ANA CLÁUDIA ALENCAR LOPES

DEVELOPMENT OF BIOTECHNOLOGICAL PRODUCTS FROM COFFEE BY-PRODUCTS USING THE BIOREFINERY CONCEPT

LAVRAS – MG
2021

ANA CLÁUDIA ALENCAR LOPES

DEVELOPMENT OF BIOTECHNOLOGICAL PRODUCTS FROM COFFEE BY-PRODUCTS USING THE BIOREFINERY CONCEPT

Tese apresentada à Universidade Federal de Lavras, como parte das exigências do Programa de Pós-Graduação em Microbiologia Agrícola, para obtenção do título de Doutor.

Prof. Dr. Whasley Ferreira Duarte
Orientador

LAVRAS – MG
2021

Ficha catalográfica elaborada pelo Sistema de Geração de Ficha Catalográfica da Biblioteca Universitária da UFLA, com dados informados pelo(a) próprio(a) autor(a).

Lopes, Ana Claudia Alencar.
Development of biotechnological products from coffee by-products using the biorefinery concept / Ana Claudia Alencar Lopes. - 2021.
93 p.

Orientador(a): Whasley Ferreira Duarte.

Tese (doutorado) - Universidade Federal de Lavras, 2021.
Bibliografia.

1. Café. 2. Fermentação. 3. Subprodutos. I. Duarte, Whasley Ferreira. II. Título.

ANA CLÁUDIA ALENCAR LOPES

DESENVOLVIMENTO DE PRODUTOS BIOTECNOLÓGICOS A PARTIR DE SUBPRODUTOS DO CAFÉ USANDO O CONCEITO DE BIORREFINARIA

DEVELOPMENT OF BIOTECHNOLOGICAL PRODUCTS FROM COFFEE BY-PRODUCTS USING THE BIOREFINERY CONCEPT

Tese apresentada à Universidade Federal de Lavras, como parte das exigências do Programa de Pós-Graduação em Microbiologia Agrícola, para obtenção do título de Doutor.

APROVADA em 9 de setembro de 2021.

Dr. Whasley Ferreira Duarte, UFLA

Dr. Eustáquio Souza Dias, UFLA

Dra. Patrícia Gomes Cardoso, UFLA

Dr. Wilder Douglas Santiago, UFLA

Dra. Zlatina Asenova Genisheva, UMINHO Portugal

Prof. Dr. Whasley Ferreira Duarte

Orientador

LAVRAS – MG

2021

AGRADECIMENTOS

À Deus e a Nossa Senhora.

À minha família por tornar esse sonho possível mesmo diante de todos os desafios.

Ao meu companheiro Carlos Eduardo, pelo amor, suporte e paciência.

À minha parceira Rafaela, pela amizade, companheirismo, paciência e risadas.

Aos amigos que ficaram no Tocantins e aos muitos amigos conquistados em Lavras.

Ao meu orientador Whasley por todos ensinamentos, conselhos, amizade e paciência.

Ao professor Mário Lúcio, INCT-Café e Cooxupé pela parceria.

À banca examinadora por aceitar o convite.

À Universidade Federal de Lavras, em especial ao Departamento de Microbiologia Agrícola, pela oportunidade de crescimento intelectual e pessoal.

À CAPES, pela concessão da bolsa de doutorado. O presente trabalho foi realizado com o apoio da Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Código de Financiamento 001.

Enfim a todos que contribuíram direta ou indiretamente para a concretização deste trabalho.

RESUMO GERAL

O café é uma *commodity* mundialmente importante, representando uma porção significativa da economia de diversos países. O processamento dos frutos de café gera uma grande variedade e volume de subprodutos, como a casca, polpa e a borra de café. Nos últimos anos tem crescido a preocupação ambiental e social em relação ao direcionamento e aproveitamento destes subprodutos. São substratos ricos em nutrientes que podem ser convertidos em produtos de maior valor agregado via ação microbiana. Logo, este trabalho teve como objetivo avaliar subprodutos do processamento do café como substratos alternativos na fermentação alcoólica e no desenvolvimento de novos produtos biotecnológicos. O primeiro artigo avaliou o uso da polpa de café úmida e seca, água residuária do processamento do café via úmida, melaço e sacarose comercial como substratos em fermentações alcoólicas. O tratamento com polpa de café úmida e sacarose resultou no melhor desempenho fermentativo e as condições foram aplicadas para a produção de uma bebida destilada. A bebida apresentou teor alcoólico de 38% (v/v) e 48 compostos voláteis foram identificados, sendo a maioria ésteres normalmente associados com aromas florais e frutados. O segundo artigo consistiu na produção em escala piloto de duas bebidas destiladas utilizando o subproduto gerado na produção de óleo de café. Foram conduzidas fermentações de 40 litros com 10% e 20% (m/v) do resíduo de grãos de café seguidas por destilação em alambique de cobre. Um total de 62 compostos voláteis foram identificados, sendo a maioria afetada pela variação na concentração do resíduo de grãos de café. Na análise sensorial por painel treinado, a bebida com a concentração de 10% (m/v) foi caracterizada por aromas florais, lácteos e de amêndoas, enquanto a bebida com 20% foi relacionada a aromas de café, vegetais, repolho cozido, avelãs e nozes. Ambas bebidas apresentaram resultados satisfatórios, principalmente a com 10% (m/v) do resíduo, demonstrando o potencial de utilização desse resíduo na produção de aguardente. Além disso, a vinhaça obtida no segundo trabalho foi utilizada em estudos preliminares com o intuito de recuperar ácido clorogênico. A vinhaça tinha concentração inicial de 3,16 g/L de ácido clorogênico e o extrato final tinha 11,96 g/L do mesmo, chegando a concentrar o composto de interesse 3,9 vezes. Por fim, tem-se a demonstração do uso de um destilado obtido a partir de grãos de café como solução atrativa no controle da broca-de-café. A invenção possibilitou o monitoramento e controle da praga sem necessidade da utilização de inseticidas e compostos tóxicos em campo.

Palavras-chave: Café. Leveduras. Fermentação alcóolica. Subprodutos.

ABSTRACT

Coffee is an important commodity worldwide, representing a significant portion of economy of several countries. The processing of coffee fruits generates a great variety and volume of by-products, such as husk, pulp, and coffee grounds. In recent years, environmental and social concerns have grown in relation to the direction and use of these by-products. They are nutrient-rich substrates that can be converted into products with higher added value via microbial action. Therefore, this study aimed to evaluate coffee by-products as alternative substrates in alcoholic fermentation and the development of new biotechnological products. The first article evaluated the use of dry and wet coffee pulp, wastewater from wet coffee processing, molasses, and commercial sucrose as substrates in alcoholic fermentation. The wet coffee pulp and sucrose treatment resulted in the best fermentation performance, and the conditions were applied to produce a distilled beverage. The beverage had an alcohol content of 38 % (v/v) and 48 volatile compounds were identified, the majority being esters normally associated with floral and fruity aromas. The second article consisted of a pilot-scale production of two distilled beverages using the by-product generated during the production of coffee oil. Fermentations of 40 liters were carried out with 10% e 20% (m/v) of green coffee seed residue, followed by distillation in copper alembic. A total of 62 volatile compounds were identified, the majority being affected by variation in the concentration of green coffee seed residue. In the sensory analysis, the beverage with a concentration of 10% (m/v) was characterized by floral, dairy, and almond aromas, while the beverage with 20% was related to coffee, vegetables, cooked cabbage, hazelnuts, and nuts aromas. Both beverages showed satisfactory results, especially with 10% (m/v) of the residue, demonstrating the potential for using this residue in the production of distilled beverages. In addition, the vinasse obtained from the second study was used in preliminary studies in order to recover chlorogenic acid. The vinasse had an initial concentration of 3.16 g/L of chlorogenic acid, and the final extract had 11.96 g/L of it, concentrating the compound of interest 3.9 times. Lastly, there is the application for filing a patent where a distillate obtained from coffee beans was applied as an attractive solution to control the coffee berry borer. Lastly, there is the use of distillate obtained from coffee beans as an attractive solution to control the coffee berry borer. The invention made it possible to monitor and control the pest without the need to use insecticides and toxic compounds in the field.

Keywords: Coffee. Yeast. Alcoholic fermentation. By-products.

LISTA DE ILUSTRAÇÕES

PRIMEIRA PARTE

SEGUNDA PARTE

ARTIGO 1

ARTIGO 2

ANEXO 1

LISTA DE TABELAS

SUMÁRIO

PRIMEIRA PARTE

1 INTRODUÇÃO

O café é cultivado em cerca de 80 países e teve uma produção mundial de mais de 175 milhões de sacas (60 kg) em 2020 (ICO, 2021). Porém, estes valores de produção consideram somente os grãos processados, sendo mais de 50% do volume do fruto do café removido durante o seu processamento (GUARDIA PUEBLA et al., 2013).

Diversos subprodutos são gerados durante o processamento e consumo do café. Os frutos de café podem ser processados via seca, úmida ou semisseca. A casca é removida ao término da secagem dos frutos no processamento via seca, enquanto a polpa é retirada ainda úmida no processamento via úmida. Após a obtenção dos grãos secos, os grãos defeituosos e de baixa qualidade são removidos, resultando no que é chamado de PVA (grãos pretos, verdes e ardidos) (ECHEVERRIA; NUTI, 2017). Além disso, há a borra de café gerada durante a produção de café solúvel e após infusão dos grãos torrados, e a torta resultante do processo de extração de óleo dos grãos verdes**.** A polpa de café pode ser facilmente fermentada por leveduras e bactérias devido a disponibilidade de monossacarídeos, como glicose e frutose. Enquanto a borra, resíduo sólido da extração de óleo e grãos defeituosos são ricos em carboidratos mais complexos, cafeína e polifenóis que podem ser utilizados para o desenvolvimento de produtos com alto valor agregado.

Nos últimos anos tem crescido a preocupação ambiental e social em relação ao direcionamento e aproveitamento destes resíduos. Dentre as principais aplicações para estes subprodutos, destaca-se a produção de etanol (GOUVEA et al., 2009) e extração de compostos fenólicos (BALLESTEROS; TEIXEIRA; MUSSATO, 2017; BURNIOL-FIGOLS et al., 2016). A aplicação de produtos gerados a partir da fermentação alcóolica podem entrar no cenário da cafeicultura como uma forma de agregação de valor a esses subprodutos. Trabalhos anteriores já avaliaram a utilização da polpa (BONILLA-HERMOSA; DUARTE; SCHWAN, 2013) e borra de café (OLIVEIRA et al., 2018; SAMPAIO et al., 2013) para a produção de bebidas destiladas, mas para o nosso conhecimento este é o primeiro estudo fazendo uso da polpa combinada com água residuária da lavagem do café. Este também será o primeiro trabalho com a utilização do resíduo da extração de óleo de café para a produção de aguardente e a recuperação de compostos fenólicos a partir da vinhaça gerada.

2 REFERENCIAL TEÓRICO

2.1 Origem e importância econômica do café

Café é uma das bebidas mais populares no mundo e ganhou grande importância comercial durante os últimos 150 anos. O café é originado da palavra arábica *Quahweh*. Os grãos são advindos do cafeeiro, o qual é pertencente à família *Rubiaceae* e ao gênero *Coffea.* Atualmente, mais de 80 espécies já foram identificadas, porém a *Coffea arabica* e *Coffea canephora* são as espécies com maior importância econômica mundialmente (CHU, 2012).

A primeira plantação de café arábica foi estabelecida durante o século XIII em Yemen, província arábica) pelos árabes. Porém, os grãos de café arábica foram originados da província Kaffa na Etiópia, sendo até hoje conhecida como o habitat natural destes grãos. Já o café robusta (*Coffea canephora)* é considerado nativo da África Central (MURTHY; NAIDU, 2012). As primeiras mudas de café entraram no Brasil durante o século XVII através de países da América Central e Guiana. Sendo que somente a partir do século XIX ganhou a atenção de grandes produtores. O café ganhou importância econômica rapidamente no país, tornando o Brasil o maior produtor de café durante grande parte do século XX (DEMARCHI, 2003).

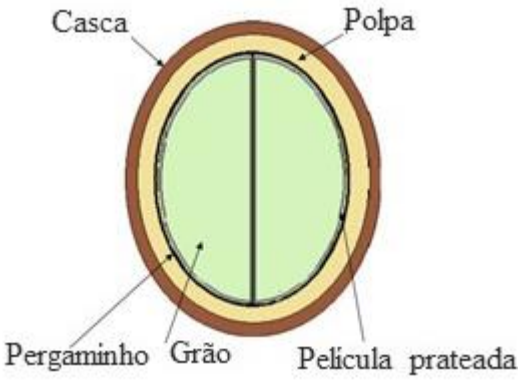
Segundo a International Coffee Organization (2021), a produção global de café em 2020 foi de 175,3 milhões de sacas (60 kg), sendo 58,6% deste valor correspondente ao café Arabica (*Coffea arabica)* e restante da produção de café robusta. Nesta mesma safra, o Brasil foi responsável pela produção de 69 milhões de sacas (60 kg), o que corresponde a 76% da produção de café da América do Sul e 36% da produção mundial. O Brasil foi o maior produtor de café no mundo durante esta última safra de café, seguido pelo Vietnã (29 milhões de sacas), Colômbia (14,3 milhões de sacas), Indonésia (12,1 milhões de sacas) e Etiópia (7,3 milhões de sacas).

2.2 Composição do fruto e grão de café

O fruto do café é composto, normalmente, por dois grãos cobertos por uma fina membrana denominada película prateada, seguida por outra fina camada de endocarpo amarelado, conhecida como pergaminho. Quando o fruto está maduro, o pergaminho é envolto por uma camada amarelada, fibrosa e adocicada conhecida como polpa ou mesocarpo.

Por fim, este mesocarpo é coberto pela casca ou pericarpo (FIGURA 1) (GHOSH; VENKATACHALAPATHY, 2014; MUSSATTO et al., 2011).

Figura 1 – Camadas do fruto do café



Fonte: Musatto et al. (2011)

A tabela 1 apresenta a composição dos grãos verdes de café. Os grãos são compostos por uma porção não-volátil, constituída por água, carboidratos, fibras, proteínas, aminoácidos, lipídeos, minerais, ácidos orgânicos, trigonelina e cafeína. Dentre os carboidratos, polissacarídeos insolúveis, como a celulose e hemicelulose, compõe cerca de 50% do peso seco do grão. Outros carboidratos solúveis, como a frutose, glicose, galactose, arabinose, sacarose, rafinose e manose também estão presentes no grão. Os lipídeos são a segunda classe de compostos mais abundantes, representando cerca de 15 – 20% do peso seco de grão. A porção volátil dos grãos verdes é pequena, mas confere aos mesmos seu aroma característico. As classes de voláteis mais abundantes são os álcoois, ésteres, hidrocarbonetos e aldeídos (CHU, 2012; GHOSH; VENKATACHALAPATHY, 2014).

Tabela 1 – Principais componentes do grão verde de café

Composto	**Concentração** (g/100 g)
Carboidratos/Fibras	
Sacarose	6,0 – 9,0
Açúcares redutores	0,1
Polissacarídeos	34 – 44
Lignina	3,0
Pectina	2,0
Compostos nitrogenados	
Proteínas	10,0 – 11,0
Aminoácidos livres	0,5
Cafeína	0,9 – 1,3
Trigonelina	0,6 – 2,0
Lipídeos	
Óleos (triglicerídeos insaponificáveis, esteróis e tocoferóis)	15 – 17,0
Diterpenos (livres e esterificados)	0,5 – 1,2
Minerais	3,0 – 4,2
Ácidos	
Ácido clorogênico	4,1 – 7,9
Ácidos alifáticos	1,0

Fonte: Adaptada de Chu (2012) e Musatto et al. (2011)

2.3 Processamento do café

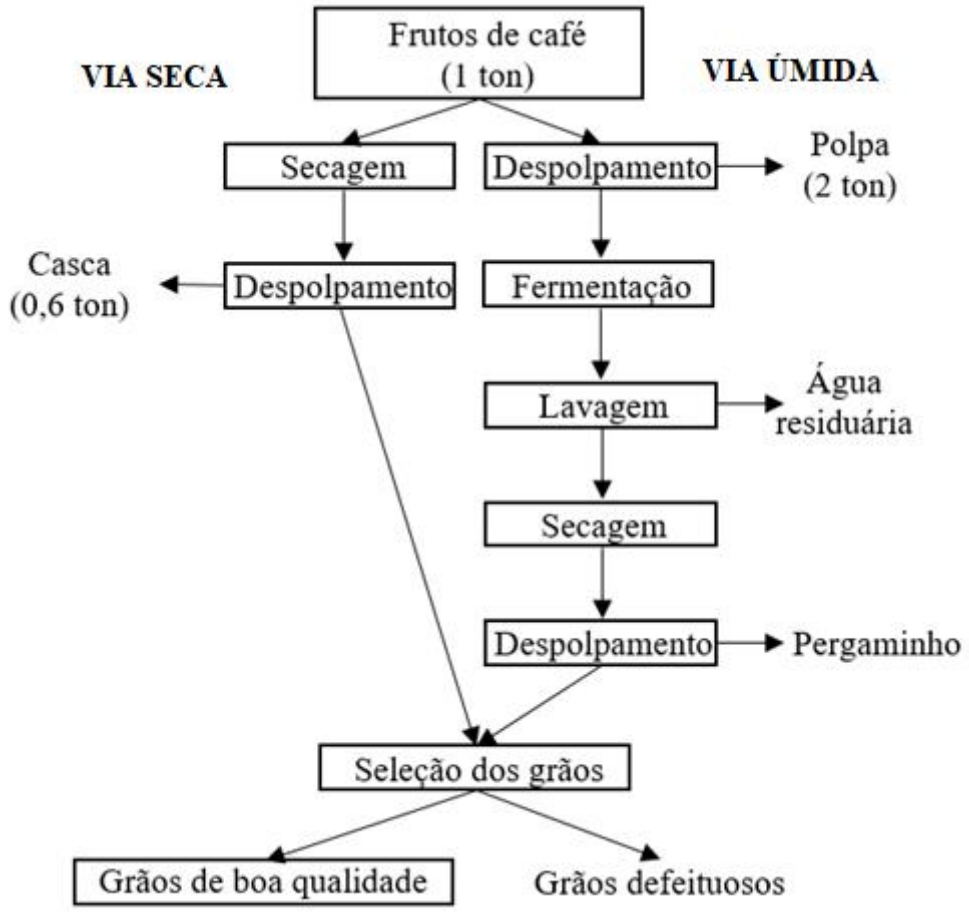
O processamento dos frutos de café pode ser divido em processamento primário e secundário. O processamento primário é referente aos procedimentos para obtenção dos grãos verdes, o secundário inclui a torra, moagem dos grãos, e processos para agregação de valor, como a produção de café instantâneo e extração de óleo (CHANAKYA; DE ALWIS, 2004).

O processamento primário do fruto do café tem o intuito de remover a mucilagem e diminuir a umidade dos grãos para cerca de 10 a 12% (p/p). Os frutos podem ser processados por métodos via seca, úmida ou semisseca. A figura 2 esquematiza o processamento de frutos de café via seca e úmida, incluindo os resíduos gerados durante o processo. O processamento via seca consiste na secagem dos frutos inteiros em terreiros ou bandejas aeradas por cerca de 14 a 30 dias. Os frutos são distribuídos em uma camada com cerca de 8 cm e revirados em

intervalos regulares. Durante este período ocorre a fermentação espontânea e secagem dos grãos. Após a secagem, a casca e polpa são removidas de forma mecânica (DE BRUYN et al., 2017).

No processamento úmido os frutos são inicialmente despolpados através da remoção mecânica da casca e polpa. Após esta etapa o grão ainda está coberto por um uma camada de mucilagem, a qual é removida durante a fermentação submersa dos grãos durante cerca de 24 horas. Após a fermentação, os grãos são lavados para a remoção completa da mucilagem e secos em terreiros ao sol. Devido a remoção das camadas externas do fruto, a secagem se dá de forma mais rápida, diminuindo o risco de fermentações excessivas ou crescimento de fungos durante a secagem. (DE BRUYN et al., 2017). O processamento via semisseca é uma variação do processamento via úmida. Neste caso, os frutos também são despolpados mecanicamente, porém a fermentação ocorre diretamente no terreiro. Por fim, os grãos defeituosos, como pretos, verdes e ardidos são removidos, e os grãos de qualidade são comercializados (POLTRONIERI; ROSSI, 2016).

