solids form green beans, which could be further aplied in the industry.

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References

[1] E. Dentan, Etude microscopique du dévelopement et de la maturation du grain de café́, in: 11th Int. Sci. Colloq. Coffee, ASIC, Paris, 1987: pp. 381–398.

[2] N. D'Amelio, E. De Angelis, L. Navarini, E. Schievano, S. Mammi, Green coffee oil analysis by high-resolution nuclear magnetic resonance spectroscopy, Talanta. 110 (2013) 118–127. doi:10.1016/j.talanta.2013.02.024.

[3] K. Speer, I. Kölling-Speer, The lipid fraction of the coffee bean, Braz. J. Plant Physiol. 18 (2006) 201–216.

[4] A.J. Wilson, M. Petracco, E. Illy, Some preliminary investigations of oil biosynthesis in the coffee fruit and its subsequent re-distribution within green and roasted beans, in: 17th Int. Sci. Colloq. Coffee, ASIC, Paris, 1997: pp. 92–99.

[5] T.A.L. Wagemaker, C.R.L. Carvalho, N.B. Maia, S.R. Baggio, O. Guerreiro Filho, Sun protection factor, content and composition of lipid fraction of green coffee beans, Ind. Crops Prod. 33 (2011) 469–473. doi:10.1016/j.indcrop.2010.10.026.

[6] G.N. Jham, R. Velikova, H. Vidal Muller, B. Nikolova-Damyanova, P.R. Cecon, Lipid classes and triacylglycerols in coffee samples from Brazil: Effects of coffee type and drying procedures, Food Res. Int. 34 (2001) 111–115. doi:10.1016/S0963-9969(00)00137-X.

[7] B. Nikolova-Damyanova, R. Velikova, G.N. Jham, Lipid classes, fatty acid composition and triacylglycerol molecular species in crude coffee beans harvested in Brazil, Food Res. Int. 31 (1998) 479–486. doi:10.1016/S0963-9969(99)00016-2.

[8] E.R. Oliveira, G.R. Carvalho, P.R. Santos, F. Queiroz, Green coffee and its residual biomass: Characterization for its industrial approach, J. Food Nutr. Res. (2018).

[9] S. Besbes, C. Blecker, C. Deroanne, G. Lognay, N.E. Drira, H. Attia, Quality characteristics and oxidative stability of date seed oil during storage, Food Sci. Technol. Int. 10 (2004) 333–338. doi:10.1177/1082013204047777.

[10] I. Nehdi, S. Omri, M.I. Khalil, S.I. Al-Resayes, Characteristics and chemical composition of date palm (*Phoenix canariensis*) seeds and seed oil, Elsevier Science, 2010.

[11] M. Biglar, M. Khanavi, M. Hajimahmoodi, S. Hassani, G. Moghaddam, N. Sadeghi, M.R. Oveisi, Tocopherol content and fatty acid profile of different Iranian date seed oils, J. Pharm. Res. 11 (2012) 873–878.

[12] M. Kazemi, A. Dadkhah, Antioxidant activity of date seed oils of fifteen varieties from Iran, Orient. J. Chem. 28 (2012) 1201–1205.

[13] L. Wang, C.L. Weller, Recent advances in extraction of nutraceuticals from plants, Trends Food Sci. Technol. 17 (2006) 300–312. doi:10.1016/J.TIFS.2005.12.004.

[14] J. Azmir, I.S.M. Zaidul, M.M. Rahman, K.M. Sharif, A. Mohamed, F. Sahena, M.H.A. Jahurul, K. Ghafoor, N.A.N. Norulaini, A.K.M. Omar, Techniques for extraction of bioactive compounds from plant materials: A review, J. Food Eng. 117 (2013) 426–436. doi:10.1016/j.jfoodeng.2013.01.014.

[15] D. Arslan, M.M. Özcan, Dehydration of red bell-pepper (<I>Capsicum annuum</i> L.): Change in drying behavior, colour and antioxidant content, Food Bioprod. Process. 89 (2011) 504–513. doi:10.1016/J.FBP.2010.09.009.

