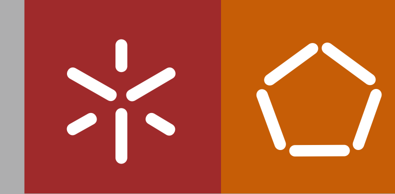
Ercília Maria dos Santos Machado
EXTRACTION OF VALUABLE COMPOUNDS FROM SPENT COFFEE GROUNDS AND ITS USE IN THE PREPARATION OF FERMENTED AND DISTILLED BEVERAGES

UMinho|2019

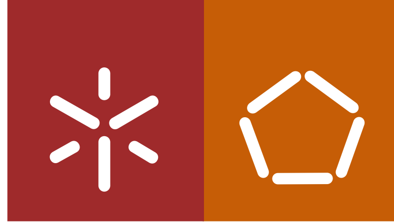


Universidade do Minho
Escola de Engenharia

Ercília Maria dos Santos Machado

EXTRACTION OF VALUABLE COMPOUNDS FROM SPENT COFFEE GROUNDS AND ITS USE IN THE PREPARATION OF FERMENTED AND DISTILLED BEVERAGES

setembro de 2019



Universidade do Minho
Escola de Engenharia

Ercília Maria dos Santos Machado

EXTRACTION OF VALUABLE COMPOUNDS FROM SPENT COFFEE GROUNDS AND ITS USE IN THE PREPARATION OF FERMENTED AND DISTILLED BEVERAGES

Tese de Doutoramento em Engenharia Química e Biológica

Trabalho efetuado sob a orientação do
Professor Doutor José António Couto Teixeira
e da
Professora Doutora Solange Inês Mussatto Dragone

setembro de 2019

DIREITOS DE AUTOR E CONDIÇÕES DE UTILIZAÇÃO DO TRABALHO POR TERCEIROS

Este é um trabalho académico que pode ser utilizado por terceiros desde que respeitadas as regras e boas práticas internacionalmente aceites, no que concerne aos direitos de autor e direitos conexos.

Assim, o presente trabalho pode ser utilizado nos termos previstos na licença abaixo indicada.

Caso o utilizador necessite de permissão para poder fazer um uso do trabalho em condições não previstas no licenciamento indicado, deverá contactar o autor, através do RepositóriUM da Universidade do Minho.

Licença concedida aos utilizadores deste trabalho

Atribuição-NãoComercial-SemDerivações
CC BY-NC-ND

https://creativecommons.org/licenses/by-nc-nd/4.0/

AGRADECIMENTOS

Para cumprir mais uma etapa da minha vida, um desafio diferente do que já tinha passado até hoje, um doutoramento, foi necessário muitos sacrifícios, empenho e dedicação, mas acima de tudo um prazer por desenvolver este trabalho e disciplina, para conjugar com as outras atividades na minha vida. Sem dúvida que houve momentos bons e menos bons, mas ao olhar para o resultado final e ver que, orgulhosamente, se converteu numa grande vitória. Nunca poderei deixar de agradecer do fundo do meu coração a todas as pessoas que fizeram parte deste trabalho, ao longo dos vários anos do programa doutoral.

Aos meus orientadores, Professor Doutor José António Couto Teixeira e Doutora Solange Inês Mussatto Dragone, que dedicaram o seu tempo e conhecimento a este trabalho, assim como pelos seus ensinamentos, ideias, conselhos e, por me terem incentivado e acreditado nas minhas capacidades.

Um agradecimento especial à Professora Doutora Maria Gabriela Bernardo–Gil, à Professora Doutora Mar Vilanova de la Torre e ao Professor Doutor José Maria Oliveira que também ajudaram na concretização deste projeto.

Aos meus colegas e amigos do Laboratório de Fermentações (CEB, UM), um muito obrigado pelo apoio demonstrado e também pelos momentos de diversão proporcionados durante todos estes anos de convivência no laboratório, não esquecendo a amizade que contribuiu para um ótimo ambiente de trabalho. Assim como a todas as pessoas que de uma forma ou outra, contribuíram à sua maneira na realização e conclusão deste projeto.

À Universidade do Minho e ao Departamento de Desporto da universidade, por tudo o que fizeram por mim para concluir esta etapa da minha vida. Sem eles, não era possível. Foram compreensíveis e, acima de tudo por me terem apoiado nos momentos mais difíceis, nos quais era necessário conciliar a vida académica com a vida de desportista.

Por último, agradeço à minha mãe, irmã e cunhado por todo o apoio que me prestaram, assim como pela paciência e compreensão demonstrada na concretização desta etapa da minha vida.

Este estudo foi financiado pela Fundação Portuguesa de Ciência e Tecnologia (FCT) no âmbito do financiamento estratégico da unidade UID/BIO/04469/2013 e COMPETE 2020 (POCI-01-0145-FEDER-006684) e operação da BioTecNorte (NORTE -01-0145-FEDER-000004) financiado pelo Fundo Europeu de Desenvolvimento Regional no âmbito do Norte2020 - Programa Operacional Regional do Norte.

STATEMENT OF INTEGRITY

I hereby declare having conducted this academic work with integrity. I confirm that I have not used plagiarism or any form of undue use of information or falsification of results along the process leading to its elaboration.

I further declare that I have fully acknowledged the Code of Ethical Conduct of the University of Minho.

EXTRAÇÃO DE COMPOSTOS DE VALOR ACRESCENTADO A PARTIR DA BORRA DE CAFÉ E A SUA UTILIZAÇÃO NA ELABORAÇÃO DE BEBIDAS FERMENTADAS E DESTILADAS

O café é uma das bebidas mais consumidas em todo o mundo, obtida a partir da mistura de água quente com café em pó. Durante o processamento do café solúvel são gerados resíduos, as borras de café (SCG), que possuem uma composição química rica em açúcares e compostos fenólicos antioxidantes que podem ter potencial como matéria–prima nas indústrias de alimentos e bebidas. O principal objetivo do presente projeto foi a extração de compostos fenólicos antioxidantes a partir da borra de café e avaliar a sua aplicação na produção de bebidas fermentadas e destiladas.

Inicialmente, a extração dos compostos fenólicos antioxidantes foi realizada utilizando a extração com CO_2 supercrítico. Para as condições que permitiram a maior extração de fenólicos, as quantidades de compostos fenólicos extraídos, flavonoides e proteínas foram de 0.36 mg GAE/g SCG, 1.55 mg QE/g SCG e 1.15 mg BSA/g SCG, respetivamente. Relativamente à atividade antioxidante, os valores mais altos obtidos foram de 0.0050 mM Fe(II)/g SCG (método de FRAP) e de 2.58 mg α–tocoferol/g SCG (método da capacidade antioxidante total). Os valores obtidos permitiram concluir que a extração supercrítica de CO_2 não foi a técnica mais eficiente para extrair compostos antioxidantes fenólicos das borras de café.

Numa segunda etapa, foi avaliada a recuperação dos compostos fenólicos antioxidantes através da extração assistida por microondas, utilizando a água como solvente. Para as condições que permitiram a máxima extração de compostos fenólicos antioxidantes, o valor máximo dos compostos fenólicos extraídos, flavonoides, proteínas e açúcares foi de 32.33 mg GAE/g SCG, 4.86 mg QE/g SCG, 31.82 mg BSA/g SCG e 49.78 mg Glucose/g SCG, respetivamente. Relativamente à atividade antioxidante os valores mais altos obtidos foram de 0.2692 mM Fe(II)/g SCG (método de FRAP) e de 48.45 mg α–tocoferol/g SCG (método da capacidade antioxidante total). Estes resultados demonstram a eficácia da extração assistida por microondas na extração de compostos fenólicos das borras de café.

Numa fase final, foram realizadas quatro fermentações alcoólicas com os extratos recuperados através da extração assistida por microondas, sob condições ótimas, com a adição de sacarose ao meio de cultura. Das quatro fermentações foram produzidas duas bebidas fermentadas (com uma percentagem volumétrica de etanol entre 10.4 % e 10 %) e duas bebidas destiladas, após a destilação do caldo fermentado (com uma percentagem volumétrica de etanol entre 38.1 % e 40.2 %). As bebidas foram submetidas a uma análise sensorial, mostrando que a bebida fermentada e destilada obtida pela incorporação das borras de café no meio de fermentação possui características agradáveis, diferentes das bebidas até o momento disponíveis no mercado.

Palavras-chave: Borra de café, Extração com fluidos supercríticos, Extração assistida por microondas, Compostos fenólicos, Bebidas fermentadas e destiladas.

EXTRACTION OF VALUABLE COMPOUNDS FROM SPENT COFFEE GROUNDS AND ITS USE IN THE PREPARATION OF FERMENTED AND DISTILLED BEVERAGES

Coffee is one of the most consumed beverages in the world, obtained from mixing hot water with coffee powder. During the processing of soluble coffee are generated residues, spent coffee grounds (SCG), which have a chemical composition rich in sugars and phenolic antioxidant compounds that may have potential as a raw material in the food and beverage industries. The main objective of this project was the extraction of phenolic antioxidant compounds from the spent coffee grounds and to evaluate its application in the production of fermented and distilled beverages.

Initially, the extraction of the antioxidant phenolic compounds was performed using supercritical CO_2 extraction. For the conditions that allowed for the highest extraction of phenolics, the amounts of extracted phenolic compounds, flavonoids and proteins were 0.36 mg GAE/g SCG, 1.55 mg QE/g SCG and 1.15 mg BSA/g SCG, respectively. Regarding the antioxidant activity, the highest values obtained were 0.0050 mM Fe (II)/g SCG (FRAP method) and 2.58 mg α–tocopherol/g SCG (antioxidant capacity method). The obtained values allowed to conclude that supercritical CO_2 extraction was not the most efficient technique to extract phenolic antioxidant compounds from the spent coffee grounds.

In a second phase, the recovery of phenolic antioxidant compounds through microwave–assisted extraction was evaluated, using water as solvent. For the conditions that allowed for the maximum extraction of phenolic antioxidant compounds, the maximum value of extracted phenolic compounds, flavonoids, proteins and sugars was 32.33 mg GAE/g SCG, 4.86 mg QE/g SCG, 31.82 mg BSA/g SCG and 49.78 mg Glucose/g SCG, respectively. Regarding the antioxidant activity, the highest values obtained were 0.2692 Mm Fe (II)/g SCG (FRAP method) and 48.45 mg α–tocopherol/g SCG (antioxidant capacity method). These results demonstrate the effectiveness of microwave–assisted extraction on the extraction of phenolic compounds from the spent coffee grounds.

In a final stage, were carried out four alcoholic fermentations with extracts recovered by microwave–assisted extraction, under optimum conditions, with the addition of sucrose to the culture medium. Out of these four alcoholic fermentations two fermented beverages were produced (with a volumetric percentage of ethanol between 10.4 % and 10 %) and two distilled beverages, after the distillation of the fermented broth (with a volume percentage of ethanol between 38.1 % and 40.2 %).Beverages were submitted to sensorial analysis, being shown that the fermented and distilled beverage obtained by the incorporation of the spent coffee grounds in the fermentation medium have pleasant characteristics, different from the drinks up till now available in the market.

Keywords: Spent coffee grounds, Supercritical fluid extraction, Microwave–assisted extraction, Phenolic compounds, Fermented and distilled beverage, Sensory descriptive analysis.

TABLE OF CONTENTS

CHAPTER 1 ..... **1**

MOTIVATION AND OBJECTIVES **1**

1. Motivation and objectives **2**

1.1. Motivation 2

1.2. Objectives 3

1.3. References 4

CHAPTER 2 ..... **6**

LITERATURE REVIEW **6**

2. Literature Review **7**

2.1. Coffee production 7

2.2. Spent coffee grounds 9

2.3. Phenolic compounds 11

2.4. Extraction techniques 12

2.4.1. Supercritical fluid extraction (SFE) 13

2.4.2. Microwave–assisted extraction (MAE) 16

2.5. Fermented and distilled beverages 18

2.5.1. Production of fermented and distilled beverages 19

2.6. Volatile compounds 26

2.6.1. Volatile compounds of coffee 29

2.7. References 30

CHAPTER 3 ..... **41**

EXTRACTION OF VALUE–ADDED COMPOUNDS FROM SPENT COFFEE GROUNDS USING SUPERCRITICAL FLUID EXTRACTION **41**

Abstract **42**

3. Introduction **43**

3.1. Material and methods 44

3.1.1. Raw materials and chemicals 44

3.1.2. Supercritical fluid extraction 44

3.1.3. Analytical methodology 47

3.1.3.1. Determination of total phenolic compounds 47
3.1.3.2. Determination of proteins 47
3.1.3.3. Determination of reducing sugars content 47
3.1.3.4. Determination of total flavonoid content 48
3.1.3.5. Determination of antioxidant activity 48
3.2. Results and discussion 49
3.3. Conclusions 54
3.4. References 56
CHAPTER 4 58
EXTRACTION OF VALUE–ADDED COMPOUNDS FROM SPENT COFFEE GROUNDS USING MICROWAVED–ASSISTED EXTRACTION 58
Abstract 59
4. Introduction 60
4.1. Material and methods 61
4.1.1. Raw materials and chemicals 61
4.1.2. Extraction procedure 61
4.1.3. Analytical methodology 61
4.1.3.1. Determination of total phenolic compounds 61
4.1.3.2. Determination of proteins 62
4.1.3.3. Determination of reducing sugars content 62
4.1.3.4. Determination of total flavonoid content 63
4.1.3.5. Determination of antioxidant activity 63
4.1.3.6. Statistical analyses 64
4.2. Results and discussion 66
4.3. Conclusions 80
4.4. References 81
CHAPTER 5 83
CHEMICAL COMPOSITION AND SENSORY PROFILE OF FERMENTED AND DISTILLED BEVERAGES FROM SPENT COFFEE GROUNDS 83
Abstract 84
5. Introduction 85

5.1. Material and methods 86
5.1.1. Raw material and chemicals 86
5.1.2. Extraction process and fermentation medium 87
5.1.3. Microorganism and inoculum 87
5.1.4. Fermentation and distillation conditions 88
5.1.5. Analytical methods 90
5.1.6. Sensory analysis 91
5.2. Results and discussion 92
5.2.1. Beverage production 92
5.2.2. Volatile composition of fermented and spirit beverages 96
5.2.3. Sensory analysis of fermented and spirit beverages 102
5.3. Conclusions 105
5.4. References 106
CHAPTER 6 109
CONCLUSIONS AND SUGGESTIONS FOR FUTURE WORK 109
6. General conclusions and future perspectives 110
6.1. Conclusions 110
6.2. Guidelines for future work 111

LIST OF GENERAL NOMENCLATURE AND ABBREVIATIONS

Abbreviation/Symbol	Description
ASE	Accelerated solvent extraction
bC	Before Christ
BSA	Bovine serum albumin
C	Concentration
CSE	Conventional solvent extraction
CE	Conventional extraction
CEB	Centre of biological engineering
CS	Coffee silverskin
ρ	Density
$\rho_{critical}$	Density critical
D	Diffusion coefficient
D	Distilled
D1	Distilled beverage 1
D2	Distilled beverage 2
DNS	3,5–dinitrosalicylic acid
μ	Dynamic viscosity
F	Fermented
F	Frequency
F1	Fermented beverage 1
F2	Fermented beverage 2
FID	Flame ionization detector
FLA	Flavonoids
FRAP	Ferric reducing antioxidant power
GAE	Gallic acid equivalent
GM	Geometric mean
GS-MS	Gas chromatography–mass spectrometry
HPLC	High performance liquid chromatography

I	Intensity
ICO	International coffee organization
ISO	International organization for standardization
L	Linear
LLE	Liquid–liquid extraction
MAE	Microwave–assisted extraction
$P_{critical}$	Pressure critical
PRO	Proteins
QE	Quercetin
SCFs	Supercritical fluids
SCG	Spent coffee ground
SCGs	Spent coffee grounds
SD	Standard deviation
SFE	Supercritical fluid extraction
SOX	Soxhlet
SPE	Solid phase extraction
SPME	Solid phase microextraction
SUG	Sugars
TAC	Total antioxidant activity
$T_{critical}$	Temperature critical
TP	Total phenolics
UAE	Ultrasound–assisted extraction
USE	Ultrasonication extraction
UM	University of Minho

LIST OF FIGURES

Figure 2.1. Appearance and structures of a coffee bean (Teixeira *et al.*, 2014). 8

Figure 2.2. Spent coffee grounds. 9

Figure 2.3. Pressure–temperature phase diagram for CO_2 (Sapkale *et al.*, 2010). 15

Figure 2.4. Schematic of a simple alembic: 1 – boiler; 2 – dome; 3 – tube of condensing; 4 – cooler; 5 – steam pipe; 6 – input of wine; 7 – discharge of wine; 8 – valve equalizing the pressure; 9 – channeling of distillate (Filho, 2010). 25

Figure 3.1. Schematic diagram of the experimental apparatus used for the extractions assays. 44

Figure 3.2. Supercritical fluid extraction installation: a) Behind is possible to see the ice bath and haskell pump and in front is possible to see the thermostated bath and vacuum pump; b) Extraction installation and the Bottle of CO_2; c) Heat exchanger, extractor and thermostated bath; d) Vessel of extractor; e) Extraction installation before extraction; f) First collector, second collector, thermometer and ariete compact gas flow meter. 45

Figure 3.3. Accumulated mass of extract as a function of extraction time. 50

Figure 4.1. Pareto chart for the effects of power (X1), solvent/solid ratio (X2), extraction time (X3), pressure (X4) and the interaction between them, during microwave–assisted extraction of spent coffee grounds, on the total phenolics (a), proteins (b), sugars (c), flavonoids (d), antioxidant activity - FRAP (e), and total antioxidant capacity (f) contents in the produced extracts. L and Q correspond to the effects at linear and quadratic levels, respectively. 71

Figure 4.1. (Conclusion) Pareto chart for the effects of power (X1), solvent/solid ratio (X2), extraction time (X3), pressure (X4) and the interaction between them, during microwave–assisted extraction of spent coffee grounds, on the total phenolics (a), proteins (b), sugars (c), flavonoids (d), antioxidant activity - FRAP (e), and total antioxidant capacity (f) contents in the produced extracts. L and Q correspond to the effects at linear and quadratic levels, respectively. 72

Figure 4.2. Response surfaces described by the models representing the total phenolics (a), proteins (b), sugars (c), flavonoids (d), antioxidant activity – FRAP (e) and total antioxidant capacity (f) of spent coffee grounds extracts obtained by microwave–assisted extraction. 75

Figure 4.2. (Cont.) Response surfaces described by the models representing the total phenolics (a), proteins (b), sugars (c), flavonoids (d), antioxidant activity – FRAP (e) and total antioxidant capacity (f) of spent coffee grounds extracts obtained by microwave–assisted extraction. 76

Figure 4.2. (Conclusion) Response surfaces described by the models representing the total phenolics (a), proteins (b), sugars (c), flavonoids (d), antioxidant activity – FRAP (e) and total antioxidant capacity (f) of spent coffee grounds extracts obtained by microwave–assisted extraction........ 77

Figure 4.3. Optimum region by overlaying the curves of the different responses as a function of the solvent/solid ratio and time used for spent coffee grounds extraction. 78

Figure 5.1. Equipment used for the fermentations assays in the first method..................................... 88

Figure 5.2. Equipment used for the distillation of fermented broth. 89

Figure 5.3. Sugars consumption (a) and cell growth (b) of *S. cerevisiae* (RL–11) from spent coffee ground extract using the first method of fermentation.. 93

Figure 5.4. Sugars consumption (a) and cell growth (b) of *S. cerevisiae* (RL–11) from spent coffee ground extract using the second method of fermentation. 94

Figure 5.5. Sensory profile (*GM* > 50 %) of fermented and distillate samples. (V – visual analysis; O – olfactory analysis; G – gustatory analysis). 104

LIST OF TABLES

Table 2.1. Annual worldwide coffee production (2014–2017) ... 7

Table 2.2. Chemical composition of spent coffee grounds (Mussatto *el al.*, 2011b) ... 10

Table 2.3. Properties of supercritical fluids, gases and liquids (Teixeira *et al.*, 2014) ... 14

Table 2.4. Critical properties (temperature, pressure and density) of various solvents (Sapkale *et al.*, 2010) ... 14

Table 2.5. Volatile compounds of roasted coffee (Trugo *et al.*, 1999; Trugo *et al.*, 2000) ... 29

Table 3.1. Conditions of pressure, temperature used in the extractions assays, and CO_2 density (ρ CO_2) for the respective conditions ... 46

Table 3.2. Total phenolic compounds, proteins and flavonoids contents in the different extracts obtained by SFE of SCG ... 51

Table 3.3. Antioxidant activity of the different extracts obtained by SFE of SCG ... 52

Table 4.1. Experimental conditions used for MAE of antioxidant phenolic compounds from SCG according to a 2^4 full experimental design. Real and coded values of the operational variables power (x1), solvent/solid ratio (x2), extraction time (x3) and pressure (x4) ... 65

Table 4.2. Results obtained for the different analyzes: Total phenolics, total proteins content and reducing sugars content for the different extracts obtained from SCG ... 67

Table 4.3. Results obtained for the different analyzes: FRAP and total antioxidant capacity for the different extracts obtained from SCG ... 70

Table 4.4. Polynomial equations fitted to the experimental data of antioxidant phenolics compounds of spent coffee grounds extracts, and their respective regression coefficients R^2 ... 74

Table 4.5. Result obtained in the assay for validation of the condition optimized for extraction of antioxidant phenolic compounds from spent coffee grounds ... 79

Table 5.1. Portions collected during the distillations of the first fermented SCG extract (D1) ... 95

Table 5.2. Portions collected during the distillations of the second fermented SCG extract (D2) ... 96

Table 5.3. Concentration (*C*) and standard deviation (*SD*) of major volatile compounds identified and quantified in fermented and distillate samples ... 97

Table 5.4. Concentration (*C*) and standard deviation (*SD*) of minor volatile compounds identified and quantified in fermented and distillate samples ... 99

Table 5.4. (Conclusion) Concentration (*C*) and standard deviation (*SD*) of minor volatile compounds identified and quantified in fermented and distillate samples 100

Table 5.5. Intensity (*I*), frequency (*F*), and geometric mean (*GM*) to each descriptor of spent coffee grounds (SCG) fermented (F) and distillated (D) 103

CHAPTER 1

MOTIVATION AND OBJECTIVES

1. Motivation and objectives

1.1. Motivation

Coffee is the most consumed beverage in the world being served more than 400 billion cups per year. Coffee beverage is formed by more than 700 compounds, including alkaloids like caffeine, minerals, chlorogenic acids, aliphatic acids, lipids, carbohydrates and amino acids (Mussatto *el al.*, 2011; Sobolík *et al.*, 2002). The coffee grain has not relevant nutritional value by itself, but as a drink produced by extraction with hot water is largely consumed mainly due to the pleasure aroma and flavor that contains. The characteristic aroma of the coffee is caused by the presence of volatile compounds (Trugo *et al.*, 2000).

Large amount of wastes are generated in the coffee industry every year, being the spent coffee grounds (SCG), a solid residue obtained in the preparation of instant coffee, one of the wastes obtained in larger amount. SCG does not have commercial value, and is currently discarded as conventional solid waste or used as fuel feeding boilers in industry. It's formed by carbohydrates, lipids and proteins, and also contains caffeine, tannins and polyphenols but in lesser amounts (Bravo *et al.*, 2013; Couto *et al.*, 2009; Vegro *et al.*, 2006). The bioactive compounds are products of value–added, emerging as an alternative source for obtaining natural antioxidants (Murthy *et al.*, 2012). Therefore, the extraction of value–added compounds from SCG could be an interesting alternative for value addition of this agro–industrial waste (Andrade *et al.*, 2012; Zuorro *et al.*, 2011).The quality of the extracts varies according to the technique and conditions used for extraction (Andrade *et al.*, 2017a).

Different techniques can be used for the extraction of compounds from the agro–industrial residues. Among them, supercritical fluid extraction (SFE) and microwave–assisted extraction (MAE) should be considered, once they possess several advantages comparing to conventional extraction methods (Andrade *et al.*, 2017b; Moreira *et al.*, 2017). SFE allows the processing of materials at low temperatures, which is especially suitable when thermo–sensitive compounds are present, preventing its degradation, what would compromise the quality of the final product. Another advantage is the possibility to recover the solvent after the extraction process (Reverchon *et al.*, 2008). The technique of MAE significantly decreases the time of extraction process and allows obtaining the extraction high yields in several plants (Kumar *et al.*, 2018; Martins *et al.*, 2010).

The beverage industry has shown a high interest in the development of new products from different raw materials. In this way, the development of products with flavor extracts and natural flavors has received great emphasis due to restrictions on the use of synthetic chemicals in foods and beverages (Coelho *et al.*, 2015; Raventós *et al.*, 2002). The production of fermented and distilled beverages from the SCG extracts recovered by the SFE and MAE processes, arise as novelty in terms of product, as, nowadays, such beverages, produced from the residue of coffee beans, do not exist on the market If successful, the application of these compounds in beverage production, will open doors for future developments of different and innovative products, which is of great interest, not only for the coffee industry, but also for other industrial sectors.

1.2. Objectives

The main goal of this work program is to extract the valuable compounds, including sugars and phenolic compounds present in SCG and evaluate its application in the development of fermented and distilled beverages as a way to add value to a product with, so far, has been underused and shows a reduced economic interest. In summary, the objectives of this work are:

- Evaluate the extraction of value–added compounds from spent coffee grounds by supercritical fluid extraction and determine the operating conditions that maximize the release of the compounds.

- Evaluate the extraction of value–added compounds from spent coffee grounds by microwave–assisted extraction and determine the operating conditions that maximize the release of the compounds.

- Use the extracts obtained (in the optimized conditions) in fermentation processes to obtain fermented and distilled beverages, which will be characterized chemically and submitted to a sensory analysis to verify their aroma and flavor characteristics as well as their consumer acceptance.

1.3. References

Andrade, K. S., Gonçalves, R. T., Maraschin, M., Ribeiro–do–Valle, R. M., Martínez, J., Ferreira, S. R. S. (2012). Supercritical fluid extraction from spent coffee grounds and coffee husks: antioxidant activity and effect of operational variables on extract composition. *Talanta*, 88, 544–52.

Andrade, K. S., Poncelet, D., Ferreira, S. R. S. (2017a). Sustainable extraction and encapsulation of pink pepper oil. *Journal of Food Engineering*, 204, 38–45.

Andrade, K. S., Trivellin, G., Ferreira, S. R. S. (2017b). Piperine–rich extracts obtained by high pressure methods. *The Journal of Supercritical Fluid*, 128, 370–377.