Figura 2 – Processamento do café via úmida e seca



Fonte: Adaptado de Echeverria e Nutti (2017)

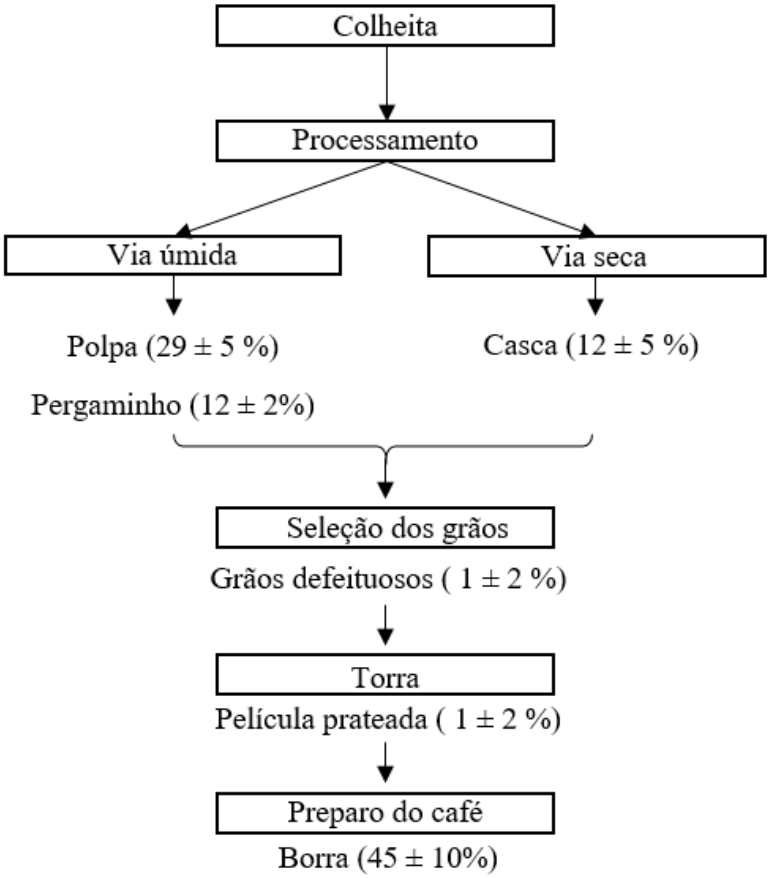
A torra é um dos principais processamentos secundários, consistindo na secagem, pirolise e resfriamento dos grãos. Durante a secagem o restante de umidade presente nos grãos é liberado lentamente, tornando os grãos levemente amarelados. As reações de pirólise convertem os compostos naturalmente presentes nos grãos em misturas complexas decorrentes da reação de Maillard (MURTHY; NAIDU, 2012). Dentre outros processamentos secundários dos grãos, destaca-se a produção do óleo de café através da prensagem mecânica de grãos verdes. O resíduo gerado apresenta composição similar aos grãos verdes, com exceção do óleo (CASTRO et al., 2018).

2.4 Subprodutos do processamento do café

As estatísticas referentes a produção mundial de café consideram somente o produto final, ou seja, o grão. Porém, os grãos compreendem aproximadamente 20% do volume total do fruto, sendo o restante removido durante o processamento do café (PUEBLA et al. 2013). Desta forma, cerca de quatro vezes o volume de grãos de café produzidos mundialmente é removido durante o processamento do fruto, o que pode ter resultado em aproximadamente 40 milhões de toneladas de subprodutos gerados em 2020, sem incluir a água residuária (ICO, 2021). Nos últimos anos houve um aumento na busca de aplicações alternativas para estes subprodutos devido ao grande volume que é gerado no processamento do café e a preocupação com o destino dos mesmos no meio ambiente.

A depender do método utilizado para processamento primário do fruto do café, diferentes resíduos, ou subprodutos, são gerados. Dentre eles estão a casca, polpa, pergaminho, película prateada e água residuária**.** A etapa de descascamento dos frutos é uma das principais diferenças entre os métodos via seca e via úmida. A casca de café é removida ao término da secagem dos frutos no processamento via seca. Já no processamento via úmida ou semisseca os frutos são descascados ainda úmidos, gerando a polpa de café (ECHEVERRIA; NUTI, 2017).

Figura 3 - Subprodutos gerados no processamento do café



Fonte: Adaptado de Murthy e Naidu (2012)

A figura 3 apresenta resumidamente a origem dos principais subprodutos dentro do processamento e consumo do café. Os subprodutos mais abundantes durante o processamento, em ordem decrescente, são a polpa (29 ± 5 % do peso seco do fruto), casca (12 ± 5 %), pergaminho (12 ± 2 %), película prateada (1 ± 2 %). Em relação aos subprodutos gerados pós-processamento, a borra corresponde a 45 ± 10 % do peso seco inicial do fruto. Os grãos defeituosos (pretos, verdes e ardidos) são um subproduto do café com baixo valor agregado, os mesmos correspondem a cerca de 15% do volume da produção nacional de grãos (MURTHY; NAIDU, 2012).

A cada duas toneladas de café processado via úmida, uma tonelada de polpa é gerada. A composição química da polpa e da casca de café não apresentam diferenças drásticas em relação aos grãos verdes, exceto pelo menor teor de lipídeos e maiores concentrações de minerais. A polpa é rica em carboidratos, proteínas e minerais, apresentando cerca de 12,4 % de açúcares redutores em seu peso seco (MURTHY; NAIDU, 2012)**.** O uso direto de polpa e

casca de café na alimentação animal ainda é de difícil implementação devido a presença de fatores antifisiológicos e antinutricionais, como taninos e cafeína (BOUAFOU et al., 2011). No entanto, a casca e a polpa são uma fonte promissora de fitoquímicos de interesse farmacêutico, como os compostos fenólicos (ESQUIVEL; JIMENEZ, 2012).

Nos últimos anos houve um aumento na busca de aplicações alternativas para estes subprodutos devido ao grande volume gerado no processamento do café e a preocupação com o destino dos mesmos no meio ambiente. As principais aplicações são o uso direto como energia (ZUORRO; LAVECCHIA, 2012), alimentação animal (BOUAFOU et al., 2011), e a produção de compostos de interesse industrial como etanol (GOUVEA et al., 2009), compostos fenólicos (BALLESTEROS; TEIXEIRA; MUSSATO, 2017a; BALLESTEROS et al., 2017b; BURNIOL-FIGOLS et al., 2016), enzimas (MURTHY; NAIDU; SRINIVAS, 2009) e açúcares (ECHEVERRIA; NUTI, 2017 ; MAYANGA-TORRES et al., 2017).

REFERÊNCIAS

BALLESTEROS, L. F.; TEIXEIRA, J. A.; MUSSATTO, S. I. Extraction of polysaccharides by autohydrolysis of spent coffee grounds and evaluation of their antioxidant activity. **Carbohydrate Polymers**, v. 157, p. 258–266, 2017a.

BALLESTEROS, L. F. et al. Optimization of autohydrolysis conditions to extract antioxidant phenolic compounds from spent coffee grounds. **Journal of Food Engineering**, v. 199, p. 1–8, 2017b.

BOUAFOU, K. G. et al. Potential food waste and by-products of coffee in animal feed. **Electronic Journal of Biology**, v. 7, p. 74-80, 2011

BONILLA-HERMOSA, V. A.; DUARTE, W. F.; SCHWAN, R. F. Utilization of coffee by-products obtained from semi-washed process for production of value-added compounds. **Bioresource Technology**, v. 166, p. 142–150, 2014.

BURNIOL-FIGOLS, A. et al. Integration of chlorogenic acid recovery and bioethanol production from spent coffee grounds. **Biochemical Engineering Journal**, v. 116, p. 54–64, 2016.

CAMPOS-VEGA, R. et al. Spent coffee grounds: A review on current research and future prospects. **Trends in Food Science and Technology**, v. 45, n. 1, p. 24–36, 2015.

CASTRO, A. C. C. M. et al. Green coffee seed residue : A sustainable source of antioxidant compounds. **Food Chemistry**, v. 246, n. November 2017, p. 48–57, 2018.

CHANAKYA, H. N.; DE ALWIS, A. A. P. Environmental issues and management in primary coffee processing. **Process Safety and Environmental Protection**, v. 82, n. 4 B, p. 291–300, 2004.

CHU, Yi-Fang (Ed.). **Coffee**: e**merging health effects and disease prevention.** John Wiley & Sons, 2012.

DE BRUYN, F. et al. Exploring the Impacts of Postharvest Processing on the Microbiota and. **Applied and environmental microbiology**, v. 83, n. 1, p. 1–16, 2017.

DEMARCHI, M. **Café: aspectos econômicos**. [Curitiba]: SEAB, departamento De Economia Rural, 2003.

ECHEVERRIA, M. C.; NUTI, M. Valorisation of the Residues of Coffee Agro-industry: Perspectives and Limitations. **The Open Waste Management Journal**, v. 10, n. 1, p. 13–22, 2017.

ESQUIVEL, P; JIMENEZ, V. M. Functional properties of coffee and coffee by-products. **Food Research International**, v. 46, n. 2, p. 488-495, 2012.

GHOSH, P.; VENKATACHALAPATHY, N. Processing and Drying of Coffee - A review. **International Journal of Engineering Research & Technology**, v. 3, n. 12, p. 784–794, 2014.

GOUVEA, B. M. et al. Feasibility of ethanol production from coffee husks. **Biotechnology Letters**, v. 31, n. 9, p. 1315–1319, 2009.

ICO. International Coffee Organization. Disponível em: <https://www.ico.org/historical/1990%20onwards/PDF/1a-total-production.pdf`>. Accesso: Julho 2021.

MAYANGA-TORRES, P. C. et al. Valorization of coffee industry residues by subcritical water hydrolysis: recovery of sugars and phenolic compounds. **The Journal of Supercritical Fluids**, v. 120, p. 75-85, 2017.

MURTHY, P. S.; NAIDU, M. M.; SRINIVAS, P. Production of α-amylase under solid-state fermentation utilizing coffee waste. **Journal of Chemical Technology & Biotechnology: International Research in Process, Environmental & Clean Technology**, v. 84, n. 8, p. 1246-1249, 2009.

MURTHY, P. S.; NAIDU, M. M. Sustainable management of coffee industry by-products and value addition - A review. **Resources, Conservation and Recycling**, v. 66, p. 45–58, 2012.

MUSSATTO, S. I. et al. Production, Composition, and Application of Coffee and Its Industrial Residues. **Food and Bioprocess Technology**, v. 4, n. 5, p. 661–672, 2011.

OLIVEIRA, J. et al. Increasing the Sustainability of the Coffee Agro-Industry: Spent Coffee Grounds as a Source of New Beverages. **Beverages**, v. 4, n. 4, p. 105, 2018.

POLTRONIERI, P.; ROSSI, F. Challenges in Specialty Coffee Processing and Quality Assurance. **Challenges**, v. 7, n. 2, p. 19, 2016.

PUEBLA, Y. G.; PÉREZ, S. R.; HERNÁNDEZ, J. J., & Renedo, V. S. G. Performance of a UASB reactor treating coffee wet wastewater. **Revista Ciencias Técnicas Agropecuarias**, v. 22, n, 3, p. 35-41, 2009.

SAMPAIO, A. et al. Production , chemical characterization , and sensory pro fi le of a novel spirit elaborated from spent coffee ground. **LWT - Food Science and Technology**, v. 54, n. 2, p. 557–563, 2013.

ZUORRO, A.; LAVECCHIA, R. Spent coffee grounds as a valuable source of phenolic compounds and bioenergy. **Journal of Cleaner Production**, v. 34, p. 49–56, 2012.

SEGUNDA PARTE

ARTIGO 1 – Production and characterization of a new distillate obtained from fermentation of wet processing of coffee by-products

Artigo publicado no periódico Journal of Food Science and Technology
doi: https://doi.org/10.1007/s13197-020-04485-4

Production and characterization of a new distillate obtained from fermentation of wet processing coffee by-products

Abstract

Coffee is one of the most important commodities worldwide. The industrial processing of coffee cherries generates a considerable volume of by-products such as wastewater, coffee pulp, mucilage, and husk. These by-products have sugars and nutrients that can be converted into value-added products via microbial action. In this study, for the first time, we evaluated the potential of coffee pulp and coffee wastewater as substrate for alcoholic fermentation produce a distilled beverage. The must composed by dry or wet coffee pulp and coffee wastewater added of commercial sucrose or sugarcane molasses was fermented by *S. cerevisiae*. After a screening step, a larger fermentation was carried out with the wet pulp added of sucrose due to its higher alcoholic fermentation efficiency. The distilled beverage contained 38% (v/v) ethanol and 0.2 g/L of acetic acid. The contaminants furfural, hydroxymethylfurfural and ethyl carbamate were below detection level. Among the 48 volatile compounds detected, the majority (21) were ethyl esters usually associated with floral and sweet aromas. Ethyl decanoate (996.88 µg/L) and ethyl dodecanoate (1088.09 µg/L) were the most abundant esters. Coffee spirit presented taste acceptance of 80% and sugarcane spirit, 70%. The tasters indicated an aroma acceptance of 86% for the coffee spirit and 78% for the sugarcane spirit. The results of this work demonstrate the potential for using coffee by-products to produce a good quality distilled beverage. Considering our results, especially sensorial analysis, we can infer that the produced coffee beverage represents a new alternative for adding value to the coffee production chain.

Keywords: coffee pulp, fermentation, wastewater

Introduction

Coffee is an important global commodity and represents a significant fraction of the economy in many countries. According to the International Coffee Organization (2019), the global coffee output of 2017/18 was 168 million bags (60 kg). However, this output considers only the coffee bean, which corresponds to about 20% of the total volume of the cherry. The remaining 80% of the cherry is skin, pulp, and mucilage, which are removed during the coffee processing (Guardia et al. 2013). Coffee cherries are processed either by dry, wet, or semi-dry method, depending on climate characteristics of the production regions. The dry method consists of direct drying of whole cherries. The wet method mechanically removes pulp and husk of the cherries; mucilage is then removed by spontaneous fermentation in water tanks, and beans are washed to remove any mucilage left and dried. Alternatively, the semi-dry is an intermediary method that also uses depulpers to remove the husk and part of the mucilage (Poltronieri and Rossi 2016).

The wet and semi-dry methods include stages where water is used to wash away the undesirable parts of the cherry. The semi-washed coffee generates about 1 m^3 of wastewater per ton of fresh fruit, without including finish fermentation and washing, while the fully washed method results in more than 20 m^3 of wastewater per ton of cherry (Chanakya and Alwis 2004). This wastewater used to carry away the coffee husk and pulp is rich in suspended organic material. The main constituents of coffee pulp are carbohydrates, fibers, and protein, that represents, respectively, 50, 18 and 10% of its dry weight. Besides these components, coffee pulp also contains tannins, pectin, polyphenols and minerals (Pandey et al. 2000).

If not treated properly these coffee by-products are easily susceptible to spontaneous fermentations, pH decrease, and can cause the eutrophication of receiving waterbodies. The development of added value products from coffee by-products has been studied by many

authors such as the production of enzymes (Cerda et al. 2017) and phenolic compounds (Burniol-Figols et al. 2016). Coffee by-products have also been reported as substrates with potential for alcoholic fermentation, for example, the spent coffee grounds (Machado et al. 2018; Sampaio et al. 2013) was used to produce distilled beverages with good sensory acceptance and desirable volatile compounds profile. In the work of Bonilla-Hermosa et al. (2014), our group verified that the sugar and nutrient of the coffee wastewater, coffee husk and pulp could be fermented by different yeasts, leading to a satisfactory ethanol yield and production of aromatic volatile compounds. To the best of our knowledge, the present work is the first report on the use of pulp and wastewater from coffee processing as a substrate for fermentation and production of a distilled beverage. Therefore, the aim of this study was to evaluate the use of coffee pulp and coffee wastewater mixture as substrate for alcoholic fermentation and production of a distilled beverage.

Materials and Methods

Raw material

Coffee wastewater and coffee pulp from the wet processing of coffee beans variety Catuaí 99 vermelho were supplied by a coffee-producing unit located in the municipality of Machado, Southern of the Minas Gerais state (Brazil). The Catuaí variety was chosen because is one of the main variety of *Coffea arabica* grown in the Minas Gerais state (Botelho et al., 2010). The sugar content (sucrose, glucose and fructose) was determined by HPLC as described further here. The obtained materials were packed in sterile plastic bags and frozen at -20 ºC. In the case of treatments using dried coffee pulp, the drying process was performed at 65 ºC until constant weight followed by manual grinding and storing in hermetic glass flasks (Bonilla-Hermosa et al. 2014).

Yeast strain and inoculum preparation

The yeast used in this study was *Saccharomyces cerevisiae* LNF CA11 (LNF - Latino América®, Bento Gonçalves – Brazil) in its active dry form, which is widely used in Brazil for cachaça production. To reactivate the yeast, 0.1 g was added in 1 mL of YPD (Yeast Extract-Peptone-Dextrose) broth (10 g/L yeast extract, 20 g/L peptone, and 20 g/L glucose) and kept at 28 ºC for 60 min. After reactivation, the yeast adaptation to the medium was performed using YPD containing coffee wastewater and increasing concentrations of glucose (YPDCoffee). First, the yeast was transferred to a flask containing 1 mL of YPDCoffee 2 ºBrix, where it remained for 24 h at 28 ºC. After 24 h of incubation, the material was transferred to a flask containing 9 mL of YPDCoffee at 8 ºBrix and incubated at 28 ºC/24h. Afterward, the content was added to an Erlenmeyer with 90 mL of YPDCoffee at 12 ºBrix and incubated at 28 ºC/24h. Lastly, the Erlenmeyer content was centrifuged at 4 ºC/6805 RCF for 10 min to obtain the biomass which was washed twice with 0.1% sterile peptone water with subsequent centrifugation (Andrade et al. 2017).

Fermentation for must selection

Different musts composed by coffee wastewater, dry or wet coffee pulp, commercial sugar (sucrose) or sugarcane molasses were inoculated with *S. cerevisiae* LNF CA11 to evaluate the fermentation efficiency. Considering the initial sugar content of the raw material (coffee pulp: 4,7% glucose, 6,4% fructose, 0,5% sucrose; coffee pulp: 0,49% glucose, 0,66% fructose and 0,04% sucrose), commercial sucrose or sugarcane molasses were added until the must reached 160 g/L of total sugars. Specifically, the studied musts were: must 1 (M1), 100 mL of coffee wastewater, 10 g of dried coffee pulp with sucrose addition; must 2 (M2), 100 mL of coffee wastewater, 10 g of dried pulp with sugarcane molasses; must 3 (M3), 100 mL of coffee wastewater, 10 g of wet pulp with sucrose; must 4 (M4), 100 mL of coffee

wastewater, 10 g of wet pulp with sugarcane molasses. All experiments were performed in duplicate.

The musts were sterilized in an autoclave for 15 min at 121 ºC, then inoculated with the yeast biomass obtained as described above and incubated at 28 ºC. Samples were collected right after the inoculation and at the end of the fermentation for the analysis of sugar and ethanol by high-performance liquid chromatography (HPLC). Ethanol yield ($Y_{P/S}$), ethanol conversion efficiency (Ef), sugar conversion (Conv) and ethanol volumetric productivity (Qp) were calculated as described below to evaluate the fermentation kinetics (Duarte et al. 2010).

$$[Y_{P/S} = (P_f)/(S_i - S_f)];\ [Ef = (Y_{P/S} * 100)/0.51];$$

$$[Conv = (S_i * 100)/(S_i - S_f)];[Q_p = (P_f)/t_f].$$

where P_f is the ethanol concentration, S_i and S_f are the initial and final concentration of sugars, and t_f the fermentation time.

Distilled beverage production

The larger volume fermentation for the production of the distilled beverage was carried out with the must that presented the highest sugar consumption and ethanol production, as well as the best fermentation kinetics parameters. Inocula for this fermentation was prepared as described in item 2.3 and the fermentation was carried out according to Amorim et al. (2016). Samples were collected at the beginning and the end of the fermentation for analyses in liquid chromatography (sugars, acetic acid, and ethanol). All experiments were performed in duplicate.

Distillation

The fermented must was distilled as described by Amorim et al. (2016) and Campos et al. (2010). The 3 fractions of the distillate were collected separately, being the first fraction

126 “head” corresponding to 10% of the distillate; the second fraction “heart” corresponding to
127 the beverage itself was collected and stored in glass bottles; the third fraction “tail”
128 corresponding to 10 % of the distillate was discarded. The distilled beverage was submitted to
129 the analyses of the volatile compounds by gas chromatography (HS SPME GC-MS); sugar,
130 ethanol, acetic acid, furfural, hydroxymethylfurfural, and ethyl carbamate by HPLC and
131 finally, sensory analysis.
132
133 **Sugars, acids and alcohols analyses**
134 Samples of the musts were analyzed to determine sugars (glucose, fructose, and
135 sucrose) and ethanol while the distillate was analyzed for its composition of ethanol and
136 acetic acid. Prior to analyses, the musts samples were centrifuged twice at 6805 RCF, 4 ºC/10
137 min, and filtered in 0.22 µm filters (Duarte et al. 2010). For the analyses, it was used a
138 Shimadzu chromatographer (Shimadzu Corp. Japan) equipped with a UV-Vis (SPD-10Ai)
139 detector and a refractive index detector (RID-10A). Separations occurred on a Supelcogel 8H
140 (7,8 mm x 30 cm) column using sulfuric acid 0.005 M as mobile phase in a flow of 0.5
141 mL/min with the oven maintained at 30 ºC. The identification of the compounds was
142 performed by comparing the retention time of standards with the retention time of peaks in the
143 samples injected under the same conditions. Quantification was done by external calibration
144 (Andrade et al. 2017; Duarte et al. 2010).
145
146 **Furfural, hydroxymethylfurfural and ethyl carbamate analyses**
147 The distilled beverage was analyzed to verify the presence of furfural,
148 hydroxymethylfurfural and ethyl carbamate. For the determination of ethyl carbamate, it was
149 used the methodology proposed by Santiago et al. (2014), with the previous derivatization of
150 the sample. Analyses were carried out using a Shimadzu high-performance liquid

chromatographer equipped with two high-pressure pumps model LC 6AD and an RF-10AXL fluorescence detector. Separations were performed using an Agilent – Zorbax Eclipse AAA (4.6 x 150 mm, 5μm) column connected to an Agilent - Zorbax Eclipse AAA (4.6 x 12.5 mm, 5μm) pre-column. Excitation and emission wavelengths employed were 233 and 660 nm, respectively. The mobile phase was composed of 20 mmol/L sodium acetate solution (Solvent A) and acetonitrile (Solvent B). The flow used throughout the analysis was 0.75 mL/min with elution in a gradient from 0 to 5 min (40-60% B); 5 to 10 min (60-70% B); 10 to 18 min (70-80% B); 18 to 19.5 min (80-90% B); 19.5 to 25 min (90-40% B); 25 to 30 min (40% B). Quantification of ethyl carbamate was done using external calibration curves.