[16] K. Ameer, H.M. Shahbaz, J.H. Kwon, Green extraction methods for polyphenols from plant matrices and their byproducts: A review, Compr. Rev. Food Sci. Food Saf. 16 (2017) 295–315. doi:10.1111/1541-4337.12253.

[17] J. Asghari, B. Ondruschka, M. Mazaheritehrani, Extraction of bioactive chemical compounds from the medicinal Asian plants by microwave irradiation, Academic Journals, 2011.

[18] F. Chemat, V. Tomao, M. Virot, Ultrasound-assisted extraction in food analysis, in: S. Otles (Ed.), Handb. Food Anal. Instruments, CRC Press, Boca Raton, 2008: pp. 85–94.

[19] B. Verhaagen, D. Fernández Rivas, Measuring cavitation and its cleaning effect, Ultrason. Sonochem. 29 (2016) 619–628. doi:10.1016/J.ULTSONCH.2015.03.009.

[20] M. Abdullah, A. Bulent Koc, Oil removal from waste coffee grounds using two-phase solvent extraction enhanced with ultrasonication, Renew. Energy. 50 (2013) 965–970. doi:10.1016/j.renene.2012.08.073.

[21] F. Jalili, S.M. Jafari, Z. Emam-Djomeh, N. Malekjani, V. Farzaneh, Optimization of ultrasound-assisted extraction of oil from canola seeds with the use of response surface methodology, Food Anal. Methods. 11 (2018) 598–612. doi:10.1007/s12161-017-1030-z.

[22] H.-Z. Li, Z.-J. Zhang, T.-Y. Hou, X.-J. Li, T. Chen, Optimization of ultrasound-assisted hexane extraction of perilla oil using response surface methodology, Ind. Crops Prod. 76 (2015) 18–24. doi:10.1016/J.INDCROP.2015.06.021.

[23] Z. Li, F. Yang, L. Yang, Y.-G. Zu, Ultrasonic extraction of oil from *Caesalpinia spinosa* (Tara) seeds, J. Chem. 2016 (2016) 1–6. doi:10.1155/2016/1794123.

[24] A. Shalmashi, Ultrasound-assisted extraction of oil from tea seeds, J. Food Lipids. 16 (2009) 465–474. doi:10.1111/j.1745-4522.2009.01159.x.

[25] S. Boonkird, C. Phisalaphong, M. Phisalaphong, Ultrasound-assisted extraction of

capsaicinoids from *Capsicum frutescens* on a lab- and pilot-plant scale, Ultrason. Sonochem. 15 (2008) 1075–1079. doi:10.1016/j.ultsonch.2008.04.010.

[26] ASTM International, ASTM E1757 - 01(2015) Standard practice for preparation of biomass for compositional analysis, ASTM Int. (2015). https://www.astm.org/Standards/E1757.htm (accessed July 18, 2018).

[27] C.E.C. Rodrigues, K.K. Aracava, F.N. Abreu, Thermodynamic and statistical analysis of soybean oil extraction process using renewable solvent, Int. J. Food Sci. Technol. 45 (2010) 2407–2414. doi:10.1111/j.1365-2621.2010.02417.x.

[28] StatSoft Inc., STATISTICA (Data analysis software system) version 8.0, (2007).

[29] É.R. Oliveira, G.R. Carvalho, F. Queiroz, Solid-liquid extraction of green coffee beans and its press cake using bio-renewable solvents as an alternative to the mechanical pressing, Innov. Food Sci. Emerg. Technol. (2018).

[30] É.R. Oliveira, G.R. Carvalho, F. Queiroz, Green coffee press cake: Green solvent extraction and thermodinamic parameters, Ind. Eng. Chem. Res. (2018).

[31] E.C. Garcia, Solid-liquid extraction of green coffee and of its press cake: Effect of the solvent mixture on the soluble solids yield, Federal University of Lavras, 2018.

[32] M. de los Á. Fernández, M. Espino, F.J.V. Gomez, M.F. Silva, Novel approaches mediated by tailor-made green solvents for the extraction of phenolic compounds from agro-food industrial by-products, Food Chem. 239 (2018) 671–678. doi:10.1016/j.foodchem.2017.06.150.