Bravo, J., Monente, C., Juániz, I., de Peña, M. P., Cid, C. (2013). Influence of extraction process on antioxidant capacity of spent coffee. *Food Research International*, 50(2), 610–616.

Coelho, E., Vilanova, M., Genisheva, Z., Oliveira, J. M., Teixeira, J. A., Domingues, L. (2015). Systematic approach for the development of fruit wines from industrially processed fruit concentrates, including optimization of fermentation parameters, chemical characterization and sensory evaluation. *Food Science and Technology (LWT)*, 62, 1043–1052.

Couto, R. M., Fernandes, J., Silva, M. D. R. G., Simões, P. C. (2009). Supercritical fluid extraction of lipids from spent coffee grounds. *The Journal of Supercritical Fluids*, 59, 159–166.

Kumar, R. C., Benal, M. M., Prasada, B. D., Krupashankarac, M. S., Kulkarnic, R. S., Siddaligaswamy, N. H. (2018). Microwave–assisted extraction of oil from pongamia pinnata seeds. *Materials Today: Proceedings*, 5, 2960–2964.

Martins, S., Aguilar, C. N., de la Garza–Rodriguez, I., Mussatto, S. I., Teixeira, J. A. (2010). Kinetic study of nordihydroguaiaretic acid recovery from Larrea tridentata by microwave–assisted extraction. *Journal of Chemical Technology and Biotechnology*, 85, 1142–1147.

Moreira, M. M., Barroso, M. F., Boeykens, A., Withouck, H., Morais, S., Delerue–Matos, C. (2017). Valorization of apple tree wood residues by polyphenols extraction: Comparison between conventional and microwave–assisted extraction. *Industrial Crops & Products*, 104, 210–220.

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

Raventós, M., Duarte, S., Alarcon, R. (2002). Application and possibilities of supercritical CO_2 extraction in food processing industry: an overview. *Food Science and Technology International*, 8, 269–284.

Reverchon, E., I. De Marco. (2008). Essential oils extraction and fractionation using supercritical fluids, in *Supercritical fluid extraction of Nutraceuticals and bioactive compounds*. Ed. J. L. Martinez, 305–335. Boca Raton, FL: CRC Press, Taylor & Francis Group.

Sobolík, V., Zitný, R., Tovcigrecko, V., Delgado, M., Allaf, K. (2002). Viscosity and electrical conductivity of concentrated solutions of soluble coffee. *Journal of Food Engineering*, 51, 93–98.

Trugo, L. C., Moreira, R. F. A., de Maria, C. A. B. (2000). Componentes voláteis do café torrado. Parte II. Compostos alifáticos, alicíclicos e aromáticos. *Química Nova*, 23(2), 195–203.

Vegro, C. L. R., Carvalho, F. C. (2006). Disponibilidade e utilização de resíduos gerados no processamento agroindustrial do café. *Informações Económicas*, 24, 9–16.

Zuorro, A., Lavecchia, R. (2011). Polyphenols and energy recovery from spent coffee grounds. *Chemical Engineering Transactions*, 25, 285–290.

CHAPTER 2

LITERATURE REVIEW

2. Literature Review

2.1. Coffee production

Coffee is one of the most popular and appreciated beverages in the entire world, being consumed for its stimulating and refreshing properties, which are defined by the green beans composition and changes occurring during the roasting process (Mussatto *et al.*, 2011a).

There are 70 different species of the botanical plant genus *Coffea*. The two most usually grown species are *Coffea arabica* (Arabica) and *Coffea canephora* (Robusta). For the coffee beverage production is normally used the variety *Coffea arabica* which is considered the noblest of all coffee plants and the one with better sensory quality, that matches to approximately 75 % of the worldwide coffee production. Robusta represents around 25 %, is a more acid variety, stronger and hardy (Teixeira *et al.*, 2014).

Currently, 56 countries around the world are producers of coffee, and for some of them, coffee is the main agricultural export product. The 10 countries that produced more coffee in the last four years (2014–2017) are shown in Table 2.1.

Table 2.1. Annual worldwide coffee production (2014–2017)

Countries	2014	2015	2016	2017
Brazil	52299	50388	55000	51500
Vietnam	26500	28737	25540	28500
Colombia	13339	14009	14634	14000
Indonesia	11418	12317	11491	10800
Ethiopia	6575	6714	7297	7650
Honduras	5268	5786	7457	8349
India	5450	5800	5200	5840
Uganda	3744	3650	4962	5100
Mexico	3591	2903	3781	4000
Guatemala	3310	3410	3684	3800

Source: ICO (2018).
Values in thousand 60 kg bags.

Brazil, Vietnam and Colombia were the three biggest producers of coffee in the world in 2017. Peculiarly Brazil as the largest producer is also the largest exporter of this product, but in terms of consume is in the fifteenth place, being Finland the biggest consumer of coffee drinks (ICO, 2018). According to International Coffee Organization (ICO), the world production of coffee in 2017 increased 7 % when compared to the production achieved in 2014 (Table 2.1).

Coffee fruits are usually harvested when the bear fruit turns red, which generally occurs after 5 years of coffee tree plantation. This fruit is composed of two beans covered by a thin parchment, a silverskin, and further surrounded by a pulp and a husk (outer skin) (Teixeira *et al.*, 2014). Figure 2.1 illustrates a coffee bean and its structures.

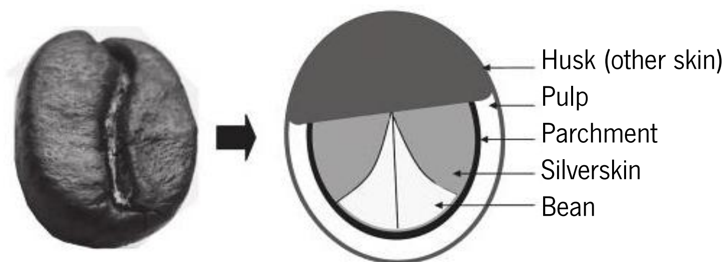


Figure 2.1. Appearance and structures of a coffee bean (Teixeira *et al.*, 2014).

In the coffee industry, for the treatment of coffee cherries to obtain green coffee are implemented two different methods, the dry process and the wet process. The first method is simpler and normally used for Robusta variety (Mussatto *et al.*, 2011a). Wet method is used for Arabica coffee beans, which involves several stages including a microbial fermentation that provides a better aroma quality (Gonzalez–Rios *et al.*, 2007). These technologies generate every year large amounts of by–products, as husks, pulp, parchment, silverskin (CS) and spent coffee grounds (SCG). Coffee silverskin (outer grain wrap) and spent coffee are the residues generated in larger scale. SCG is the residual waste obtained after aqueous extraction is the main by–product generated during the instant coffee preparation (Teixeira *et al.*, 2014). All residues originated during the processing of coffee are rich in phenolic compounds, and are thus potential raw material for obtaining valuable compounds (Campos–Vega *et al.*, 2015; Mussatto *et al.*, 2011a).

2.2. Spent coffee grounds

Spent Coffee Grounds (SCG) are the main by–product of the coffee brewing process and are obtained by both domestic brew preparation (at coffee shops, restaurants, homes) or during the industrial preparation of instant coffee (Galanakis, 2017). Nestlé, a Swiss company, was the maker of the world's first instant coffee in 1938 under the brand name NESCAFÉ (Somnuk *et al.*, 2017).

SCG consist of a dark brown solid residue with high moisture, being those from the first origin richer in chemical compounds compared to spent coffee instant coffee industries. This is understandable since for soluble coffee production, extraction is maximized in order to obtain the highest yields (Galanakis, 2017; Teixeira *et al.*, 2014). Figure 2.2 shows how SCGs look like.

Figure 2.2. Spent coffee grounds.

One ton of green coffee used for the production of instant coffee generates about 650 kg of spent coffee grounds. This value is still more significant when taking into account that from the worldwide coffee production, about 50 % is used for soluble coffee preparation (Teixeira *et al.*, 2014). Generally, SCG from the coffee industry are used as a renewable energy resource, and this waste is collected by specialized agencies, which sell the residues for different purposes such as composting, gardening, bioenergy production and mushroom growth (Campos–Vega *et al.*, 2015).

Spent coffee grounds usually have an elevated moisture, in the range of 80 % to 85 % (Mussatto *et al.*, 2011a) and, on a dry weight basis, are normally composed of 45.3 % of sugars (mannose, galactose and arabinose) and 13.6 % of proteins (Mussatto *et al.*, 2011b). Table 2.2 shows the chemical composition of spent coffee grounds.

Table 2.2. Chemical composition of spent coffee grounds (Mussatto *el al.*, 2011b)

Components	Dry weight (g/100 g)
Cellulose (glucan)	8.6
Hemicellulose	36.7
Arabinan	1.7
Galactan	13.8
Mannan	21.2
Proteins (*N* x 6.25)	13.6
Acetyl groups	2.2
Ashes	1.6

Spent coffee ground, as contains large amounts of organic compounds (*i.e.* fatty acids, amino acids, polyphenols, minerals and polysaccharides) has been highly valorized as a source of value–added products (Caetano *et al.*, 2012). Ballesteros *et al.* (2014a) analyzed the nutritional composition of spent coffee grounds derived from mixtures of Arabica and Robusta coffee varieties, provided by a coffee roaster industry, highlighting the richness of this by–product in polysaccharides, lignin and protein.

Others studies have shown other alternatives to reuse this waste, such as the studies that was published suggesting the use of SCG for the production of biodiesel (Caetano *et al.*, 2012; Kondamudi *et al.*, 2008), fuel ethanol (Mussatto *et al.,* 2012) and precursor for actived carbon production (Kante *et al.*, 2012). SCG has been also used as substrate for cultivation of microorganisms (Machado *et al.*, 2012) and as support for anaerobic microorganisms in the treatment of wastewater (Hein *et al.*, 2006). Ramalakshim *et al.* (2009) studied the bioactive compounds available in the SCG and evaluated the amino acids present, in order to find alternatives for the reuse of SCG.

SCG have been studied mainly for their antioxidant activities (Esquivel *et al.*, 2012). This residue is rich in sugars and phenolic compounds with value–added making SCGs suitable for applications in the food and drink's industry and as raw material to produce fuel ethanol or distilled beverage with aroma of coffee (Machado, 2009; Mussatto *et al.*, 2011b; Sampaio *et al.*, 2013). Extracting antioxidant phenolic compounds from SCG can be thus considered an interesting alternative to obtain these important industrial compounds in order to add value to SCG.

2.3. Phenolic compounds

The phenolic compounds are secondary metabolites synthesized by different plants during their normal development or as a response to environmental stress conditions (Beckman, 2000). These compounds present important functional properties with great interest for chemical, pharmaceutical and food industries. Some works identified phenolic compounds in green coffee where these compounds are partially transformed during the coffee roasting process (Mussatto *et al.*, 2015).

Phenolic compounds are the major determinant of antioxidant potentials found in high concentrations in the plants and have received considerable attention due to their beneficial effects on human health (Mussatto *et al.*, 2011c). Antioxidants are important for food and biological systems because they are substances able to inhibit or retard oxidative degradation (Lima *et al.*, 2010).

Since 1990, there has been an increased interest in the antioxidant activity of natural products. Some studies showed a beneficial influence of food and beverages (fruit, tea, red wine and coffee) on human health, which is associated with the antioxidant activity of polyphenols of these foods (Roginsky *et al.*, 2005). Moreover, these compounds, in addition to have a pharmacological and anti–nutritional activity, also inhibit the lipid oxidation and multiplication of the fungi, once they participate in the processes responsible for the color and flavor development in many foods (Angelo *et al.*, 2007).

These compounds are defined as substances having an aromatic ring with one or more hydroxyl substituents, including its functional groups. They include from simple phenolic molecules to compounds highly polymerized. There are about five thousand phenols, but the main ones are the flavonoids, phenolic acids, simple phenols, tannins, lignins and tocopherols. The phenolic compounds act as protective factors in the prevention of chronic degenerative diseases, cancer and cardiovascular diseases due to their antioxidant activity (Balasundram *et al.*, 2006; Mussatto *et al.*, 2011c; Soares, 2002).

Yen *et al.* (2005) have investigated the existence of antioxidants of roasted coffee residues, which of was confirmed with the presence of caffeine, trigonelline, caffeic acid, volatiles compounds, and polyphenols as chlorogenic acids and flavonoids. A study conducted by Ramalakshmi *et al.* (2009) and Murthy *et al.* (2012), demonstrated that extracts produced from SCG showed anti–tumor and anti–allergic activity , which were related to the presence of phenolic compounds such as chlorogenic acid in their composition. Chlorogenic acid, which is one of the most abundant phenolic compounds in SCG, has been reported to have several properties beneficial to human health, as their potent antioxidant

activity as well as anti–allergic activity, anti–inflammatory vasodilator, antibiotic, anti–thrombotic and cardioprotective (Mussatto *et al.*, 2011c).

2.4. Extraction techniques

There are different techniques that can be used to obtain compounds of interest from raw materials (Coelho *et al.*, 2015), solid-liquid extraction with organic solvents being one extensively used (Simões *et al.*, 2010; Mussatto *et al.*, 2011c). Modern techniques have been increasingly developed with a high degree of technology, speed, precision and accuracy (Mussatto *et al.*, 2011c). The extraction technique used to obtain extracts from natural sources directly influences the quality and composition of the final product (Andrade *et al.*, 2017a). The extraction procedure must be chosen according to the family of compounds to be extracted, the process yield and composition of extracts depending on the solvent and extraction technique employed (Tsao *et al.*, 2004).

Several extraction techniques have been proposed as an alternative to the traditional extraction procedures, as liquid–liquid extraction (LLE) and soxhlet extraction used for liquid and solid samples, respectively. Among these techniques, solid phase extraction (SPE) and solid phase microextraction (SPME) have been currently used to replace most of the applications of liquid–liquid extraction, while microwave–assisted extraction (MAE), supercritical fluid extraction (SFE), ultrasonication extraction (USE) and accelerated solvent extraction (ASE) have been used as an alternative to soxhlet extraction (Andrade *et al.*, 2012; Biernacka *et al.*, 2012; Dahmoune *et al.*, 2015; Jardim *et al.*, 2006; Martins *et al.*, 2010; Nayak *et al.*, 2015).

In the coffee and coffee by-products, different extraction techniques have been applied in order to recover antioxidant phenolic compounds, polysaccharides, oils, etc., such as solid-liquid extraction (Mussatto *et al.*, 2011c; Ballesteros *et al.*, 2014b), alkali pretreatment (Simões *et al.*, 2010; Ballesteros *et al.*, 2015), dilute acid hydrolysis (Mussatto *et al.*, 2011b), autohydrolysis (Ballesteros *et al.*, 2017a and 2017b), microwave-assisted extraction (Passos *et al.*, 2013; Passos *et al.*, 2014; Machado *et al.*, 2018) and supercritical fluid extraction (Barbosa *et al.*, 2014). An example of this are the works developed by Mussatto et al. (2011c) and Ballesteros et al. (2014b), which have studied the extraction of antioxidant phenolic compounds from SCG or CS through the conventional solid–liquid method with

methanol, ethanol and/or acetone. Whether SCG or CS have demonstrated to be an agro-industrial waste rich in antioxidant phenolic compounds (Mussatto *et al.*, 2011c; Ballesteros *et al.*, 2014b).

Mussatto *et al.* (2011b) used sulfuric acid to recover carbohydrates (hemicellulose sugars) by dilute acid hydrolysis of SCG. In the work developed by Ballesteros *et al.* (2017a and 2017b), was demonstrated that it is possible to recover polysaccharides from SCG using an environmentally friendly technique as autohydrolysis. Simões *et al.* (2010) and Ballesteros *et al.* (2015) have studied the extraction of polysaccharides through alkali treatments using sodium hydroxide from SCG. These studies exposed the potential of polysaccharides presented in coffee, showing that they can provide enormous functional properties as immunostimulatory activity and possible applications in the food industry (Simões *et al.*, 2010; Ballesteros *et al.*, 2015; Passos *et al.*, 2019).

The methods evaluated in this study to extract antioxidant phenolic compounds from SCG are supercritical fluid extraction (SFE) and microwave–assisted extraction (MAE), two modern techniques, less aggressive to the environment and human health and, thus, must be considered as alternative in the extraction of value–added compounds from SCGs, that are briefly described below.

2.4.1. Supercritical fluid extraction (SFE)

Supercritical fluids (SCFs) have been investigated since 1970 with the strongest commercial interest initially focusing on the use of supercritical toluene in petroleum and shale oil refining (Sapkale *et al.*, 2010). Supercritical fluids have properties between those of a gas and a liquid, in other words, the densities of SCFs are similar to those of liquids, and the viscosities are similar to those of gases.

Table 2.3 shows properties of SCFs when compared to those of gases and liquids (Teixeira *et al.*, 2014). Generally, the fluid most commonly used in processes of SFE is carbon dioxide (CO_2) alone or with the addition of small amounts of a solvent, referred to as co–solvent (Bernardo–Gil *et al.*, 2002; Toss, 2010).

Table 2.3. Properties of supercritical fluids, gases and liquids (Teixeira *et al.*, 2014)

Physical Properties	Liquids	SCFs	Gases
Density, ρ (kg m^{-3})	600 to 1600	100 to 1000	0.6 to 2
Dynamic viscosity, μ (mPa s)	0.2 to 1200	0.01 to 0.03	0.01 to 0.3
Diffusion coefficient, D (10^{6} m^{2} s^{-1})	0.0002 to 0.002	0.001 to 0.1	1 to 40

CO_2 is the most appropriate solvent for application in SFE (Table 2.4) due to its low values of critical temperature and pressure, allowing its use in the extraction of thermolabile compounds. Its non–toxic and non–flammable, exists in abundance, is practically chemically inert, and is cheap with high purity when compared with other solvents. The extraction efficiency by SFE depends on the density of the supercritical fluid, temperature and pressure used in the extraction (Passos *et al.*, 2009; Teixeira *et al.*, 2014).

Table 2.4. Critical properties (temperature, pressure and density) of various solvents (Sapkale *et al.*, 2010)

Solvents	$T_{critical}$ (K)	$P_{critical}$ (MPa)	$\rho_{critical}$ (g/cm^3)
Carbon Dioxide (CO_2)	304.1	7.38	0.449
Water (H_2O)	647.10	22.06	0.322
Methane (CH_4)	190.4	4.60	0.162
Ethane (C_2H_6)	305.3	4.87	0.203
Propane (C_3H_8)	369.8	4.25	0.217
Ethylene (C_2H_4)	282.4	5.04	0.215
Propylene (C_3H_6)	364.9	4.60	0.232
Methanol (CH_3OH)	512.6	8.09	0.272
Ethanol (C_2H_5OH)	513.9	6.14	0.278
Acetone (C_3H_6O)	508.1	4.70	0.278

Figure 2.3 shows the pressure–temperature phase diagram for CO_2. The boiling line separates the gas and liquid region and ends in the critical point, where the liquid and gas phases disappear to become a single supercritical phase.

Supercritical fluid extraction is the process of separating one component (the extractant) from another (the matrix) using supercritical fluids as the extracting solvent. SFE has been used in industrial

level in order to obtain high value–added compounds such as oils, fragrances and aroma compounds from natural sources, as well as to remove impurities or certain compounds from food or other products for human consumption, like the case of the caffeine removal from coffee (Maul, 1999).

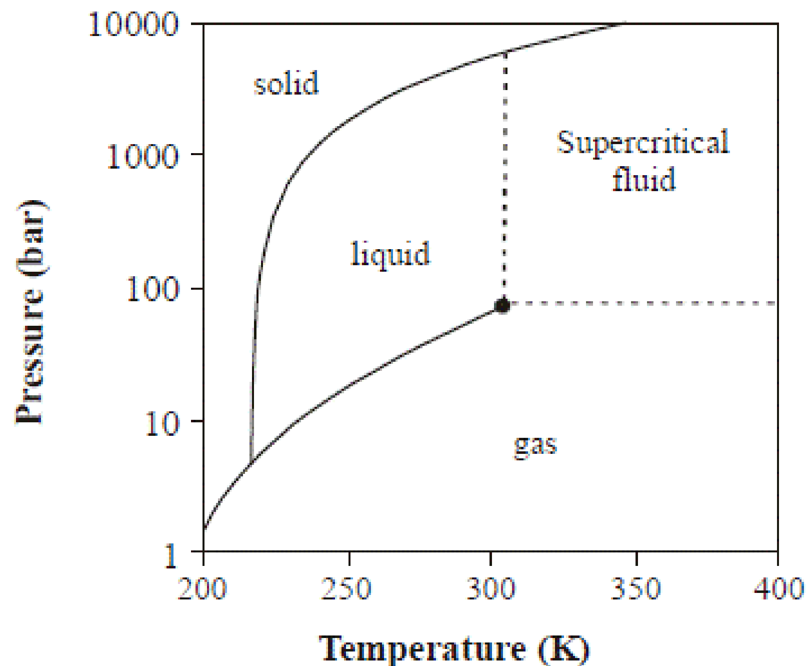


Figure 2.3. Pressure–temperature phase diagram for CO_2 (Sapkale *et al.*, 2010).

This technique uses supercritical fluids with high density approaching to the density of state liquid, the diffusion coefficient is between the gases and liquids, and has a low viscosity (Díaz–Reinoso *et al.*, 2006). The use of supercritical fluids in industrial processes have been highlighted due to the quality of the extracts obtained, and also by positive environmental aspects of this technology such as cleanness, selectiveness and toxic residues free. In addition, the extracts obtained do not undergo thermal degradation when using lower temperatures, and is not needed to apply a subsequent treatment for the solvent elimination, once this is eliminated by depressurization of the system (Maul *et al.*, 1996; Maul, 1999; Melecchi, 2005).

Some authors have studied the application of the SFE process in plants and micro–algae, as shown in the following examples: extraction of essential oils (Andrade *et al.*, 2017a; Costa *et al.*, 2012; Kemzuraité, *et al.*, 2014; Uquiche *et al.*, 2012), vegetable oils (Mhemdi *et al.*, 2011), volatile oils (Andrade *et al.*, 2017b; Palavra *et al.*, 2011; Zhi–ling *et al.*, 2011), antioxidants (Bernardo–Gil *et al.*,

2011; Moura *et al.*, 2012; Pereira *et al.*, 2013), flavonoids (Bimakr *et al.*, 2011; Lepojević *et al.*, 2017) and other compounds of interest (Dias *et al.*, 2012; Felföldi–Gáva *et al.*, 2012).

In the last few years, has also been studied the extraction of bioactive compounds and others compounds of interest, using the SFE, from industrial by–products, such as: fatty acids (Nguyen *et al.*, 2011; Pederssetti *et al.*, 2011; Rubio–Rodríguez *et al.*, 2012), antioxidants (Cavalcanti *et al.*, 2011; Povilaitis *et al.*, 2015), caffeine (Park *et al.*, 2012; Tello *et al.*, 2011), polyphenols (Moreira *et al.*, 2017) and other compounds of interest (Albuquerque *et al.*, 2012; Chu *et al.*, 2012; del Valle *et al.*, 2012; Romo–Hualde *et al.*, 2012). Another example is the work elaborated by Passos *et al.* (2009), in which was studied the extraction of grape seed oil through the supercritical fluid extraction, with carbon dioxide as a solvent, using enzymatically pre-treated seeds.

The extraction of oils from SCG was also studied through the supercritical fluid extraction. Andrade *et al.* (2012) analyzed the chemical composition and the antioxidant activity of spent coffee grounds and coffee husks extracts, obtained by supercritical fluid extraction (SFE) with CO2 and with CO2 and co-solvent. SFE extracts presented lower yields when compared to soxhlet and ultrasound method, besides good results of antioxidant activity by ABTS method were detected in the supercritical extracts.

In the work elaborated by Barbosa *et al.* (2014), was studied the optimization of the supercritical fluid extraction of spent coffee grounds (SCG) oil that was carried out considering three process variables, namely pressure (140–190 bar), temperature (40–70°C) and co-solvent (ethanol) content in the CO2 stream (0–5 wt.%). The highest total extraction yield was 11.97%, obtained by SFE at 190 bar, 55° C and 5 wt.% EtOH.

However, this process becomes expensive due to high cost of investment, once the equipment must be able of withstanding high pressures, and also due to the high operating costs for solvent compression (Maul *et al.*, 1996).

2.4.2. Microwave–assisted extraction (MAE)

The microwave technology has been used in chemistry since 1970. Salgo *et al.* (1986 and 1987), published studies using a conventional microwave oven to enhance the extraction of organic compounds from solid matrices such as soil, seeds, food and feedstuffs. In 1993, Onsuka and Terry used microwaves to extract organochlorine pesticides in sediment samples.

The microwave energy is a non–ionizing electromagnetic radiation that interacts directly as raw material causing heat due to interaction of the electric field of molecules with the electromagnetic waves in different phases. This leads to a migration of ions and dipolar rotation but without causing changes in the molecular structure (Oliveira *et al.*, 2000). The microwave energy has a frequency which varies between 300 MHz (radio radiation) and 300 GHz. There are two frequencies used in chemistry research: 2450 MHz for laboratory equipment and 915 MHz usually for industrial equipment (Kumar *et al.*, 2018; Vinatoru *et al.*, 2017).

The efficiency of the MAE is based on the selection of operating conditions and parameters that affect the mechanisms of extraction and production. The factors that may influence the performance of this technique are: the solvent properties (nature and volume), extraction time, microwave power, temperature, sample characteristics and the effect of agitation (Chan *et al.*, 2011; Dahmoune *et al.*, 2015; Tatke *et al.*, 2011). The Solvents frequently used in MAE are ethanol, methanol, acetone, hexane, ethyl acetate and water (Armstrong, 1999). This technique is very attractive due to the speed of the extraction process, to the reduced use of organic solvents, to the possibility of multiple extractions when using the system in carousel and to the simplicity of use along with its good reproducibility (Chan *et al.*, 2011; Kumar *et al.*, 2018). This method extracts the compounds in a more selective and more rapid way, with similar or best yields in comparison with conventional extraction processes, but using less power and less volume of solvent, which makes it friendlier to the environment (Rodriguez–Jasso *et al.*, 2011).

Microwave–assisted extraction is a powerful tool for natural compounds extraction from plants. Several works have been published applying the MAE, for the extraction of bioactive compounds from a large variety of natural resources (Chemat *et al.*, 2013), such as antioxidants (Martins *et al.*, 2010; Pandey *et al.*, 2018; Thirugnanasambandham *et al.*, 2017; Upadhyay *et al.*, 2012), polysaccharides (Rodriguez–Jasso *et al.*, 2011), polyphenols (Calinescu *et al.*, 2017; Dahmoune *et al.*, 2014; Dahmoune *et al.*, 2015; Drosou *et al.*, 2015; Moreira *et al.*, 2017; Nayak *et al.*, 2015; Setyaningsih *et al.*, 2015), essential oils (Kumar *et al.*, 2018). One more example is the work performed by Coelho *et al.* (2014), where was evaluated the feasibility of microwave superheated water extractions to recover two kind of polysaccharides from brewer's spent grain. Some of this studies reported that microwave–assisted extraction, compared with conventional methods, is more effective for extracting bioactive compounds (Dahmoune *et al.*, 2015; Du *et al.*, 2010; Moreira *et al.*, 2017).