Furthermore, furfural and hydroxymethylfurfural were analyzed according to the methodology described by Sousa et al. (2009) with some minor modifications. Samples and standards were filtered on a 0.45 μm polyethylene membrane and directly injected into the Shimadzu chromatographic system, equipped with two high-pressure pumps model SPD-M20A, a diode array detector and a Zorbax Eclipse XDB-C18 (4.6 x 250 mm, 5μm) column connected to an Agilent - Zorbax Eclipse XDBC18 4-Pack (4.6 x 12.5 mm, 5μm) pre-column. The solvents used as mobile phase were: 2% acetic acid solution in water (Solvent A) and methanol:water:acetic acid (70:28:2% v/v/v) (Solvent B). Elution was in a 0.8 mL/min flow and a gradient from 0 to 25 min (0-40% B); 25 to 40 min (40-55% B); 40 to 43 min (55-60% B); 43 to 50 min (60-100% B); 50 to 55 min (100-0% B); 55 to 60 min (0% B). The wavelength used was 280 nm. The compounds quantification was done by external calibration with analytical curves obtained from a stock solution diluted to concentrations ranging from 0.1 to 25 mg/L. The identity of the analytes was confirmed by the retention time and peak profile of the samples compared to the standards. All injections were done in triplicate.

Volatile compounds analysis by HS SPME GC-MS

Volatile organic compounds (VOCs) were analyzed from 1 mL of sample diluted in 4 mL of distilled water containing 0.25 g NaCl. The 15 mL vials containing the samples were kept at 60 ºC and the VOCs extraction from the headspace was performed with a DVB/CAR/PDMS 50-30 µm (Supelco) fiber in a manual holder for 25 min. After extraction, the fiber was maintained in the injector for 5 min for the desorption of the VOCs. The analyses were carried out in a gas chromatograph GC-MS QP2010SE (Shimadzu) coupled to a mass spectrometer equipped with a Carbowax (30 m x 0.20 mm id x 0.25 µm) column. The injector temperature was maintained at 230 ºC and injections were in splitless mode. Helium was used as the carrier gas with a flow rate of 1.2 mL/min. Oven temperature was set at 50 ºC/5 min, followed by a heating ramp of 5 ºC/min until 200 ºC, keeping the final temperature for 10 min. The mass spectra were acquired using scan mode (45 a 1000 m/z) from 5 min of analysis (solvent cut time) (Amorin et al. 2016). Compounds were identified using the NIST library version 2011 and the identity confirmed by linear retention index calculated using an alkanes homologous series (C8-C40). Concentrations were expressed as equivalents of 4-nonanol, used as the internal standard at a final concentration of 125 µg/L (Duarte et al. 2010).

Sensory analysis

The sensory analysis was performed by 50 untrained testers, 29 women and 21 men with ages ranging from 18 to 52 years. Besides the beverage produced with coffee by-product, it was included a sample of sugarcane spirit produced in the study of Amorim et al. (2016). This beverage was used as a beverage with recognized quality due to its high acceptance scores in the sensory analysis. Each taster received two random samples containing 5 mL of each beverage and analyzed them according to their appearance, aroma, taste and global

impression, according to a hedonic scale ranging from 9 to 1: (9) like extremely, (8) like very much, (7) like moderately, (6) like slightly, (5) neither like nor dislike, (4) dislike slightly, (3) dislike moderately, (2) dislike very much and (1) dislike extremely. The acceptance percentage, percentage of tasters who did not reject the product, was obtained considering scores higher than 5.

Statistical analysis

HPLC data were analyzed with ANOVA and Scott-Knott test using the Sisvar 5.6 software (Lavras, Brazil). Sensory analysis data were submitted to principal component analysis using XLSTAT® software (Addinsoft).

Results and discussion

Must selection

Before the fermentation in a larger volume to produce the distilled beverage, the screening step was carried out to verify which of the musts would allow a better alcoholic fermentation efficiency. For this evaluation, the musts were characterized by HPLC before and after their fermentation. Overall, the total sugar concentration in the musts was higher for those supplemented with commercial sucrose and, among the sugars, sucrose was the most abundant in all musts (Table 1). Similar to that observed for sucrose, the monosaccharides glucose and fructose were also detected in higher concentrations in musts added of commercial sucrose, but without significant difference ($p<0.05$). Considering the type of coffee pulp used (dry or wet), musts prepared with dry pulp (M1 and M2) presented higher initial sugar concentration ($p<0.05$) than musts M3 and M4 prepared with wet pulp (Table 1). This higher sugar concentration in the musts with dry pulp occurred due to the greater amount

(in weight) of coffee pulp added, since in the case of the wet pulp the moisture content is approximately 82%, as previously reported by Bonilla-Hermosa et al. (2014). After 48 h of fermentation, the residual sugars of the fermented musts followed the same profile found at the beginning of the fermentation with 7.94 g/L and 4.77 g/L for musts M1 (dried pulp+sucrose) and M3 (wet pulp+sucrose) (Table 1). The ethanol production after 48 h of fermentation was in general proportional to the sugar concentration in each must. Those musts (M1 and M3) supplemented with commercial sucrose resulted in significantly higher ethanol concentrations ($p<0.05$) (Table 1). The fermentation of musts M1 and M3 resulted in 69.07 g/L and 67.19 g/L of ethanol, respectively; while in M2 (dried pulp+sugarcane molasses) and M4 (wet pulp+sugarcane molasses), the ethanol concentrations were respectively, 33.24 g/L and 30.41 g/L (Table 1).

From the obtained data it was possible to notice that the supplementation with commercial sucrose would be preferential when compared to the addition of sugarcane molasses, once the ethanol content was higher for supplementation with sucrose. However, to obtain a more detailed view on sugars consumption, ethanol production and consequently, a more accurate decision about the better must for fermentation, the parameters sugar conversion (Conv), ethanol yield ($Y_{P/S}$), ethanol conversion efficiency (Ef) and ethanol volumetric productivity (Qp) were analyzed. The “Conv” parameter was similar and did not showed statistical difference ($p<0.05$) for all musts with values around 96%, reinforcing that (based on the total sugar consumption) is possible to cultivate *S. cerevisiae* in musts containing coffee pulp and wastewater. This value is similar to that reported in studies using *S. cerevisiae* in musts such as sugarcane (Amorim et al. 2016), grapes (Vernocchi et al. 2015) and honey (Pereira et al. 2013), which are fermentative process focused on the yield of ethanol. As Conv only indicates the total sugar used by yeast, the ethanol conversion efficiency (Ef) and ethanol yield (Yp/s) were calculated to verify how much of the sugars

were specifically converted into ethanol. While the fermentations of musts M1, M2 and M4 resulted in Ef values around 80-82% without significant difference ($p>0.05$), the fermentation Ef of must M3 (wet pulp + sucrose) was approximately 96%, corresponding to a Yp/s of 0.49 g/g (Table 1). These values found for the fermentation of must M3 are indicative of a high efficiency alcoholic fermentation and comparable to fermentation of sugarcane juice reported by Amorim et al. (2016) using the same *Saccharomyces* strain. Being the efficient alcoholic fermentation the focus of the study, the must M3 (wet pulp+sucrose) was selected for the production of a wastewater and coffee pulp distillate.

Ethanol and acetic acid

The distilled beverage obtained from the fermentation of must M3 presented an ethanol content of 38% v/v. The Brazilian legislation defines that to be named “aguardente” or spirit (of any substrate) the distilled beverage must present an alcoholic content ranging from 38 to 54% v/v at 20 ºC (Brasil 2009); therefore, the produced distilled beverage is in agreement with the standards required by the Brazilian legislation.

Among the acids produced during fermentation, acetic acid has been, quantitatively, the main component of the acidic fraction, and is expressed as volatile acidity. The acetic acid content detected in the distillate was 0.2 g/L (52.36 mg/100 mL anhydrous alcohol – a.a.). In general, acetic acid affects the acidity of the beverage and contributes to an undesirable aroma due to its “vinegar” aroma descriptor (Czerny et al. 2008). Compared to the sugarcane spirit produced by Amorim et al. (2016) using the same strain *S. cerevisiae*, which presented the acetic acid concentration of 0.018 g/L (4.74 mg/100 mL a.a.), the beverage produced with wastewater and coffee pulp resulted in a higher volatile acidity. However, the beverage is still within the Brazilian legislation requirement where the acetic acid concentration should not exceed 150 mg/100 mL a.a. (Brasil 2005).

276

277 **Furfural, hydroxymethylfurfural and ethyl carbamate quantification**

278 Considering the substrates used in this work, the produced distillate was analyzed to
279 check the presence of organic contaminants, furfural, hydroxymethylfurfural, and ethyl
280 carbamate. All analyzed contaminants were below the detection limit which was respectively
281 for furfural, hydroxymethylfurfural and ethyl carbamate, 0.017 mg/100 mL a.a., 0.011
282 mg/100 mL a.a. and 1.86 µg/L.

283 Some aldehydes are considered organic contaminants in alcoholic beverages, which is
284 the case of furfural and hydroxymethylfurfural, and their presence is undesirable. They are
285 formed by the chemical decomposition of pentoses and hexoses, or by pyrogenation of
286 organic matter deposited on the bottom of distillers. The contaminations can be avoided by
287 keeping the must to be distilled clean and free of organic matter in suspension. Before the
288 distillation, the must was left undisturbed to allow the sedimentation of solid material, a
289 procedure that probably helped in the non-detection of these contaminants in the wastewater
290 and coffee pulp beverage. Another procedure that assists in the elimination of these beverage
291 contaminants is the separation of the fractions with the discard of the “head” which
292 correspond to 10% of the distillate volume.

293 Another contaminant analyzed in the beverage was ethyl carbamate, an organic
294 contaminant that has been widely studied in various beverages. This carcinogenic compound
295 is naturally found in low concentrations in different alcoholic beverages and some fermented
296 foods (D’Avila et al. 2016; Santiago et al. 2014). Due to its high toxicity and common
297 presence in alcoholic beverages, its detection and quantification has become relevant.
298 According to the Brazilian legislation (Brasil 2005), the maximum limit for ethyl carbamate
299 content is 210 µg/L. This compound is produced in low levels (ng/L or ng/kg or mg/L) from
300 several precursors, such as hydrocyanic acid, urea, citrulline, and N-carbamyl amino acids

(including carbamyl phosphate by reaction with ethanol) (Beland et al. 2005). However, the pathways of formation and precursors of ethyl carbamate in foods and beverages have not been completely elucidated yet, as they depend on the type of food and their processing.

Evaluation of volatile compounds by HS-SPME-GC-MS

The HS-SPME-GC- MS analysis resulted in the identifications of 48 compounds (Table 2). Different chemical classes were detected, such as higher alcohols, terpenes, volatile acids, aldehydes, ketones, and esters, being esters the most abundant group.

The higher abundance of esters is generally associated with superior quality beverages. Among the ethyl esters were detected 21 compounds, being ethyl dodecanoate (1088.09 µg/L), ethyl octanoate (996.88 µg/L) and ethyl 9-decenoate (850.98µg/L) the most abundant. Esters are associated with pleasant descriptions, such as roses, fruity and floral (Hu et. al. 2017). These compounds are common in many alcoholic beverages, including wine, other spirits and fruit distillates (Amorim et al. 2016; Palassarou et al. 2017; Vernocchi et al. 2015). Ethyl octanoate was reported by Sampaio et al. (2013) in similar concentration to the distillate produced in this study, 842 µg/L (Table 2). On the other hand, this concentration was higher than the 239.4 µg/L and 698.0 µg/L found in two different spent coffee ground spirits by Machado et al. (2018). The profile of these three main ethyl esters reinforces the potential of studied coffee by-products for use in alcoholic fermentation and generation of desirable volatile aromatic compounds.

Besides ethyl esters, five acetates were also found in the beverage, with phenylethyl acetate being the most abundant (296.17 µg/L) (Table 2). This concentration was more than twice as high as that found by Sampaio et al. (2013) in the spent coffee grounds distillate. Phenylethyl acetate is an impactful compound in the beverages with aroma descriptor associated with roses. Isoamyl acetate, responsible for conferring banana aroma in to the

distillate (Hu et al. 2017) was found in the concentration of 62 µg/L. Other authors also reported the presence of this compound in other distilled beverages such as, whey and cachaça (Amorim et al. 2016; Dragone et al. 2009). It is also worth mentioning that the high concentration of ethyl esters found in the beverage produced with coffee pulp is similar to those reported in studies such as Sampaio et al. (2013) and Santiago et al. (2014), in which high-quality sugarcane spirits also presented esters as the main group of volatile compounds.

Among other compounds that positively influence the aroma of distilled beverages, there are higher alcohols. Higher alcohols can be synthesized by yeasts through an anabolic glucose pathway or a catabolic pathway of corresponding amino acids (valine, leucine, iso-leucine and phenylalanine). Consequently, higher alcohols are released to the medium as secondary products from yeast metabolism and are responsible for secondary aroma in beverages. The higher alcohols that are formed by the metabolism of yeasts form amino acids – naturally, occur in higher concentrations in distilled beverages (Sampaio et al. 2013). In the beverage produced in this study, the higher alcohols with highest concentrations were 2-methyl-1-butanol and 3-methyl-1-butanol (isoamyl alcohols), with a concentration of 1269.16 µg/L. These compounds constitute most of the higher alcohols in distilled beverages and define the sensory character of the beverage (Czerny et al. 2008). In a distilled beverage produced from spent coffee grounds by Sampaio et al. (2013) these compounds were found high concentrations, as well as in the sugarcane spirit produced by Amorim et al. (2016) with the same yeast used in this study. In both studies, the final beverages were submitted to sensory analysis and presented a good acceptance by the trained and untrained tasters. As the major alcohols in the beverage, 2-methyl-1-butanol and 3-methyl-1-butanol can be considered positive contributors to the sensorial quality of the beverage as discussed below. The positive impact of these is associated with their aroma descriptors such as “banana” described by Czerny et al. (2008). Another higher alcohol also detected in a high concentration was the 2-

phenylethanol, with 226.76 µg/L (Table 2). This compound presence in low concentrations may contribute to the floral and sweet aroma of the distillate (Amorim et al. 2016; Hu et al. 2017). The aroma character of this compound changes with its oxidation and additional oxidation produces esters with honey aroma. Besides alcohols with a positive impact in the sensorial characteristics of distilled beverages, an interesting fact is that 1-butanol was found in the lowest concentration (2.45 µg/L) among the measured alcohols. In beverages, this compound is associated with "solvent" odor (Czerny et al. 2008) and may exert a negative effect on the aroma of the final product. The concentration found in distilled coffee pulp beverage was lower than the 4.89 µg/L reported by Amorim et al. (2016) in the sugarcane spirit fermented by the same strain used here.

The monoterpene alcohols found in the beverage were linalool, citronellol, nerolidol, α-terpineol and D-nerolidol (Table 2). The monoterpene alcohols are described with "citrus", "bergamot", "pinus" and "citronella" aromas by Czerny et al. (2008), and strongly impact the final aroma of beverages due to their low perception threshold. Among them, the linalool was detected in the highest concentration, 36.52 µg/L (Table 2). Considering that terpenes are either derived from the substrate or released by enzymatic reactions during the fermentation as described by Penã-Alvarez et al. (2004), the linalool content found in the beverage may be from the used substrate. The concentration found in our beverage is higher than the value found by Sampaio et al. (2013) in coffee spent ground distillate. Citronellol was detected in a concentration of 22.75 µg/L, almost two times more than the concentration reported by Machado et al. (2018) in spent coffee ground distilled beverage. Unlike citronellol, nerolidol was found in the distillate of spent coffee ground by Machado et al. (2018) in an amount approximately 8 times higher than the one we measured in our distillate (16.94 µg/L). α-terpineol, which common aromatic descriptor is "pinus", was found in a concentration of 11.91 µg/L (Table 2).

Regarding the volatile acids, interestingly, only 2 compounds were found, 1-decanoic acid (456.98 µg/L) and octanoic acid (200.85 µg/L) (Table 2). Their concentrations were lower than those reported in other coffee by-products by Sampaio et al. (2013) and Machado et al. (2018). These compounds are frequently associated with negative impacts on the sensorial quality of beverages. The aroma of octanoic acid is described as "rancid" while decanoic acid descriptors are "waxy, rancid and tallow". These acids are related to rancid and fat aromas such as in the whey distillate produced by Dragone et al. (2009).

Aldehydes (related to the hangover) and ketones were also detected in the beverage. According to Perestrelo et al. (2006), aldehydes are formed from unsaturated fatty acids while ketones are formed by the condensation of active fatty acids. Aldehydes may also be produced from their corresponding alcohols during fermentation (Perestrelo et al. 2006), so they were identified in low concentrations.

Sensory analysis

The sensory analysis was performed in comparison to a sugarcane spirit previously produced by Amorim et al. (2016). This sugarcane spirit showed a desirable aromatic compounds profile and good acceptance among the tasters in its previous sensory analysis. From the comparative sensory analysis, it was verified that the coffee pulp beverage presented a considerable higher acceptance (scores higher than 5 in the hedonic scale) percentage for aroma, taste and global impression. While the sugarcane spirit presented 70% of acceptance in relation to "taste", the coffee pulp spirit showed 80%. In the "aroma" evaluation, the tasters indicated an acceptance of 86% for the coffee pulp spirit and 78% for the sugarcane spirit. Similarly, to the "aroma", the "global impression" of the coffee pulp spirit (84%) was 8% higher than the sugarcane spirit (76%). The difference between both beverages for the "appearance" attribute was 4% (74% for the coffee pulp spirit and 78% for the sugarcane

spirit). These differences, found mainly for the “aroma” and “taste” attributes, are directly related to the composition of volatiles previously described, which when compared to that reported by Amorim et al. (2016) shows differences in the diversity as well as the concentration of the common compounds in the two beverages.

It was detected a large number of esters and terpenes in the distilled beverage of coffee pulp, which are compounds associated with floral and fruity aromas. Even more, many of these compounds present a low perception threshold that strongly impacts the sensory quality of the beverage. Terpene-like notes have already been related to the aroma of green coffee beans (Zellner et al. 2008). As shown in the volatile compound profile of the coffee pulp spirit, it was identified a considerable abundance of terpenes in the beverage. When questioned as to which aroma could be used to describe the coffee pulp spirit, a large percentage of the tasters pointed out the presence of an aroma that recalled them to brewed coffee. Also, considering the general acceptance for coffee, probably this aroma of coffee in the coffee pulp spirit positively impacted in its higher acceptance.

The Principal Component Analysis (PCA) of the sensory evaluation data (Fig. 1) demonstrated that the first two components PC1 and PC2 accounted for 72.21% of the variance. The “aroma”, “taste” and “global impression” attributes of the coffee pulp spirit were in the right lower quadrant (positive side of PC1 and negative of PC2), while these same attributes for the sugarcane spirit were grouped in the right superior quadrant (positive side of PC1 and PC2). For the “appearance” attribute, both beverages were grouped together, which is due to the fact that they are distilled beverages with identical clear visual; thus, they did not generate a different perception by the tasters.