[33] L.C.B.A. Bessa, M.C. Ferreira, C.E.C. Rodrigues, E.A.C. Batista, A.J.A. Meirelles, Simulation and process design of continuous countercurrent ethanolic extraction of rice bran oil, J. Food Eng. 202 (2017) 99–113. doi:10.1016/j.jfoodeng.2017.01.019.

[34] K.N. Prasad, K.W. Kong, R.N. Ramanan, A. Azlan, A. Ismail, Determination and optimization of flavonoid and extract yield from brown mango using response surface methodology, Sep. Sci. Technol. 47 (2012) 73–80. doi:10.1080/01496395.2011.606257.

[35] X. Wang, Y. Wu, G. Chen, W. Yue, Q. Liang, Q. Wu, Optimisation of ultrasound assisted extraction of phenolic compounds from *Sparganii rhizoma* with response surface methodology, Ultrason. Sonochem. 20 (2013) 846–854. doi:10.1016/J.ULTSONCH.2012.11.007.

[36] M. Bimakr, R.A. Rahman, F.S. Taip, N.M. Adzahan, M.Z.I. Sarker, A. Ganjloo, Optimization of ultrasound-assisted extraction of crude oil from winter melon (*Benincasa hispida*) seed using response surface methodology and evaluation of its antioxidant activity, total phenolic content and fatty acid composition, Molecules. 17 (2012) 11748–

11762. doi:10.3390/molecules171011748.

[37] T.J. Mason, L. Paniwnyk, J.P. Lorimer, The uses of ultrasound in food technology, Ultrason. Sonochem. 3 (1996) S253–S260. doi:10.1016/S1350-4177(96)00034-X.

[38] S. Zhao, K.C. Kwok, H. Liang, Investigation on ultrasound assisted extraction of saikosaponins from *Radix bupleuri*, Sep. Purif. Technol. 55 (2007) 307–312. doi:10.1016/j.seppur.2006.12.002.

[39] J. Dong, Y. Liu, Z. Liang, W. Wang, Investigation on ultrasound-assisted extraction of salvianolic acid B from *Salvia miltiorrhiza* root, Ultrason. Sonochem. 17 (2010) 61–65. doi:10.1016/j.ultsonch.2009.05.006.

[40] X.-S. Wang, Y.-F. Wu, S.-L. Dai, R. Chen, Y. Shao, Ultrasound-assisted extraction of geniposide from *Gardenia jasminoides*, Ultrason. Sonochem. 19 (2012) 1155–1159. doi:10.1016/J.ULTSONCH.2012.03.012.

[41] S. Jang, A.Y. Lee, A.R. Lee, G. Choi, H.K. Kim, Optimization of ultrasound-assisted extraction of glycyrrhizic acid from licorice using response surface methodology, Integr. Med. Res. 6 (2017) 388–394. doi:10.1016/j.imr.2017.08.003.

[42] D.T. Veličković, D.M. Milenović, M.S. Ristić, V.B. Veljković, Ultrasonic extraction of waste solid residues from the *Salvia* sp. essential oil hydrodistillation, Biochem. Eng. J. 42 (2008) 97–104. doi:10.1016/J.BEJ.2008.06.003.

[43] R. Sun, J. Tomkinson, Comparative study of lignins isolated by alkali and ultrasound-assisted alkali extractions from wheat straw, Ultrason. Sonochem. 9 (2002) 85–93. doi:10.1016/S1350-4177(01)00106-7.

[44] T. V. Suchinina, T.S. Shestakova, V.M. Petrichenko, V. V. Novikova, Solvent polarity effect on the composition of biologically active substances, UV spectral characteristics, and antibacterial activity of Euphrasia brevipila herb extracts, Pharm. Chem. J. 44 (2011) 683–686. doi:10.1007/s11094-011-0542-x.

[45] L.R. Snyder, Classification of the solvent properties of common liquids, J. Chromatogr. A. 92 (1974) 223–230. doi:10.1016/S0021-9673(00)85732-5.