Another author has also studied the extraction of polysaccharides from SCG by using microwave-assisted extraction. Passos *et al.* (2013 and 2014) studied the feasibility of microwave superheated water extraction of polysaccharides from SCG, being the highest effectiveness obtained when a sequential microwave-assisted extraction is used. More recently, Passos *et al.* (2019) have studied the microwave-assisted extraction for recovery of polysaccharides from spent coffee grounds taking into account three variables: temperature, which has the most significant impact; time and alkali treatments. They used this method as more environmentally friendly with the advantage of using only water as solvent and a shorter operation time.

2.5. Fermented and distilled beverages

The alcoholic beverages preparation is one of the oldest processes that goes along with the civilization. The beverage industry has shown a high interest in the development of new products from different raw materials. For that reason, the development of products with flavor extracts and natural flavors has received great emphasis due to restrictions on the use of synthetic chemicals in foods and beverages (Coelho *et al.*, 2015; Sampaio *et al.*, 2013).

The production of alcohol by fermentation of cereal grains was already known before the year 6000 bC (Pacheco, 2010). The alcoholic beverage is defined as a refreshing product, appetizer or stimulant, designed for human ingestion in liquid state, without the intention to use as a medicament and containing more than half a degree Gay–Lussac ethanol (Aquaroni *et al.*, 1986). It is obtained from the alcoholic fermentation of any fruit juice or cereal that has present in its constitution fermentable sugars (Asquieri *et al.*, 2008).

Fruits are foods that provide a wide range of sensory attractions, such as color, texture, flavor and aroma (Filho, 2010). The use of fruits in the preparation of fermented alcoholic beverages and/or distilled, is a process developed in order to avoid waste when consumption is not immediate, as well as to generate new applications and technologies. In the 1970s, Garcia *et al.* (1976) have used banana, buko water, and tomato, as a substrate in the production of fermented beverages. Several studies have been published on the preparation and characterization of fermented fruits, such as hog plum (Dias *et al.*, 2003), pine cone fruit, siriguela, mangaba (Muniz *et al.,* 2002), cashew (Neto *et al.*, 2006), cherry (Sun *et al.*, 2013) and raspberry (Duarte *et al.*, 2010a). Duarte *et al.* (2010b) have produced fruit

wines, using pulp of tropical fruits as gabiroba, cacao, umbu, cupuassu and jaboticaba. In the same way, Coelho *et al.* (2015) have elaborated four fruit wines, using fruit concentrates such as orange, banana, cherry and mango. Already exist recent studies have been made to create alcoholic beverages from fruit for fruit wastes re–valorization (Isitua *et al.*, 2010).

The first distilled beverage, the vodka made from rye, appeared around the year 1100, in Poland or Russia. It was used for medicinal purpose as an anesthetic and disinfectant (Santos *et al.*, 2005). The distillation is defined as the concentration of the alcohol of a fermented liquid in order to get a new drink. The distilled beverages have assumed such importance that it became the official drink in some countries, such as cachaça (from sugar cane) for Brazil, vodka (cereals and tubers) for Russia, tequila (piña) for Mexico, rum (molasses) for Cuba, whiskey (cereals) for Scotland, cognac (wine) for France, soju (rice) for Korea, among others (Santos *et al*,, 2006). The production of cachaça in Brazil is considered an important economic activity, once is produced over 1.3 billion liters per year, placing it as the third most consumed distilled beverage in the world, only behind the vodka and the soiu (Silva *et al.*, 2011). Some fruits have been used to produce distillates beverages, such as orange (Santos *et al.*, 2013), jabuticaba (Duarte *et al.*, 2011), black mulberries and blackcurrants (González *et al.*, 2010), blueberry (González *et al.*, 2016) and others fruits. Sampaio *et al.* (2013) have successfully elaborated a new distilled beverage from spent coffee grounds.

2.5.1. Production of fermented and distilled beverages

a) Raw material

The fermented and distilled beverages are produced from a wide variety of cereals and/or fruits, such as: grapes, apples, pears, cherries, strawberries, raspberries, oranges, currants, honey, sugar cane, cassava, potatoes, soybeans, corn, rice, wheat, among others (Azevedo *et al.*, 2007). The use of fruit for the preparation of beverages is a way of using the excess production of fruit or as a technological innovation for the use, as example, in the production of fermented beverages.

Most fruits are constituted by water, carbohydrates, vitamins, proteins, mineral salts and fat, which makes it a very rich source of nutrients for human metabolism (Filho, 2010). Theoretically, any fruit or vegetable which contains moisture, nutrients and sugar for the yeasts can be used as feedstock

for the production of fermented and distilled beverages (Filho, 1983). These raw materials are subjected to steps of crushing, grinding or even hydrolysis to release the sugars, which are mixed with water in order to form a mash rich in sugars which will be used for fermentation. Subsequently, the fermented juice is distilled and the fraction corresponding to what will be the liquor is then separated (Sampaio, 2010).

Variety of studies have been published for the production of fermented and distilled beverages with different varieties of fruits as yellow mombin (*Spondias mombin*), cocoa (*Theobroma cacao*), siriguela (*Spondias purpurea*), soursop (*Annona muricata*), acerola (Malpighia punicifolia) kiwi (*Actinidia deliciosa*), camu-camu (*Myrciaria dubious*), gabiroba, umbu, cupuassu, jabuticaba, hog plum, pine cone fruit, siriguela, mangaba, cashew, orange, banana, cherry and mango showing promising results relatively to the production of new beverages (Bortolini *et al.*, 2001; Coelho *et al.*, 2015; Dias *et al.*, 2003; Duarte *et al.*, 2010b; Duarte *et al.*, 2011; Maeda *et al.*, 2003; Muniz *et al.,* 2002; Neto *et al.*, 2006; Santos *et al.*, 2005; Sun *et al.*, 2013).

Actually there are studies for the production of new fermented beverages and distilled using waste from other industrial processes (Isitua *et al.*, 2010), such as: seed and pulp of melon (Briones *et al.*, 2002), skin and sediments of jabuticaba (Asquieri *et al.*, 2009), orange and tangerine peels (Alvarez *et al.*, 2004), among others.

b) Yeasts

Yeast fermentation of different plant carbohydrate sources is one of the oldest human technologies. At the present time, yeasts are essential for many biotechnological processes, such as beer, wine, and biofuel fermentations (Dashko *et al.*, 2014).

The yeast species more used in the production of alcoholic beverages worldwide is *Saccharomyces cerevisiae* and the type of yeast employed in fermentation exert a profound influence on the flavor and aroma characteristics of different beverages. In the production of wine and distilled beverages the yeast usually used is *Saccharomyces cerevisiae*. For the production of beer is possible used two different yeast, as *Saccharomyces cerevisiae* (in ale beer) and *Saccharomyces pastorianus* (in lager beer) (Walker *et al.*, 2016a; Walker *et al.*, 2016b).

Yeasts are able to unfold the glucose to produce ethanol and carbon dioxide. Its use in the fermentation depends on several factors such as the substrate or the raw material employed, the

alcohol content desired in the final product, the duration of the fermentation and product properties (Walker *et al.*, 2016a; Walker *et al.*, 2016b). To ensure a better performance of the fermentation yeasts should have the following characteristics: high rate of fermentation, tolerance to alcohol, resistance to high temperature, resistance to acid, and genetic stability (Filho, 2010).

c) Fermentation

The fermentation is the process where a substrate rich in sugars is converted in ethanol and carbon dioxide (CO_2), by action of yeasts (Walker *et al.*, 2016a). It is a process that consists in three stages: the preliminary stage, the tumultuous phase and complementary phase. In the preliminary phase it is observed that the consumption of sugar results in a very high multiplication of cells. There is no alcohol production or release of CO_2, only a small rise in temperature. This step should be short for yeast adaptation to the culture medium. In the second stage, the tumultuous phase, occurs the main fermentation, in which there is already a large formation of ethanol and CO_2, the temperature rises quickly, there is no foaming and there is a density reduction of the wort due to the transformation the sugars into alcohol. In the complementary phase, there is the consumption of sugars that are still available in the culture medium and the surface of fermented foam stays quiet and clean, thus completing the fermentation. The nutritional composition of fermented product depends on, besides the raw material which contributes to the characteristics of the beverage, the, inoculum type, conditions of fermentation and procedures post-fermentation (Filho, 2010; Walker *et al.*, 2016a).

- **Conduction of the alcoholic fermentation**

The fermentation may be conducted in discontinuous, semi–continuous or continuous (Mears *et al.*, 2017; Pacheco, 2010):

(1) Discontinuous process: initially is prepared a medium culture suitable for nutrition and development of the organism, which is placed in the reactor together with the microorganism responsible for the biological process. After fermentation, the fermented broth is removed from the reactor, being subjected to some operations in order to recover the product. The discontinuous fermentation can result in low yields and low productivity, due to the inhibitory effect of the substrate when entirely added at the beginning the fermentation. On the other hand, presents a lower risk of

contamination, as well as a great flexibility of operation due to the fact of allowing the use of the same reactors for different products.

(2) Consecutive discontinuous processes: in this process, there is an initial fermentation step using a fermentation medium as in the discontinuous process. At the end of this initial fermentation step, a part of the fermented medium is removed and a new volume of fermentation medium is added to the reactor, equal to the volume of fermentation medium that was removed. Thus the fermented mash that was inside the reactor serves as inoculum to the medium that was added after. The process is restarted and repeated until there is a drop in the productivity of the process

(3) Continuous process: characterized by having a continuous feed of culture medium at a constant flow rate, the reaction volume is maintained constant through a continuous withdrawal of fermentation broth. It is important that the system reaches the steady state condition, so that the state variables (concentration of cells, limiting substrate and product) remain constant during the system operation. It is an advantageous process because it allows the optimization of process conditions for greater productivity.

The discontinuous processes are frequently used for alcoholic fermentation, and within them four types of industrial fermentation are identified (Filho, 2010; Genisheva *et al.*, 2014):

i) Cutting processes, aimed at the multiplication of the yeast;

ii) Decanting process, proposes the reuse of the ferment, separated at a previous fermentation by decantation of the cells that are settled to the bottom of the reactor;

iii) Process Melle–Boinot, consists in the reuse of the cells separated by centrifugation and treated for later use. It has a higher cost and therefore is used in production units of medium and large size;

iv) Process Melle–Boinot–Almeida, is a combination of decantation and process Melle–Boinot, with the advantage of using concentrated musts, thereby producing fermentation product with high alcohol levels.

- **Control parameters of fermentation**

The fermentation should be carefully monitored in order to eliminate the possibility of contamination. The control parameters are (Filho, 2010; Walker *et al.*, 2016b):

(1) Concentration of sugars: Usually, there is a continuing decline of the sugars, which shows that sugars are being converted into alcohol, but if there is a low consumption of sugars or even a stop in consumption, it can mean that exists signs of contamination;

(2) Temperature: The ideal temperature is between 26 °C and 32 °C;

(3) Time of fermentation: the fermentation should last between 12 hours and 8 days (for wine). If the fermentation time increases, it may be a sign of contamination;

(4) Smell: fermentation has a pleasant smell with fruit flavor, but if it's distasteful indicates that there is the possibility of contamination;

(5) Aspect of the foam: should be light and can easily rupture. In the presence of the contamination the aspect of the foam is thick;

(6) Acidity and pH: the concentration of acid, expressed as H_2SO_4, in the medium should be between 2.5 g/L and 3.0 g/L. For wine, the ideal pH of fermentation is 3. But if the values are low, it may indicate the presence of microbial contamination;

(7) Yield: yield is the most important parameter to consider in a fermentation process, which is calculated by the ratio between the sugar consumed and the alcohol produced.

To prevent contamination it is necessary to maintain aseptic conditions and adequate values for pH and temperature, in order to provide good performance of the microorganism of interest. The monitoring of these parameters allows the formation of a fermented product with good organoleptic characteristics.

d) Distillation

Distillation is a process in which a liquid, by heating effect, passes into the gas phase, and then, back to a liquid state by cooling in which the objective is the purification or formation of a new product by separation of the fractions (Filho, 2010). In consists in concentrating the alcohol contained in a fermented liquid (Santos *et al.*, 2006). After fermentation of the wort, it is obtained a fermented broth (referred to as "wine") constituted by 5 % to 10 % of ethanol, 89 % to 94 % of water and 2 % to 4 % of other components (such as succinic acid and acetic acid, furfural, glycerol, higher alcohols, acetaldehyde, among others). The fermented broth is subjected to a distillation process where the constituents are separated through the differences in their ebullition temperatures (Filho, 2010).

The quality of the distillate obtained depends on the qualitative composition of the constituents present in minimum amounts, but especially on the proportion of the mixed components, that determine the flavor and aroma of the beverage (Walker *et al.*, 2016b). The distillation must be performed slowly and gradually to allow the formation and separation of aromatics inside the distiller, descendant of the fermentation (Filho, 2010). The alcoholic strength, by volumes of the distilled beverages is normally between 39 % and 47 %, but there are some that are marketed with an alcohol content of 60 % (Santos *et al.*, 2006).

The equipment used in the distillation varies accordingly with the product to be distilled. The equipment generally used for the production of brandy, for example, is a simple alembic as shown in Figure 2.4, which is normally constituted by a copper boiler heated in a furnace or through tubes where the steam is transported. The vapour resulting of the heating of the fermented broth is directed by the dome to the condensing tube, where the distillate begins to condensate. It is then directed to a cooling vessel that can be typically a water reservoir where the vapors stops to condensate, giving origin to a distillate which exits in the opposite extremity (Sampaio, 2010).

Initially, the distillate possesses a high alcohol content (65 % to 70 %, by volume), that will decrease as the volume of liquid in the boiler and the amount of ethanol available decreases (Walker *et al.*, 2016b). There are three types of the distillates: distillate from the head, heart and tail (Filho, 2010). The distillate's head is the fraction corresponding to the initial 5 % to 10 % of volume of distilled brandy and will be discarded once they are high in esters, aldehydes, acetaldehyde, methanol, ethyl acetate and other volatile compounds (Walker *et al.*, 2016b). The second fraction, designated by the heart, represents about 80 % of the volume of the distillate. This fraction is richer in ethanol and contains a lesser proportion of the lighter elements and the higher alcohols, volatile acids and other by–products

formed during fermentation, which corresponds to brandy. The last fraction, designated by tail, corresponds to 10 % of the distillate's total volume. It is richer in water and undesirable compounds; however, it can be added once again in the fermented broth in order to be redistilled that will increases the yield of production (Filho, 2010).

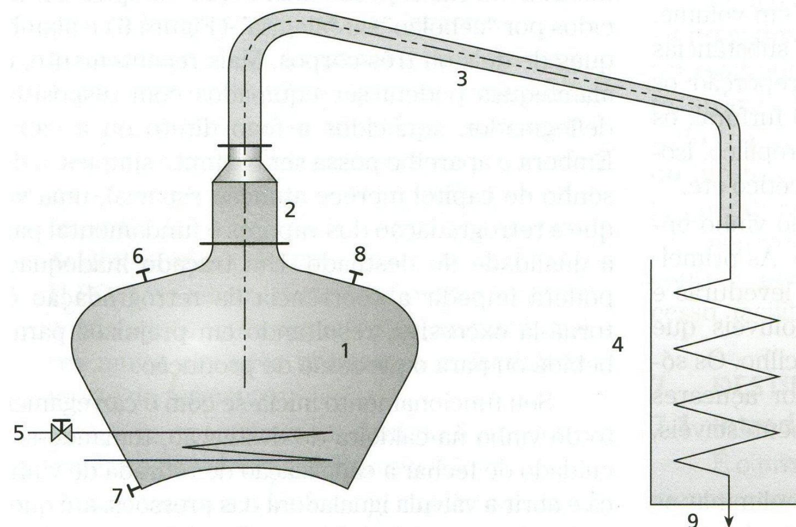


Figure 2.4. Schematic of a simple alembic: 1 – boiler; 2 – dome; 3 – tube of condensing; 4 – cooler; 5 – steam pipe; 6 – input of wine; 7 – discharge of wine; 8 – valve equalizing the pressure; 9 – channeling of distillate (Filho, 2010).

For large scale industrial distillations, are also used the alembics with three bodies and distillation columns, because they allow a greater yield in the distillate's production as well as the production of distillates with low and high levels of alcohol. In the production of brandy, such as cachaça, after the distillation a beverage with ethanol content, by volumes, of 50 % is obtained, which can be used to aging and commercialization. There is also the possibility of a new distillation, increasing the alcoholic level of the new beverage distilled, from 70 % to 80 %, in order to obtain a better quality product (Filho, 2010).

- **Control parameters of the distillation**

In the distillation process, there are some parameters to take into account to control: the time of distillation, the volume of the distillate and alcohol level (Filho, 2010).

e) Maturation

At the end of distillation it is obtained a beverage represented by water (59 %), ethanol (40 %) and 1 % of other compounds such as acetaldehyde, ethyl acetate, propanol, butanol, isoamyl alcohol, acetic acid. The newly distilled brandy does not have color, but have an aggressive flavor and an irregular aroma, which is not recommended for immediate consumption. For this reason, the aging of the distillate is considered a major factor in the quality of the beverage, being the type of barrel used (wood container) and maturation duration determinative for the final product. At this stage, the drink acquires positive attributes of aroma and flavor, characteristic of a higher quality beverage (Mosedale *et al.*, 1998; Pandey *et al.*, 2017; Walker *et al.*, 2016b). The type of wood varies according to the place where the aging is done. The wood most used in the Europe is the oak. At the end of aging is obtained a distillate with characteristics different from the initial, resulting in a product with a smoother taste and more appealing flavor (Sampaio, 2010).

The time required for the maturation stage depends on the characteristics of the distillate, the origin of the wood and treatment of the barrel. The change in the flavor of the distillate occurs due to changes in composition and concentration of compounds that influence the taste and flavor, and may be caused by: direct extraction of compounds of the wood; by decomposing of macromolecules of the wood and the extraction of its products for the distillate; by reactions between the compounds of wood and distillate; by reactions involving only the extractives of the wood; by reactions involving only compounds of distillate; by the evaporation of volatile compounds, as well as the input of oxygen (Mosedale *et al.*, 1998; Pandey *et al.*, 2017; Sampaio, 2010).

The aging must be made in a fresh and clean place because when realized in an environment whose air is dry, there will be a tendency for the evaporation of water, resulting in a higher alcoholic level of the beverage. If it is stored in a humid environment, there will be a tendency for the evaporation of the alcohol, resulting in an alcoholic beverage with less level of alcohol (Filho, 2010; Sampaio, 2010).

2.6. Volatile compounds

Flavor is one of the most important factors in sensory quality identity of fruits and food products. Food flavor can be explained and identified by its volatile chemical compounds content. These

volatiles are sensed and the aroma perceived by olfactory sensory receptors either when food are tasted or by its smell (Correa *et al.*, 2010). Qualitative and quantitative analyses of these compounds are needed in monitoring product quality and for flavor developing in fresh and processed products (Bertagnolli, 2014).

Volatile compounds are especially important in alcoholic beverages because they contribute to the quality of the final product. They result from biochemical reactions that occur during the fermentation process to fermented beverages or which arise during fermentation, distillation and storage processes for distilled beverages (Dragone *et al.*, 2009; Moreira *et al.*, 2012). Alcohols, acids and esters form, quantitatively and qualitatively, the major groups present in the volatile fraction of alcoholic beverages. The carbonyl compounds (as aldehydes and ketones), acetals, sulfur compounds and phenols are minor, but may be of great importance for the aroma and taste of these products (Duarte *et al.*, 2010a; Moreira *et al.*, 2012; Walker *et al.*, 2016a; Walker *et al.*, 2016b). The identification of these compounds is of major importance to determine the flavor characteristics of the drink as well as to detect illicit spirits, and to identify anomalies that are indicative of inconsistent manufacturing practices (Sampaio *et al.*, 2013).

Alcohols are produced by yeast as by–products of reactions involving amino acids and carbohydrates. Higher alcohols usually have a great influence on the taste of alcoholic beverages, presenting characteristic odors traditionally associated with them. At low levels higher alcohols have a positive effect on the sensory properties of distillates but at higher concentrations iso–amyl alcohol in particular becomes unpleasant. Sugar cane spirits may also contain small amounts of methanol. The presence of this substance is undesired in alcoholic beverages as it may cause toxic symptoms (Moreira, *et al.*, 2012; Walker *et al.*, 2016a).

Aldehydes are formed from the oxidative degradation of amino acids, oxidation of alcohols or auto–oxidation of fatty acids. They have great influence on the aroma and taste of alcoholic beverages. The larger aldehydes of the C8–C11 series are present in nature and have a pleasant aroma, giving an odor of citrus fruit to the aroma of several products. On the other hand, aldehydes with up to eight carbon atoms (*e.g.* acetaldehyde and furfural) exhibit penetrating odors, which are considered undesirable for being toxic to humans due the unpleasant effects of excessive consumption of alcoholic beverages (Moreira, *et al.*, 2012; Walker *et al.*, 2016b).

Although ketones are present in small amounts in alcoholic beverages, they may play an important role in the taste and aroma of these beverages. The origin of ketones in sugar cane spirits is

related to secondary fermentation processes and possible contamination during production (Moreira, *et al.*, 2012).

Esters and fatty acids are formed primarily by the action of yeast via fatty acid esterification and/or amino acid degradation. The lower the acidity of the sugar cane spirit, the better its acceptance by the consumers. Acetic acid is a very important compound for the quality of sugar cane spirit because it has a great influence on the taste and aroma of this type of beverage, being related to a penetrating and irritating odor, described as a vinegar odor. In addition to the higher alcohols, the esters are, quantitatively and qualitatively, one of the largest groups of compounds in the volatile fraction of alcoholic beverages. Ethyl esters of fatty acids and acetates are considered to be the most important in alcoholic beverages, because their concentrations are relatively high, their aroma characteristics are usually pleasant and their odor thresholds are relatively low. For example ethyl acetate, impart fruity and floral flavors and aromas to fermented beverages, especially beers and wines (Moreira, *et al.*, 2012; Walker *et al.*, 2016a).

Acetals are formed through the reaction between aldehydes and alcohols, causing the pungent odor of the aldehydes to be reduced as these reactions develop. For example, 1,1–diethoxyethane may contribute to the final flavor of these beverages directly, by their abovementioned flavor characteristics or, indirectly, by reducing the levels of acetaldehyde (Moreira, *et al.*, 2012)

The origin of sulfur compounds can be to be related both to microbial metabolism and also to the degradation of the sulfur amino acids (methionine and cysteine) of yeast proteins. These compounds, particularly dimethyl sulfide (DMS), are generally responsible for unpleasant aroma (sulphide odor) in food and beverages. In beer, dimethyl sulphide (DMS) if present in low concentrations is a desirable attribute of lagers, but higher concentrations impart off–flavors (Moreira, *et al.*, 2012; Walker *et al.*, 2016a).

The presence of phenolic compounds (*e.g.* phenolic acids) in sugar cane spirits is mostly associated with the aging process of these beverages in wooden barrels, from which these compounds are slowly extracted. As example of phenolic compounds is 4–vinylguaiacol. Some yeasts, can produce undesirable phenolic flavors and aromas. However, the 4–vinylguaiacol is desirable in certain beer styles (Moreira, *et al.*, 2012; Walker *et al.*, 2016a).

2.6.1. Volatile compounds of coffee

The aroma of coffee is formed by an extremely complex mixture of several volatile compounds that feature differences in aroma qualities, intensities and concentrations. Most of these compounds are generated during the green coffee roasting. The coffee drink is reported to contain more volatile compounds than any other food or drink (Trugo *et al.*, 2000). Table 2.5 shows the volatile constituents of roasted coffee.

Table 2.5. Volatile compounds of roasted coffee (Trugo *et al*., 1999; Trugo *et al*., 2000)

Volatile compounds	
Furans	Alcohols
Pyrazines	Ethers
Pyrroles	Hydrocarbons
Oxazoles	Carboxylic Acids
Thiazoles	Anhydrides
Thiophenes	Esters
Pyridines	Lactones
Phenols	Amines
Aldehydes	Sulfur Compounds
Ketones	

Depending on the species/variety of coffee, conditions used for cultivation, harvesting and processing method, it may have significant differences in the volatile compounds (Trugo *et al.*, 2000).

The study done by Sampaio *et al*. (2013) showed that spent coffee grounds has in its composition some volatile compounds, which are important for color, taste and flavor of a drink. They identified seventeen volatile compounds in the distillate such as alcohols, esters, aldehydes, and acids in concentrations able to promote pleasant characteristics to the product. Based on the chemical composition of SCG spirit, it was considered as having organoleptic quality acceptable for human consumption.

2.7. References

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

Alvarez, M. J. M., Rodríguez, G., Aponte, H., Camacho, D. R. B. (2004). Cambios fisicoquímicos en dos aguardientes dulces aromatizados con cáscaras de mandarina y naranja. *Revista de la Facultad de Agronomía*, 21, 285–296.

Andrade, K. S., Gonçalves, R. T., Maraschin, M., Ribeiro–do–Valle, R. M., Martínez, J., Ferreira, S. R. S. (2012). Supercritical fluid extraction from spent coffee grounds and coffee husks: antioxidant activity and effect of operational variables on extract composition. *Talanta*, 88, 544–52.

Andrade, K. S., Poncelet, D., Ferreira, S. R. S. (2017a). Sustainable extraction and encapsulation of pink pepper oil. *Journal of Food Engineering*, 204, 38–45.

Andrade, K. S., Trivellin, G., Ferreira, S. R. S. (2017b). Piperine–rich extracts obtained by high pressure methods. *The Journal of Supercritical Fluid*, 128, 370–377.

Angelo, P. M., Jorge, N. (2007). Compostos fenólicos em alimentos – Uma breve revisão. *Revista Instituto Adolfo Lutz*, 66(1), 232–240.

Aquaroni, E., Lima, U. A., Borzani, W. (1986). *Alimentos e Bebidas Produzidos por Fermentação*, Edgar Bucher, São Paulo.

Armstrong, S. D. (1999). *Microwave–assisted extraction for the isolation of trace systemic fungicides from Woody plant material*. Virginia Polytechnic Institute and State University, 23–60.

Asquieri, E. R., Rabêlo, A. M. S., Silva, A. G. M. (2008). Fermentado de jaca: estudo das características físico–químicas e sensoriais. *Ciência e Tecnologia de Alimentos*, 28(4), 881–887.