The world coffee production in 2017/18 was 168 million 60 kg bags (IOC, 2019). Brazil was responsible for 76% of this production. However, this value considers only the final green coffee beans, being most part of the cherry weight removed during the coffee

426 processing. Indeed, for every 2 tons of processed coffee, about 1 ton of pulp is generated
427 (Murthy and Naidu 2012). Also, the wet processing releases up to 20 m^3 of wastewater per ton
428 of cherry in the fully washed method (Chanakya and Alwis 2004). Considering the volume of
429 coffee produced in Brazil, the volume of by-products generated, the amount of fermentable
430 sugar available in these by-products and the quality of the distilled beverage produced in this
431 work, the use of by-products represents a great economic potential for generation of profit in
432 the coffee production chain.
433
434 **Conclusions**
435 Considering the results found in this work, especially in the analysis of volatile and
436 sensory compounds, we can conclude that the evaluated coffee by-products can be used to
437 produce a good quality distilled beverage. Also, we can infer that the production of a distilled
438 beverage represents an interesting alternative for adding value to the coffee production chain
439 since currently, the by-products used here do not represent a source of profit for coffee
440 farmers. In a scenario of search for sustainability and value aggregation to the coffee
441 production chain, the use of coffee pulp and wastewater for alcoholic fermentation represents
442 itself as an interesting alternative to be exploited, for example, in the coffee producing units,
443 generating a differentiated product that can be attractive to the industry because it is a distilled
444 beverage with coffee aroma.
445
446 **Acknowledgments**
447 This study was financed in part by the Coordenação de Aperfeiçoamento de
448 Pessoal de Nível Superior – Brasil (CAPES) – Finance Code 001. The authors also would
449 like to thank Conselho Nacional de Desenvolvimento Científico e Tecnológico do Brasil

(CNPq), Fundação de Amparo à Pesquisa de MG (FAPEMIG) and National Institute of Coffee Science and Technology for their support.

References

Amorim JC, Schwan RF, Duarte WF (2016) Sugar cane spirit (cachaça): Effects of mixed inoculum of yeasts on the sensory and chemical characteristics. Food Res Int 85:76-83

Andrade RP, Melo CN, Genisheva Z, Schwan RF, Duarte WF (2017) Yeasts from Canastra cheese production process: Isolation and evaluation of their potential for cheese whey fermentation. Food Res Int 91:72-79

Beland FA, Benson RW, Mellick PW, Kovatch RM, Roberts DW, Fang JL et al (2005) Effect of ethanol on the tumorigenicity of urethane (ethyl carbamate) in B6C3F1 mice. Food Chem Toxicol 43:1–19

Bonilla-Hermosa VA, Duarte WF, Schwan RF (2014) Utilization of coffee by-products obtained from semi-washed process for production of value-added compounds. Bioresour Technol 166:142-150

Botelho CA, Rezende JC, Carvalho GR, Carvalho AM, Andrade VT, Barbosa CR. Adaptabilidade e estabilidade fenotípica de cultivares de café arábica em Minas Gerais. Pesquisa Agropecuária Brasileira, Brasília, v. 45, n. 12, p. 1404-1411, dez. 2010.

BRASIL. Ministério da Agricultura Pecuária e Abastecimento. Decreto n° 6.871 de 4 de junho de 2009.

BRASIL. Ministério da Agricultura Pecuária e Abastecimento. Instrução Normativa n◦. 13 de 29 de junho de 2005.

473 Burniol-Figols A, Cenian K, Skiadas IV, Gavala HN (2016) Integration of chlorogenic acid
474 recovery and bioethanol production from spent coffee grounds. Biochem Eng
475 J 116:54-64
476 Campos CR, Silva CF, Dias DR, Basso LC, Amorim HV, Schwan RF (2010). Features of
477 *Saccharomyces cerevisiae* as a culture starter for the production of the distilled sugar
478 cane beverage, cachaça in Brazil. J Appl Microbiol 108(6):1871-1879
479 Cerda A, Gea T, Vargas-García MC, Sánchez A (2017) Towards a competitive solid-state
480 fermentation: Cellulases production from coffee husk by sequential batch operation
481 and role of microbial diversity. Sci Total Environ 589:56-65
482 Chanakya HN, Alwis AAP (2004) Environmental issues and management in primary coffee
483 processing. Process Saf Environ 82(4):291-300
484 Czerny M, Christlbauer M, Christlbauer M, Fischer A, Granvogl M, Hammer M et al (2008)
485 Re-investigation on odour thresholds of key food aroma compounds and development
486 of an aroma language based on odour qualities of defined aqueous odorant
487 solutions. Eur Food Res Technol 228(2):265-273
488 de Souza PP, de Oliveira LC, Catharino RR, Eberlin MN, Augusti DV, Siebald HG, Augusti
489 R (2009) Brazilian cachaça: “Single shot” typification of fresh alembic and industrial
490 samples via electrospray ionization mass spectrometry fingerprinting. Food Chem
491 115(3):1064-1068
492 d'Avila GB, Cardoso MDG, Santiago WD, Rodrigues LMA, da Silva BL, Cardoso RR et al
493 (2016). Quantification of ethyl carbamate in cachaça produced in different
494 agro-industrial production systems. J I Brewing 122(2):299-303
495 Dragone G, Mussatto SI, Oliveira JM, Teixeira JA (2009) Characterisation of volatile
496 compounds in an alcoholic beverage produced by whey fermentation. Food
497 Chem 112(4):929-935

498 Duarte WF, Dias DR, Oliveira JM, Vilanova M, Teixeira JA, Silva JBA, Schwan RF (2010)
499 Raspberry (*Rubus idaeus L.*) wine: Yeast selection, sensory evaluation and
500 instrumental analysis of volatile and other compounds. Food Res Int 43(9):2303-2314
501 Guardia Puebla Y, Rodríguez Pérez S, Jiménez Hernández J, Sánchez-Girón Renedo V (2013)
502 Performance of a UASB reactor treating coffee wet wastewater. Revista Ciências
503 Técnicas Agropecuárias 22(3)
504 Gurbuz O, Rouseff JM, Rouseff RL (2006) Comparision of aroma volatiles in commercial
505 Merlot and Carbenet Sauvignon wines using gas chromatography – olfactometry and
506 gas chromatography-mass spectrometry. J Agric Food Chem 54:3990-3996
507 Hu K, Jin GJ, Mei WC, Li T, Tao YS (2018) Increase of medium-chain fatty acid ethyl ester
508 content in mixed *H. uvarum/S. cerevisiae* fermentation leads to wine fruity aroma
509 enhancement. Food Chem 239:495-501
510 ICO (2019). International Coffee Organization. Available at: http://www.ico.org. Accessed 15
511 February 2019.
512 Machado E, Mussatto S, Teixeira J, Vilanova M, Oliveira J (2018) Increasing the
513 Sustainability of the Coffee Agro-Industry: Spent Coffee Grounds as a Source of New
514 Beverages. Beverages 4(4):105
515 Martines N, Garcia R, Davide M, Freintas AMC, da Silva MG, Cabrita MJ (2018) An anciant
516 winemaking tehcnology: Exploring the volatile composition of amphora wines. LWT
517 – Food Sci Technol 96:288-295
518 Meilgaard MC (1975) Flavor chemistry of beer. II. Flavor and threshold of 239 aroma
519 volatiles. Tech Quart Master Brew Assoc Am 12:151-168
520 Murthy PS, Naidu MM (2012) Suistainable management of coffee industry by-products and
521 value addition – A review. Resour, Conserv Recy 66:45-58

522 Palassarou M, Melliou E, Liouni M, Michaelakis A, Balayiannis G, Magiatis P (2017)
523 Volatile profile of Greek dried white figs (*Ficus carica L.*) and investigation of the
524 role of β-damascenone in aroma formation in fig liquors. J Sci Food Agric 97:5254–
525 5270
526 Pandey A, Soccol CR, Nigam P, Brand D, Mohan R, Roussos S (2000) Biotechnological
527 potential of coffee pulp and coffee husk for bioprocesses. Biochem Eng J 6(2):153-162
528 Peña-Alvarez A, Díaz L, Medina A, Labastida C, Capella S, Vera LE (2004) Characterization
529 of three Agave species by gas chromatography and solid-phase microextraction–gas
530 chromatography–mass spectrometry. J Chromatogr A 1027(1-2):131-136
531 Pereira AP, Mendes-Ferreira A, Oliveira JM, Estevinho LM, Mendes-Faia A (2013) High-
532 cell-density fermentation of *Saccharomyces cerevisiae* for the optimisation of mead
533 production. Food microbiol 33(1):114-123
534 Perestrelo R, Fernandes A, Albuquerque, FF, Marques JC, Câmara JS (2006) Analytical
535 characterization of the aroma of Tinta Negra Mole red wine: Identification of the main
536 odorants compounds. Analytica Chimica Acta 563(1-2):154-164
537 Pino JA, Queris O (2011) Characterization of odor-active compounds in guava wine. J Agric
538 Food Chem 59(9):4885-4890
539 Poltronieri P, Rossi F (2016) Challenges in specialty coffee processing and quality
540 assurance. Challenges 7(2):19
541 Ribéreau-Gayon P, Glories Y, Maujean A, Dubourdieu D (2006) Alcohols and other volatile
542 compounds. In: Handbook of Enology, vol2, 2nd ed. Wiley, Bordeaux, pp 51-61
543 Sampaio A, Dragone G, Vilanova M, Oliveira JM, Teixeira JA, Mussatto SI (2013)
544 Production, chemical characterization, and sensory profile of a novel spirit elaborated
545 from spent coffee ground. LWT-Food Sci Technol 54(2):557-563

546 Santiago WD, Das Graças Cardoso M, Duarte FC, Saczk AA, Nelson DL (2014) Ethyl
547 carbamate in the production and aging of cachaça in oak (*Quercus sp*.) and amburana
548 (*Amburana cearensis*) barrels. J I Brewing 120(4):507-511
549 Vernocchi P, Patrignani F, Ndagijimana M, Lopez CC, Suzzi G, Gardini F, Lanciotti R (2015)
550 Trebbiano wine produced by using *Saccharomyces cerevisiae* strains endowed with β-
551 glucosidase activity. Ann Microbiol 65(3):1565-1571

552

553 **Table 1** Concentrations of sugars and ethanol by HPLC in fermented coffee pulp and kinetics
554 parameters for S. cerevisiae CA11

555

Non-fermented musts (0 h)				
Compounds	M1	M2	M3	M4
Sucrose	103.82±5.54^{a}	47.06±0.78^{d}	92.82±1.25^{b}	58.83±0.43^{c}
Glucose	26.03±0.22^{a}	13.30±0.57^{a}	18.00±0.89^{a}	6.66±0.06^{a}
Fructose	37.98±0.88^{a}	20.43±1.51^{a}	25.65±0.79^{a}	8.04±0.60^{b}
Total	173.33±5.17^{a}	83.28±2.90^{c}	141.38±0.36^{b}	76.64±0.98^{c}
Fermented musts (48 h)				
Compounds	M1	M2	M3	M4
Sucrose	0.65±0.03^{a}	0.18±0.00^{c}	0.39±0.07^{b}	0.23±0.05^{c}
Glucose	0.18±0.02^{b}	0.11±0.00^{b}	0.26±0.02^{a}	0.03±0.00^{c}
Fructose	7.07±1.19^{a}	3.01±0.05^{b}	4.09±0.31^{b}	2.25±0.04^{b}
Total	7.94±1.17^{a}	3.32±0.05^{b}	4.77±0.36^{a}	2.53±0.10^{b}
Ethanol	69.07±2.42^{a}	33.24±2.47^{b}	67.19±0.26^{a}	30.41±0.27^{b}
Kinetics parameters				
Yp/s (g/g)	0.42±0.03^{b}	0.42±0.02^{b}	0.49±0.00^{a}	0.41±0.01^{b}
Efic (%)	82.0±6.01^{b}	81.45±3.14^{b}	96.43±0.38^{a}	80.47±1.69^{b}
Conv (%)	95.41±0.81^{a}	96.02±0.08^{a}	96.63±0.24^{a}	96.70±0.08^{a}
Qp (g/L/h)	1.44±0.05^{a}	0.69±0.05^{b}	1.40±0.01^{a}	0.63±0.01^{b}

556 *Considering sucrose mathematically converted to fructose and glucose
557 Data expressed as mean value ± standard deviation of duplicates.
558 Values followed by the same latter in the superscript do not statistically differ among the
559 treatments by the Scott-Knott test ($p>0.05$).

560 **Table 2** Concentration of volatile compounds (μg/L) in distilled beverage produced from coffee pulp by HS SPME GC-MS

Number	Compound	LRI calc	LRI lit	Concentration (μg/L)	Descriptors
	Alcohols (9)				
1	2-Methyl-1-propanol	1095	1048[f]	44.63±0.21	Malty[a], unpleasant
2	1-Butanol	1146	1145[f]	2.45±0.02	Solvent[a]
3	2-Methyl-1-butanol 3-Methyl-1-butanol	1212	1212[f]	1269.16±5.23	Banana[a]
4	2-Methyl-1-decanol	1500	N.I.	13.28±0.56	-
5	1-Octanol	1562	1567[f]	8.15±0.12	Coco, walnut oil[b]
6	1-Decanol	1767	1809[f]	8.75±0.31	Sweet, fatty[g]
7	2-Phenylethanol	1925	1931[f]	226.79±4.74	sweet, roses[a]
8	1-Dodecanol	1973	1940[f]	24.16±1.41	Floral, waxy[g]
9	1-Eicosanol	2179	N.I.	6.34±0.27	-
	Monoterpene alcohols (5)				
10	Citronellol	1172	N.I.	22.75±0.11	Citrus[c]
11	Linalool	1516	1550[f]	36.52±1.02	Bergamot[a]
12	Nerolidol*	2407	N.I.	16.94±0.38	-
13	D-Nerolidol*	1673	1634[f]	5.81±0.09	-
14	α-Terpineol	1706	1696[g]	11.91±0.20	Pinus[b]
	Volatile acids (2)				
15	1-Decanoic acid	2329	2278[g]	454.98±5.83	Waxy, rancid, tallow[b]
16	Octanoic acid	2098	2047[f]	200.85±0.92	Rancid[b]
	Esters (26)				
17	Linalool acetate	1735	N.I.	1.57±0.10	Citrus[a]
18	Phenylethyl acetate	1830	1820[f]	296.17±7.54	Roses[b]
19	Farnesyl acetate	2282	N.I.	13.43±1.24	
20	Isoamyl acetate	1126	1105[f]	62.04±0.54	Banana, apple[d]

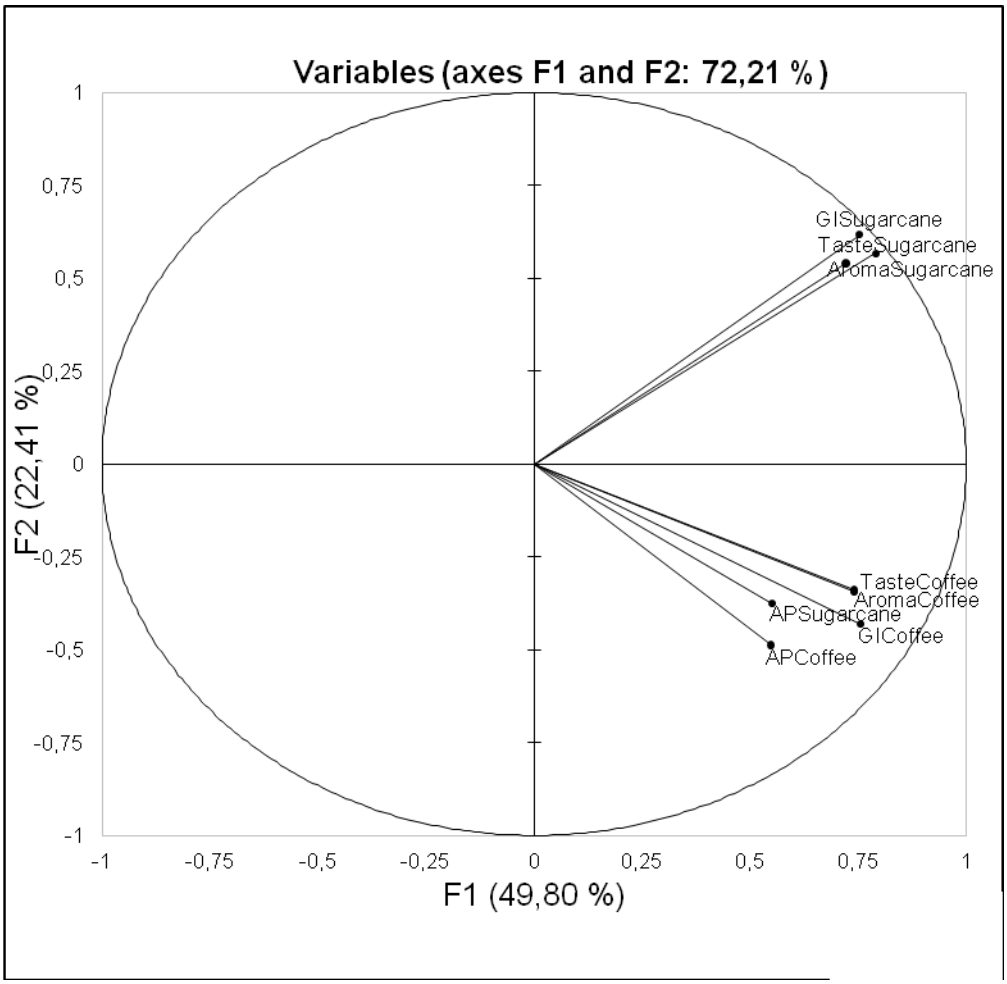
Number	Compound	LRI calc	LRI lit	Concentration (µg/L)	Descriptors
21	Citronellol acetate	1670	N.I.	10.69±1.20	Citronella[c]
22	Ethyl butanoate	1036	1031[h]	25.28±0.42	Fruity, sweet, apple[a]
23	Ethyl 3-metylbutanoate	1138	1035[h]	5.21±.012	Fruity, berries[a]
24	Ethyl decanoate	1543	1620[f]	39.67±.84	Fruity[b]
25	Butyl octanoate	1559	N.I.	6.25±0.05	-
26	Methyl decanaote	1602	1603[f]	20.98±0.57	-
27	Isoamyl octanoate	1666	1658[f]	141.08±0.90	Oily[g]
28	Ethyl 9-decenoate	1699	1694[f]	850.98±5.31	Rose[g]
29	Propyl decanoate	1731	N.I.	2.34±0.05	-
30	Ethyl undecanoate	1748	1725[f]	13.18±0.70	-
31	Isobutyl decanoate	1762	1773[f]	11.84±0.21	-
32	Methyl salicylate	1792	1820[g]	48.57±0.98	-
33	Ethyl dodecanoate	1852	1848[f]	1088.09±6.83	Floral, fruity[d]
34	Isoamyl decanoate	1780	1779[f]	109.58±0.51	-
35	Ethyl hydrocinnamato	1900	N.I.	26.24±0.90	-
36	Ethyl 9-hexadeceoate*	1905	N.I.	22.45±0.18	-
37	Ethylicosanoate	2056	N.I.	11.71±0.14	-
38	Ethyl hexanoate	1239	1241[f]	193.89±2.30	Green apple[b]
39	Ethyl heptanoate	1339	1338[f]	8.55±0.06	Fruity, pineapple[d]
40	Methyl octanaote	1395	1385[f]	5.18±0.21	-
41	Ethyl octanoate	1443	1445[f]	996.88±1.42	Fruity[b]
42	Isoamyl hexanoate	1465	1445[f]	8.28±0.08	Sweet, fruity[g]
	Aldehydes (2)				
43	Decanal	1507	1500[g]	9.97±0.14	Sweet waxy, orange[g]
44	Dodecanal	1720	1729[g]	4.65±0.04	Floral, waxy[g]

561

Number	Compound	LRI calc	LRI lit	Concentration (µg/L)	Descriptors
	Ketones (2)				
45	1-Menthone*	1472	N.I.	15.2±0.06	-
46	β-Damascenone	1835	1805[h]	55.81±0.48	Honey, sweet[g]
	Others (2)				
47	D-Limonene	1198	1222[f]	17.83±0.33	Citrus, herbal[e]
48	2,3-Dihydrofarnesol*	2285	N.I.	13.43±0.65	-

Data expressed as mean value ± standard deviation of duplicates.
LRI_{calc}, linear retention index based on a series of n-hydrocarbons reported according to their elution order on a Carbowax column
$LRI_{lit,}$ linear retention index from literature
[a]Czerny et al. (2008)**,** [b]Meilgaard (1975), [c]Ribéreau-Gayon et al. (2000), [d]HU et al. (2017), [e]Palassarou et al. (2017), [f]Martines et al. (2018), [g]Gurbuz et al. (2006), and [h]Pino and Queris (2011).

570



571

572 Figure 1 Principal component analysis (PCA) of sensory attributes of coffee pulp and
573 wastewater spirit and sugarcane spirit. AP: appearance, GI: Global impact

574

575

ARTIGO 2 – Production and characterization of a new distillate obtained from green coffee seed residue

Normas de formatação do periódico Food Chemistry

Artigo submetido e sob revisão no periódico Food Chemistry

Production and characterization of a new distillate obtained from green coffee seed residue

Abstract

This study evaluated green coffee seed residue (GCSR) as alternative substrate to produce a distilled beverage. Two proportions of GCSR, 10 % and 20 % (w/v) were fermented and distilled in cupper alembic. Spirits were characterized by GC-FID, HS-SPME GC-MS and sensory analysis by trained panelists. Most of 62 identified volatile compounds were affected by GCSR concentration. Total terpenes, higher alcohols and acetals showed the highest concentrations in the 10 % GCSR spirit. Esters, acetates and aldehydes were higher in the 20 % GCSR. In the sensory analysis, 10 % GCSR spirit was characterized by floral, dairy and almond aromas; while 20 % GCSR was related to coffee, vegetable, hazelnut, cooked cabbage and nuts descriptors. Results demonstrate the GCSR potential as substrate to produce coffee spirits with chemical and sensory quality, being 10 % GCSR the better option for fermentation.