Asquieri, E. R., Silva, A. G. M., Cândido, M. A. (2009). Aguardente de jabuticaba obtida da casca e da borra da fabricação de fermentado de jabuticaba. *Ciência e Tecnologia de Alimentos*, 29(4), 896–904.

Azevedo, A. W. M., Santa, H. S. D., Alvarez, D. C., Rigo, M., Bastos, R., Santa, O. R. D. (2007). Estudo da viabilidade de obtenção de etanol por *Saccharomyces cereviseae* em mosto de frutos de caqui (*Diospyros kaki* L.). *Revista Ciências Exatas e Naturais*, 9(2), 268–278.

Balasundram, N., Sundram, K., Samman, S. (2006). Phenolic compounds in plants and agro–industrial by–products: antioxidant activity, occurrence, and potential uses. *Food Chemistry,* 99(1), 191–203.

Ballesteros, L. F., Teixeira, J. A., Mussatto, S. I. (2014a). Chemical, functional, and structural properties of spent coffee grounds and coffee silverskin. *Food and Bioprocess Technology*, 7(12), 3493–3503.

Ballesteros L.F., Teixeira, J. A., Mussatto, S. I. (2014b). Selection of the Solvent and Extraction Conditions for Maximum Recovery of Antioxidant Phenolic Compounds from Coffee Silverskin. *Food and Bioprocess Technology*, 7, 1322–1332.

Ballesteros, L. F., Cerqueira, M. A., Teixeira, J. A., Mussatto, S. I. (2015). Characterization of polysaccharides extracted from spent coffee grounds by alkali pretreatment. *Carbohydrate Polymers*, 127, 347–354.

Ballesteros L.F., Teixeira, J. A., Mussatto, S. I. (2017a). Extraction of polysaccharides by autohydrolysis of spent coffee grounds and evaluation of their antioxidant activity. *Carbohydrate Polymers*, 157, 258–266.

Ballesteros L.F., Ramirez, M. J., Orrego, C. E., Teixeira, J. A., Mussatto, S. I. (2017b). Optimization of autohydrolysis conditions to extract antioxidante phenolic compounds from spent coffee grounds. *Journal of Food Engineering*, 199, 1–8.

Barbosa, H. M. A., Melo, M. M. R., Coimbra, M. A., Passos, C. P., Silva, C. M. (2014). Optimization of the supercritical fluid coextraction of oil and diterpenes from spent coffee grounds using experimental design and response surface methodology. *Journal of Supercritical Fluids*, 85, 165– 172.

Beckman, C. H. (2000). Phenolic–storing cells: keys to programmed cell death and periderm formation in wilt disease resistance and in general defence responses in plants. *Physiological and Molecular Plant Pathology*, 57(3), 101–110.

Bernardo–Gil, M. G., Ribeiro, M. A., Esquível, M. M. (2002). Produção de extractos para a indústria alimentar: uso de fluidos supercríticos. *Boletim de Biotecnologia*, 73, 14–21.

Bernardo–Gil, M. G., Roque, R., Roseiro, L. B., Duarte, L. C., Gírio, F., Esteves, P. (2011). Supercritical extraction of carob kibbles (*Ceratonia siliqua* L.). *Journal of Supercritical Fluids*, 59, 36–42.

Bertagnolli, S. M. M. (2014). Bebidas fermentadas de goiaba: Compostos bioactivos, caracterização volátil e aproveitamento de resíduos. *Tese de doutoramento em ciência e tecnologia de alimentos, Universidade Federal de Santa Maria.*

Biernacka, P., Wardencki, W. (2012). Volatile composition of raw spirits of different botanical origin. *The Journal of the Institute of Brewing*¸ 118, 393–400.

Bimark, M., Rahman, R. A., Taip, F. S., Ganjloo, A., Salleh, L. M., Selamat, J., Hamid, A., Zaidul, I. S. M. (2011). Comparison of different extraction methods for the extraction of major bioactive flavonoid compounds from spearmint (*Mentha spicata* L.) leaves. *Food and Bioproducts Processing*, 89, 67–72.

Bortolini, F., Sant'Anna, E. S., Torres, R. C. (2001). Comportamento das fermentações alcoólica e acética de sucos de kiwi (*Actinidia deliciosa*); composição dos mostos e métodos de fermentação acética. *Ciência e Tecnologia de Alimentos*, 21, 236–243.

Briones, A. I., Hernández–Gómez, L. F., Ubeda, J. F. (2002). Elaboración de aguardiente de melón. *Alimentación, Equipos y Tecnología*, 171, 47–52.

Caetano, N. S., Silva, V. F., Mata, T. M. (2012). Valorization of coffee grounds for biodiesel production. *Italian Association of Chemical Engineering*, 26.

Calinescu, I., Lavric, V., Asofiei, I., Gavrila, A. I., Trifan, A., Ighigeanu, D., Martin, D., Matei, C. (2017). Microwave–assisted extraction of polyphenols using a coaxial antenna and a cooling system. *Chemical Engineering and Processing: Process Intensification*, 122, 373–379.

Campo–Vega, R., Loarca–Piña, G., Vergara–Castañeda, H. A., Oomah, B. D. (2015). Spent coffee grounds: A review on current research and future propects. *Food Science & Technology*, 45, 24–36.

Cavalcanti, R. N., Veggi, P. C., Meireles, M. A. A. (2011). Supercritical fluid extraction with a modifier of antioxidant compounds from jabuticaba (*Myrciaria cauliflora*) by–products: economic viability. *Procedia Food Science*, 1, 1672–1678.

Chan, C.–C., Yusoff, R., Ngoh, G.–C., Kung, F. W.–L. (2011). Microwave–assisted extractions of active ingredients from plants. *Journal of Chromatography A*, 1218, 6213–6225.

Chu, Y.–F., Chen, Y., Brown, P. H., Lyle, B. J., Black, R. M., Cheng, I. H., Ou, B., Prior, R. L. (2012). Bioactivities of crude caffeine: Antioxidant activity, cyclooxygenase–2 inhibition, and enhanced glucose uptake. *Food Chemistry*, 131, 564–568.

Chemat, F., Cravotto, G. (2013), *Microwave–assisted Extraction for Bioactive Compounds*, Springer. New York.

Coelho, E., Angélica, M., Rocha, M., Saraiva, J. A., Coimbra, M. A. (2014). Microwave superheated water and dilute alkali extraction of brewers' spent grain arabinoxylans and arabinoxylo-oligosaccharides. *Carbohydrate Polymers*, 99, 415– 422.

Coelho, E., Vilanova, M., Genisheva, Z., Oliveira, J. M., Teixeira, J. A., Domingues, L. (2015). Systematic approach for the development of fruit wines from industrially processed fruit concentrates, including optimization of fermentation parameters, chemical characterization and sensory evaluation. *Food Science and Technology (LWT)*, 62, 1043–1052.

Correa, M. I. C., Chaves, J. B. P., Jham, G. N., Ramos, A. M., Minim, V. P. R., Yokota, S. R. C. (2010). Changes in guava (*Psidium guajava* L. *var. Paluma*) nectar volatile compounds concentration due to thermal processing and storage. *Ciência e Tecnologia de Alimentos*, 30(4), 1061–1068.

Costa, P., Gonçalves, S., Grosso, C., Andrade, P. B., Valentão, P., Bernardo–Gil, M. G., Romano, A. (2012). Chemical profiling and biological screening of *Thymus lotocephalus* extracts obtained by supercritical fluid extraction and hydrodistillation. *Industrial Crops and Products*, 36, 246–256.

Dahmoune, F., Spigno, G., Moussi, K., Remini, H., Cherbalc, A., Madani, K. (2014). *Pistacia lentiscus* leaves as a source of phenolic compounds: Microwave–assisted extraction optimized and compared with ultrasound–assisted and conventional solvent extraction. *Industrial Crops and Products*, 61, 31–40.

Dahmoune, F., Nayak, B., Moussi, K., Remini, H., Madani, K. (2015). Optimization of microwave–assisted extraction of polyphenols from *Myrtus communis* L. leaves. *Food Chemistry*, 166, 585–595.

Dashko, S., Zhou, N., Compagno, C., Piškur, J. (2014). Why, when, and how did yeast evolve alcoholic fermentation?. *FEMS Yeast Research*, 14(6), 826–832.

del Valle, J. M., Glatzel, V., Martínez, J. L. (2012). Supercritical CO_2 extraction of allicin from garlic flakes: Screening and kinetic studies. *Food Research International*, 45, 216–224.

Dias, D. R., Schwan, R. F., Lima, L. C. O. (2003). Metodologia para elaboração do fermentado de cajá (*Spondias mombin* L.). *Ciência e Tecnologia de Alimentos*, 23(3), 342–350.

Dias, A. M. A., Santos, P., Seabra, I. J., Júnior, R. N. C., Braga, M. E. M., de Sousa, H. C. (2012). Spilanthol from *Spilanthes acmella* flowers, leaves and stems obtained by selective supercritical carbon dioxide extraction. *Journal of Supercritical Fluids*, 61, 62–70.

Díaz–Reinoso, B., Moure, A., Domíngues, H., Parajó, J. C. (2006). Supercrítical CO_2 extration and purification of compounds with antioxidant activity. *Journal of Agricultural and Food Chemistry*, 54, 2441–2469.

Dragone, G., Mussatto, S. I., Oliveira, J. M., Teixeira, J. A. (2009). Characterisation of volatile compounds in an alcoholic beverage produced by whey fermentation. *Food Chemistry*, 112, 929–935.

Drosou, C., Kyriakopoulou, K., Bimpilas, A., Tsimogiannis, D., Krokida, M. (2015). A comparative study on different extraction techniques to recover red grape pomace polyphenols from vinification byproducts. *Industrial Crops and Products*, 75, 141–149.

Du, G., Zhao, H. Y., Zhang, Q. W., Li, G. H., Yang, F. Q., Wang, Y., Li, Y. C, Wang, Y. T. (2010). A rapid method for simultaneous determination of 14 phenolic compounds in *Radix puerariae* using microwave–assisted extraction and ultra–high performance liquid chromatography coupled with diode array detection and time–of–flight mass spectrometry. *Journal of Chromatography A*, 1217(5), 705–714.

Duarte, W. F., Dias, D. R., Oliveira, J. M., Vilanova, M., Teixeira, J. A., Almeida e Silva, J. B., Schwan, R. F. (2010a). Raspberry (*Rubus idaeus* L.) wine: Yeast selection, sensory evaluation and instrumental analysis of volatile and other compounds. *Food Research International*, 43, 2303–2314.

Duarte, W. F., Dias, D. R., Oliveira, J. M., Vilanova, M., Teixeira, J. A., Almeida e Silva, J. B., Schwan, R. F. (2010b). Characterization of different fruit wines made from cacao, cupuassu, gabiroba, jaboticaba and umbu. *Food Science and Technology (LWT)*, 43, 1564–1572.

Duarte, W. F., Amorim, J. C., Lago, L. A., Dias, D. R., Schwan, R. F. (2011) Optimization of fermentation conditions for production of the jabuticaba (*Myrciaria cauliflora*) spirit using the response surface methodology. *Journal of Food Science*, 76(5), 782–790.

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

Felföldi–Gáva, A., Szarka, S., Simándi, B., Blazics, B., Simon, B., Kéry, Á. (2012). Supercritical fluid extraction of *Alnus glutinosa* (L.) Gaertn. *Journal of Supercritical Fluids*, 61, 55–61.

Filho, A. M. (1983). *Tecnologia de vinhos e vinagres de frutas*. Departamento de Tecnologia Rural da ESALQ/USP. Piracicaba, São Paulo, 130.

Filho, V. G. V. (2010). *Bebidas alcoólicas: Ciência e tecnologia*, 1. Blucher, São Paulo.

Galanakis, C. M. (2017). *Handbook of coffee processing by–products: Sustainable applications*, 1–26. Academic Press, United Kingdom.

Garcia, E. H., Cahanap, A. C., Cabrera, M. P. (1976). Organoleptic and chemical porperties of four new Philippine fruit wines II (banana, buko water, pineapple and tomato). *The Philippine Journal of Plant Industry*, 40, 29–35.

Genisheva, Z., Mota, A., Mussatto, S. I., Oliveira, J. M., Teixeira, J. A. (2014). Integrated continuous winemaking process involving sequential alcoholic and malolactic fermentations with immobilized cells. *Process Biochemistry*, 49, 1–9.

González, E. A., Agrasar, A. T., Castro, L. P., Fernández, I. O., Guerra, N. P. (2010) Production and characterization of distilled alcoholic beverages obtained by solid–state fermentation of black mulberry (*Morus nigra* L.) and black currant (*Ribes nigrum* L.). *Journal of Agricultural and Food Chemistry*, 58, 2529–2535.

González, E. A., Fernández, I. O., Castro, L. P., Guerra, N. P. (2016) Production and characterization of a novel distilled alcoholic beverage produced from blueberry (*Vaccinium corymbosum* L.). *Fruits*, 71(4), 215–220.

Gonzalez–Rios, O., Suarez–Quiroz, M. L., Boulanger, R., Barel, M., Guyot, B., Guiraud, J.–P., Schorr–Galindo, S. (2007). Impact of "ecological" post–harvest processing on coffee aroma: II. Roasted coffee. *Journal of Food Composition and Analysis*, 20(3), 297–307.

Hein, L., Gatzweiler, F. (2006). The economic value of coffee (*Coffea arabica*) genetic resources. *Ecological Economics*, 60(1), 176–185.

ICO – International Coffee Organization. (2018). Available at: http://www.ico.org/. Accessed 23 February 2018.

Isitua, C., Ibeth, I. (2010). Novel method of wine production from banana (*Musa acuminata*) and pineapple (*Ananas comosus*) wastes. *African Journal of Biotechnology*, 9(44), 7521–7524.

Jardim, I. C. S. F., Pinto, G. M. F., Pinto, J. F. (2006). Extração com fluido supercrítico. *Chemkeys – liberdade de aprender,* 1–12.

Kante, K., Nieto–Delgado, C., Rangel–Mendez, J. R., Bandosz, T. J. (2012). Spent coffee–based activated carbon: specific surface features and their importance for H_2S separation process. *Journal of Hazardous Materials*, 201, 141–147.

Kemzuraité, A., Venskutonis, P. R., Baranauskiené, R., Navikiené, D. (2014), Optimization of supercritical CO_2 extraction of different anatomical parts of lovage (*Levisticum officinale* Koch.) using response surface methodology and evaluation of extracts composition. *The Journal of Supercritical Fluids*, 87, 93–103.

Kondamudi, N., Mohapatra, S. K., Misra, M. (2008). Spent coffee grounds as a versatile source of green energy. *Journal of Agricultural and Food Chemistry*, 56, 11757–11760.

Kumar, R. C., Benal, M. M., Prasada, B. D., Krupashankarac, M. S., Kulkarnic, R. S., Siddaligaswamy, N. H. (2018). Microwave–assisted extraction of oil from pongamia pinnata seeds. *Materials Today: Proceedings*, 5, 2960–2964.

Lepojević, I., Lepojević, Z., Pavlić, B., Ristić, M., Zeković, Z., Vidović, S. (2017). Solid–liquid and high–pressure (liquid and supercritical carbon dioxide) extraction of *Echinacea purpurea* L. *The Journal of Supercritical Fluids*, 119, 159–168.

Lima, A. R., Pereira, R. G. F. A., Abrahão, S. A., Duarte, S. M. S., Paula, F. B. A. (2010). Compostos bioativos do café: atividade antioxidante *in vitro* do café verde e torrado antes e após a descafeinação. *Quimica Nova*, 33(1), 20–24.

Machado, E. M. S. (2009). Reaproveitamento de resíduos da indústria do café como matéria–prima para a produção de etanol. *Dissertação de Mestrado em Engenharia Biológica, Universidade do Minho,* Portugal.

Machado, E. M., Rodriguez–Jasso, R. M., Teixeira, J. A., Mussatto, S. I. (2012). Growth of fungal strains on coffee industry residues with removal of polyphenolic compounds. *Biochemical Engineering Journal*, 60, 87–90.

Machado, E., Mussatto, S. I., Teixeira, T., Vilanova, M., Oliveira, J. (2018). Increasing the Sustainability of the Coffee Agro-Industry: Spent Coffee Grounds as a Source of New Beverages. *Beverages*, 4, 105.

Maeda, R. N., Andrade, J. S. (2003). Aproveitamento do camu–camu (*Myrciaria dubia*) para produção de bebida alcoólica fermentada. *Acta Amazonica*, 33(3), 489–498.

Martins, S., Aguilar, C. N., de la Garza–Rodriguez, I., Mussatto, S. I., Teixeira, J. A. (2010). Kinetic study of nordihydroguaiaretic acid recovery from *Larrea tridentata* by microwave–assisted extraction. *Journal of Chemical Technology and Biotechnology*, 85, 1142–1147.

Maul, A. A., Wasicky, R., Bacchi, E. M. (1996). Extração por fluido supercrítico. *Revista Brasileira de farmacognosia*, 5(2), 185–200.

Maul, A. A. (1999). Fluidos supercríticos, situação atual e futuro da extração supercrítica. *Biotecnologia Ciência & Desenvolvimento*, 2(11), 42–46.

Mears, L., Stocks, S. M., Sin, G., Gernaey, K. V. (2017). A review of control strategies for manipulating the feed rate in fed–batch fermentation processes. *Journal of Biotechnology*, 245, 34–46.

Melecchi, M. I. S. (2005). Caracterização química de extratos de *Hibiscus tiliaceus* L: Estudo comparativo de métodos de extração. *Tese de Doutoramento em Química*, *Universidade Federal do Rio Grande do Sul*, 11–148.

Mhemdi, H., Rodier, E., Kechaou, N., Fages, J. (2011). A supercritical tuneable process for the selective extraction of fats and essential oil from coriander seeds. *Journal of Food Engineering*, 105, 609–616.

Moreira, R. F. A., Netto, C. C., de Maria, C. A. B. (2012). A fração volátil das aguardentes de cana produzidas no Brasil. *Química Nova*, 35(9), 1819–1826.

Moreira, M. M., Barroso, M. F., Boeykens, A., Withouck, H., Morais, S., Delerue–Matos, C. (2017). Valorization of apple tree wood residues by polyphenols extraction: Comparison between conventional and microwave–assisted extraction. *Industrial Crops & Products*, 104, 210–220.

Mosedale, J., Puech, J. (1998). Wood maturation of distilled beverages. *Food Science and Technology*, 9, 95–101.

Moura, P. M., Prado, G. H. C., Meireles, M. A. A., Pereira, C. G. (2012). Supercritical fluid extraction from guava (*Psidium guajava*) leaves: Global yield, composition and kinetic data. *Journal of Supercritical Fluids*, 62, 116–122.

Muniz, C. R., Borges, M. F., Abreu, F. A. P., Nassu, R. T., Freitas, C. A. S. (2002). Bebidas fermentadas a partir de frutos tropicais. *Boletim do Centro de Pesquisa de Processamento de Alimentos*, 20(2), 309–322.

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

Mussato, S. I., Carneiro, L. M., Silva, J. P. A., Roberto, I. C., Teixeira, J. A. (2011b). A study on chemical constituents and sugars extraction from spent coffee grounds. *Carbohydrate Polymers,* 83, 368–374.

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

Mussatto, S. I., Machado, E. M., Carneiro, L. M., Teixeira, J. A. (2012). Sugars metabolism and ethanol production by different yeast strains from coffee industry wastes hydrolysates. *Applied Energy*, 92, 763–768.

Mussatto S.I. (2015). Generating biomedical polyphenolic compounds from spent coffee or silverskin. In: V.R. Preedy (Ed.), *Coffee in Health and Disease Prevention*, 93–106. Elsevier.

Nayak, B., Dahmoune, F., Moussi, K., Remini, H., Dairi, S., Aoun, O., Khodir, M. (2015). Comparison of microwave, ultrasound and accelerated–assisted solvente extraction for recovery of polyphenols from *Citrus sinensis* peels. *Food Chemistry*, 187, 507–516.

Neto, A. B. T., da Silva, M. E., Silva, w. B., Swarnakar, R., da Silva, F. L. H. (2006). Cinética e caracterização físico–química do fermentado do pseudofruto do caju (*Anacrdium occidentale* L.). *Química Nova*, 29(3), 489–92.

Nguyen, H. N., Gaspillo, P–a. D., Maridable, J. B., Malaluan, R. M., Hinode, H., Salim, C., Huynh, H. K. P. (2011). Extraction of oil from *Moringa oleifera* kernels using supercritical carbon dioxide with ethanol for pretreatment: Optimization of the extraction process. *Chemical Engineering and Processing*, 50, 1207–1213.

Oliveira, C. C., Sartini, R. P., Zagatto, E. A. G. (2000). Microwave–assisted sample preparation in sequential injection: Spectrophotometers determination of magnesium, calcium and iron in food. *Analytica Chimica Acta*, 413, 41–48.

Pacheco, T. F. (2010). Fermentação alcoólica com leveduras de características floculantes em reator tipo torre com escoamento ascendente. *Dissertação de Mestrado em Engenharia Química, Universidade Federal de Uberlândia*, Brasil.

Palavra, A. M. F., Coelho, J. P., Barroso, J. G., Rauter, A. P., Fareleira, J. M. N. A., Mainar, A., Urieta, J. S., Nobre, B. P., Gouveia, L., Mendes, R. L., Cabral, J. M. S., Novais, J. M. (2011). Supercritical carbon dioxide extraction of bioactive compounds from microalgae and volatile oils from aromatic plants. *Journal of Supercritical Fluids*, 60, 21–27.

Pandey, A., Sanromán, M. A., Du, G., Soccol C. R., Dussap, C. G. (2017). Current Developments in Biotechnology and Bioengineering. *Food and Beverages Industry*, Elsevier.

Pandey, R., Shrivastava, S. L. (2018). Comparative evaluation of rice bran oil obtained with two–step microwave–assisted extraction and conventional solvent extraction. *Journal of Food Engineering*, 218, 106–114.

Park, H. S., Im, N. G., kim, K. H. (2012). Extraction behaviors of caffeine and chlorophylls in supercritical decaffeination of green tea leaves. *Food Science and Technology (LWT)*, 45, 73–78.

Passos, C. P., Silva, R. M., da Silva, F. A., Coimbra, M. A., Silva, C. M. (2009). Enhancement of the supercritical fluid extraction of grape seed oil by using enzymatically pre-treated seed. *Journal of Supercritical Fluids*, 48, 225–229.

Passos, C. P., Coimbra, M. A. (2013). Microwave superheated water extraction of polysaccharides from spent coffee grounds. *Carbohydrate Polymers*, 94, 626–633.

Passos, C. P., Moreira, A. S. P., Domingues, M. R. M., Evtuguin, D. V., Coimbra, M. A. (2014). Sequential microwave superheated water extraction of mannans from spent coffee grounds. *Carbohydrate Polymers*, 103, 333–338.

Passos, C. P., Rudnitskaya, A., Neves, J. M. M. G. C., Lopes, G. R., Evtuguin, D. V., Coimbra, M. A. (2019). Structural features of spent coffee grounds water-soluble polysaccharides: Towards tailor-made microwave assisted extractions. *Carbohydrate Polymers*, 214, 54–61.

Pederssetti, M. M., Palú, F., da Silva, E. A., Rohling, J. H., Cardozo–Filho, L., Dariva, C. (2011). Extraction of canola seed (*Brassica napus*) oil using compressed propane and supercritical carbon dioxide. *Journal of Food Engineering*, 102, 189–196.

Pereira, P., Bernardo–Gil, M. G., Cebola, M. J., Mauricio, E., Romano, A. (2013). Supercritical fluid extracts with antioxidant and antimicrobial activities from myrtle (*Myrtus communis* L.) leaves. Response surface optimization. *The Journal of Supercritical Fluids*, 83, 57–64.

Povilaitis, D., Venskutonis, P. R. (2015), Optimization of supercritical carbon dioxide extraction of rye bran using response surface methodology and evaluation of extract properties. *The Journal of Supercritical Fluids*, 100, 194–200.

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

Rodriguez–Jasso, R. M., Mussatto, S. I., Pastrana, L., Aguilar, C. N., Teixeira, J. A. (2011). Microwave–assisted extraction of sulfated polysaccharides (fucoidan) from *Brown seaweed*. *Carbohydrate Polymers*, 86, 1137–1144.

Roginsky, Y. V., Lissi, E. A. (2005). Review of methods to determine chain–breaking antioxidant activity in food. *Food Chemistry*, 92, 235–254.

Romo–Hualde, A., Yetano–Cunchillos, A. I., González–Ferrero, C., Sáiz–Abajo, M. J., González–Navarro, C. J. (2012). Supercritical fluid extraction and microencapsulation of bioactive compounds from red pepper (*Capsicum annum* L.) by–products. *Food Chemistry*, 133(3), 1045–1049.

Rubio–Rodríguez, N., de Diego, S. M., Beltrán, S., Jaime, I., Sanz, M. T., Rovira, J. (2012). Supercritical fluid extraction of fish oil from fish by–products: A comparison with other extraction methods. *Journal of Food Engineering*, 109, 238–248.

Salgo, A., Ganzler, K. (1986). MAE: Novel Sample Preparation Method for Chromatography. *Journal of Chromatographic Science*, 371, 299–306.

Salgo, A., Ganzler, K. (1987). MAE: Novel Sample Preparation Method for Chromatography. *Z. Lebensm. Uters. Forsch*, 184, 247.

Sampaio, A. R. M. (2010). Desenvolvimento de tecnologias para produção de etanol a partir do hidrolisado da borra de café. *Dissertação de Mestrado, Departamento de Engenharia Biológica, Universidade do Minho*, Portugal.

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 elaborated from spent coffee ground. *Food Science and Technology (LWT)*, 54(2), 557–563.

Santos, S. C., Almeida, S. S., Toledo, A. L., Santana, J. C. C., Souza, R. R. (2005). Elaboração e Análise Sensorial do Fermentado de Acerola (*Malpighia Punicifolia* L.). *Brazilian Journal of Food Technology*, 5° SIPAL, 47–50.

Santos, J., Dinham, R. (2006). *O Essencial em cervejas e destilados.* 1–135. SENAC SP, São Paulo.

Santos, C. C., Duarte, W. F., Carreiro, S. C., Schwan, R. F. (2013). Inoculated fermentation of orange juice (*Citrus sinensis* L.) for a production of a citric fruit spirit. *Journal of the Institute of Brewing*, 119(4), 280–287.

Sapkale, G. N., Patil, S. M., Surwase, U. S., Bhatbhage, P. K. (2010). Supercritical Fluid Extraction. *International Journal of Chemical Sciences*, 8(2), 729–743.

Setyaningsih, W., Saputro, I. E., Palma, M., Barroso, C. G. (2015). Optimisation and validation of the microwave–assisted extraction of phenolic compounds from rice grains. *Food Chemistry*, 169, 141–149.

Silva, M. C. Azevêdo, L. C., Carvalho, M. M., Sá, A. G. B., Lima, M. S. (2011). Elaboração e avaliação da qualidade de aguardentes de frutas submetidas a diferentes tratamentos. *Revista Seminário De Visu*, 1(2), 92–106.

Simões, J., Nunes, F. M., Maria do Rosário, M. D., Coimbra, M. A. (2010). Structural features of partially acetylated coffee galactomannans presenting immunostimulatory activity. *Carbohydrate polymers*, 79, 397–402.

Soares, S. E. (2002). Ácidos fenólicos como antioxidantes. *Revista de Nutrição*, 15(1), 71–81.

Somnuk, K., Eawlex, P., Prateepchaikul, G. (2017). Optimization of coffee oil extraction from spent coffee grounds using four solvents and prototype–scale extraction using circulation process. *Agriculture and Natural Resources*, 51, 181–189.

Sun, S. Y., Che, C. Y., Sun, T. F., Lv, Z. Z., He, S. X., Gu, H. N., Shen, W. J., Chi, D. C., Gao, Y. (2013). Evaluation of sequential inoculation of *Saccharomyces cerevisiae* and *Oenococcus oeni* strains on the chemical and aromatic profiles of cherry wines. *Food Chemistry*, 138, 2233–2241.

Tatke, P., Jaiswal, Y. (2011). An overview of microwave–assisted extraction and its applications in herbal drug research. *Research Journal of Medicinal Plant*, 5(1), 21–31.

Teixeira, J. A., Vicente, A. A. (2014). Adding value to food processing by–products – The role of biotechnology, in *Engineering Aspects of Food Biotechnology*. Ed. Da–Wen Sun, 413–428. Boca Raton, FL: CRC Press, Taylor & Francis Group.

Tello, J., Vigueira, M., Calvo, L. (2011). Extraction of caffeine from Robusta coffee (*Coffea canephora* var. Robusta) husks using supercritical carbon dioxide. *Journal of Supercritical Fluids*, 59, 53–60.

Thirugnanasambandham, K., Sivakumar, V. (2017). Microwave assisted extraction process of betalain from dragon fruit and its antioxidant activities. *Journal of the Saudi Society of Agricultural Sciences*, 16, 41–48.

Toss, D. (2010). Extração de compostos fenólicos de *Butia capitata* utilizando dióxido de carbono supercrítico, *Dissertação de Mestrado em Engenharia Química*, *Universidade Federal do Rio Grande do Sul*, Brasil.

Trugo, L. C., Moreira, R. F. A., de Maria, C. A. B. (1999). Componentes voláteis do café torrado. Parte I. Compostos heterocíclicos. *Química Nova*, 22(2), 209–217.

Trugo, L. C., Moreira, R. F. A., de Maria, C. A. B. (2000). Componentes voláteis do café torrado. Parte II. Compostos alifáticos, alicíclicos e aromáticos. *Química Nova*, 23(2), 195–203.

Tsao, R., Deng, Z. (2004). Separation procedures for naturally occurring antioxidant phytochemicals. *Journal of Chromatography B*, 812, 85–99.

Upadhyay, R., Ramalakshmi, K., Rao, L. J. M. (2012). Microwave–assisted extraction of chlorogenic acids from green coffee beans. *Food Chemistry*, 130, 184–188.

Uquiche, E., Huerta, E., Sandoval, A., del Valle, J. M. (2012). Effect of boldo (*Peumus boldus* M.) pretreatment on kinetics of supercritical CO_2 extraction of essential oil. *Journal of Food Engineering*, 109, 230–237.

Vinatoru, M., Mason, T. J., Calinescu, I. (2017). Ultrasonically assisted extraction (UAE) and microwave–assisted extraction (MAE) of functional compounds from plant materials. *Analytical Chemistry*, 97, 159–178.

Walker, G. M., Stewart, G. G. (2016a). *Saccharomyces cerevisiae* in the production of fermented beverages. *Beverages*, 2(4), 30.

Walker, G. M., Hill, A. E. (2016b). *Saccharomyces cerevisiae* in the production of whisk(e)y. *Beverages*, 2, 38.

Yen, W. J. (2005). Antioxidant properties of roasted coffee residues. *Journal of Agricultural and Food Chemistry*, 53(7), 2658–2663.

Zhi–ling, C., Jian–ping, C., Hui–lin, C., Wei–tao, B., Hai–yan, C., Mo–lin, L. (2011). Research on the extraction of plant volatile oils. *Procedia Environmental Sciences*, 8, 426–432.

CHAPTER 3

EXTRACTION OF VALUE–ADDED COMPOUNDS FROM SPENT COFFEE GROUNDS USING SUPERCRITICAL FLUID EXTRACTION

Abstract

In this work, supercritical fluid extraction (SFE) with carbon dioxide (CO_2) as solvent was used to recover antioxidant phenolic compounds from spent coffee grounds (SCG). Extraction assays were carried out under different conditions of temperature and pressure. The conditions for the SFE were: temperatures of 313.15 K, 323.15 K and 333.15 K and pressures from 150 bar to 250 bar. The extracts obtained in this process were characterized regarding the contents of total phenolic compounds, proteins, sugars, flavonoids, and antioxidant activity, which was determined by two methods (ferric reducing antioxidant power – FRAP – assay and the phosphomolybdenum complex – total antioxidant capacity – method). The highest contents of flavonoids (164.57 mg QE/g extract and 1.55 mg QE/g SCG), protein (160.97 mg BSA/g extract and 1.15 mg BSA/g SCG), and antioxidant activity: FRAP (0.5843 mM Fe(II)/g extract and 0.0050 mM Fe(II)/g SCG) and total antioxidant capacity (272.26 mg α–tocopherol/g extract and 2.58 mg α–tocopherol/g SCG) were obtained using 250 bar and 333.15 K. The Supercritical fluid extraction at 150 bar and 313.15 K was the condition that has released the highest contents of sugars (0.0610 mg glucose/g extract and 0.0007 mg glucose/g SCG). On the other hand, SFE at 200 bar and 313.15 K was the best condition to recover phenolic compounds from SCG (33.63 mg GAE/g extract and 0.36 mg GAE/g SCG).

Keywords: Supercritical fluid extraction; Phenolic compounds; Sugars; Antioxidant activity; Spent coffee grounds.

3. Introduction

Coffee is the most consumed beverage in the world being served more than 400 billion cups per year. As a consequence, large amount of wastes are generated in the coffee industry every year. Among these wastes, the spent coffee grounds (SCG) are one of the most important in terms of volume generated. One ton of green coffee generates about 650 kg of SCG, and about 2 kg of wet SCG (70 % to 80 % moisture) are obtained to each 1 kg of soluble coffee produced (Mussatto *et al.*, 2011a). SCG does not have commercial value, and is currently discarded as conventional solid waste. However, this waste is a rich source of compounds with industrial interest such as carbohydrates, proteins and phenolic compounds (Mussatto *et al.*, 2011b). Therefore, the extraction of volatile compounds from SCG could be an interesting alternative to valorize this agro–industrial waste (Andrade *et al.*, 2012; Zuorro *et al.*, 2011).

Nowadays, there are different techniques to recover antioxidant phenolic compounds such as solid–liquid extraction with organic solvents, ultrasound–assisted extraction, microwave–assisted extraction, supercritical fluid extraction and high pressure processes (Andrade *et al.*, 2017a; Andrade *et al.*, 2017b; Moreira *et al.*, 2017; Mussatto *et al.*, 2011b). Supercritical fluid extraction (SFE) is a technology that presents many advantages over conventional methods of extraction, especially because it uses as solvents harmless substances to the environment and human health. This technology has been used industrially for the extraction of valuable compounds from plants, such as oils, fragrances, and active components (flavors), as well as to remove impurities and other in food products for human consumption, as is the case of the removal of caffeine from coffee (Perrut *et al.*, 2000). The use of supercritical fluids in industrial processes has been highlighted by the quality of the extracts as well as the environmental aspects, because it is a clean method, selective and free of toxic residues. The extracts produced don't undergo thermal degradation as mild temperatures are used and it is not necessary to apply a further treatment to remove the extraction solvent, since this will be eliminated by the depressurization system (Maul *et al.*, 1996; Maul, 1999).

This study aimed to evaluate the extraction of antioxidant phenolic compounds from SCG by SFE. The extractions were performed using carbon dioxide as solvent under temperature and pressure different conditions to determine the operating conditions that maximize the release of such compounds.

3.1. Material and methods

3.1.1. Raw materials and chemicals

The raw material used was spent coffee grounds (SCG) which was supplied by a Portuguese company of reference in this sector NovaDelta – Comércio e Indústria de Cafés, Lda (Campo Maior, Portugal). The provided material was dried in an oven at 60 °C until approximately 10 % moisture content and stored afterwards for use in the following steps. Phenolic standards including gallic acid and quercetin, methanol, α–tocopherol and Folin–Ciocalteu phenol reagent were purchased from Sigma (Sigma–Aldrich GmbH, Sternheim, Germany); Coomassie (Bradford) protein assay kit was got from Thermo Scientific. The carbon dioxide used in this work was CO_2 N45 (purity ≥ 99.995 %) and supplied by Air Liquide (Lisbon, Portugal).

3.1.2. Supercritical fluid extraction

The extraction experiments were carried out in a semi–batch flow extraction apparatus built in Instituto Superior Técnico (Lisbon, Portugal) and supercritical carbon dioxide was the solvent used (Esquível *et al.*, 1993). The experimental diagram of the supercritical apparatus used in this work is shown in Figure 3.1.

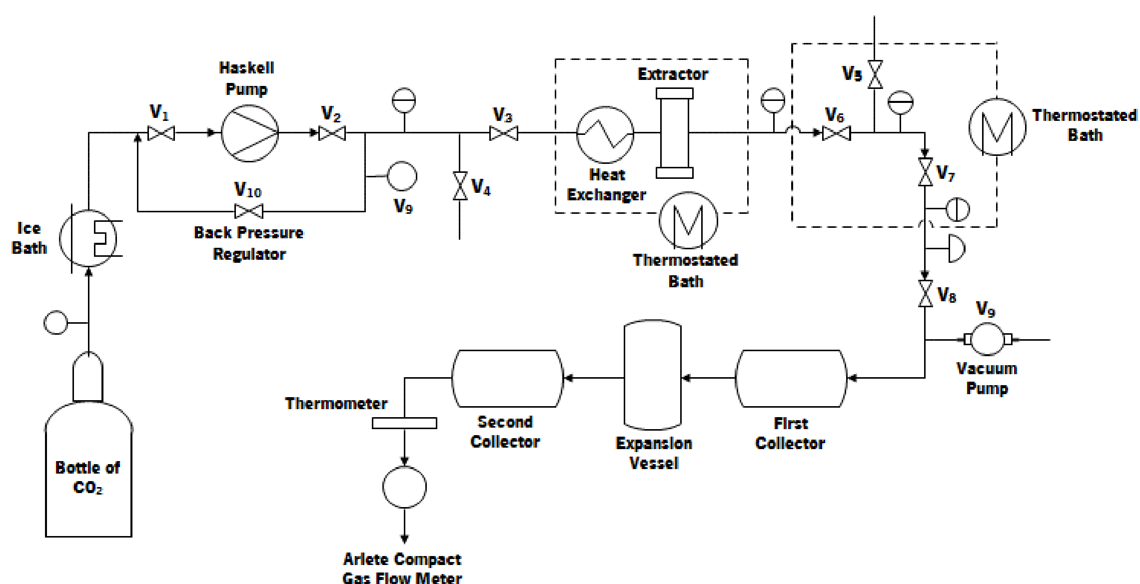


Figure 3.1. Schematic diagram of the experimental apparatus used for the extractions assays.

Initially, the carbon dioxide from one of the pressed bottle (6 MPa) passes through a cold bath (near 273 K) and is fed to a "Haskell" pump that presses it to the desired pressure. Then, the liquefied carbon dioxide passes through a heat exchanger (being pre-heated to the temperature of extraction), prior of being fed to a 100 mL tubular extractor in stainless steel containing SCG. This extractor will be immersed in a thermostatic water bath, with the desired temperature for each extraction. Figure 3.2 shows detailed photographs of the supercritical fluid extraction installation.

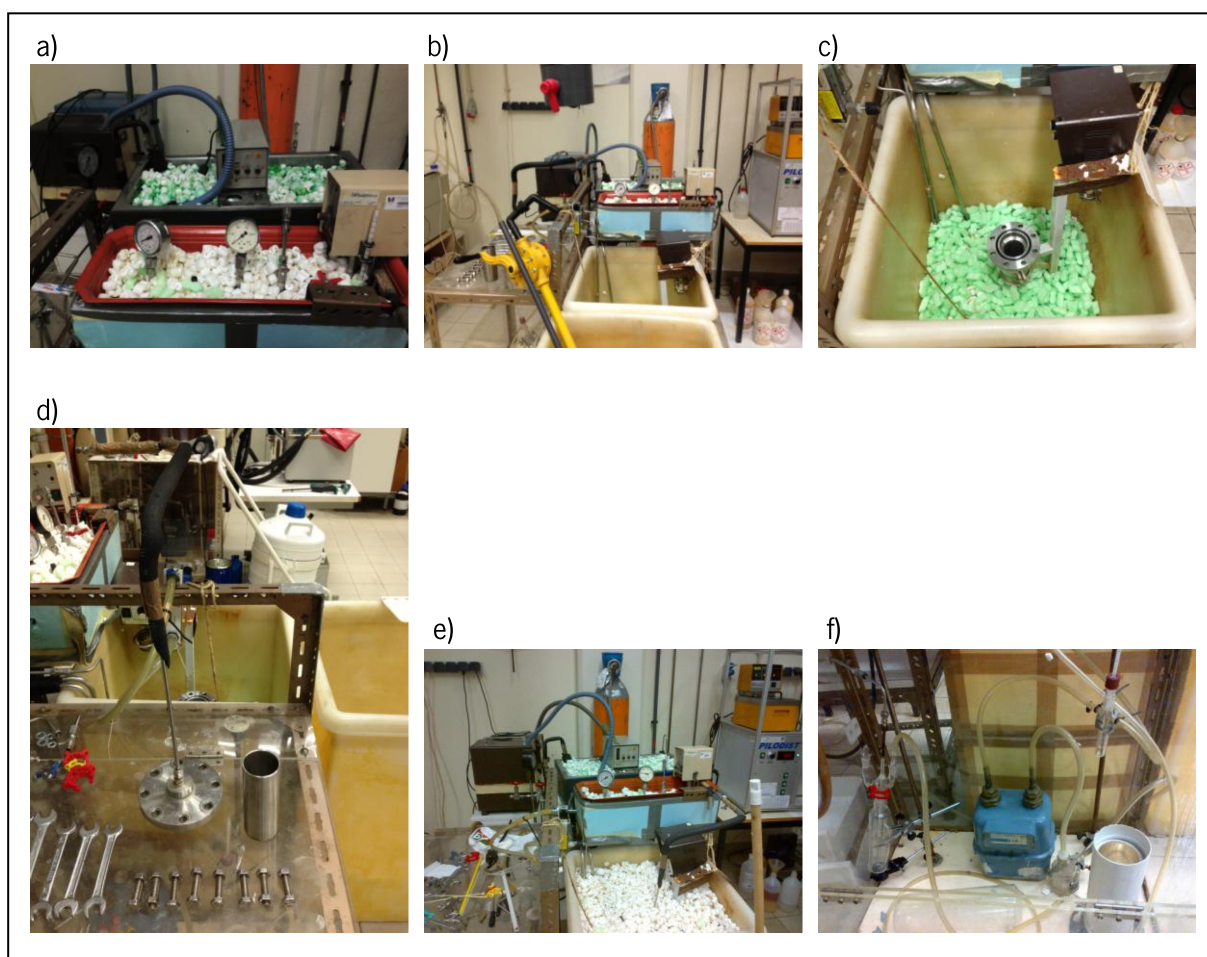


Figure 3.2. Supercritical fluid extraction installation: a) Behind is possible to see the ice bath and haskell pump and in front is possible to see the thermostated bath and vacuum pump; b) Extraction installation and the Bottle of CO_2; c) Heat exchanger, extractor and thermostated bath; d) Vessel of extractor; e) Extraction installation before extraction; f) First collector, second collector, thermometer and ariete compact gas flow meter.

The needle valve V8 controls the exit flow of the solvent. In the valves V6 to V8, the pressure is reduced several levels up to atmospheric pressure, recovering the residual SCG oil in the first collector.

Water and volatile components are separated in the second collector at a temperature of 203 K. During each extraction some samples were taken, and the extract obtained measured by weighing. The carbon dioxide quantity was determined by an "Ariete Compact" gas flowmeter and the pressure and temperature conditions were measured at the end of the assembly. After each extraction, the pipes around the extractor were washed with a volatile solvent (ethanol) which is fed through valve V5 and then sucked out using a vacuum pump. During this operation, valve V6 is closed. The cleaning of the extractor is made manually at the end of each run.

Preliminary tests were performed in order to establish extraction conditions, such as temperature, pressure and extraction time. For each experiment of extraction was used 30 g of SCG. The conditions used for the different extractions assays of supercritical are represented in Table 3.1. In each test, after reaching the stabilization in 30 min of the defined supercritical conditions, the extraction process and the respective time started to count. At the end of each extraction was recovered and saved the solid residue which was stored inside a freezer. The overall extraction yield was determined through the ratio between the extracted mass obtained and raw material mass used for extraction.

Table 3.1. Conditions of pressure, temperature used in the extractions assays, and CO_2 density (ρ CO_2) for the respective conditions

Assay	Pressure (bar)	Temperature (K)	ρ CO_2 (Kg/m^3)
1	150	313.15	780.3
2	150	323.18	699.8
3	150	333.17	603.9
4	200	313.18	839.9
5	200	323.19	784.4
6	200	333.20	723.8
7	250	313.21	879.6
8	250	323.22	834.4
9	250	333.23	786.8

3.1.3. Analytical methodology

3.1.3.1. Determination of total phenolic compounds

The phenolic compounds in SCG extracts were determined using a 96–well microplate–adapted colorimetric assay that uses Folin–Ciocalteu reagent according to the colorimetric method described by Singleton *et al.* (1965). Initially, 5 μL of each filtered extract were mixed with 60 μL of sodium carbonate solution at 7.5 % (w/v) and 15 μL of Folin–Ciocalteu reagent. Then, 200 μL of distilled water were added and the solutions were mixed. After standing for 5 min at 60 °C samples were allowed to cool at room temperature. The absorbance was measured by means of a spectrophotometric microplate reader set at 700 nm. A calibration curve was made from a gallic acid standard solution (200 mg/L, 400 mg/L, 600 mg/L, 800 mg/L, 1000 mg/L, 2000 mg/L and 3000 mg/L) and the blank was prepared with distilled water. The phenolic compounds content was expressed as milligram gallic acid equivalent per dry weight of material or per gram of extract (mg GAE/g SCG or mg GAE/g extract).

3.1.3.2. Determination of proteins

Proteins in SCG extracts were measured using the Coomassie G–250 dye in a colorimetric reagent described by Bradford (1976) with some modifications. A volume of 10 μL of each filtered extract was added to 300 μL of Coomassie Blue reagent in a 96–well microplate. Then, the plate is shaked for 30 s and allowed to rest for 10 min at room temperature. The absorbance of the mixture was then measured at 595 nm against a blank with distilled water. A calibration curve was prepared with BSA solution (25 mg/L, 125 mg/L, 250 mg/L, 500 mg/L, 750 mg/L and 1000 mg/L). The total proteins content was expressed as milligram protein equivalent per dry weight of material or per gram of extract (mg BSA/g SCG or mg BSA/g extract).

3.1.3.3. Determination of reducing sugars content

The reducing sugars content of SCG extracts was estimated using the DNS (3,5–dinitrosalicylic acid) method, adapted to a 96–well microplate. Initially, 25 μL of each filtered extract was added to

25 µL of DNS in a 96–well microplate and incubated at 100 °C for 5 min. After, was added 250 µL of distilled water in the samples and the microplate was placed on ice. Then, the samples were cooled to room temperature. The absorbance of the mixture was then measured at 540 nm against a blank with distilled water. A calibration curve was prepared with a standard solution of glucose (0 mg/mL, 0.2 mg/mL, 0.4 mg/mL, 0.6 mg/mL, 0.8 mg/mL, 1.0 mg/mL, 1.2 mg/mL, 1.4 mg/mL, 1.6 mg/mL, 1.8 mg/mL and 2.0 mg/mL). The reducing sugars content was expressed as milligram glucose equivalent per dry weight of material or per gram of extract (mg glucose/g SCG or mg glucose/g extract).

3.1.3.4. Determination of total flavonoid content

The flavonoids in SCG extracts were estimated using the colorimetric assay previously described by Chang *et al.* (2002) with some modifications. Initially, 30 µL of each filtered extract was added to 90 µL of methanol in a 96–well microplate. After, the addition of 6 µL of aluminum chloride at 10 % (w/v), 6 µL of potassium acetate (1 mol/L), and 170 µL of distilled water to each extract sample was performed. Samples were maintained in the dark, at room temperature, during 30 min. The absorbance of the mixture was then measured at 415 nm against a blank with distilled water. A calibration curve was prepared with a standard solution of quercetin (25 mg/L, 50 mg/L, 100 mg/L, 150 mg/L and 200 mg/L). The total flavonoid content was expressed as milligram quercetin equivalent per dry weight of material or per gram of extract (mg QE/g SCG or mg QE/g extract).

3.1.3.5. Determination of antioxidant activity

The antioxidant activity of SCG extracts was estimated by two different methods: the ferric reducing antioxidant power (FRAP) assay, and the phosphomolybdenum complex method (total antioxidant capacity). The FRAP assay was performed according to the method described by Benzie *et al.* (1996) with some modifications. Initially, 10 µL aliquot of filtered extract was mixed with 290 µL of FRAP reagent in a 96–well microplate, and incubated at 37 °C for 15 min. Then, the absorbance was determined at 593 nm against a blank that was prepared using distilled water. A calibration curve was constructed using an aqueous solution of ferrous sulfate – $FeSO_4.7H_2O$ (200 µM, 400 µM, 600 µM,

800 μM and 1000 μM). The FRAP values were expressed as millimoles of ferrous equivalent per dry weight of material or per gram of extract (mM Fe(II)/g SCG or mM Fe(II)/g extract).

To determine the total antioxidant capacity of SCG extracts by the phosphomolybdenum complex method described by Prieto *et al.* (1999), 100 μL of each filtered extract was added to 1 mL of a reaction solution containing 0.6 M of sulfuric acid, 28 mM of sodium phosphate, and 4 mM of ammonium molybdate, in tubes. After, the samples were placed in a water bath at 95 °C during 90 min. Then, the samples were cooled to room temperature. The absorbance was then measured at 695 nm against a blank with distilled water. The calibration curve was prepared using a solution "stock" of α–tocopherol (25 μg/mL, 75 μg/mL, 125 μg/mL, 250 μg/mL, 375 μg/mL and 500 μg/mL). The total antioxidant capacity was expressed as milligram α–tocopherol equivalent per dry weight of material or per gram of extract (mg α–tocopherol/g SCG or mg α–tocopherol/g extract).

3.2. Results and discussion

In an initial phase preliminary tests were carried out with the objective of defining the most suitable operating conditions to be used in the supercritical fluid extractions, such as temperature, pressure and extraction time. The tests were done under two different conditions, the first at a pressure of 150 bar and a temperature of 313.15 K and the second at a pressure and temperature of 200 bar and 333.15 K, respectively, for a total extraction time of 210 min with a carbon dioxide flow rate of 4 g/min. The pressure and temperature are key factors in extraction processes, so they affect both the kinetics of phenolics release from the solid matrix and the antioxidant activity of the extract. Therefore, this study consisted in evaluating the effect of these variables on the recovery of antioxidant phenolic compounds from SCG.

Figure 3.3, shows the accumulated mass of extract as a function of extraction time obtained for the preliminary tests. With these results it was possible to define the conditions, as pressure, temperature and extraction time, to use in the different extractions assays of supercritical as shown in Table 3.1. For each experiment of extraction was used 30 g of SCG, a supercritical carbon dioxide flow of 4 g/min and an extraction time of 3 h.

Table 3.2 and 3.3 summarize the extraction conditions used in each experimental assay and the results obtained for total phenolic compounds, proteins, flavonoids and antioxidant activity.

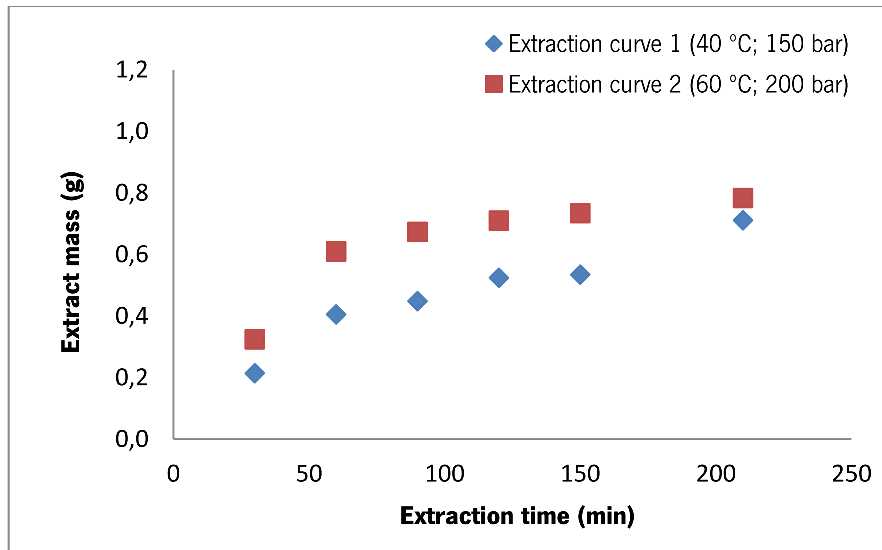


Figure 3.3. Accumulated mass of extract as a function of extraction time.