Keywords: *Coffee by-products; Coffee spirit; Volatile compounds.*

1. Introduction

Coffee is grown in more than 80 countries, being the second largest commodity in the world. The global coffee output in 2018/19 was 170 million bags (60 kg). Brazil was responsible for 36.8 % of this production with 62,925 million bags (60 kg) (International Coffee Organization, 2020). Coffee is commercialized as green coffee beans, with or without roasting. Green coffee beans are composed of a non-volatile portion, including water, carbohydrates, fibers, proteins, amino acids, lipids, organic acids, trigonelline, caffeine and chlorogenic acid. Insoluble polysaccharides, such as cellulose and hemicellulose, make up about 50 % of the dry weight of the grain. Other soluble carbohydrates such as, fructose, glucose, galactose, arabinose, raffinose and mannose are also present. Lipids are the second most abundant compounds, representing from 15 % to 20 % of the dry weight (Esquivel et al., 2012; Oliveira et al., 2019). A wide variety of volatile compounds, such as acids, alcohols, aldehydes, alkanes, alkenes, esters, furans, furanones, ketones, lactones, pyrazines, pyridines and terpenes are present in the coffee beans. These compounds may be naturally formed in green coffee beans or converted during post-harvest processing, such as mucilage removal, drying and storage (Lee et al., 2017).

Green coffee oil has gained attention of the cosmetic industry due to its composition of triglycerides and free fatty acids (Castro et al., 2018). The oil is produced by mechanical pressing of green coffee beans, which generates a defatted biomass as by-product. This defatted biomass, named here as green coffee seed residue (GCSR), is usually disposed in landfills or used to generate bioenergy without a significant added value (Mayanga-Torres et al., 2017). GCSR presents a great technological potential because it shows chemical composition similar to green coffee, with exception of the extracted oil.

The coffee industry generates considerable amounts of residues during the coffee cherry processing. Environmental and social concerns have increased in relation to the

reutilization of these coffee by-products. Among the main applications for coffee by-products, such as pulp, husk and wastewater are ethanol production (Gouvea et al., 2009), extraction of antioxidants (Burniol-Figols et al., 2016), and production of bioenergy (Zuorro & Lavecchia, 2012). Few studies have also reported the recovery of sugars (Mayanga-Torres et al., 2017) and bioactive compounds from GCSR, mainly phenolic compounds (Oliveira et al., 2019; Castro et al., 2018).

In recent years, studies about raw materials as alternative to produce beverages have increased. Alcoholic fermentation enters this scenery as an alternative way to aggregate value to coffee by-products such as, coffee pulp (Bonilla-Hermosa et al., 2014), spent coffee grounds (Sampaio et al., 2013) and coffee pulp and wastewater (Lopes et al., 2020). All these coffee by-produces were reported as suitable substrates to produce spirits with good chemical and sensory quality. In this context, our study aimed to evaluate the use of green coffee seed by-product to produce coffee spirits, and characterize the chemical and sensory profile of the beverages. To our knowledge this is the first study on the green coffee seed residue as a substrate for alcoholic fermentation to produce a distilled beverage.

2. Materials and Methods

2.1. Raw material and microorganism

Green coffee seed residue (GCSR) was supplied by Cooxupé (Guaxupé, Minas Gerais, Brazil). GCSR chemical composition was N 19.91 g/kg, P 1.38 g/kg, K 7.07 g/kg, Mg 0.78 g/kg, S 1.3 g/kg, B 7.34 mg/kg, Cu 24.47 mg/kg, Mn 10.92 mg/kg, Zn 1.75 mg/kg and Cu 26.67 mg/kg. The commercial *Saccharomyces cerevisiae* strain CA11 (LNF Latino America, Bento Gonçalves, Rio Grande do Sul, Brazil) was used in all fermentations

considering previous studies that showed its applicability to produce sugarcane (Amorim et al., 2016) and coffee by-products spirits (Lopes et al., 2020).

2.2. Spirit production

Fermentations were performed in 70 L stainless steel vats. Two beverages were produced, using 10 % and 20 % (w/v) green coffee seed residue. The fermentations were carried out in fed batch to facilitate the cell adaption to the must as reported by Amorim et al. (2016). Commercial sucrose was added to adjust the Brix must to 16. All fermentations were performed in duplicate, at room temperature, and considered finished after °Brix stabilization, cell decantation and decreased CO_2 liberation. The fermented musts were transferred to a 40 L copper alembic, temperature was kept at 91-97 °C to maintain an approximate distillation rate of 1 L per hour. The 'head' fraction (corresponding to 10 % of the expected volume of the distillate) was discarded and the 'heart' fraction (or spirit) was collected up to 42 % (v/v) ethanol (Amorim et al., 2016). All spirits were stored in 500 mL glass bottles until chemical and sensory analyses.

2.3. Chemical characterization of GCSR spirits

All spirits were evaluated in relation to their identity and standard quality as set by Brasil (2005a). The parameters evaluated were relative density, actual alcoholic degree, volatile acidity, copper - colorimetric, total aldehydes, total esters, methyl alcohol - chromatographic, dry extract and furfural. All analyses were carried out in triplicate.

Major higher alcohols (1-propanol, 2-methyl-1-propanol, 2-methyl-1-butanol and 3-methyl-1-butanol) and methyl were determined by GC-FID. Injections were in split mode 1:10. Separation of the compounds was carried out in a DBWax column (30 m x 0.25 mm, 0.25 µm) and helium gas (1.4 mL/min) as mobile phase. The temperature of the injector and

92 detector were, respectively, 150 and 170 °C. The oven was heated from 55 °C to 70 °C at 1
93 °C/min. Compounds were identified and quantified by external calibration (Brasil, 2005a).
94
95 **2.4. Ethyl carbamate determination**
96 Samples were previously derivatized and evaluated as described by Santiago et al.
97 (2014). Derivation was performed in amber flasks, in which 0.8 mL of xanthydrol (0.02
98 mol/L) in propanol was added to 4 mL of sample. After homogenization, 0.4 mL of HCl (1.5
99 mol/L) was added and stirred for 1 min. The mixture was kept at rest for 60 min, filtered
100 through a 0.45 µm polyethylene membrane and 20 µL was injected in the system. Separation
101 was carried out in an Agilent – Zorbax Eclipse AAA (4.6 x 150 mm, 5µm) column connected
102 to an Agilent - Zorbax Eclipse AAA (4.6 x 12.5 mm, 5µm) pre-column. Wavelengths of
103 excitation and emission were, respectively, 233 and 660 nm. The mobile phase was 20
104 mmol/L sodium acetate (Solvent A) and acetonitrile (Solvent B) at a flow of 0.75 mL/min.
105 The elution was carried out with a gradient from 0 to 5 min (40-60 % B); 5 to 10 min (60-70
106 % B); 10 to 18 min (70-80 % B); 18 to 19.5 min (80-90 % B); 19.5 to 25 min (90-40 % B);
107 25 to 30 min (40 % B). Quantification and identification of peaks was performed by external
108 calibration curves in triplicate.
109
110 **2.5. Volatile compounds by HS SPME GC MS**
111 Samples were prepared by adding 4 mL of deionized water, 0.25 of NaCl, and 1 mL
112 of the spirit in 15 mL vials. 4-Nonanol at a final concentration of 125 µg/L was used as
113 internal standard. The headspace solid phase microextraction (HS SPME) was performed at
114 60 °C for 25 min with a 50/30 µm DVB/Carboxen/PDMS Stable flex SPME fiber (Supelco,
115 Bellefonte, PA, USA) in a manual holder (Amorim et al., 2016). The compounds were
116 separated using a Rtx-5MS (30 m x 0.25 mm x 0.25 µm) column in a GC-MS-QP2010 Plus.

The injector temperature was kept at 270 °C and injections were in splitless mode (30 s at 25 psi) with thermal desorption of 100 s. The mobile phase was helium gas at 1.78 mL/min. Oven was operated from 35 to 240 °C with an increment of 4 °C/min. Detector interface and ion source temperature were 240 and 200 °C, respectively (Zacaroni et al., 2017). Identification was performed by comparing mass spectra of the compounds with the NIST library 2011. Linear retention indexes of the compounds were calculated using retention data of a *n*-alkanes (C8-C40) series injected under the same conditions as the samples, and values were compared to literature in order to confirm identification.

2.6. Sensory analysis

The green coffee seed residue (GCSR) spirits, with replicates, were submitted to sensory analysis by seven trained panelists, 5 males and 2 females, ranging in age from 40 to 60 years, all of them members of the official panel of "Geographic Indication Protected of the Spirits and Traditional Liqueurs from Galicia (Spain)". The panel of Spirits and Traditional Liqueurs from Galicia is the official panel composed by expert professionals trained and with high experience in sensory analyses of distillates from grape and herbs pomace and coffee liquor and all of them have previously taken part in similar studies.

The sensory analysis was performed in a professional-standard room in agreement with the ISO Norm 8589 (1988). The evaluation was carried out using the QDA method (Lawless & Heymann, 1998) in order to establish the descriptors of the distillates. A constant sample volume of 30 mL of each spirit was evaluated in spirit-taster glasses at 12 ºC. During the analysis, the judges smelled and tasted the samples, and the perceived descriptors were indicated. Then, they scored the intensity of each attribute using a 10-point scale, where 10 indicated a very high intensity. The relative frequency (F), relative intensity (I) and geometric mean (GM) of the different descriptors were calculated for each spirit. GM was calculated as

the square root of the product between I and F, *i.e.* $\mathrm{GM}\ (\%) = \sqrt{I \times F} \times 100$, where I corresponds to the sum of the intensities given by the panel for a descriptor, divided by the maximum possible intensity for this descriptor; and F is the number of times that the descriptor was mentioned divided by the maximum number of times that it could be mentioned.

The descriptors were classified for each spirit by using the GM according to the International Organization for Standardization–ISO Norm 11035 (1994), which made possible to eliminate the descriptors whose geometric means were relatively low. This method allowed taking into account descriptors which were rarely mentioned but which were very important in terms of the perceived intensity, and descriptors with a low perceived intensity but which are mentioned often (Dravnieks et al., 1978).

2.7. Statistical analyses

The sensory and instrumental data were analyzed using XLSTAT statistical and data solution, 19.1.1 (Addinsoft, 2020). Analysis of variance (ANOVA) was carried out to test significant differences among the spirits composition. Also, relative intensity (I), frequency (F) and geometric mean (GM) for each aroma descriptor were calculated in the sensory analysis. To show the relationship between data of sensory and volatile analyses of wines, partial least squares regression (PLSR) was applied on volatile compounds as independent variables (*X*-matrix) and sensory attributes as dependent variables (*Y*-matrix). The data were standardized by mean-centered to get all values at the same scale. PLSR creates a set of components starting from a table with several observations described by several variables. This is a data reduction technique that reduces the X variables to a set of noncorrelated factors that describe the variation in the data. (Cozzolino et al., 2009).

3. Results and discussion

3.1. Physical chemical, contaminants and ethyl carbamate characterization of GCSR spirits

In this study two spirits with different proportions of green coffee seed residue (10 % and 20 % GCSR) were produced to evaluate the effect of this novel substrate on the chemical and sensory composition of the beverages. To our knowledge, there is not an international legislation about the commercialization of distilled beverages produced from coffee or coffee by-produces. Here, we considered the Brazilian legislation that sets identity and quality standards for sugarcane spirit to characterize the spirits produced with green coffee seed residue. Brazil is a great exporter of distilled beverages, mainly sugarcane spirit. The market demands a rigorous control over the chemical characteristics of the product, especially about the contaminants that could invalidate the exportation of the beverage. Table 1 shows the physical-chemical profile of the GCSR spirits according to requirements of the Normative instruction nº 13 from the Ministério de Agricultura, Pecuária e Abastecimento (MAPA), (Brasil, 2005b).

The distillation of 10 % GCSR fermented must resulted in a heart fraction yield of 14.8 % v/v (distillate yield considering the fermented must volume used for distillation) with real alcoholic degree of 42 % v/v. The 20 % GCSR fermentation resulted in a distillate yield of 13.1 % with 41 % v/v of ethanol content. The alcoholic degree is within the limits set by the MAPA for the characterization of distilled beverages (Brasil, 2005).

The spirit produced with 20 % GCSR presented a volatile acidity of 82.6 mg/100 mL (expressed per 100 mL of anhydrous alcohol – a.a), considerably higher than 13.7 mg/100 mL of a.a found for 10 % GCSR spirit (Table 1). High volatile acidity during fermentation affects the cell viability and fermentative capacity, and it should be at low concentrations to

avoid vinegar-like *off-flavor* and sensory imbalance (Santiago et al., 2016, Czerny et al., 2008). Acetic acid is mainly formed by *S. cerevisiae* during the catabolism of sugar in the presence of oxygen, contamination of the must with acetic or other bacteria, or oxidation and esterification reactions during storage (Masson et al., 2012). However, Bortoletto & Alcarde (2013) showed that acidity of alcoholic beverages can also be influenced by the presence of phenolic acids (gallic, tannic, ferulic, syringic and vanillic acids) in the wood used for aging. Green coffee beans contain non-volatile aliphatic acids, such as citric, malic and quinic acid and phenolic acids, mainly chlorogenic acid (Esquivel et al., 2012). In this scenery, it is possible that the increase in GCSR resulted in a higher volatile acidity because of the higher concentration of phenolic acids from the raw material.

As expected, sec-butyl was not detected in none of the samples and n-butyl and methyl alcohol were detected in both spirits within the limit allowed by legislation (Brasil, 2005). Butyl, sec-butyl and methyl alcohols are organic contaminants formed during fermentation and distillation, and they should not be detected, or detected in low concentrations. Butyl alcohol may result from the contamination of acetobutylic bacteria during fermentation (Masson et al., 2012). Copper and furfural, were detected at considerable low concentrations in both spirits (Table 1). Copper is an inorganic contaminant from the distillation apparatus while furfural is an organic contaminant from Maillard reactions during distillation (Bortoletto et al., 2016).

Major higher alcohols (or fusel alcohols) were considered the sum of n-propyl, isobutyl and isoamyl alcohols. The 10 % GCSR spirit showed higher concentration of fusel alcohols (306.7 mg/100 ml of a.a.) than 20 % GCSR spirit (283.4 mg/100 ml of a.a.) (Table 1). Fusel alcohols are the main higher alcohols in fermented beverages and can have both positive and negative impacts on aroma and flavor. For concentrations below 300 mg/L, higher alcohols are considered desirable to fermented beverages, whereas concentrations

exceeding this concentration are regarded to contribute negatively (Olaniran et al., 2017). The formation of higher alcohols depends on the yeast strain, yeast performance during fermentation, and fermentation conditions (pH, temperature, nitrogen content). Considering that both spirits were produced with the same yeast and under similar environmental conditions, it is possible to notice that the increase in GCSR not necessarily results in higher concentrations of these compounds.

The spirit produced with 20 % GCSR showed a higher concentration of esters expressed as ethyl acetate (25.4 mg/100 mL of a.a) than the 10 % GCSR spirit (13.1 mg/100 mL of a.a.) (Table 1). All concentrations were within the limit allowed by MAPA legislation (Brasil, 2005). Ethyl acetate is the main ester in spirits and may correspond to more than 80 % of total esters, being formed by the esterification between ethanol and acetic acid during maturation (Bortoletto & Alcarde, 2013).

The International Agency for Research on Cancer classifies ethyl carbamate as ‘possibly carcinogenic to humans’ (class 2A) (IARC, 2007). Ethyl carbamate results from the reaction of ethanol and compounds containing carbamyl groups. These main EC precursors are commonly generated from arginine metabolism by *S. cerevisiae* or lactic acid bacteria accompanied by the fermentation process (Jiao et al., 2014), so it is crucial to trace it in these types of products. There is no international consensus about the maximum levels of ethyl carbamate. However, major countries producers of distilled beverages such as, Canada, EUA, Czech Republic, France and Brazil allow up to 150 µg/L of ethyl carbamate in distilled spirits (EFSA, 2007; Brasil, 2005). The chromatographic method had a limit of quantification (LOQ) of 5.69 µg/L and limit of detection (LOD) of 1.71 µg/L. The 10 % GCSR spirit showed an ethyl carbamate content of 3.54 µg/L, which was below the limit of quantification of the chromatographic method. In 20 % GCSR, ethyl carbamate was found at a

concentration of 11.70 µg/L. Both concentrations were considerably below the allowed limit of ethyl carbamate in distilled beverages (Table 1).

3.4. Volatile compounds

Table 2 shows the volatile profile of the green coffee seed residue spirits. Overall, 62 compounds were detected among esters, terpenes, higher alcohols, aldehydes, acetates, acetals and miscellaneous. The ANOVA analysis showed that 87.1 % of the volatile compounds were modified by % GCSR.

Esters from medium-chain fatty acids (or ethyl esters) were the most abundant group of volatiles in both spirits (Table 2). The 20 % GCSR spirit showed a higher content of esters (30688.1 µg/L) than the 10 % GCSR spirit (21050.2 µg/L). There were significant differences for most analyzed esters (83.3%), with the exception of ethyl butanoate, ethyl 2-methylbutanoate, and ethyl dodecanoate. Ethyl decanoate was the most abundant ester ($p<0.01$) in both beverages, 10 % and 20 % GCSR with concentrations of 10320.9 µg/L and 12908.3 µg/L, respectively. In the same way, ethyl octanoate showed high concentrations in both beverages (4488.9 µg/L and 9829.8 µg/L for 10 % and 20 % GCSR respectively). From esters with significant differences between spirit samples, five compounds (ethyl nonanoate, 2-methylpropyl benzoate, ethyl 3-phenylpropanoate, 3-methylbutyl benzoate and ethyl hexadecanoate) showed higher concentrations in the 10 % GCSR than 20 % GCSR spirit, whereas the 20 % GCSR spirit exhibited higher concentrations in the other ten esters with significant differences between samples. Flavor-active esters may be divided in two groups: ethyl esters (the alcohol group is ethanol or a higher alcohol and the acid group is a medium-chain fatty acid) and acetate esters (in which the alcohol group is ethanol or a higher alcohol and the acid group is an acetate) (Cacho et al., 2013). This group of compounds are usually associated with pleasant descriptors, such as ‘fruity’ and ‘floral’, and are desirable to the

aroma bouquet of the beverage (Czerny et al., 2008). Esters may be produced during fermentation or by fatty acid esterification with ethanol and acetic acid during storage (Bortoletto et al., 2016). The role of ester production in the yeast metabolism is still unclear. However, it is possible that esterification may occur to remove toxic fatty acids from the cell (Olaniran et al., 2017). The 20 % GCSR spirit showed a higher volatile acidity and lower ethanol content than the 10 % GCSR spirit. Also, fatty acids were detected only in the 20 % GCSR spirit, being 376.6 µg/L of decanoic acid (Table 2). In this scenery, the spirit obtained from a higher content of GCSR (20 %) could have been a more stressful environment for the yeast than the spirit produced with 10% GCSR. These key differences between both spirits may be responsible for a higher frequency of esterification reactions during fermentation.