The extraction yield is accounted for by the ratio of the extract mass obtained at the end of each extraction to the initial extract mass. Table 3.2 shows that the highest yield was 2.82 %, obtained by SFE at 250 bar and 323.15 K and the lower yield (1.98 %) was obtained under condition at 150 bar and 323.15 K. Comparing these results with other works reported, we can verify that this work obtained the lowest yield. Andrade *et al.* (2012) obtained yields of 9.1 % and 9.7 % in oils extracts from SCG using a co–solvent at 200 bar and temperatures of 313.15 K and 323.15 K, respectively, but without addition of a co–solvent the results were 9.1 % at 200 bar and 333.15 K. For the same conditions of pressure (200 bar) and temperature (313.15 K, 323.15 K and 333.15 K) but without addition of a co–solvent, the results obtained in this work were 2.29 %, 2.78 % and 2.09 %. In another work, Couto *et al.* (2009) obtained 13.1 %, 14.0 % and 8.1 % yields but in oil extraction front SCG, at 200 bar and 313.15 K, 323.15 K and 333.15 K with addition of ethanol as co–solvent, respectively.

The difference between these results can be explained by several factors, such as: the different coffee varieties, pretreatment of the raw materials or different equipment. In both studies, it's possible to see a yield increase with the enhancement of pressure, keeping the temperature constant, and is possible to verify that this happens in this work only for the temperature of 323.15 K. In two works published we can verify that the yield of extraction also increased, with the increase of the pressure (Passos *et al.*, 2009; Barbosa *et al.*, 2014). This can too be explained by the increase in solvent density with the enhancement of pressure, which subsequently increases the solvation power of CO_2 (Andrade *et al.*, 2012).

Table 3.2. Total phenolic compounds, proteins and flavonoids contents in the different extracts obtained by SFE of SCG

	Extraction conditions		% Yield (g/ 30g SCG)	Solvent flow rates CO_2 (g/min)	Total phenolic		Proteins		Sugars	
	Pressure (bar)	Temperature (K)			(mg GAE/g extract)	(mg GAE/g SCG)	(mg BSA/g extract)	(mg BSA/g SCG)	(mg Glucose/g extract)	(mg Glucose/g SCG)
1	150	313.15	2.36	10.28	32,00 ± 1,84	0,39 ± 0,02	120,13 ± 4,05	1,40 ± 0,04	0,0610 ± 0,0022	0,0007 ± 0,0000
2	150	323.15	1.98	9.61	17,93 ± 0,82	0,17 ± 0,01	80,26 ± 7,52	0,90 ± 0,10	0,0463 ± 0,0018	0,0005 ± 0,0000
3	150	333.15	2.51	8.58	26,43 ± 1,90	0,32 ± 0,02	114,87 ± 2,78	1,33 ± 0,04	0,0526 ± 0,0031	0,0006 ± 0,0000
4	200	313.15	2.29	9.70	33,63 ± 2,03	0,36 ± 0,02	130,70 ± 4,13	1,46 ± 0,06	0,0549 ± 0,0010	0,0006 ± 0,0000
5	200	323.15	2.29	9.89	21,84 ± 2,49	0,25 ± 0,03	114,64 ± 4,96	1,44 ± 0,05	0,0583 ± 0,0020	0,0007 ± 0,0000
6	200	323.15	2.67	10.26	26,43 ± 3,10	0,38 ± 0,05	93,20 ± 4,18	1,24 ± 0,07	0,0559 ± 0,0012	0,0007 ± 0,0000
7	200	323.15	2.78	10.04	24,34 ± 0,69	0,33 ± 0,01	97,87 ± 2,66	1,30 ± 0,02	0,0488 ± 0,0012	0,0007 ± 0,0000
8	200	333.15	2.09	9.38	29,34 ± 3,43	0,26 ± 0,01	116,64 ± 4,81	1,27 ± 0,03	0,0478 ± 0,0004	0,0005 ± 0,0000
9	250	313.15	2.07	10.40	24,01 ± 0,80	0,24 ± 0,01	136,53 ± 3,10	1,65 ± 0,04	0,0518 ± 0,0012	0,0005 ± 0,0000
10	250	323.15	2.82	9.95	25,99 ± 0,68	0,38 ± 0,01	99,25 ± 2,24	1,59 ± 0,04	0,0558 ± 0,0011	0,0008 ± 0,0000
11	250	333.15	2.14	9.84	27,05 ± 0,80	0,25 ± 0,01	160,97 ± 4,07	1,15 ± 0,04	0,0593 ± 0,0024	0,0006 ± 0,0000

*GAE – Gallic acid equivalent, BSA – Bovine serum albumin equivalent.

Table 3.3. Antioxidant activity of the different extracts obtained by SFE of SCG

	Extraction conditions		Flavonoids		FRAP		Total Antioxidant Capacity	
	Pressure (bar)	Temperature (K)	(mg QE/g extract)	(mg QE/g SCG)	(mM Fe (II)/g extract)	(mM Fe (II)/g SCG)	(mg α-Tocoferol/g extract)	(mg α-Tocoferol/g SCG)
1	150	313.15	56,30 ± 2,15	0,70 ± 0,03	0,2911 ± 0,0167	0,0035 ± 0,0002	252,9 ± 18,03	2,95 ± 0,24
2	150	323.15	45,00 ± 1,43	0,38 ± 0,01	0,2844 ± 0,0108	0,0033 ± 0,0001	182,3 ± 6,02	1,81 ± 0,06
3	150	333.15	44,37 ± 1,36	0,52 ± 0,02	0,3154 ± 0,0102	0,0037 ± 0,0001	229,3 ± 4,48	2,84 ± 0,06
4	200	313.15	86,76 ± 1,29	0,87 ± 0,01	0,3072 ± 0,0084	0,0036 ± 0,0001	252,1 ± 7,33	2,87 ± 0,09
5	200	323.15	73,82 ± 3,94	0,93 ± 0,07	0,2985 ± 0,0167	0,0038 ± 0,0002	237,1 ± 10,49	3,24 ± 0,13
6	200	323.15	77,56 ± 3,91	0,92 ± 0,05	0,2806 ± 0,0098	0,0039 ± 0,0001	238,6 ± 9,37	2,90 ± 0,14
7	200	323.15	62,70 ± 6,59	0,89 ± 0,05	0,2795 ± 0,0048	0,0038 ± 0,0001	207,9 ± 12,72	2,83 ± 0,21
8	200	333.15	44,88 ± 4,98	0,49 ± 0,07	0,3604 ± 0,0110	0,0039 ± 0,0001	218,8 ± 7,36	2,47 ± 0,07
9	250	313.15	118,54 ± 4,43	1,29 ± 0,06	0,2471 ± 0,0055	0,0025 ± 0,0001	212,6 ± 8,23	2,25 ± 0,08
10	250	323.15	89,38 ± 1,74	1,40 ± 0,03	0,2378 ± 0,0089	0,0035 ± 0,0001	201,9 ± 5,13	3,18 ± 0,08
11	250	333.15	164,57 ± 5,41	1,55 ± 0,02	0,5843 ± 0,0120	0,0050 ± 0,0002	272,3 ± 17,14	2,58 ± 0,09

*****QE – Quercetin equivalent, Fe(II) – Ferrous equivalent.

According to Campos *et al.* (2008) and Mezzomo *et al.* (2009), increased pressure can lead to disruptions in the plant cells, which will facilitate the release of compounds which were not previously available and consequently increasing the yield of the process.

The highest value of total phenolic compounds (33.63 mg GAE/g extract) was obtained at 200 bar and 313.15 K. When compared to the results reported by Andrade *et al.* (2012), that obtained 24.1 mg GAE/g extract during the SFE of SCG at 200 bar and 323.15 K, the result obtained in the present study under the same extraction conditions is similar (26.43 mg GAE/g extract). Andrade *et al.* (2017a) reported total phenolic contents of 16 mg GAE/g extract for the pink pepper (*Schinus terebinthifolius R.*) oil obtained by SFE at 300 bar and 40 °C. Comparing the values obtained at 200 bar and 60 °C in the study by Andrade *et al.* (2017a) and this work it is possible to verify that are lower, 14.4 mg GAE/g extract against 29.34 mg GAE/g extract, respectively. In another work by Andrade *et al.* (2017b) evaluated the recovery of compounds of high value–added from black pepper (*Piper nigrum*) through the performance of supercritical fluid extraction (SFE). The maximum values of total phenolic content for SFE with CO_2 as a solvent were obtained at 200 bar and 60 °C (26 mg GAE/g extract) similar than this work. But when used SFE with 5 % ethanol as co–solvent at 150 bar and 50 °C they obtained the highest result of total phenolic content (46 mg GAE/g extract). This behavior can be explained by the increase in the solubility of polar compounds in the mixture ethanol + CO_2, compared to the solubility in pure CO_2 (Pereira *et al.*, 2010).

However, when compared to the results reported for other extraction techniques, the use of SFE provided lower values of phenolic compounds extraction. For example, a phenolic compounds content of 587 mg GAE/g extract was obtained when SCG was submitted to ultrasound–assisted extraction with ethanol as solvent (Andrade *et al.*, 2012). Andrade *et al.* (2017b) showed that the others methods used, as soxhlet (SOX) and ultrasound–assisted extraction (UAE), in the recovery of compounds of high value–added from black pepper (*Piper nigrum*) obtained better results than SFE. The best values reported in the work were 55 mg GAE/g extract and 42 mg GAE/g extract for SOX and UAE method, respectively. Only using SFE with addition of 5 % ethanol as co–solvent produced similar values of the total phenolic content that obtained in the UAE (Andrade *et al.*, 2017b).

The highest contents of proteins (1.65 mg BSA/g SCG) in the extract were obtained when the SFE was performed at 250 bar and 313.15 K (Table 3.2). For the extraction of reducing sugars, this technique was not the best one, because the quantified values were very low, as is show in Table 3.2. The maximum value was 0.0008 mg glucose/g SCG at 250 bar, 323.15 K.

The highest value of flavonoids compounds (1.55 mg QE/g SCG) in the extract were obtained when the SFE was performed at 250 bar and 333.15 K (Table 3.3). When compared to the results reported by Mussatto *et al.* (2011c), who tried to extract the antioxidant phenolic compounds from spent coffee grounds through conventional solid–liquid method, the best result was 2.50 mg QE/g SCG, using 80 % methanol concentration as solvent and 40 mL/g SCG as solvent/solid ratio, in which we can verify that the results were similar.

The results from Table 3.3 also show the values for antioxidant activity from all extracts. The antioxidant activity of the SCG extracts using the FRAP method fluctuated between 0.2471 mM Fe(II)/g extract to 0.5843 mM Fe(II)/g extract, or 0.0025 mM Fe(II)/g SCG to 0.0050 mM Fe(II)/g SCG. These results are very low compared with the results obtained by Mussatto *et al.* (2011c). They obtained, as best result, 0.102 mM Fe (II)/g SGC using 50 % methanol concentration as solvent and 40 mL/g SCG as solvent/solid ratio (Mussatto *et al.*, 2011c).

The best result for total antioxidant capacity, using the second method was 272.26 mg α–tocopherol/g extract or 2.58 mg α–tocopherol/g SCG. Such conditions are different of those that maximized the extraction of total phenolic compounds (200 bar and 313.15 K). The best results of antioxidant activity were also found in the extracts produced at 250 bar and 333.15 K (Table 3.3).

Unfortunately, it exists a high cost associated to supercritical fluid extractions processes with high-pressures. Their operation is more expensive, owing to equipment design and construction, mainly due the additional safety features that are indispensable (Passos *et al.*, 2009).

3.3. Conclusions

Antioxidant phenolic compounds, proteins and flavonoids were extracted from SCG by supercritical fluid extraction using CO_2 as solvent. The optimal parameters that were found using the results of the extraction yield were 250 bar and 323.15 K. When the results of the antioxidant activity were used, the ideal parameters were: pressure 250 bar, temperature of 333.15 K. The highest amount of phenolic compounds (33.63 mg GAE/g extract) was extracted when the process was carried out at 200 bar and 313.15 K; while the maximum values of proteins, flavonoids and antioxidant activity

were obtained in the extracts produced at 250 bar and 333.15 K. These results suggest that other substances than phenolic compounds may be contributing to the antioxidant potential of the extracts.

The results obtained in the present study for the extraction of antioxidant phenolic compounds from SCG are lower when compared to others already published in the literature using different extraction techniques. Additionally, higher costs are also reported for implementation of SFE on industrial scale. However, SFE is a more environmental friendly technique of extraction when compared to other methods that use organic solvents, for example. Further studies using different supercritical solvents are needed to improve the extraction results obtained by SFE in order to make this technique more competitive to the technologies currently used for extraction of antioxidant phenolic compounds from natural resources.

Acknowledgements

This study was funded by Portuguese National Fund through FCT – Fundação para a Ciência e a Tecnologia (grant SFRH/BD/75195/2010).

3.4. References

Andrade, K. S., Gonçalvez, R. T., Maraschin, M., Ribeiro–do–Valle, R. M., Martínez, J., Ferrreira, S. R. S. (2012). Supercritical fluid extraction from spent coffee grounds and coffee husks: Antioxidant activity and effect of operational variables on extract composition. *Talanta*, 88, 544–552.

Andrade, K. S., Poncelet, D., Ferreira, S. R. S. (2017a). Sustainable extraction and encapsulation of pink pepper oil. *Journal of Food Engineering*, 204, 38–45.

Andrade, K. S., Trivellin, G., Ferreira, S. R. S. (2017b). Piperine–rich extracts obtained by high pressure methods. *The Journal of Supercritical Fluid*, 128, 370–377.

Barbosa, H. M. A., Melo, M. M. R., Coimbra, M. A., Passos, C. P., Silva, C. M. (2014). Optimization of the supercritical fluid coextraction of oil and diterpenes from spent coffee grounds using experimental design and response surface methodology. *Journal of Supercritical Fluids*, 85, 165– 172.

Benzie, I. F. F., Strain, J. J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of “antioxidant power”: The FRAP assay. *Analytical Biochemistry*, 239, 70–76.

Bradford, M. (1976). A rapid and sensitive method for the quantification of microgram quantities of protein using the principles of protein–dye binding. *Analytical Biochemistry.,* 72, 248–254.

Campos, L. M. A. S., Leimann, F. V., Pedrosa, R. C., Ferreira, S. R. S. (2008). Free radical scavenging of grape pomace extracts from Cabernet sauvingnon (*Vitis vinifera*). *Bioresource Technology*, 99, 8413–8420.

Couto, R. M., Fernandes, J., Gomes da Silva, M. D. R., Simões, P. C. (2009). Supercritical fluid extraction of lipids from spent coffee grounds. *The Journal of Supercritical Fluids*, 51, 159–166.

Chang, C.–C., Yang, M.–H., Wen, H.–M., Chern, J.–C. (2002). Estimation of total flavonoid content in propolis by two complementary colorimetric methods. *Journal of Food and Drug Analysis*, 10, 178–182.

Esquível, M., Bernardo–Gil, M. G. (1993). Extraction of olive husk oil with compressed carbon dioxide. *Journal of Supercritical Fluids*, 6, 1993, 91–94.

Maul, A. A., Wasicky, R., Bacchi, E. M. (1996). Extração por fluido supercrítico. *Revista Brasileira de farmacognosia*, 5(2), 185–200.

Maul, A. A. (1999). Fluidos supercríticos, situação atual e futuro da extração supercrítica. *Biotecnologia Ciência & Desenvolvimento*, 2(11), 42–46.

Mezzomo, N., Martinéz, J., Ferreira, S. R. S. (2009). Supercritical fluid extraction of peach (Prunus persica) almond oil: Kinetics, mathematical modeling and scale–up. *The Journal of Supercritical Fluids*, 51, 10–16.

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

Mussato, S. I., Carneiro, L. M., Silva, J. P. A., Roberto, I. C., Teixeira, J. A. (2011b). A study on chemical constituents and sugars extraction from spent coffee grounds. *Carbohydrate Polymers,* 83, 368–374.

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

Passos, C. P., Silva, R. M., da Silva, F. A., Coimbra, M. A., Silva, C. M. (2009). Enhancement of the supercritical fluid extraction of grape seed oil by using enzymatically pre-treated seed. *Journal of Supercritical Fluids*, 48, 225–229.

Pereira. C. G., Meireles, M. A. A. (2010). Supercritical fluid extraction of bioactive compounds: fundamentals, applications and economic perspectives. *Food Bioprocess Technology*, 3, 340–372

Perrut, M. (2000). Supercritical Fluid Applications: Industrial development and economic issues. *Industrial & Engineering Chemistry Research*, 39, 4531–4535.

Prieto, P., Pineda, M., Aguilar, M. (1999). Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. *Analytical Biochemistry*, 269, 337–341.

Singleton, V. L., Rossi Jr, J. A. (1965). Colorimetry of total phenolics with phosphomolybdic–phosphotungstic acid reagents. *American Journal of Enology and Viticulture*, 16, 144–158.

Zuorro, A., Lavecchia, R. (2011). Polyphenols and energy recovery from spent coffee grounds. *Chemical Engineering Transactions*, 25, 285–290.

CHAPTER 4

EXTRACTION OF VALUE–ADDED COMPOUNDS FROM SPENT COFFEE GROUNDS USING MICROWAVED–ASSISTED EXTRACTION

Abstract

Microwave–assisted extraction (MAE) has been used for the extraction of antioxidant phenolic compounds from spent coffee grounds (SCG) using water as solvent. Various experimental conditions, such as power (50 % to 100 %), solvent/solid ratio (10 to 40 mL/SCG), extraction time (1 min to 31 min) and pressure (60 psi to 120 psi) were investigated to find the optimal condition for the extraction. The extracts obtained in this process were characterized regarding the contents of total phenolic compounds, proteins, sugars, flavonoids, and antioxidant activity, which was determined by two methods (ferric reducing antioxidant power (FRAP) assay and total antioxidant capacity with α–tocopherol). The highest contents of phenolic compounds (32.33 mg GAE/g SCG), sugars (49.78 mg Glucose/g SCG) and flavonoids (4.86 mg QE/g SCG) were obtained for MAE at 90 psi, power at 75 %, 16 min and using a solvent/solid ratio of 25 mL/g SCG. The microwave–assisted extraction at 120 psi, 31 min, power at 100 % and utilizing a solvent/solid ratio of 10 mL/g SCG was the condition that released the highest contents of protein (31.82 mg BSA/g SCG). In terms of antioxidant activity, MAE at 90 psi, power at 75 %, 31 min and utilizing a solvent/solid ratio of 25 mL/g SCG has showed the highest value of the FRAP (0.2692 mM Fe (II)/g SCG) and with a MAE at 120 psi, power at 75 %, 16 min using a solvent/solid ratio of 25 mL/g SCG was the condition that obtained the best values of total antioxidant capacity (48.45 mg α–tocopherol/g SCG).

Keywords: Microwave–assisted extraction; Phenolic compounds; Antioxidant activity; Proteins; Spent coffee grounds.

4. Introduction

In the coffee industry are generated large amounts of waste, being the SCG the most important on due to the large annual volume in which it is obtained (6 000 000 tons/year). For each ton of processed green coffee are generated around 650 kg of SCG, and for a pound of coffee produced, are generated 2 kg of SCG wet, which have been disposed of as a conventional solid waste (Mussatto *et al.*, 2011a). All wastes of coffee processing are rich in phenolic compounds, so they are raw materials with the potential to obtain valuable compounds (Murthy *et al.*, 2012). Some studies published earlier have showed that this residue is rich in sugars and phenolic compounds (Machado, 2009; Mussatto *et al.*, 2011b; Mussatto *et al.*, 2011c).

The Microwave–assisted extraction (MAE) emerges as a technology of interest to the industry, which representing an alternative to conventional extraction processes (Dahmoune *et al.*, 2015; Drosou *et al.*, 2015; Moreira *et al.*, 2017). This technique is very attractive because of the speed of the extraction process, the reduced use of organic solvents, the possibility of multiple extractions when using the system with carousel, simplicity of use and good reproducibility (Chan *et al.*, 2011). Several authors have investigated the use of this technique to obtain bioactive compounds from other wastes, for the example plants, microalgae and fruits (Calinescu *et al.*¸2017; Dahmoune *et al.*, 2015; Drosou *et al.*, 2015; Kumar *et al.*, 2018; Moreira *et al.*, 2017; Pandey *et al.*, 2018; Thirugnanasambandham *et al.*, 2017; Upadhyay *et al.*, 2012). Because is a technique that has little impact on the environment, comparing to traditional techniques, the MAE arouses great interest for its application in the extraction of high–value compounds.

The purpose of this study aims to evaluate the extraction of sugars and antioxidant phenolic compounds from SCG by the technique of MAE and determine the operating conditions that maximize the release of the compounds. The experiments will be performed in accordance with a statistical experimental design (in section 4.1.3.6.) and operating conditions that maximize the extraction of the respective compounds and will be identified through the statistical methodology of response surface.

4.1. Material and methods

4.1.1. Raw materials and chemicals

The raw material used was spent coffee grounds (SCG) which was supplied by a Portuguese company of reference in this sector NovaDelta – Comércio e Indústria de Cafés, Lda (Campo Maior, Portugal). The provided material was dried in an oven at 60 °C until approximately 10 % moisture content and stored afterwards for use in the following steps. Phenolic standards including gallic acid and quercetin; methanol, α–tocopherol and Folin–Ciocalteu phenol reagent were purchased from Sigma (Sigma–Aldrich GmbH, Sternheim, Germany); Thermo Scientific provided Coomassie (Bradford) protein assay kit.

4.1.2. Extraction procedure

The experiments were performed in a digestion oven model MDS2000 (CEM Corporation, Matthews, NC). In each experiment were placed reaction vessels interconnected with tubing, in the sample holder placed in a rotating carousel. Only one of the vessels was equipped with pressure sensor that measured and controlled the set point within the cell. For each extraction, spent coffee grounds was suspended in a certain amount of distilled water and placed inside the extraction vessel. These extractions were irradiated under different powers, solid–liquid ratios, extraction times and pressures. After irradiation, the vessels were immediately cooled in ice bath and the suspensions were filtrated to separate the spent coffee grounds of the extract obtained. All extractions were performed in duplicate.

4.1.3. Analytical methodology

4.1.3.1. Determination of total phenolic compounds

The phenolic compounds in SCG extracts were determined using a 96–well microplate–adapted colorimetric assay that uses Folin–Ciocalteu reagent according to the colorimetric method described by Singleton et al. (1965). Initially, 5 μL of each filtered extract were mixed with 60 μL of sodium carbonate solution at 7.5 % (w/v) and 15 μL of Folin–Ciocalteu reagent. Then, 200 μL of distilled water

were added and the solutions were mixed. After standing for 5 min at 60 °C samples were allowed to cool at room temperature. The absorbance was measured by means of a spectrophotometric microplate reader set at 700 nm. A calibration curve was made from a gallic acid standard solution (200 mg/L, 400 mg/L, 600 mg/L, 800 mg/L, 1000 mg/L, 2000 mg/L and 3000 mg/L) and the blank was prepared with distilled water. The phenolic compounds content was expressed as milligram gallic acid equivalent per dry weight of material or per gram of extract (mg GAE/g SCG or mg GAE/g extract).

4.1.3.2. Determination of proteins

Proteins in SCG extracts were measured using the Coomassie G–250 dye in a colorimetric reagent described by Bradford (1976) with some modifications. A volume of 10 μL of each filtered extract was added to 300 μL of Coomassie Blue reagent in a 96–well microplate. Then, the plate is shaked for 30 s and allowed to rest for 10 min at room temperature. The absorbance of the mixture was then measured at 595 nm against a blank with distilled water. A calibration curve was prepared with BSA solution (25 mg/L, 125 mg/L, 250 mg/L, 500 mg/L, 750 mg/L and 1000 mg/L). The total proteins content was expressed as milligram protein equivalent per dry weight of material or per gram of extract (mg BSA/g SCG or mg BSA/g extract).

4.1.3.3. Determination of reducing sugars content

The reducing sugars content of SCG extracts was estimated using the DNS (3,5–dinitrosalicylic acid) method, adapted to a 96–well microplate. Initially, 25 μL of each filtered extract was added to 25 μL of DNS in a 96–well microplate and incubated at 100 °C for 5 min. After, was added 250 μL of distilled water in the samples and the microplate was placed on ice. Then, the samples were cooled to room temperature. The absorbance of the mixture was then measured at 540 nm against a blank with distilled water. A calibration curve was prepared with a standard solution of glucose (0 mg/mL, 0.2 mg/mL, 0.4 mg/mL, 0.6 mg/mL, 0.8 mg/mL, 1.0 mg/mL, 1.2 mg/mL, 1.4 mg/mL, 1.6 mg/mL, 1.8 mg/mL and 2.0 mg/mL). The reducing sugars content was expressed as milligram glucose

equivalent per dry weight of material or per gram of extract (mg glucose/g SCG or mg glucose/g extract).

4.1.3.4. Determination of total flavonoid content

The flavonoids in SCG extracts were estimated using the colorimetric assay previously described by Chang *et al.* (2002) with some modifications. Initially, 30 μL of each filtered extract was added to 90 μL of methanol in a 96–well microplate. After, the addition of 6 μL of aluminum chloride at 10 % (w/v), 6 μL of potassium acetate (1 mol/L), and 170 μL of distilled water to each extract sample was performed. Samples were maintained in the dark, at room temperature, during 30 min. The absorbance of the mixture was then measured at 415 nm against a blank with distilled water. A calibration curve was prepared with a standard solution of quercetin (25 mg/L, 50 mg/L, 100 mg/L, 150 mg/L and 200 mg/L). The total flavonoid content was expressed as milligram quercetin equivalent per dry weight of material or per gram of extract (mg QE/g SCG or mg QE/g extract).

4.1.3.5. Determination of antioxidant activity

The antioxidant activity of SCG extracts was estimated by two different methods: the ferric reducing antioxidant power (FRAP) assay, and the phosphomolybdenum complex method (total antioxidant capacity). The FRAP assay was performed according to the method described by Benzie *et al.* (1996) with some modifications. Initially, 10 μL aliquot of filtered extract was mixed with 290 μL of FRAP reagent in a 96–well microplate, and incubated at 37 °C for 15 min. Then, the absorbance was determined at 593 nm against a blank that was prepared using distilled water. A calibration curve was constructed using an aqueous solution of ferrous sulfate – $FeSO_4.7H_2O$ (200 μM, 400 μM, 600 μM, 800 μM and 1000 μM). The FRAP values were expressed as millimoles of ferrous equivalent per dry weight of material or per gram of extract (mM Fe(II)/g SCG or mM Fe(II)/g extract).

To determine the total antioxidant capacity of SCG extracts by the phosphomolybdenum complex method described by Prieto *et al.* (1999), 100 μL of each filtered extract was added to 1 mL of a reaction solution containing 0.6 M of sulfuric acid, 28 mM of sodium phosphate, and 4 mM of ammonium molybdate, in tubes. After, the samples were placed in a water bath at 95 °C during 90 min. Then, the samples were cooled to room temperature. The absorbance was then measured at

695 nm against a blank with distilled water. The calibration curve was prepared using a solution "stock" of α–tocopherol (25 μg/mL, 75 μg/mL, 125 μg/mL, 250 μg/mL, 375 μg/mL and 500 μg/mL). The total antioxidant capacity was expressed as milligram α–tocopherol equivalent per dry weight of material or per gram of extract (mg α–tocopherol/g SCG or mg α–tocopherol/g extract).