Terpenes and C_{13} norisoprenoids in fermented beverages are usually derived from free and glycosylated conjugates from the raw material, and transformation of precursors by the yeast during fermentation. It has also been shown that some *S. cerevisiae* can synthesize via *de novo* some important monoterpenes enhanced by fermentation conditions (Carrau et al., 2005). Among the twelve terpenes quantified in the GCSR spirits, significant differences were found for 75 % of them. Overall, 10 % GCSR spirit showed a higher terpene content (14215.3 µg/L) than the 20 % GCSR spirit (10586.3 µg/L). β-Damascenone was the most abundant terpene in both spirits (6890.7 µg/L and 5030.7 µg/L in the 10 % and 20 % GCSR spirit, respectively) followed by linalool, being only β-damascenone significantly higher ($p<0.001$) in 20 % GCSR spirit (Table 2). β-Damascenone is a key odor in several fruits (peaches, lychees, and grapes) and beverages (coffee, beer and wine), being associated with 'fruity-flowery', 'woody', 'honey' and 'baked apple' descriptors (Czerny et al., 2009; Gao et al., 2014). Vilanova et al. (2012) reported that nitrogen may induce changes in glycosidase activity, with the lowest residual concentration of β-damascenone precursors in wine musts with low to moderate nitrogen. Considering the positive impact of terpenes and C_{13}

290 norisoprenoids in the aroma of beverage, 10 % GCSR would be the best proportion to
291 produce the spirit, which also relates to the results of sensory analysis discussed below.
292 Twelve higher alcohols were detected in the GCSR spirits, and all of them showed
293 significant differences between the beverages, reaching the highest total concentration for 10
294 % GCSR spirit (81226.7 µg/L). The 10 % GCSR spirit presented significant higher levels of
295 3-methyl-1-butanol (52411.2 µg/L), 2-methyl-1-butanol (10894.2 µg/L), 2-phenylethanol
296 (17115.9 µg/L) and 1-nonanol (129.6 µg/L). However, the 20 % GCSR spirit showed higher
297 concentrations of the other compounds (Table 2). Isoamyl alcohols (3-methyl-1-butanol and
298 2-methyl-1-butanol) and 2- phenylethanol are the most common alcohols in distilled
299 beverages, and define their sensory character (Czerny et al., 2008). Higher alcohols are those
300 compounds with over two carbons which result from yeast utilization of nitrogen-containing
301 compounds. Formation of higher alcohols is influenced by environmental conditions during
302 fermentation, distillation process and by the apparatus of distillation. Overall, they are
303 desirable compounds because contribute to the sensory profile of the beverage with ‘fruity’
304 and ‘floral’ descriptors (Bortoletto et al., 2016).
305 Nine aldehydes were identified in the GCSR spirits and 88.9 % showed significant
306 difference between produced beverages. The increase of GCSR in the beverages resulted in a
307 higher concentration of aldehydes. The 20 % GCRS spirit showed a total aldehyde
308 concentration (2915.6 µg/L) higher than 10 % GCSR spirit (1930.2 µg/L). Nonanal was the
309 most abundant aldehyde in both beverages, but significant difference between spirits were not
310 observed. All aldehydes showed higher concentrations for 20 % GCSR spirit with exception
311 to benzaldehyde which was higher for 10 % GCSR spirit ($p<0.01$, 379.0 µg/L) (Table 2).
312 Aldehydes are formed from unsaturated fatty acids or their corresponding alcohol. They
313 usually negatively affect to the flavor and aroma of beverages, being associated with
314 undesirable descriptors (Lopes et al., 2020).

315 **3.5. Sensory analysis**

316 The GCSR spirits were evaluated by sensory descriptive analysis to obtain the
317 aromatic descriptors. Descriptive analysis revealed that these spirits were characterized with
318 twenty-two descriptors: 2, 11 and 8 for visual, olfactory and gustatory phases respectively
319 (Table 3). Global value was also evaluated. All visual and gustatory descriptors reached the
320 highest frequency (100 %) in both beverages. Also, olfactory intensity, quality and coffee
321 showed 100 % of relative frequency in both spirits. Regarding the olfactory phase, it is also
322 valid to notice that fineness was 86 % frequent in the 10 % GCSR spirit, and 71 % in the 20
323 % GCSR spirit (Table 3). With respect to relative intensity, the highest values found for 10
324 % GCSR spirit descriptors were sparkly (75 %), clean (74 %), gustatory persistence (73 %)
325 and Olfactory intensity and quality (66 % and 68 % respectively); while the highest relative
326 intensities for 20 % GCSR spirit were sparkly (77 %), clean (75 %) and olfactory intensity
327 (66 %) and gustatory persistence (61 %).

328 Frequency and intensity were evaluated to calculate the geometric mean (GM) of the
329 different descriptors (Table 3). The GM was used to classify the descriptors, which allowed
330 to amplify the effects of small divergences between the perceived intensity of descriptors, and
331 at the same time, reduces the effects of the large divergences (Dravnieks et al., 1978). Fifteen
332 and sixteen sensory descriptors for 10 % and 20 % GCSR spirit respectively reached GM >
333 20 %.

334 From visual phase, clean and sparkly reached GM > 86 %. From olfactory phase,
335 intensity, quality, fineness and coffee aroma descriptors showed the highest values of GM
336 (GM > 54 %) in both 10 % and 20 % GCSR spirits. However, vegetable reached GM > 20 %
337 only for the 20 % GCSR spirit. From gustatory phase, all descriptors reached high values of
338 GM (GM > 43 %) in both spirit beverages. Similar results were obtained for global value
339 (GM > 72 %) being higher in the 10 % GCSR spirit than the 20 % GCSR.

Comparing both spirits, it was observed highest values (GM > 20 %) for the 10 % GCSR vs 20 % GCSR spirit. Ten from sixteen descriptors with GM > 20 % reached highest values in the 10 % GCSR spirit compared to the 20 % GCSR. Among these ten descriptors are include aroma intensity and quality, aromatic fineness, and global quality characterizing the 10 % GCSR spirit (Figure 1a). Coffee and vegetable aroma descriptor characterized 20 % GCSR spirit, which might be expected considering the higher concentration of GCSR during fermentation. The gustatory profile (Figure 1b) shows highest values of quality, salty, acidity, body and persistence for 10 % GCSR spirit. On the other hand, 20 % GCSR spirit was characterized by sweet and bitter taste descriptors.

3.6. Correlations between volatile composition and sensory data of spirits

Three partial least squares regression (PLSR) analyses were carried out with the aim to assess the correlation between volatile composition and aroma sensory attributes of the two spirits samples with replications, A (10 % GCSR) and B (20 % GCSR) (Figure 2). In Figure 2, the small ellipse represented 50 % of the explained variances and the big ellipse showed 100% of explained variances.

The first PLSR (Figure 2a) was performed on 62 volatile compounds designed as the *X*-matrix (coded as indicated in table 1) while the *Y*-matrix consisted in intensity of 12 aroma sensory descriptors of spirits and replications: 10 % GCSR spirit (A1, A2, A3) and 20 % GCSR spirit (B1, B2, B3). The two first factors explained 77 % of the *X*-variables (R^2X accumulated) and 45 % of *Y*-variables. (R^2Y accumulated). The first PLSR dimension shows a good separation between the distillates A and B where the descriptor of coffee characterized the distillate B, however quality, aroma intensity, fineness and dairy characterize to A distillate samples. It was observed that most of volatile compounds were located outside the small ellipse and correlated with some sensory attributes (global value, floral, hazelnut,

365 vegetable, almond and quality). The floral aroma in the 10 % GCSR (A) may be a
366 consequence of the higher content of alcohols such as 2-phenylethanol and acetals like ethyl
367 2-phenylacetate, usually described with ‘rose’ and ‘floral’ aroma. While the higher content of
368 aldehydes such as 2-nonenal and 2-decenal, and esters like ethyl hexanoate with ‘green’
369 descriptors may be related to the vegetable descriptor in the 20 % GCSR spirit (B). In the
370 small ellipse, high correlations were detected between some volatiles: B (ethyl butanoate), Q
371 (ethyl dodecanoate), and BM (2,4-di-t-butylphenol) with coffee sensory descriptor.

372 The second PLSR (Figure 2b) was carried out with volatile families designed as *X*-
373 matrix and intensity sensory descriptors as *Y*-matrix. The two first factors explained 78 % of
374 the variation in volatile composition (R^2X accumulated) and 44 % in sensory descriptors.
375 (R^2y accumulated). In this case the first PLSR dimension shows also a good separation
376 between the distillates A and B in basis to volatiles and sensory descriptors. The 10 % and 20
377 % GCSR spirit are located on the positive and negative side of first component (t1)
378 respectively. High correlation was shown between global value and floral descriptors with
379 terpenes, higher alcohols and acetals (100 % of explained variances) characterizing the
380 distillates A. However, B distillates, in the opposite side, were characterized by aldehydes,
381 esters, acetates and other volatile compounds which showed high correlations with hazelnut
382 and vegetable descriptors (100 % of explained variance).

383 The last PLSR (Fig 2c) was carried out with volatile families designed as *X*-matrix
384 and data for three sensory descriptors, global value (GV), quality (Q) and fineness (F) given
385 by the seven panelists as *Y*-matrix. The two first factors explained 79 % of the variation in
386 volatile composition (R^2X accumulated) and 69 % in sensory descriptors. (R^2Y accumulated).
387 In the same way that Figure 2a and b, a good separation of A and B samples was observed in
388 basis to first dimension of PLSR. In this case, PLSR allowed to know the grade of consensus
389 among the panelists (1-7) to evaluate the distillates the three descriptors proposed (GV, Q and

F). The panelists reached high consensus on the global value (GV) and quality (Q) sensory descriptors grouped in the positive side on the first factor (t1) giving higher value to the A spirits)10 % GCSR). However, this consensus was not shown on fineness (F), because 3 from 7 judges (F4, F6 and F7) sited in the negative side of t1, gave the higher value for A spirits (20 % GCSR).

From the PLSRs data sets, global value, quality, floral and almond descriptors of distillates could be predicted by high level of terpenes, higher alcohols and acetals while esters, aldehydes, acetates and other volatile compounds groups could predict descriptors as vegetable and hazelnut. However, fineness, intensity, coffee, nuts, cooked cabbage and dairy were not able to sufficiently predicted by the effect of flavor compounds on the perception of these sensory attributes of distillates by the panelists.

4. Conclusions

Green coffee seed residue presented itself as a promising substrate to produce a distilled beverage. The change in concentration of GCSR resulted in significant changes in the chemical and sensory profile of the final beverages. Although GCSR does not have a significant production cost, the processing and time consumption to manage 20 % GCSR during the spirit production has a considerable economic impact in the final product. The increase in the proportion of GCSR did not result in a better yield, or improved the sensory profile and acceptance of the beverage. Indeed, during the sensory evaluation the spirit produced with 10 % GCSR received the highest relative intensity, relative frequency and geometric mean scores regarding the quality, salty, acidity, body and persistence parameters. While the 20 % GCSR was characterized with higher scores to the sweet and bitter taste descriptors. Also, panel of specialists described the 10 % GCSR spirit with floral, dairy and

414 almond aromas, while the 20 % GCSR spirit was associated with vegetable and cooked
415 cabbage aromas.
416
417 **Conflict of interest:** The authors declare they have no conflict of interest.
418
419 **Acknowledgments** Authors would like to thank the official panel of Geographic Indication
420 Protected of the Spirits and Traditional Liqueurs from Galicia (Spain) for sensory evaluation.
421 Conselho Nacional de Desenvolvimento Científico e Tecnológico do Brasil (CNPq),
422 Coordenação de aperfeiçoamento de Pessoal de Nível Superior (CAPES) and Fundação de
423 Amparo à Pesquisa de MG (FAPEMIG) for financial support and scholarship.
424
425 **References**
426 Alves, Z., Melo, A., Figueiredo, A. R., Coimbra, M. A., Gomes, A. C., & Rocha, S.
427 M. (2015). Exploring the Saccharomyces cerevisiae volatile metabolome: indigenous versus
428 commercial strains. *PLoS One*, *10*(11). https://doi.org/10.1371/journal.pone.0143641.
429 Amorim, J. C., Schwan, R. F., & Duarte, W. F. (2016). Sugar cane spirit (cachaça):
430 Effects of mixed inoculum of yeasts on the sensory and chemical characteristics. *Food*
431 *Research International*, *85*, 76-83. https://doi.org/10.1016/j.foodres.2016.04.014.
432 Bonilla-Hermosa, V. A., Duarte, W. F., & Schwan, R. F. (2014). Utilization of coffee
433 by-products obtained from semi-washed process for production of value-added compounds.
434 *Bioresource Technology*, 166, 142–150. https://doi.org/10.1016/j.biortech.2014.05.031.
435 Bortoletto, A. M., & Alcarde, A. R. (2013). Congeners in sugar cane spirits aged in
436 casks of different woods. *Food chemistry*, *139*(1-4), 695-701.
437 https://doi.org/10.1016/j.foodchem.2012.12.053.

438 Bortoletto, A. M., Correa, A. C., & Alcarde, A. R. (2016). Aging practices influence
439 chemical and sensory quality of cachaça. *Food research international*, 86, 46-53.
440 https://doi.org/10.1016/j.foodres.2016.05.003.
441 Brasil. (2005a). Ministério da Agricultura, Pecuária e Abastecimento. Instrução
442 normativa no. 24, 8 September 2005.
443 Brasil. (2005b). Ministério da Agricultura, Pecuária e Abastecimento. Instrução
444 normativa no. 13, 29 June 2005.Burniol-Figols, A., Cenian, K., Skiadas, I. V., & Gavala, H.
445 N. (2016). Integration of chlorogenic acid recovery and bioethanol production from spent
446 coffee grounds. *Biochemical Engineering Journal*, *116*, 54-64.
447 https://doi.org/10.1016/j.bej.2016.04.025.
448 Cacho, J., Moncayo, L., Palma, J. C., Ferreira, V., & Culleré, L. (2013). Comparison
449 of the aromatic profile of three aromatic varieties of Peruvian pisco (Albilla, Muscat and
450 Torontel) by chemical analysis and gas chromatography–olfactometry. *Flavour and*
451 *Fragrance Journal*, *28*(5), 340-352. https://doi.org/10.1002/ffj.3171.
452 Cardeal, Z. L., & Marriott, P. J. (2009). Comprehensive two-dimensional gas
453 chromatography–mass spectrometry analysis and comparison of volatile organic compounds
454 in Brazilian cachaça and selected spirits. *Food Chemistry*, *112*(3), 747-755.
455 https://doi.org/10.1016/j.foodchem.2008.06.057.
456 Carrau, F. M., Medina, K., Boido, E., Farina, L., Gaggero, C., Dellacassa, E., ... &
457 Henschke, P. A. (2005). De novo synthesis of monoterpenes by *Saccharomyces cerevisiae*
458 wine yeasts. *FEMS microbiology letters*, 243(1), 107-115.
459 https://doi.org/10.1016/j.femsle.2004.11.050
460 Castro, A. C. C. M., Oda, F. B., Almeida-Cincotto, M. G. J., Davanço, M. G., Chiari-
461 Andréo, B. G., Cicarelli, R. M. B., ... & Isaac, V. L. B. (2018). Green coffee seed residue: A

462 sustainable source of antioxidant compounds. *Food chemistry*, *246*, 48-57.
463 https://doi.org/10.1016/j.foodchem.2017.10.153.

464 Cozzolino, D., Cynkar, W. U., Shah, N., Dambergs, R. G., & Smith, P. A. (2009). A
465 brief introduction to multivariate methods in grape and wine analysis. *International Journal*
466 *of Wine Research*, *1*, 123-130. https://doi.org/10.2147/IJWR.S4585.

467 Czerny, M., Christlbauer, M., Christlbauer, M., Fischer, A., Granvogl, M., Hammer,
468 M., ... & Schieberle, P. (2008). Re-investigation on odour thresholds of key food aroma
469 compounds and development of an aroma language based on odour qualities of defined
470 aqueous odorant solutions. *European Food Research and Technology*, *228*(2), 265-273.
471 https://doi.org/10.1007/s00217-008-0931-x.

472 Dravnieks, A., Bock, F. C., Powers, J. J., Tibbetts, M., & Ford, M. (1978).
473 Comparison of odors directly and through profiling. *Chemical Senses*, *3*(2), 191-225.

474 Esquivel, P., & Jiménez, V. M. (2012). Functional properties of coffee and coffee by-
475 products. *Food Research International*, *46*(2), 488-495.
476 https://doi.org/10.1016/j.foodres.2011.05.028.

477 European Food Safety Authority (EFSA). (2007). Ethyl carbamate and hydrocyanic
478 acid in food and beverages-Scientific Opinion of the Panel on Contaminants. EFSA
479 Journal, 5(10), 551.

480 Gao, W., Fan, W., & Xu, Y. (2014). Characterization of the key odorants in light
481 aroma type Chinese liquor by gas chromatography–olfactometry, quantitative measurements,
482 aroma recombination, and omission studies. *Journal of agricultural and food*
483 *chemistry*, *62*(25), 5796-5804. https://doi.org/10.1021/jf501214c.

484 Gouvea, B. M., Torres, C., Franca, A. S., Oliveira, L. S., & Oliveira, E. S. (2009).
485 Feasibility of ethanol production from coffee husks. *Biotechnology letters*, *31*(9), 1315-1319.
486 https://doi.org/10.1007/s10529-009-0023-4.

IARC (International Agency for Research on Cancer). (2007). International Agency for Research. Volume 96: Alcoholic Beverage Consumption and Ethyl Carbamate (Urethane) 6–13 February 2007 1-5. World Health Organization, Lyon, France. Available from: http://monographs.iarc.fr/ENG/Meetings/vol96-summary.pdf. Acessed October 10 2020.

ICO (International Coffee Organization). (2020). Total coffee production from 1990 to present. Available from: http://www.ico.org/historical/1990%20onwards/PDF/1a-total-production.pdf. Acessed October 12 2020.

Jiao Z., Dong Y., & Chen Q. (2014). Ethyl Carbamate in Fermented Beverages: Presence, Analytical Chemistry, Formation Mechanism, and Mitigation Proposals. Comprehensive Reviews in Food Science and Food Safety, 13, 611-626.

Lawless H.T., & Heymann H. (1998). Sensory evaluation of food. Principle and practices. Massachussets: Kluwer Academic/Plenum Publishers.

Ledauphin, J., Guichard, H., Saint-Clair, J. F., Picoche, B., & Barillier, D. (2003). Chemical and sensorial aroma characterization of freshly distilled calvados. 2. Identification of volatile compounds and key odorants. *Journal of agricultural and food chemistry*, *51*(2), 433-442. https://doi.org/10.1021/jf020373e.

Lee, L. W., Tay, G. Y., Cheong, M. W., Curran, P., Yu, B., & Liu, S. Q. (2017). Modulation of the volatile and non-volatile profiles of coffee fermented with Yarrowia lipolytica: II. Roasted coffee. *LWT*, *80*, 32-42. https://doi.org/10.1016/j.lwt.2017.01.070.

Lopes, A. C. A., Andrade, R. P., de Oliveira, L. C. C., Lima, L. M. Z., Santiago, W. D., de Resende, M. L. V., ... & Duarte, W. F. (2020). Production and characterization of a new distillate obtained from fermentation of wet processing coffee by-products. *Journal of Food Science and Technology*, *57*(12), 4481-4491. https://doi.org/10.1007/s13197-020-04485-4.

Masson, J., Cardoso, M. D. G., Zacaroni, L. M., Anjos, J. P. D., Sackz, A. A., Machado, A. M. D. R., & Nelson, D. L. (2012). Determination of acrolein, ethanol, volatile acidity, and copper in different samples of sugarcane spirits. *Food Science and Technology*, *32*(3), 568-572. https://doi.org/10.1590/S0101-20612012005000075.

Mayanga-Torres, P. C., Lachos-Perez, D., Rezende, C. A., Prado, J. M., Ma, Z., Tompsett, G. T., ... & Forster-Carneiro, T. (2017). Valorization of coffee industry residues by subcritical water hydrolysis: recovery of sugars and phenolic compounds. *The Journal of Supercritical Fluids*, *120*, 75-85. https://doi.org/10.1016/j.supflu.2016.10.015.

Nicolli, K. P., Welke, J. E., Closs, M., Caramão, E. B., Costa, G., Manfroi, V., & Zini, C. A. (2015). Characterization of the volatile profile of Brazilian moscatel sparkling wines through solid phase microextraction and gas chromatography. *Journal of the Brazilian Chemical Society*, *26*(7), 1411-1430. https://doi.org/10.5935/0103-5053.20150110

Norme ISSO 8589 (1988). Sensory Analysis, General guidance for the design of test rooms.

Norme ISO 11035 (1994). Sensory Analysis, Identification and selection of descriptors for establishing a sensory profile by a multidimensional approach.

Olaniran, A. O., Hiralal, L., Mokoena, M. P., & Pillay, B. (2017). Flavour-active volatile compounds in beer: production, regulation and control. *Journal of the Institute of Brewing*, *123*(1), 13-23. https://doi.org/10.1002/jib.389.

Oliveira, É. R., Silva, R. F., Santos, P. R., & Queiroz, F. (2019). Potential of alternative solvents to extract biologically active compounds from green coffee beans and its residue from the oil industry. *Food and bioproducts processing*, *115*, 47-58. https://doi.org/10.1016/j.fbp.2019.02.005.

Sampaio, A., Dragone, G., Vilanova, M., Oliveira, J. M., Teixeira, J. A., & Mussatto, S. I. (2013). Production, chemical characterization, and sensory profile of a novel spirit

536 elaborated from spent coffee ground. *LWT-Food Science and Technology*, *54*(2), 557-563.
537 https://doi.org/10.1016/j.lwt.2013.05.042.

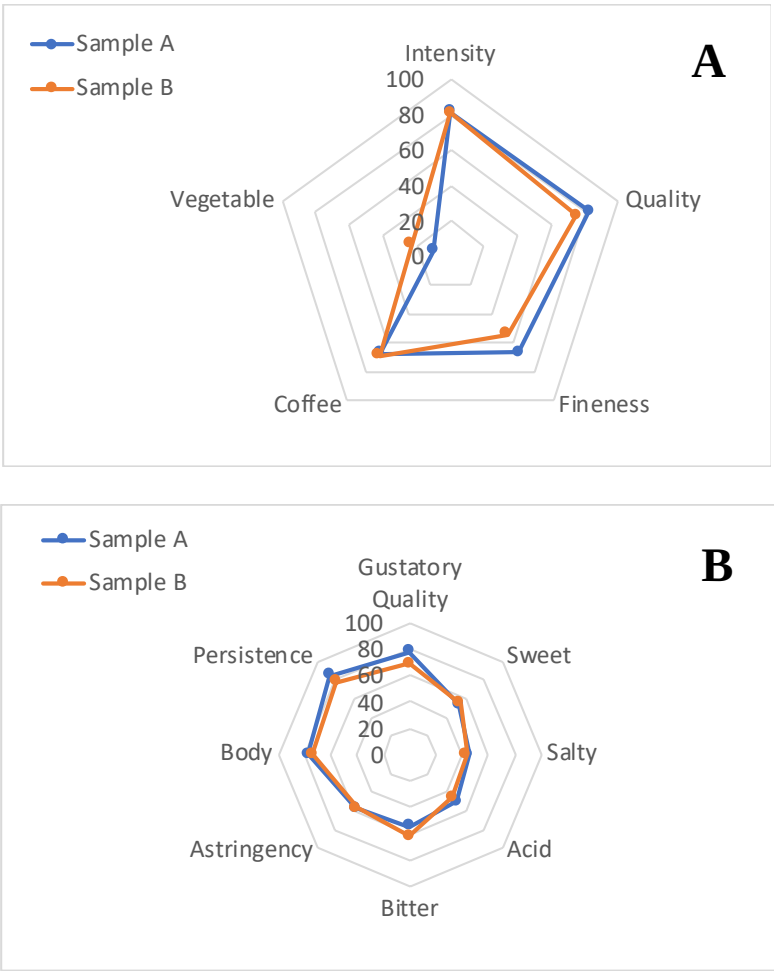
538 Santiago, W. D., Das Graças Cardoso, M., Duarte, F. C., Saczk, A. A., & Nelson, D.
539 L. (2014). Ethyl carbamate in the production and aging of cachaça in oak (*Quercus* sp.) and
540 amburana (*Amburana cearensis*) barrels. *Journal of the Institute of Brewing*, *120*(4), 507-
541 511. https://doi.org/10.1002/jib.158.

542 Santiago, W. D., Cardoso, M. D. G., de Andrade Santiago, J., Teixeira, M. L.,
543 Barbosa, R. B., Zacaroni, L. M., ... & Nelson, D. L. (2016). Physicochemical profile and
544 determination of volatile compounds in cachaça stored in new oak (*Quercus* sp.), amburana
545 (*Amburana cearensis*), jatoba (*Hymenaeae carbouril*), balsam (*Myroxylon peruiferum*) and
546 peroba (*Paratecoma peroba*) casks by SPME-GC–MS. *Journal of the Institute of*
547 *Brewing*, *122*(4), 624-634. https://doi.org/10.1002/jib.372.

548 Vilanova, M., Siebert, T. E., Varela, C., Pretorius, I. S., & Henschke, P. A. (2012).
549 Effect of ammonium nitrogen supplementation of grape juice on wine volatiles and non-
550 volatiles composition of the aromatic grape variety Albariño. *Food chemistry*, *133*(1), 124-
551 131. https://doi.org/10.1016/j.foodchem.2011.12.082.

552 Zacaroni, L. M., de Sales, P. F., Cardoso, M. D. G., Santiago, W. D., & Nelson, D. L.
553 (2017). Response surface optimization of SPME extraction conditions for the analysis of
554 volatile compounds in Brazilian sugar cane spirits by HS-SPME-GC–MS. *Journal of the*
555 *Institute of Brewing*, *123*(2), 226-231. https://doi.org/10.1002/jib.410.

556 Zuorro, A., & Lavecchia, R. (2012). Spent coffee grounds as a valuable source of
557 phenolic compounds and bioenergy. *Journal of Cleaner Production*, *34*, 49-56.
558 https://doi.org/10.1016/j.jclepro.2011.12.003



559

560 **Figure 1** Aroma (a) and Taste Profile (b) for coffee distillate samples A (10 % GCSR) and B
561 (20 % GCSR) in basis to geometric mean (GM%) of descriptors.

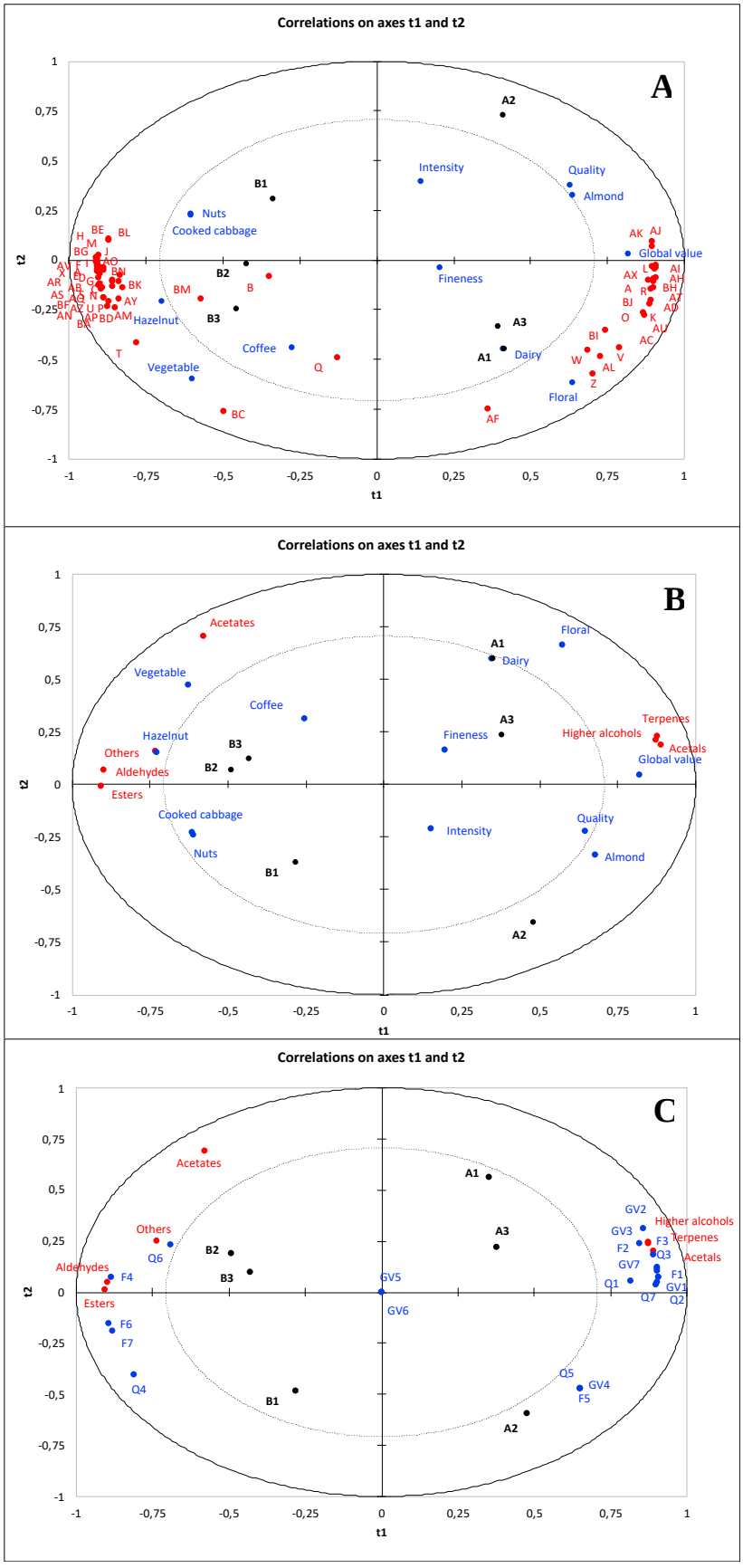


Figure 2 P Partial least squares regression (PLSR) analysis of spirits and replications, 10 % GCSR (A1, A2, A3) and 20 % GCSR (B1, B2, B3) on volatiles and sensory data of distillates. PLSR (A) volatile compounds (coded as indicated in table 1) and aroma sensory

descriptors; PLSR (B) volatile families and aroma sensory descriptors; PLSR (C) volatile families and data for sensory descriptors global value (GV), quality (Q) and fineness (F) done by each juice (1-7).

569 **Table 1.** Chemical characterization of spirits produced with 10 % and 20 % Green Coffee Seed Residue (GCSR)

No.	Items	Units	Maximum levels*	10 % GCSR spirit	20 % GCSR spirit
1	Relative density	20/20 °C	-	0.9	0.9
2	Copper	mg/L	5	0.5	2.7
3	Dry extract at 100 °C	g/L	-	0.016	0.048
4	Real alcoholic degree	% v/v	54-38	41.98	40.99
5	Volatile acidity in acetic acid	mg/100 mL of a.a.	150	13.7	82.6
6	Higher alcohols [7+8+9]	mg/100 mL of a.a.	360	306.7	283.4
7	n-Propyl alcohol	mg/100 mL of a.a.	-	86.4	76.7
8	Isobutyl alcohol	mg/100 mL of a.a.	-	104.5	97.3
9	Isoamyl alcohol	mg/100 mL of a.a.	-	115.8	109.4
10	Sec-butyl alcohol	mg/100 mL of a.a.	10	nd	nd
11	n-butyl alcohol	mg/100 mL of a.a.	3	0.3	0.6
12	Furfural	mg/100 mL of a.a.	5	0.3	0.3
13	Aldehydes in acetaldehyde	mg/100 mL of a.a.	30	5.7	6.3
14	Esters in ethyl acetate	mg/100 mL of a.a.	200	13.1	25.4
15	Sum of secondary compounds	mg/100 mL of a.a.	650-200	339.6	397.9
16	Methyl alcohol	mg/100 mL of a.a.	20	6.7	6.4
17	Ethyl carbamate	µg/L	150	3.54	11.70

570 a.a.: anhydrous alcohol

571 *Limits of quality and identity standards for spirts according to the Normative instruction nº 13 from the Ministério de Agricultura, Pecuária e Abastecimento
572 (MAPA) (Brasil, 2005).

573

574 **Table 2.** Volatile compounds of 10 % and 20 % green coffee seed residue (GCSR) spirits in
575 µg/L

	Volatile compounds	LRI_{calc}	LRI_{lit}	Concentration (µg/L)		
				10 % GCSR	**20 % GCSR**	Sig
Esters						
A	Ethyl methanoate	-	-	nd	166.7 ± 16.2	***
B	Ethyl butanoate	803	804[b]	122.8 ± 9.3	131.0 ± 23.0	ns
C	Ethyl 2-methylbutanoate	848	850[c]	nd	35.2 ± 3.5	ns
D	Ethyl 3-methylbutanoate	852	853[c]	148.1 ± 10.0	263.2 ± 13.2	***
E	Ethyl hexanoate	1004	1007[d]	436.5 ± 36.3	1961.9 ± 285.1	***
F	Ethyl heptanoate	1099	1106[a]	nd	146.7 ± 23.7	***
G	Ethyl benzoate	1174	-	193.5 ± 13.2	262.8 ± 12.1	**
H	Methyl 2-hydroxybenzoate	1196	-	353.0 ± 48.5	992.3 ± 126.8	***
I	Ethyl octanoate	1206	1201[a]	4488.9 ± 112.7	9829.8 ± 841.3	***
J	Ethyl nonanoate	1328	1296[b]	328.8 ± 36.5	452.7 ± 41.9	**
K	2-Methylpropyl benzoate	1347	-	64.2 ± 13.3	nd	**
L	Ethyl 3-phenylpropanoate	1368	1353[a]	248.4 ± 9.2	nd	***
M	Ethyl 9-decenoate	1409	1382[c]	65.0 ± 5.1	269.5 ± 5.5	***
N	Ethyl decanoate	1438	1409[b]	10320.9 ± 378.2	12908.3 ± 1274.8	**
O	3-Methylbutyl benzoate	1448	-	148.3 ± 37.0	nd	***
P	3-Methylbutyl octanoate	1453	1447[a]	147.4 ± 18.9	221.5 ± 20.4	**
Q	Ethyl dodecanoate	-	1594[a]	1783.4 ± 116.0	1804.7 ± 312.8	ns
R	Ethyl hexadecanoate	-	1963[b]	2201.0 ± 22.6	1241.8 ± 217.1	***
Acetates						
S	3-Methybutyl acetate	878	877[d]	1462.9 ± 28.1	1641.1 ± 30.6	***
T	2-Methybutyl acetate	879	-	139.6 ± 26.2	198.6 ± 33.9	*
U	Ethyl 2-phenylacetate	1252	1254[b]	855.2 ± 61.0	1103.8 ± 39.1	**
V	2-Phenylethyl acetate	1263	1254[c]	1389.3 ± 206.8	1081.1 ± 35.2	*
Terpenes and C_{13} norisoprenoids						
W	Linalool	1104	1103[d]	3881.5 ± 351.9	3536.6 ± 161.9	ns
X	Nerol oxide	1161	1158[c]	141.4 ± 35.1	727.6 ± 10.8	***
Y	Nerol	1228	1229[c]	46.5 ± 3.3	232.6 ± 45.0	***
Z	Citronellol	1230	1231[b]	149.4 ± 40.6	93.3 ± 18.0	ns
AB	Isogeraniol	1242	1248[a]	45.8 ± 10.3	228.3 ± 39.4	***
AC	Geraniol	1254	1252[c]	278.9 ± 34.3	133.0 ± 21.0	**
AD	β-Damascenone	1395	1386[c]	6890.7 ± 89.3	5030.7 ± 223.8	***
AF	Nerylacetone	1476	-	147.4 ± 27.4	127.9 ± 27.4	ns
AG	β-Farnesene	1500	1508[a]	343.2 ± 15.2	67.1 ± 7.2	***
AH	Nerolidol	-	1566[a]	432.7 ± 22.4	169.0 ± 17.6	***

AI	2,3-Dihydrofarnesol	-	1664[b]	468.1 ± 14.8	nd	***
AJ	Farnesol	-	1725[d]	1389.7 ± 224.6	240.2 ± 50.3	***
Higher alcohols						
AK	3-Methyl-1-butanol	-	740[c]	52411.2 ± 2322.8	41538.0 ± 42.5	***
AL	2-Methyl-1-butanol	-	748[c]	10894.2 ± 2638.0	7620.1 ± 1119.1	*
AM	3-Methyl-2-buten-1-ol	-	782[a]	nd	27.9 ± 6.0	*
AN	1-Hexanol	876	874[a]	84.0 ± 12.3	393.6 ± 43.1	***
AO	2-Heptanol	950	912[b]	111.6 ± 11.0	601.5 ± 83.8	***
AP	1-Heptanol	981	974[a]	52.1 ± 9.5	121.9 ± 29.3	*
AQ	Oct-1-en-3-ol	999	982[d]	175.4 ± 45.4	471.3 ± 80.6	**
AR	2-Ethylhexan-1-ol	1035	1033[a]	160.9 ± 11.9	300.1 ± 47.0	**
AS	1-Octanol	1088	1079[a]	91.8 ± 20.0	250.7 ± 20.0	***
AT	2-Phenylethanol	1123	1121[a]	17115.9 ± 1212.1	8241.9 ± 1196.2	***
AU	1-Nonanol	1176	1174[c]	129.6 ± 43.8	nd	**
AV	1-Dodecanol	1507	1479[d]	nd	169.1 ± 25.6	***
Aldehydes						
AX	Benzaldehyde	970	968[d]	379.0 ± 9.8	89.0 ± 2.7	**
AY	Octanal	1030	1006[a]	99.0 ± 6.3	173.1 ± 16.9	**
AZ	2-Phenylacetaldehyde	1073	1044[a]	93.9 ± 15.2	196.1 ± 24.8	**
BA	Nonanal	1113	-	684.9 ± 66.8	876.4 ± 7.8	ns
BC	2-Nonenal	1167	-	150.4 ± 43.1	176.5 ± 20.5	**
BD	Decanal	1215	1218[b]	286.6 ± 35.0	405.7 ± 9.7	**
BE	2,4-Dimethylbenzaldehyde	1226	-	nd	413.5 ± 27.0	***
BF	2-Decenal	1268	1266[a]	160.8 ± 33.5	330.4 ± 4.8	***
BG	Dodecanal	1446	1410[c]	75.6 ± 7.2	254.9 ± 14.7	***
Acetals						
BH	1,1-Diethoxyethane	-	726[d]	1280.9 ± 74.0	117.7 ± 26.8	***
BI	1,1-Diethoxy-3-methylbutane	961	959[b]	23.1 ± 5.9	nd	*
	Others					
BJ	2-Methoxy-3-isobutylpyrazine	1192	-	190.2 ± 22.9	nd	***
BK	Decanoic acid	1386	1379[d]	nd	376.6 ± 236.7	*
BL	Pentadecane	1561	1501[a]	272.2 ± 26.2	606.9 ± 96.7	**
BM	2,4-di-t-Butylphenol	1593	-	1895.9 ± 172.1	3329.7 ± 2465.8	ns
BN	Tetradecane	-		193.9 ± 35.6	344.7 ± 54.6	**

*, **, ***, and ns indicate significance at $P < 0.05$, 0.01, 0.001, and not significant, respectively.
GCSR: green coffee seed residue; LRIlit: linear retention index from literature; LRIcalc: calculated linear retention index. LRI data were according to [a]Alves et al. (2015) [b] Cardeal & Marriott (2009); [c] Nicolli et al. (2015); [d] Ledauphin et al. (2013).

Table 3. Relative intensity (% I), relative frequency (% F), and geometric mean (% GM) of each descriptor of 10 % and 20 % Green coffee seed residue (GCSR) spirits

	Descriptors	**10 % GCSR**			**20 % GCSR**		
		I %	F %	GM %	I %	F %	GM %
Visual	Clean	74	100	86	75	100	87
	Sparkly	75	100	87	77	100	88
Olfactory	Intensity	66	100	82	66	100	81
	Quality	68	100	82	56	100	75
	Fineness	51	86	66	41	71	54
	Coffee	46	100	68	48	100	69
	Dairy	9	14	11	-	-	-
	Vegetable	7	14	10	19	29	23
	Floral	10	14	12	-	-	-
	Cooked cabbage	-	-	-	11	29	18
	Nuts	-	-	-	7	14	10
	Hazelnut	-	-	-	9	14	11
	Almond	9	14	11	-	-	-
Gustatory	Quality	61	100	78	47	100	69
	Sweet	29	100	53	30	100	55
	Salty	20	100	45	19	100	43
	Acid	26	100	51	21	100	46
	Bitter	30	100	55	38	100	62
	Astringency	33	100	57	33	100	57
	Body	59	100	77	55	100	74
	Persistence	73	100	85	61	100	78
Global Value		69	100	83	52	100	72

ANEXO 1 – Recovery of chlorogenic acid from vinasse obtained during green coffee seed residue distillation

Recovery of chlorogenic acid from vinasse obtained during green coffee seed residue distillation

1 Introduction

Chlorogenic acids (CQAs) are phenolic compounds that naturally occur in higher plants, including coffee. They are formed by the esterification between quinic acid and hydroxycinnamic acids, mainly ferulic, and *p*-coumaric acids. CQAs are classified according to the type, number, and position of the acyl residue, being 5-caffeoylquinic acid (5-CQA) the most common in coffee (Castro et al., 2018; Gil 2017). The most reported effect of chlorogenic acid is its antioxidant activity, but its positive impact on diseases like type 2 diabetes, neurodegenerative and cardiovascular diseases have also been reported (Tomac & Seruga, 2016; Erk et al., 2014). According to the International Coffee Organization (2021), over 175 million bags (60kg) of green coffee beans were produced worldwide in 2020, making it a relevant commodity. Beyond the beverage, many products can be obtained from coffee and coffee by-products, including phenolic compounds (Budryn et al., 2009; Murthy & Naidu et al., 2012; Mussato et al., 2011; Oliveira et al., 2019).

Different methods have been used to extract, isolate, and purify phenolics compounds from coffee and coffee by-products. Solid-liquid extraction with organic solvents is the most common extraction method (Murthy & Naidu, 2012; Mussato et al., 2011). Usually, additional steps are required to remove contaminants such as sugars, fats, waxes, pigments, and alkaloids. Isolation and purification of the compound of interest are commonly done by adsorption, membrane filtration, and chromatography-based techniques (Hasbay & Galanakis, 2018). The adsorption allows the separation of target compounds from diluted solutions. Several solids can be used as sorbents, but the most used are resins and activated carbons (Ramalakshmi et al.,2011; Suárez-Quiroz et al., 2014).

Coffee oil is extracted from green coffee beans by mechanical pressing and generates a defatted coffee as by-product (Castro et al., 2018). This defatted coffee, named here as green coffee seed residue (GCSR), was used to produce a distilled beverage in a previous study (Data not published). Our study group noticed that the fermentation and distillation processes helped to extract chlorogenic acid and caffeine from the residue. In the end, we obtained a vinasse from GCSR with a considerable high concentration of 5-CQA. This study aimed to recovery chlorogenic acid (5-CQA) from the GCSR vinasse using a resin and activated

carbon. To our knowledge, this is the first report that shows the concentration of chlorogenic acid from vinasse of coffee by-products using activated carbon and resins as adsorbents.