4.1.3.6. Statistical analyses

The influence of the variables as power, water/SCG ratio, time and pressure, on the extraction of antioxidant phenolic compounds from SCG by MAE was investigated through a 2^4 full factorial design and a 2^4 central composite design. The real and coded values of the variables for the experimental designs are given in Table 4.1.

Statistical significance of the variables was determined at the 5 % probability level (p <0.05). Statistical analysis of the data and determination of the optimal condition able to maximize the extraction results were performed utilizing the Software Statistica (version 8.0), and Design Expert (version 7.0).

Table 4.1. Experimental conditions used for MAE of antioxidant phenolic compounds from SCG according to a 2^4 full experimental design. Real and coded values of the operational variables power (x1), solvent/solid ratio (x2), extraction time (x3) and pressure (x4)

Assay	Coded variables				Real variables*			
	x1	x2	x3	x4	X1	X2	X3	X4
1	-	-	-	+	50	10	1	120
2	+	-	-	+	100	10	1	120
3	-	+	-	+	50	40	1	120
4	+	+	-	+	100	40	1	120
5	-	-	+	-	50	10	31	60
6	+	-	+	-	100	10	31	60
7	-	+	+	-	50	40	31	60
8	+	+	+	-	100	40	31	60
9	-	-	-	+	50	10	1	120
10	+	-	-	+	100	10	1	120
11	-	+	-	+	50	40	1	120
12	+	+	-	+	100	40	1	120
13	-	-	+	-	50	10	31	60
14	+	-	+	-	100	10	31	60
15	-	+	+	-	50	40	31	60
16	+	+	+	-	100	40	31	60
17	0	0	0	0	75	25	16	90
18	0	0	0	0	75	25	16	90
19	0	0	0	0	75	25	16	90
20	0	0	0	0	75	25	16	90
21	-	0	0	0	50	25	16	90
22	+	0	0	0	100	25	16	90
23	0	-	0	0	75	10	16	90
24	0	+	0	0	75	40	16	90
25	0	0	-	0	75	25	1	90
26	0	0	+	0	75	25	31	90
27	0	0	0	-	75	25	16	60
28	0	0	0	+	75	25	16	120

***** x1: Power (%); x2: Solvent/solid ratio (mL g^{-1} SCG); x3: Extraction time (min); x4: Pressure (psi).

4.2. Results and discussion

This study consisted in determining the best condition for the maximum recovery of antioxidant phenolic compounds from spent coffee ground, checking the effect of the following variables: microwave power, solvent/solid ratio, time and pressure on the recovery of these compounds. The results of the extraction of the phenolic compounds, sugars, proteins and flavonoids are presented in Table 4.2 and in Table 4.3 it is possible to see the results obtained for antioxidant activity by two different methods: FRAP and total antioxidant capacity. Statistical analysis of these data revealed a significant influence (p <0.05) of all the studied variables on the extraction results.

It is possible to verify in Table 4.2, that the highest value for total phenolic contents, sugars and flavonoids obtained are 32.33 mg GAE/g SCG, 49.78 mg Glucose/mg SCG and 4.86 mg QE/g SCG, respectively for the same condition (microwave power at 75 %, extraction time 16 min, pressure 90 psi and using solvent/solid ratio of 25 mL/g SCG). For the proteins quantification the maximum value was 31.82 mg BSA/g SCG using a microwave power of 100 %, extraction time of 31 min, pressure of 120 psi and utilizing solvent/solid ratio of 10 mL/g SCG (Table 4.2).

Pintathong *et al.* (2011) studied the utilization of microwave for phenolic antioxidants extraction. The variables which were investigated in that work were microwave power, extraction time and ethanol concentration. The highest result was 6.71 mg GAE/g SCG using a microwave power of 772 w, an extraction time 30 min and an ethanol concentration 46 % v/v, which is much lower than the result obtained in this work. In another work to extract phenolic antioxidants compounds from potato peels using MAE, we can see that the highest value of total phenolic contents was 3.94 mg/g dry matter, at a microwave power of 67,33 %, an extraction time of 15 min and a solvent concentration (methanol) of 14.67 % (Singh *et al.*, 2011).

Table 4.2. Results obtained for the different analyzes: Total phenolics, total proteins content and reducing sugars content for the different extracts obtained from SCG

	Power (%)	Solvent/solid ratio (ml/g SCG)	Time (min)	Pressure (psi)	Total Phenolics		Proteins		Sugars		Flavonoids	
					(mg GAE/g extract)	(mg GAE/g SCG)	(mg BSA/g extract)	(mg BSA/g SCG)	(mg Glucose/g extract)	(mg Glucose/g SCG)	(mg QE/g extract)	(mg QE/g SCG)
1	50	40	1	60	16.53 ± 0.38	12.40 ± 0.29	23.68 ± 0.26	17.76 ± 0.20	32.43 ± 1.36	24.32 ± 1.02	2.48 ± 0.08	1.86 ± 0.06
2	100	40	1	60	19.49 ± 0.13	12.43 ± 0.08	23.27 ± 0.31	14.85 ± 0.20	32.89 ± 0.87	20.98 ± 0.55	2.57 ± 0.05	1.64 ± 0.03
3	50	10	1	60	21.14 ± 0.60	18.50 ± 0.52	33.46 ± 0.54	29.28 ± 0.47	34.98 ± 1.31	30.61 ± 1.15	3.31 ± 0.05	2.90 ± 0.05
4	100	10	1	60	19.28 ± 0.34	17.35 ± 0.30	31.79 ± 0.76	28.61 ± 0.68	31.16 ± 0.71	28.04 ± 0.63	3.07 ± 0.06	2.76 ± 0.05
5	50	40	31	60	21.20 ± 0.54	15.90 ± 0.41	20.52 ± 0.40	15.39 ± 0.30	38.16 ± 0.98	28.62 ± 0.73	2.96 ± 0.02	2.22 ± 0.01
6	100	40	31	60	28.93 ± 0.35	18.51 ± 0.23	20.66 ± 0.38	13.15 ± 0.24	51.69 ± 1.64	33.05 ± 1.06	3.54 ± 0.08	2.26 ± 0.05
7	50	10	31	60	22.04 ± 0.55	19.29 ± 0.48	34.26 ± 0.54	29.98 ± 0.47	36.15 ± 1.09	31.63 ± 0.95	3.38 ± 0.08	2.96 ± 0.07
8	100	10	31	60	24.37 ± 0.15	21.32 ± 0.13	36.01 ± 0.55	31.51 ± 0.48	38.67 ± 0.59	33.83 ± 0.51	3.87 ± 0.09	3.38 ± 0.08
9	50	40	1	120	20.98 ± 0.13	15.74 ± 0.10	25.11 ± 0.48	18.83 ± 0.36	34.12 ± 1.17	25.59 ± 0.88	3.06 ± 0.04	2.29 ± 0.03
10	100	40	1	120	21.15 ± 0.28	14.59 ± 0.19	25.44 ± 0.45	17.49 ± 0.32	36.57 ± 1.06	25.14 ± 0.73	2.99 ± 0.07	2.07 ± 0.05
11	50	10	1	120	21.86 ± 0.25	19.13 ± 0.22	33.88 ± 0.54	29.65 ± 0.47	33.68 ± 0.37	29.47 ± 0.32	3.65 ± 0.03	3.20 ± 0.03
12	100	10	1	120	20.07 ± 0.41	18.25 ± 0.37	31.95 ± 0.52	29.13 ± 0.48	31.76 ± 1.27	28.90 ± 1.16	3.52 ± 0.05	3.21 ± 0.05
13	50	40	31	120	25.01 ± 0.38	17.13 ± 0.26	12.80 ± 0.43	8.71 ± 0.28	41.16 ± 1.56	28.10 ± 1.04	2.46 ± 0.04	1.70 ± 0.03
14	100	40	31	120	25.80 ± 1.13	14.41 ± 0.63	11.19 ± 0.79	6.17 ± 0.40	43.43 ± 2.00	24.31 ± 1.18	2.37 ± 0.01	1.34 ± 0.00
15	50	10	31	120	25.89 ± 0.58	22.65 ± 0.51	32.81 ± 0.51	28.71 ± 0.44	45.79 ± 0.89	40.07 ± 0.78	4.34 ± 0.06	3.80 ± 0.05
16	100	10	31	120	24.67 ± 0.73	21.58 ± 0.64	36.37 ± 0.43	31.82 ± 0.38	40.63 ± 0.45	35.55 ± 0.40	3.97 ± 0.05	3.48 ± 0.05
17					32.77 ± 0.35	24.58 ± 0.26	31.77 ± 0.85	23.83 ± 0.64	49.04 ± 1.07	36.78 ± 0.80	4.36 ± 0.15	3.27 ± 0.12
18	75	25	16	90	40.42 ± 0.28	32.33 ± 0.22	36.08 ± 0.67	28.86 ± 0.54	62.22 ± 1.12	49.78 ± 0.90	6.08 ± 0.06	4.86 ± 0.05
19					39.63 ± 0.76	31.70 ± 0.61	28.02 ± 0.21	22.42 ± 0.17	39.16 ± 0.94	31.32 ± 0.76	6.05 ± 0.07	4.84 ± 0.06
20	50	25	16	90	29.93 ± 0.25	20.64 ± 0.15	24.55 ± 0.21	17.00 ± 0.15	41.74 ± 0.65	28.85 ± 0.50	4.31 ± 0.04	2.94 ± 0.03
21	100	25	16	90	27.87 ± 0.35	24.39 ± 0.31	25.26 ± 0.33	22.10 ± 0.29	42.03 ± 1.73	36.78 ± 1.52	4.28 ± 0.06	3.75 ± 0.05
22	75	10	16	90	24.93 ± 0.13	18.69 ± 0.10	16.38 ± 0.35	12.28 ± 0.26	37.59 ± 1.15	28.19 ± 0.86	3.52 ± 0.05	2.64 ± 0.04
23	75	40	16	90	27.08 ± 0.26	23.35 ± 0.23	31.41 ± 0.66	27.10 ± 0.58	32.89 ± 1.22	28.37 ± 1.04	3.68 ± 0.05	3.18 ± 0.04
24	75	25	1	90	23.94 ± 0.27	20.29 ± 0.23	28.08 ± 0.60	23.80 ± 0.50	33.30 ± 0.49	28.19 ± 0.41	3.89 ± 0.06	3.28 ± 0.05
25	75	25	31	90	30.63 ± 0.18	23.74 ± 0.14	23.49 ± 0.17	18.21 ± 0.14	47.58 ± 1.18	36.88 ± 0.91	4.60 ± 0.02	3.56 ± 0.02
26	75	25	16	60	27.84 ± 0.20	23.31 ± 0.17	26.72 ± 0.53	22.37 ± 0.45	36.61 ± 1.39	30.64 ± 1.16	3.91 ± 0.06	3.27 ± 0.05
27	75	25	16	120	33.41 ± 0.32	26.67 ± 0.26	23.38 ± 0.54	18.77 ± 0.42	49.92 ± 1.05	39.73 ± 0.82	4.69 ± 0.02	3.74 ± 0.02
28	75	25	16	90	33.09 ± 0.41	25.20 ± 0.31	26.77 ± 0.49	20.40 ± 0.37	43.87 ± 2.16	33.41 ± 1.65	4.85 ± 0.06	3.70 ± 0.04

Dahmoune *et al.* (2015) investigated MAE method for extraction of polyphenols from *Myrtus communis* leaves. The highest result for the recovery of total phenolic was 162.49 mg GAE/g DW and for the total flavonoids was 5.02 mg QE/g DW. These results were obtained using, as extraction conditions, 42 % of ethanol concentration, 500 W microwave power, 62 s irradiation time and 32 mL/g solvent to material ratio. Through the study by Dahmoune *et al.* (2015) is possible to verify that values presented for flavonoids are very similar with the result obtained in this work, however for the total phenolic is much higher. Comparing the results reported in the study of Dahmoune *et al.* (2015) with those of this work, it is possible to conclude that the presence of ethanol as a solvent increases the extraction efficiency of the polyphenols (Drosou *et al.*, 2015).

The use of MAE has obtained highest values of antioxidant phenolic compounds comparing with another techniques used for extraction of the same compounds. In the study of Dahmoune *et al.* (2015), previously mentioned, they compared different extraction methods as microwave–assisted extraction (MAE), ultrasound–assisted extraction (UAE) and conventional solvent extraction (CSE). They verified that MAE showed a similar extraction capacity for the total phenolic as compared to USE (144.77 mg GAE/g DW) and CSE (128.00 mg GAE/g DW). The extracted flavonoids of MAE (5.02 mg QE/g DW) were also significantly higher than UAE (3.88 mg QE/g DW) and CSE (4.15 mg QE/g DW). The results obtained in this work for the flavonoids contents are also significantly higher than UAE and CSE reported by Dahmoune *et al.* (2015).

Mussatto *et al.* (2011c) evaluated the performance of conventional solid-liquid method (methanol as solvent) in the recovery of antioxidant phenolic compounds from spent coffee grounds. The results obtained showed that the best value for total phenolic compounds was 16 mg GAE/g SCG using 60 % methanol in a solvent/solid ratio of 40 mL/g SCG, during 90 min (Mussatto *et al.*, 2011c). The best result for the flavonoids contents was 2.50 mg QE/g SCG, using 80 % methanol concentration as solvent and 40 mL/g SCG as solvent/solid ratio, which was lower than the results obtained in this work (Mussatto *et al.*, 2011c).

Another study by Moreira *et al.* (2017) also reported that the MAE was the best technique for the extraction of polyphenols comparing with conventional extraction (CE). They obtained values of phenolic compounds of the 44.7 mg GAE/g DW in the MAE and 35.8 mg GAE/g DW in the CE. These results were obtained in an optimum conditions: 20 mL ethanol:water 60:40 v/v, 20 min, 100 °C, sample weight 0.1 g for MAE and 20 mL ethanol:water 50:50 v/v, 2 h, 55 °C, sample weight 0.5 g for CE.

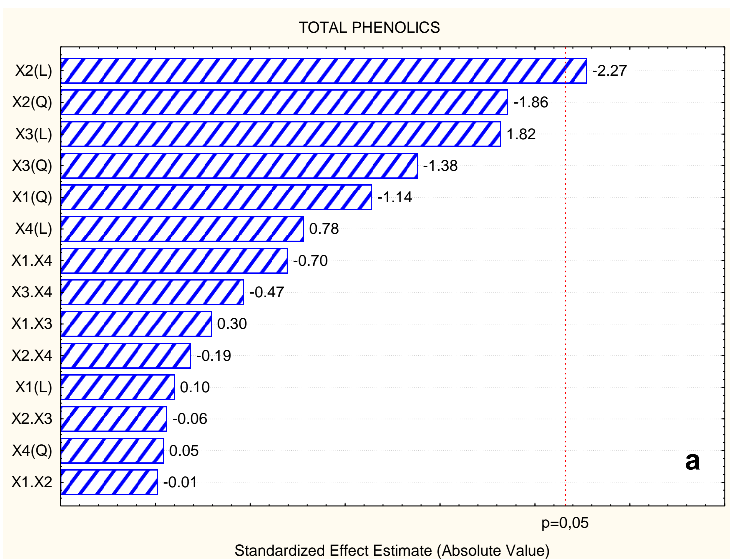
These differences in results may be related with the main MAE principle (volumetric heating–heat is originated inside the material). MAE is considered as a green extraction process as the time and amount of solvents used are lower in comparison with the other methods. Moreover, one of the greater advantages of MAE technique is that the extraction solvent penetrates more easily inside the cell walls and break those linkages between the phenolics and cell walls increasing the amount of phenolic compounds recovered (Drosou *et al.*, 2015). Therefore, the application of MAE technique despite of a high initial investment can become more profitable in terms of yield, and time and money savings (Moreira *et al.*, 2017).

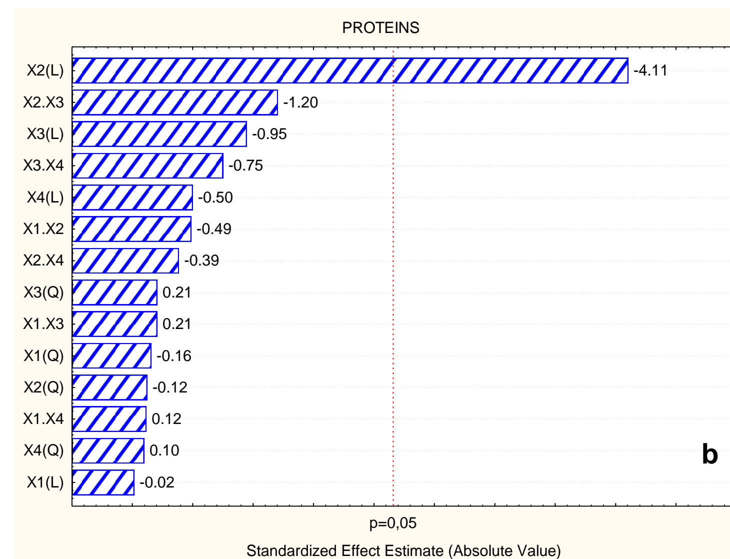
In Table 4.3, are shown the values for antioxidant activity from all extracts. We used two different methods to quantify the antioxidant activity of extracts from SCG: the first was determinate by the ferric reducing antioxidant power (FRAP) assay, and the second by the phosphomolybdenum complex method. The best value for antioxidant activity of the SCG extracts using the FRAP method was 0.2692 mM Fe(II)/g SCG with a microwave power of 75 %, extraction time of 31 min, pressure of 90 psi and using solvent/solid ratio of 25 mL/g SCG. These results are higher compared with the results obtained by Mussatto *et al.* (2011c). Their best result obtained 0.102 mM Fe (II)/g SGC using 50 % methanol concentration as solvent and 40 mL/g SCG as solvent/solid ratio (Mussatto *et al.*, 2011c). The highest result for total antioxidant capacity, using the second method was 48.45 mg α–tocopherol/g SCG, with a microwave power of 75 %, extraction time of 16 min, pressure of 120 psi and utilizing solvent/solid ratio of 25 mL/g SCG. These results demonstrate that studied operational variables had influence in the extraction of antioxidant phenolic compounds from SCG.

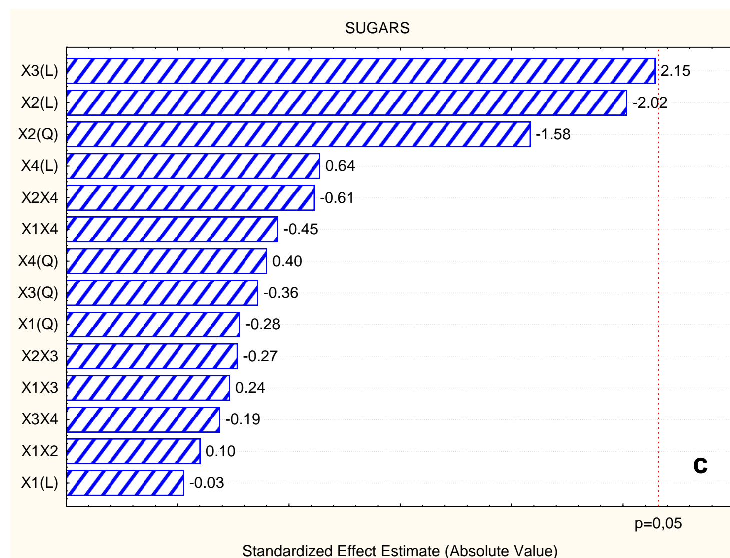
The Pareto charts in Figure 4.1 show the effect of each operational variable on the different responses. It is possible to see that the variable solvent/solid ratio (X2) exerted significant influence (p <0.05) in almost all the evaluated responses. The solvent/solid ratio (X2) was the most important variable affecting the total phenolics (Figure 4.1a), proteins (Figure 4.1b), sugars (Figure 4.1c), flavonoids (Figure 4.1d), antioxidant activity – FRAP (Figure 4.1e) and total antioxidant activity of the extracts (Figure 4.1f). One can also verify that the time (X3) is another variable that affects the total antioxidant activity (Figure 4.1f). In two works published we can verify that the solid/solvent ratio is also an important parameter when performing MAE, yielding higher recoveries when using more diluted conditions (Rodriguez-Jasso *et al.*, 2011; Passos *et al.*, 2013). According to work of Passos et al. (2019) one of the main advantages of the MAE were the short operating time because shorter times are associated with minimal processing with also reduction of the costs involved in the extraction process.

Table 4.3. Results obtained for the different analyzes: FRAP and total antioxidant capacity for the different extracts obtained from SCG

	Power (%)	Solvent/solid ratio (ml/g SCG)	Time (min)	Pressure (psi)	FRAP		Total Antioxidant Capacity	
					(mM Fe (II)/g extract)	(mM Fe (II)/g SCG)	(mg α-Tocoferol/g extract)	(mg α-Tocoferol/g SCG)
1	50	40	1	60	0.1113 ± 0.0008	0.0834 ± 0.0006	33.51 ± 0.81	25.14 ± 0.61
2	100	40	1	60	0.1078 ± 0.0008	0.0687 ± 0.0005	34.44 ± 0.75	21.94 ± 0.47
3	50	10	1	60	0.1468 ± 0.0010	0.1285 ± 0.0009	38.24 ± 0.70	31.32 ± 0.62
4	100	10	1	60	0.1313 ± 0.0022	0.1182 ± 0.0020	37.32 ± 0.92	33.10 ± 0.83
5	50	40	31	60	0.1273 ± 0.0006	0.0955 ± 0.0005	36.59 ± 0.73	30.04 ± 0.55
6	100	40	31	60	0.1925 ± 0.0024	0.1231 ± 0.0015	45.56 ± 1.09	31.09 ± 0.69
7	50	10	31	60	0.1582 ± 0.0012	0.1384 ± 0.0010	50.44 ± 0.38	38.97 ± 0.34
8	100	10	31	60	0.1784 ± 0.0019	0.1561 ± 0.0017	55.70 ± 0.49	40.99 ± 0.43
9	50	40	1	120	0.1396 ± 0.0005	0.1047 ± 0.0004	41.77 ± 0.57	33.76 ± 0.43
10	100	40	1	120	0.1189 ± 0.0015	0.0819 ± 0.0010	41.71 ± 0.69	33.98 ± 0.50
11	50	10	1	120	0.1576 ± 0.0026	0.1379 ± 0.0023	41.30 ± 0.93	34.99 ± 0.81
12	100	10	1	120	0.1375 ± 0.0019	0.1251 ± 0.0018	35.47 ± 0.63	30.17 ± 0.58
13	50	40	31	120	0.1983 ± 0.0020	0.1367 ± 0.0014	50.71 ± 0.96	31.69 ± 0.66
14	100	40	31	120	0.1848 ± 0.0015	0.1036 ± 0.0009	48.80 ± 1.03	29.40 ± 0.52
15	50	10	31	120	0.2024 ± 0.0024	0.1771 ± 0.0021	44.02 ± 0.59	38.52 ± 0.52
16	100	10	31	120	0.1895 ± 0.0015	0.1658 ± 0.0013	41.42 ± 0.94	36.24 ± 0.82
17					0.2336 ± 0.0022	0.1752 ± 0.0017	62.16 ± 0.21	46.62 ± 0.16
18	75	25	16	90	0.2669 ± 0.0000	0.2135 ± 0.0000	59.37 ± 0.48	44.52 ± 0.39
19					0.1902 ± 0.0030	0.1522 ± 0.0024	62.13 ± 0.81	38.83 ± 0.65
20	50	25	16	90	0.3279 ± 0.0015	0.2261 ± 0.0011	47.77 ± 1.10	33.21 ± 0.74
21	100	25	16	90	0.3034 ± 0.0040	0.2655 ± 0.0035	50.09 ± 1.18	40.33 ± 1.04
22	75	10	16	90	0.2366 ± 0.0013	0.1775 ± 0.0010	44.02 ± 0.54	35.73 ± 0.40
23	75	40	16	90	0.2495 ± 0.0017	0.2152 ± 0.0015	42.01 ± 1.45	31.51 ± 1.25
24	75	25	1	90	0.2356 ± 0.0027	0.1989 ± 0.0023	41.22 ± 0.40	35.39 ± 0.35
25	75	25	31	90	0.3473 ± 0.0018	0.2692 ± 0.0014	44.03 ± 1.57	35.96 ± 1.22
26	75	25	16	60	0.2691 ± 0.0040	0.2254 ± 0.0034	44.81 ± 1.61	38.33 ± 1.35
27	75	25	16	120	0.3298 ± 0.0042	0.2631 ± 0.0034	62.52 ± 1.35	48.45 ± 1.05
28	75	25	16	90	0.3262 ± 0.0028	0.2485 ± 0.0021	52.34 ± 1.54	43.85 ± 1.16







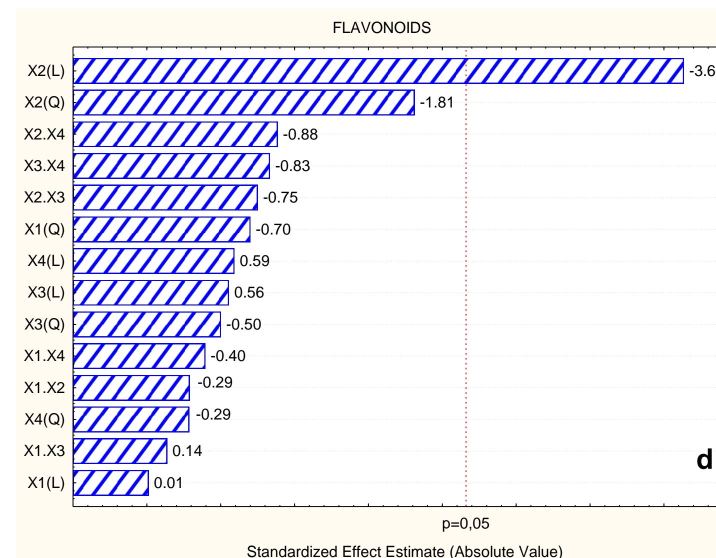
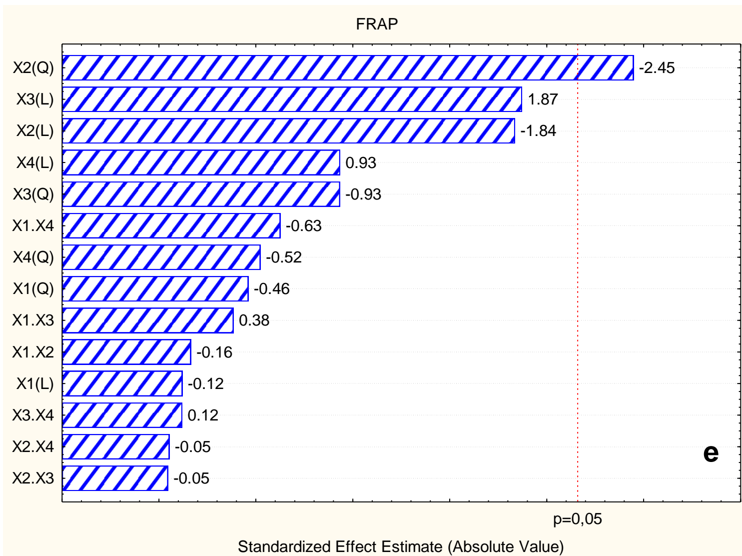


Figure 4.1. Pareto chart for the effects of power (X1), solvent/solid ratio (X2), extraction time (X3), pressure (X4) and the interaction between them, during microwave–assisted extraction of spent coffee grounds, on the total phenolics (a), proteins (b), sugars (c), flavonoids (d), antioxidant activity - FRAP (e), and total antioxidant capacity (f) contents in the produced extracts. L and Q correspond to the effects at linear and quadratic levels, respectively.