2 Materials and methods

2.1 Green coffee seed residue vinasse

The vinasse used in this study was from a previous experiment conducted by our research group (Data not published). Solid residue produced during the oil extraction of green coffee beans was used in a distilled beverage, and the vinasse showed a significant amount of chlorogenic acid. The material was stored at -20 °C until its use. The resin experiment was carried out with centrifuged vinasse (9000 rpm, 5min, 25 °C) to facilitate resin recovery. All vinasse used in this study had its pH adjusted to 3.0 with phosphoric acid.

2.2 Material and reagents

Purifica-X C18 EC (53-75 µm) resin was from Kopp Technologies, and activated carbon (67.42% granulometry in 325 mesh) from MV química. Glacial acetic acid (J.T. Baker), methanol (Merck for HPLC analyses and Dinamica ltd. for desorption tests), and ethanol (Dinamica ltd.) were all of analytical grade for HPLC. Chlorogenic acid (5-caffeoylquinic acid) and caffeine were HPLC grade from Sigma-Andrich®. Vacuum filtrations were done using N°1 filter paper (Whatman).

2.3 Chlorogenic acid recovery with resin

The resin was hydrated through immersion in ethanol for 24 h, washed twice with deionized water, and collected in paper filters by vacuum filtration before all essays (Zhang et al., 2019). After use, resins were recovered in methanol for 24 h at 150 rpm, washed, and vacuum filtered. Successive adsorption and desorption tests were performed using recovered resins.

All adsorption assays were carried out in 15 mL of centrifuged vinasse (pH 3.0) continuously stirred at 130 rpm, 25 °C, from up to 24 hours (Mu & Sun, 2019). The resin adsorption capacity was tested at 1:5, 1:4, and 1:3 (g of dry weight resin to mL of vinasse) ratios. Each assay was tested separately, from the lowest to the highest resin ratio. The sampling interval varied as it was observed the resin saturation after each test. Chlorogenic

acid content was determined by HPLC, and the most successful ratio and time were used in the subsequent tests.

It was carried a successive adsorption test because of the fast resin saturation. The same volume of vinasse was subjected to two volumes of resin subsequently. First, 15 mL of vinasse with 1:3 (g of dry weight resin to mL of vinasse) ratio of resin was incubated for 6 h. The vinasse was separated by vacuum filtration and submitted to a new adsorption essay with the same resin ratio for 18 h. Resins from the successive adsorption test were desorbed separately in 15 mL of ethanol 70% and 92% (v/v). Essays were carried out at 150 rpm for 6h (Hui et al., 2010). The final liquid was recovered by vacuum filtration, lyophilized for 48 h, resuspended in Mili Q water, and chlorogenic acid determined by HPLC.

2.4 Chlorogenic acid recovery with activated carbon

Both adsorption and desorption essays were based in Suarez-Quiroz (2014), but with modifications. Adsorption capacity was tested with 5, 10, and 20 % (w/v) of activated carbon (AC). Falcons with 25 mL of vinasse (pH 3.0) and activated carbon were incubated in a water bath at 60 °C without agitation. Residual liquid was recovered by vacuum filtration after 30, 60, and 120 min to determine chlorogenic acid by HPLC.

Adsorption was carried out with 50 mL of vinasse and 20% (w/v) of activated carbon incubated without agitation for 1 h at 60 °C. Activated carbon was recovered by vacuum filtration and used in the desorption essays. Desorption essays tested different desorption agents and agitation methods. Treatments were: I – ethanol 70% (v/v) without agitation; II – ethanol 70% (v/v) with orbital agitation at 130 rpm; III – water pH 5 without agitation; IV – water pH 5 with orbital agitation at 130 rpm; V – ethanol 70% (v/v) with strong magnetic stirring; VI - ethanol 80% (v/v) with strong magnetic stirring; VII - ethanol 90% (v/v) with strong magnetic stirring. Treatments I to IV were sampled at 1, 3, 6, and 24 h, and treatments with magnetic stirring were samples after 1 and 2 h. Chlorogenic acid was determined by HPLC.

2.4 Chlorogenic acid determination

Chlorogenic acid (5-CQA) and caffeine content were quantified as method described by Santiago et al. (2020). Before analysis, samples were centrifuged twice at 9000 rpm for 5 min and filtered through 0.22um filters. Analyses were performed in a Shimadzu chromatographer (Shimadzu Corp., Japan) equipped with a UV-Vis detector (SPD-20A)

operating at a wavelength of 272 nm. Separations occurred in a Supelcosil LC-C18 column (4.6 x 250 mn, 5 µm) connected to a C18 pre-column (4.6 x 12.5 mm, 5µm). Elution was done with an isocratic system of 1% glacial acetic acid (Solvent A), methanol:water:acetic acid (85:14:1% v/v) (Solvent B) at a flow of 1 mL/min. Identification of compounds was performed by comparing the retention time of standards and samples peaks injected under the same conditions. Quantification was done by external calibration using solutions ranging from 0.1 to 2.5 mg/L. All samples were injected in duplicate.

3 Results and discussion

3.1 Chlorogenic acid recovery with resin

The resin adsorption capacity was tested at 1:5, 1:4, and 1:3 (g of dry weight resin to mL of vinasse) ratios, and chlorogenic acid concentration was evaluated after 3 and 24 h of incubation (Figure 1).

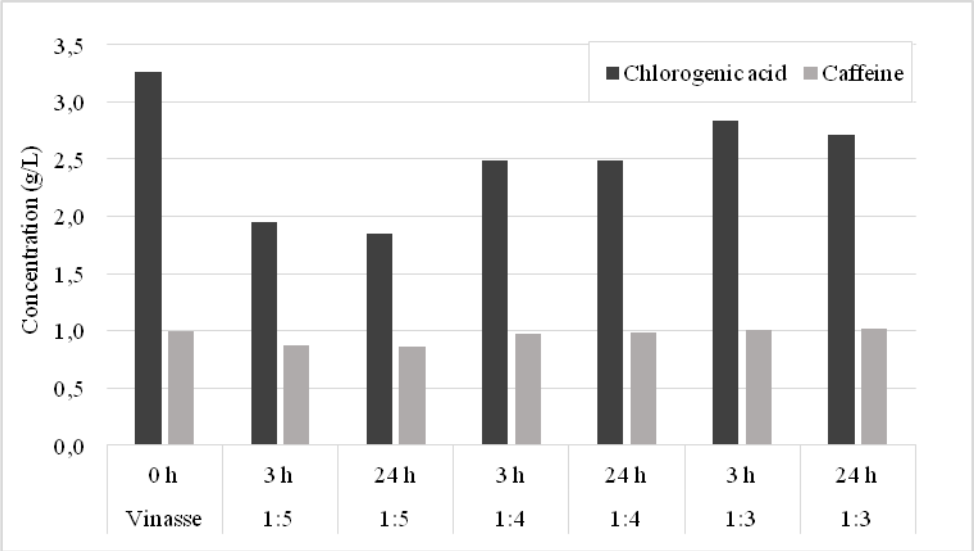


Figure 1. Adsorption of chlorogenic acid and caffeine using Purifica-X C18 resin at 1:5, 1:4, and 1:3 (g of dry weight resin to mL of vinasse) ratios

The vinasse used in the resin adsorption capacity essays had an initial concentration of 3.16 ± 0.14 g/L of chlorogenic acid and 1.02 ± 0.03 g/L of caffeine. When using the 1:5 ratio, 1.96 g/L and 1.84 g/L of chlorogenic acid were adsorbed after 3 and 24 hours of exposure, respectively. It was adsorbed 2.49 g/L of chlorogenic acid both after 3 and 24 hours of analyses when using the ratio 1:4. Last, the ratio 1:3 adsorbed 2.83 g/L and 2.71 g/L after 3

and 24 hours, respectively. The 1:3 was the most efficient because 86.9 % of the initial concentration adsorbed after only 3 h. Overall, it is possible to notice that there were no considerable differences of adsorption between the tested intervals. However, the resin was already saturated after 3 h of exposure in all tested ratios. Regarding the caffeine content: 83.9 and 82.7 % were adsorbed after 3 and 24 h at the 1:5 ratio; 93.8 and 94.2 % adsorbed after 3 and 24 h at the 1:4 ratio; 97.1 and 98.0% were adsorbed after 3 and 24 h at the 1:3 ratio. Caffeine was easily adsorbed by the resin, which may be one of the factors responsible for the fast resin saturation.

The 1:3 ratio (g of dry weight resin to mL of vinasse) was the final one to be tested due to the high solid to liquid ratio. However, it was performed a test where the same volume of vinasse was submitted twice to different resin volumes (Table 1). Overall, 1.63 ± 0.03 g/L of chlorogenic acid was desorbed during the first adsorption and 0.97 ± 0.01 g/ L after the second one. The total adsorption was 2.59 ± 0.04 g/L of chlorogenic acid, which is 84.8 % of the initial concentration. Most of the caffeine content was adsorbed during the first adsorption essay. In total, 0.96 ± 0.01 g/ L of caffeine (96.6 % of the initial concentration) was adsorbed.

Table 1 - Successive adsorption with 1:3 resin ratio (g of dry weight resin to mL of vinasse) and desorption tests using ethanol to recovery chlorogenic acid from green coffee seed residue vinasse

Treatment	92 % ethanol	70 % ethanol	92 % ethanol	70 % ethanol
Sample	Chlorogenic acid (g/L)		Caffeine (g/L)	
Vinasse	3.06	3.26	1.00	1.04
First adsorption	1.65	1.60	0.72	0.71
Second adsorption	0.97	0.96	0.24	0.24
Total adsorption	2.62	2.56	0.97	0.96
First desorption	1.09	0.75	0.50	0.40
Second desorption	0.47	0.32	0.15	0.11
Total desorption	1.56	1.07	0.65	0.51
Final recovery	9.86	11.96	4.55	4.70

The resins from the successive adsorption test were used to assess the desorption capacity of 92 and 70% (v/v) ethanol (Table 1). Overall, 1.56 g/L of chlorogenic acid and 0.65 g/L of caffeine were desorbed using 92% ethanol, which is 59.6 % and 67.0 % of the

adsorbed values, respectively. While 1.07 g/L of chlorogenic acid and 0.51 g/L of caffeine were desorbed by 70 % ethanol, which corresponds to 41.8 % and 52.9 % of the adsorbed concentration. The supernatants recovered after the desorption test were pooled, lyophilized, and the residual solid suspended in water. The final extract of the treatment with 92 % ethanol had 9.86 g/L of chlorogenic acid and 4.55 g/L of caffeine, while the 70 % ethanol had 11.96 g/L of chlorogenic acid and 4 .70 g/L of caffeine (Table 1). Despite the 70% ethanol treatment desorbed a slightly lower percentage of chlorogenic acid, the final extract had a higher concentration. Considering the initial concentration, chlorogenic acid was concentrated 3.2 times when desorbed with 92% ethanol and 3.9 times with 70% ethanol. As the values were very close, it is not possible to say which treatment was more efficient without including experimental repetitions and statistical analyses. However, both treatments were successful to concentrate the target compound. Further studies need to be done to improve the adsorption and desorption conditions.

3.1 Chlorogenic acid recovery with activated carbon

The adsorption capacity of 5, 10, and 20 % (w/v) activated carbon was tested for 30, 60, and 120 min. The initial vinasse had 3.67 ± 0.28 g/L of chlorogenic acid and 1.18 ± 0.09 g/L of caffeine. When using 5 % activated carbon, 45.3 %, 52.5 %, and 51.1 % of chlorogenic acid and 90.3 %, 93.4 %, and 89.8 % of caffeine were adsorbed after 30, 60 and 120 min, respectively. All caffeine was adsorbed using 10 % activated carbon, but low concentrations of chlorogenic acid were still detected after 30 min (0.08 g/L) and 120 min (0.01 g/L) of analysis. Note that with either 5 % or 10 % activated carbon, the highest adsorption occurred at 60 min. No traces of chlorogenic acid nor caffeine were detected using 20 % activated carbon. Therefore, the following tests were carried out with 20 % activated carbon for 60 min.

Table 2 shows the desorbed content of chlorogenic acid and caffeine using different ethanol concentrations and methods of agitation. The highest desorption using 70% ethanol without agitation was after 3 hours of immersion, which released 22.8 % of the initial chlorogenic acid. On the other hand, the highest desorption with 70% ethanol and orbital agitation was after 24 hours, which was 19.5% of initial chlorogenic acid. The treatments using magnetic stirring desorbed higher concentrations of chlorogenic acid with 1 hour of agitation. After one hour of magnetic stirring with 70 %, 80 %, and 90 % ethanol, 0.73 g/L, 0.87 g/L, and 1.49 g/L of chlorogenic acid were desorbed, respectively (Table 2). The release of chlorogenic acid increased along with the ethanol concentration, and the most successful

178 desorption was obtained using 90 % ethanol with a magnetic stirring (38.8 % of the adsorbed
179 chlorogenic acid). There is no pattern regarding the caffeine desorption. It is important to note
180 that caffeine was not released after 1 h of agitation with 90 % ethanol. It was desorbed in
181 other treatments, but no more than 0.07 g/L. Chlorogenic acid and caffeine were not desorbed
182 from the activated carbon using water (pH 5.0) with or without agitation. These treatments
183 were added expecting that the pH change would affect the ionization of the chlorogenic acid
184 and weaken the activated carbon-chlorogenic acid bond.
185
186

187 **Table 2 -** Desorption of chlorogenic acid and caffeine from activated carbon using different
188 concentrations of ethanol and agitation methods

Treatment	Time	Chlorogenic acid (g/L)	Caffeine (g/L)
Vinasse	-	3.67 ± 0.28	1.18±0.09
Ethanol 70% without agitation	1h	0.22	0.03
	3h	0.74	0.05
	6h	0.27	0.02
	24h	0.21	0.01
Ethanol 70% with orbital agitation	1h	0.31	0.01
	3h	0.39	0.02
	6h	0.57	0.02
	24h	0.63	0.01
Ethanol 70% with magnetic stirring	1h	0.73	0.06
	2h	0.14	0.01
Ethanol 80% with magnetic stirring	1h	0.87	0.07
	2h	0.22	0.01
Ethanol 90% with magnetic stirring	1h	1.49	n.d.
	2h	0.33	0.03

189
190

3 References

Budryn, G., Nebesny, E., Podsędek, A., Żyżelewicz, D., Materska, M., Jankowski, S., & Janda, B. (2009). Effect of different extraction methods on the recovery of chlorogenic acids, caffeine and Maillard reaction products in coffee beans. *European Food Research and Technology*, *228*(6), 913-922.

Castro, A. C. C. M., Oda, F. B., Almeida-Cincotto, M. G. J., Davanço, M. G., Chiari-Andréo, B. G., Cicarelli, R. M. B., ... & Isaac, V. L. B. (2018). Green coffee seed residue: A sustainable source of antioxidant compounds. Food chemistry, 246, 48-57.

Erk, T., Renouf, M., Williamson, G., Melcher, R., Steiling, H., & Richling, E. (2014). Absorption and isomerization of caffeoylquinic acids from different foods using ileostomist volunteers. *European journal of nutrition*, *53*(1), 159-166.

Gil, M., & Wianowska, D. (2017). Chlorogenic acids–their properties, occurrence and analysis. *Annales Universitatis Mariae Curie-Sklodowska, sectio AA–Chemia*, *72*(1), 61.

Hasbay, I., & Galanakis, C. M. (2018). Recovery technologies and encapsulation techniques. *Polyphenols: Properties, Recovery, and Applications*, 233-264.

Hui, Z., Jun, W., Jing, J., Ji, L., Xiuquan, L., & Dingqiang, L. (2010). Enrichment and purification of total chlorogenic acids from tobacco waste extract with macroporous resins. *Separation Science and Technology*, *45*(6), 794-800.

ICO (International Coffee Organization). (2020). Total coffee production from 1990 to present. Available from: http://www.ico.org/historical/1990%20onwards/PDF/1a-total-production.pdf. Acessed October 12 2020.

Mu, T. H., & Sun, H. N. (2019). Sweet potato leaf polyphenols: preparation, individual phenolic compound composition and antioxidant activity. In *Polyphenols in Plants* (pp. 365-380). Academic Press.

Murthy, P. S., & Naidu, M. M. (2012). Recovery of phenolic antioxidants and functional compounds from coffee industry by-products. *Food and Bioprocess Technology*, *5*(3), 897-903.

Mussatto, S. I., Ballesteros, L. F., Martins, S., & Teixeira, J. A. (2011). Extraction of antioxidant phenolic compounds from spent coffee grounds. *Separation and Purification Technology*, *83*, 173-179.

Oliveira, É. R., Silva, R. F., Santos, P. R., & Queiroz, F. (2019). Potential of alternative solvents to extract biologically active compounds from green coffee beans and its residue from the oil industry. Food and bioproducts processing, 115, 47-58.

Ramalakshmi, K., Hithamani, G., Asha, K. R., & Jagan Mohan Rao, L. (2011). Separation and characterization of chlorogenic acid-rich conserves from green coffee beans and their radical scavenging potential. *International journal of food science & technology*, *46*(1), 109-115.

Santiago, W. D., Teixeira, A. R., de Andrade Santiago, J., Cláudia, A., Lopes, A., Brandão, R. M., ... & Resende, M. L. V. (2020). Development and validation of chromatographic methods to quantify organic compounds in green coffee (Coffea arabica) beans. *Australian Journal of Crop Science*, *14*(8), 1275-1282.

Suarez-Quiroz, M. L., Campos, A. A., Alfaro, G. V., González-Ríos, O., Villeneuve, P., & Figueroa-Espinoza, M. C. (2014). Isolation of green coffee chlorogenic acids using activated carbon. *Journal of Food Composition and Analysis*, *33*(1), 55-58.

Tomac, I., & Šeruga, M. (2016). Electrochemical properties of chlorogenic acids and determination of their content in coffee using differential pulse voltammetry. *Int. J. Electrochem. Sci*, *11*, 2854-2876.

Zhang, X., Zeng, L., Sun, T., Liu, X., Hou, J., Ma, Q., ... & Chen, S. (2019). Purification of chlorogenic acid from Heijingang potatoes and evaluation of its binding properties to recombinant human serum albumin. *Journal of Chromatography B*, *1110*, 87-93.

ANEXO 2 – Patente

Pedido de depósito de patente “Solução atrativa aplicada ao controle da broca-de-café”

Patente apresentada conforme permissão do INPI – Instituto Nacional de Propriedade Industrial

INSTITUTO NACIONAL DA PROPRIEDADE INDUSTRIAL

23/09/2021 870210087773 15:20

29409161939295920

Pedido nacional de Invenção, Modelo de Utilidade, Certificado de Adição de Invenção e entrada na fase nacional do PCT

Número do Processo: BR 10 2021 018989 4

Dados do Depositante (71)

Depositante 1 de 2

Nome ou Razão Social:	UNIVERSIDADE FEDERAL DE LAVRAS
Tipo de Pessoa:	Pessoa Jurídica
CPF/CNPJ:	22078679000174
Nacionalidade:	Brasileira
Qualificação Jurídica:	Instituição de Ensino e Pesquisa
Endereço:	CAMPUS DA UFLA CX POSTAL 3037
Cidade:	Lavras
Estado:	MG
CEP:	37200-000
País:	Brasil
Telefone:	3829.1127
Fax:	3829.1127
Email:	nintec@nintec.ufla.br

PETICIONAMENTO ELETRÔNICO Esta solicitação foi enviada pelo sistema Peticionamento Eletrônico em 23/09/2021 às 15:20, Petição 870210087773

Depositante 2 de 2

Nome ou Razão Social:	FUNDAÇÃO DE AMPARO À PESQUISA DE MINAS GERAIS
Tipo de Pessoa:	Pessoa Jurídica
CPF/CNPJ:	21949888000183
Nacionalidade:	Brasileira
Qualificação Jurídica:	Órgão Público
Endereço:	AV. JOSÉ CÂNDIDO DA SILVEIRA, 1500, BAIRRO HORTO
Cidade:	Belo Horizonte
Estado:	MG
CEP:	31035-536
País:	BRASIL
Telefone:	
Fax:	
Email:	

Dados do Pedido

Natureza Patente:	10 - Patente de Invenção (PI)
Título da Invenção ou Modelo de Utilidade (54):	SOLUÇÃO ATRATIVA APLICADA AO CONTROLE DA BROCA-DE-CAFÉ
Resumo:	A presente invenção conjuga a produção de um destilado obtido através da fermentação de grãos de café que pode ser aplicado como solução atrativa da broca-de-café no campo, proporcionando assim o monitoramento e controle de uma praga sem necessidade da utilização de inseticidas e compostos tóxicos em campo. A dita solução atrativa tem grãos de café como matéria-prima e é produzida através da fermentação e destilação de uma solução de grãos de café, leveduras e açúcares. A atração da broca-de-café é possível pois a solução aromática possui composição volátil similar ao café, com a presença de ésteres, álcoois superiores e terpenos similares aos do fruto e grão verde de café. O destilado de grãos de café pode ou não ser adicionado de outros álcoois e produtos já utilizados para atração de broca-de-café.

PETICIONAMENTO ELETRÔNICO	Esta solicitação foi enviada pelo sistema Peticionamento Eletrônico em 23/09/2021 às 15:20, Petição 870210087773