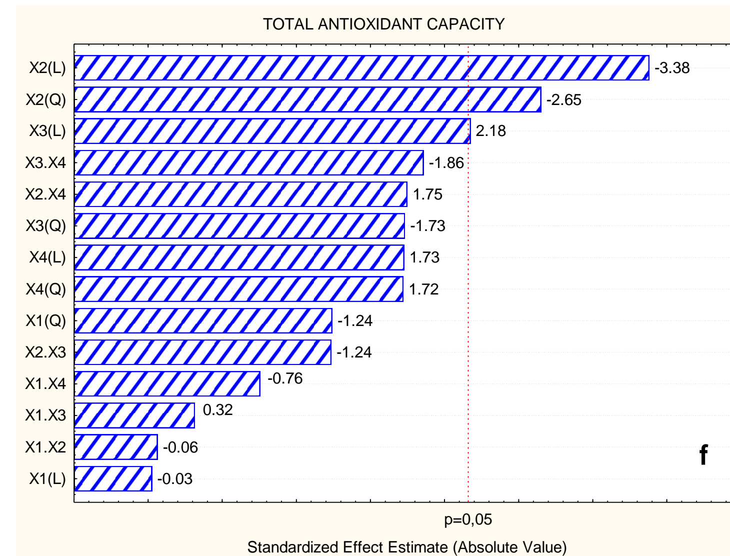


Figure 4.1. (Conclusion) Pareto chart for the effects of power (X1), solvent/solid ratio (X2), extraction time (X3), pressure (X4) and the interaction between them, during microwave–assisted extraction of spent coffee grounds, on the total phenolics (a), proteins (b), sugars (c), flavonoids (d), antioxidant activity – FRAP (e), and total antioxidant capacity (f) contents in the produced extracts. L and Q correspond to the effects at linear and quadratic levels, respectively.

In Table 4.4 are represented the polynomial equations fitted to the experimental data of antioxidant phenolic compounds in spent coffee grounds extracts. The models were simplified by elimination of statistically insignificant terms (with p >0.05). The coefficient of determinations R^2 that were established at the different models ranged between 0.56 to 0.83. In almost all mathematical models, the linear (L) term was the most important for the different responses, once that presented statistical significance at p <0.05. The quadratic (Q) term is statistically significant only in the antioxidant activities (Figure 4.1).

A three–dimensional plot for all the responses obtained is presented in Figure 4.2. When comparing the several graphs, it is possible to see that the region where the responses are maximized is almost the same in both figures. Therefore, it would really be good to determine the optimum extraction condition that maximizes the release of the compounds as mentioned at the beginning of the work. A graphical optimization based on overlaying the different response curves was made to establish an extraction condition that maximizes the release of the different compounds. In order to find the optimum condition the following criteria were imposed: total phenolics ≥ 22 mg GAE/g SCG, proteins ≥ 13 mg BSA/g SCG, sugars ≥ 35 mg Glucose/g SCG, flavonoids ≥ 2.5 mg QE/g SCG, FRAP ≥ 0.22 mM Fe(II)/g SCG and total antioxidant capacity ≥ 39 mg α–tocopherol/g SCG (Table 4.5).

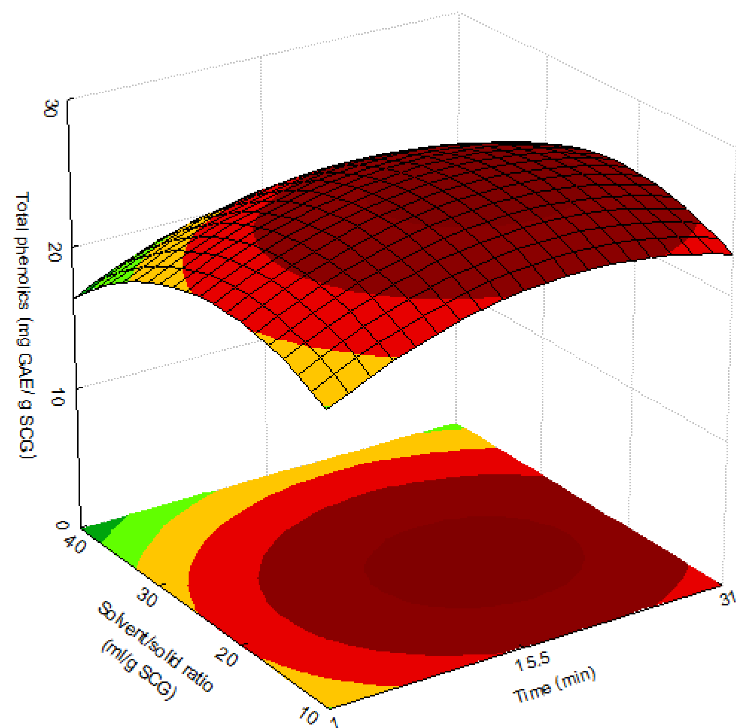
An area that meets all the criteria imposed was determined, and it was assigned a point as the optimum point (marked by the flag) (Figure 4.3). This optimum point has the following conditions: microwave power at 71 %, extraction time 20 min, pressure 120 psi and solvent/solid ratio of 20 mL/g SCG. According to this condition, it is possible to obtain: 27.26 mg GAE/g SCG, 22.62 mg BSA/g SCG, 37.78 mg Glucose/g SCG, 3.95 mg QE/g SCG, 0.230 mM Fe(II)/g SCG and 46.10 mg α–tocopherol/g SCG. Therefore, were made assays to validate the optimum extraction conditions and the results obtained revealed some similarities in some variables but others stay a bit far from results predicted by the statistical analysis (Table 4.5).

Table 4.4. Polynomial equations fitted to the experimental data of antioxidant phenolics compounds of spent coffee grounds extracts, and their respective regression coefficients R^2

Response	Model equation *	R^2
Total phenolics (TP, in mg GAE/g SCG)	$TP = 26.33+0.08X_1-1.80X_2+1.44X_3+0.62X_4+0.25X_1X_3-0.58X_1X_4-0.05X_2X_3-0.16X_2X_4-0.39X_3X_4-2.36X_1^2-3.85X_2^2-2.86X_3^2$	0.78
Proteins (PRO, in mg BSA/g SCG)	$PRO = 21.65-6.20X_2-1.43X_3-0.76X_4-0.78X_1X_2+0.33X_1X_3-1.93X_2X_3-0.62X_2X_4-1.20X_3X_4-0.67X_1^2+0.78X_3^2$	0.61
Sugars (SUG, in mg Glucose/g SCG)	$SUG = 35.14-2.66X_2+2.82X_3+0.84X_4-0.63X_1X_4-0.86X_2X_4-6.42X_2^2+0.49X_4^2$	0.56
Flavonoids (FLA, in mg QE/g SCG)	$FLA = 3.84-0.54X_2+0.08X_3+0.09X_4-0.05X_1X_2-0.06X_1X_4-0.12X_2X_3-0.14X_2X_4-0.13X_3X_4-0.28X_1^2-0.71X_2^2-0.20X_3^2-0.11X_4^2$	0.76
Antioxidant activity (FRAP, in mM Fe(II)/g SCG)	$FRAP = 0.23-0.001X_1-0.017X_2+0.018X_3+0.009X_4-0.002X_1X_2+0.004X_1X_3-0.006X_1X_4-0.0005X_2X_3-0.0005X_2X_4+0.001X_3X_4-0.011X_1^2-0.061X_2^2-0.023X_3^2-0.013X_4^2$	0.78
Total antioxidant activity (TAC, in mg α–tocopherol /g SCG)	$TAC = 41.11-2.86X_2+1.84X_3+1.46X_4-0.68X_1X_4-1.11X_2X_3+1.57X_2X_4-1.67X_3X_4-2.77X_1^2-5.92X_2^2-3.87X_3^2+3.85X_4^2$	0.83

* X_1: power; X_2: solvent/solid ratio; X_3: time; X_4: pressure. Coded values.

a)



b)

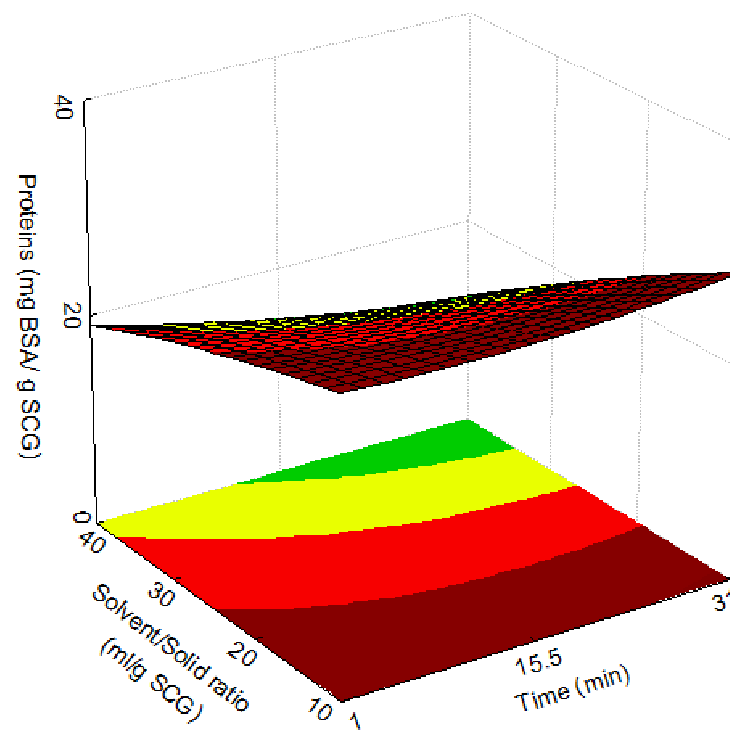
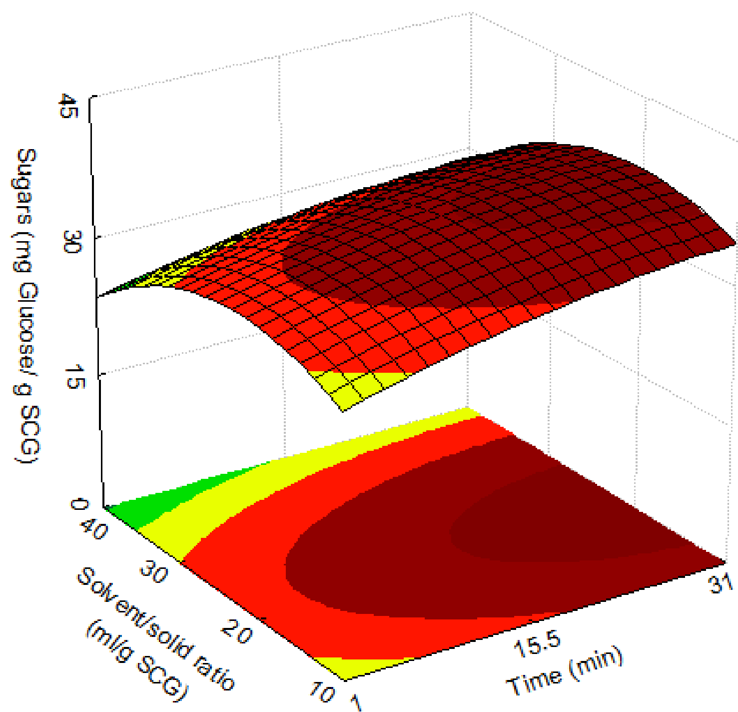


Figure 4.2. Response surfaces described by the models representing the total phenolics (a), proteins (b), sugars (c), flavonoids (d), antioxidant activity – FRAP (e) and total antioxidant capacity (f) of spent coffee grounds extracts obtained by microwave–assisted extraction.

c)



d)

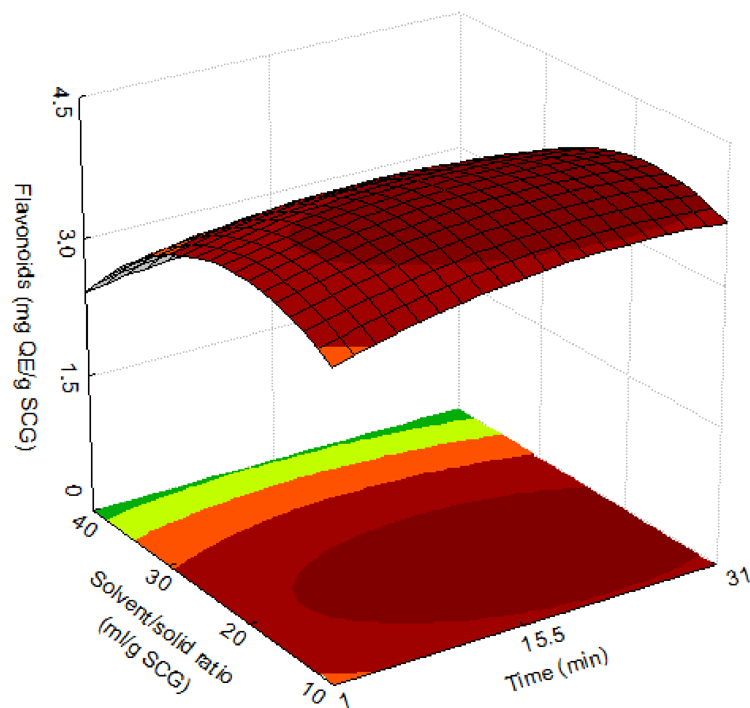
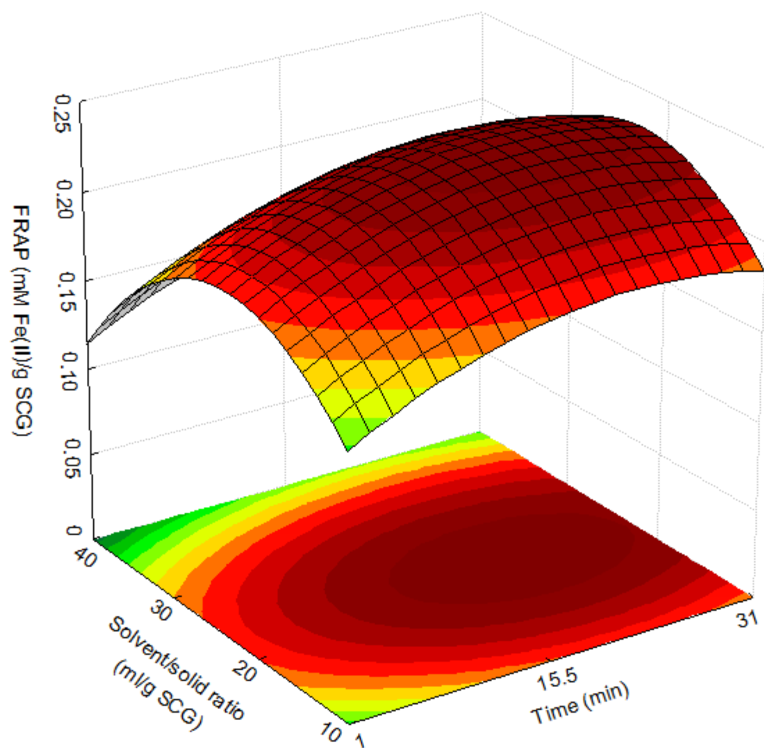


Figure 4.2. (Cont.) Response surfaces described by the models representing the total phenolics (a), proteins (b), sugars (c), flavonoids (d), antioxidant activity – FRAP (e) and total antioxidant capacity (f) of spent coffee grounds extracts obtained by microwave–assisted extraction.

e)



f)

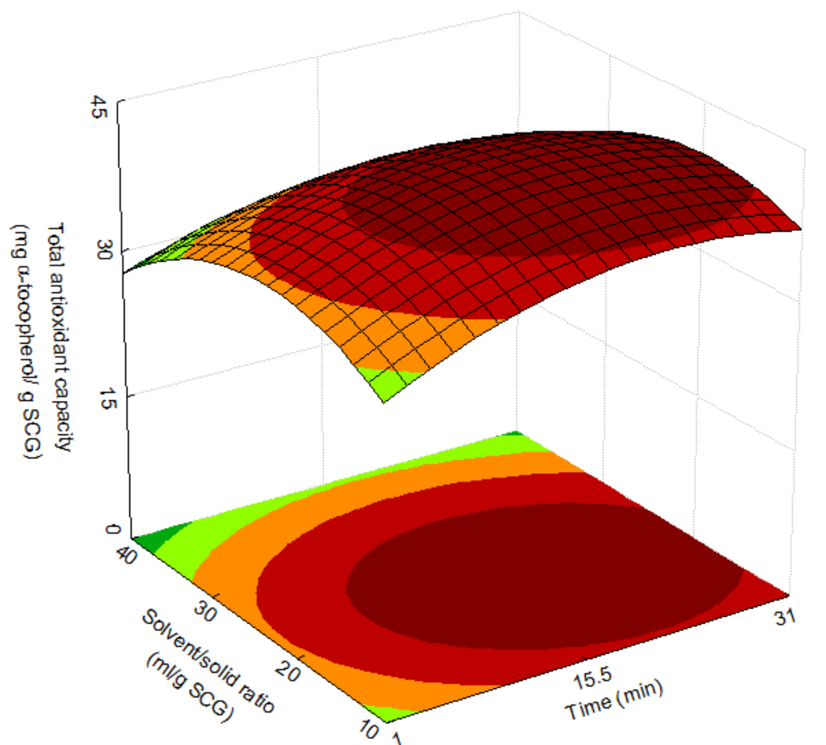


Figure 4.2. (Conclusion) Response surfaces described by the models representing the total phenolics (a), proteins (b), sugars (c), flavonoids (d), antioxidant activity – FRAP (e) and total antioxidant capacity (f) of spent coffee grounds extracts obtained by microwave–assisted extraction.

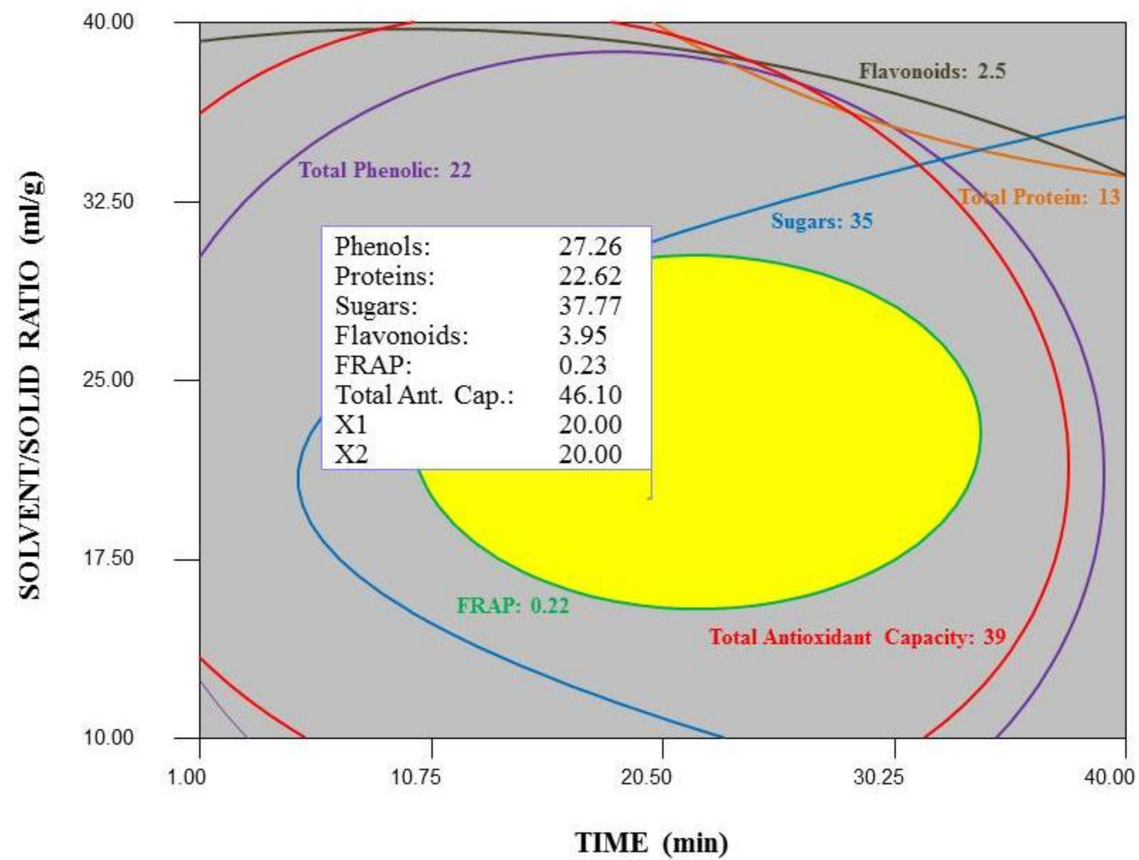


Figure 4.3. Optimum region by overlaying the curves of the different responses as a function of the solvent/solid ratio and time used for spent coffee grounds extraction.

Table 4.5. Result obtained in the assay for validation of the condition optimized for extraction of antioxidant phenolic compounds from spent coffee grounds

Assay for validation of the optimized condition	Process variables – optimum point values				Responses *					
	Power (%)	Solvent/solid ratio (mL/g SCG)	Time (min)	Pressure (psi)	Total phenolics (mg GAE/ g SCG)	Proteins (mg BSA/ g SCG)	Sugars (mg Glucose/ g SCG)	Flavonoids (mg QE/g SCG)	FRAP (mM Fe(II)/ g SCG)	Total Antioxidant Capacity (mg α–tocopherol/ g SCG)
1	71	20	20	120	22.94	13.98	55.45	2.58	0.237	40.01
Results predicted by the statistical analysis					27.26	22.62	37.78	3.95	0.230	46.10
Criteria imposed					22	13	35	2.5	0.22	39

* FRAP: antioxidant activity by the ferric reducing antioxidant power assay.

4.3. Conclusions

Microwave–assisted extraction has been considered as an alternative to traditional extraction methods. The extraction of total phenolics, proteins, sugars, flavonoids and antioxidant activities of the produced extracts were affected by the solvent/solid ratio and extraction time used. Initially, several extraction conditions were evaluated, such as microwave power, solvent/solid ratio, extraction time and pressure in order to find the optimum condition for maximum extraction of the antioxidant phenolic compounds. Maximum value of total phenolics, sugars and flavonoids were correspondent to 32.33 mg GAE/g SCG, 49.78 mg Glucose/mg SCG and 4.86 mg QE/g SCG, and were obtained when using a microwave power at 75 %, extraction time 16 min, pressure 90 psi and using solvent/solid ratio of 25 mL/g SCG. The highest protein value was 31.82 mg BSA/g SCG using a microwave power of 100 %, extraction time of 31 min, pressure of 120 psi and utilizing solvent/solid ratio of 10 mL/g SCG. The best values for the antioxidant activities were: 0.2692 mM Fe(II)/g SCG with one microwave power of 75 %, extraction time of 31 min, pressure of 90 psi and using solvent/solid ratio of 25 mL/g SCG (by FRAP method) and 48.45 mg α–tocopherol/g SCG, with one microwave power of 75 %, extraction time of 16 min, pressure of 120 psi and utilizing solvent/solid ratio of 25 mL/g SCG (by total antioxidant capacity). The optimum point for extraction was determinate (microwave power at 71 %, extraction time 20 min, pressure 120 psi and using solvent/solid ratio of 20 mL/g SCG) and showed that is possible to obtain one condition that maximizes the extraction of antioxidant phenolic compounds, proteins and sugars, simultaneously. Comparing with others methods, the MAE reduces extraction time and solvent usage (non–corrosive solvents), resulting in reduced costs. In conclusion, the results demonstrated that the developed microwave procedure could be used for extraction of phenolic antioxidants compounds from spent coffee grounds.

Acknowledgements

This study was funded by Portuguese National Fund through FCT – Fundação para a Ciência e a Tecnologia (grant SFRH/BD/75195/2010).

4.4. References

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

Bradford, M. 1976. A rapid and sensitive method for the quantification of microgram quantities of protein using the principles of protein–dye binding. *Analytical Biochemistry.,* 72, 248–254.

Calinescu, I., Lavric, V., Asofiei, I., Gavrila, A. I., Trifan, A., Ighigeanu, D., Martin, D., Matei, C. (2017). Microwave–assisted extraction of polyphenols using a coaxial antenna and a cooling system. *Chemical Engineering and Processing: Process Intensification*, 122, 373–379.

Chan, C.–H., Yusoff, R., Ngoh, G.-C., Kung, F. W.–L. 2011. Microwave–assisted extractions of active ingredients from plants. *Journal of Chromatography A*, 1218, 6213-6225.

Chang, C.–C., Yang, M.–H., Wen, H.–M., Chern, J.–C. 2002. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. *Journal of Food and Drug Analysis*, 10, 178–182.

Dahmoune, F., Nayak, B., Moussi, K., Remini, H., Madani, K. (2015). Optimization of microwave–assisted extraction of polyphenols from *Myrtus communis* L. leaves. *Food Chemistry*, 166, 585–595.

Drosou, C., Kyriakopoulou, K., Bimpilas, A., Tsimogiannis, D., Krokida, M. (2015). A comparative study on different extraction techniques to recover red grape pomace polyphenols from vinification byproducts. *Industrial Crops and Products*, 75, 141–149.

Kumar, R. C., Benal, M. M., Prasada, B. D., Krupashankarac, M. S., Kulkarnic, R. S., Siddaligaswamy, N. H. (2018). Microwave–assisted extraction of oil from pongamia pinnata seeds. *Materials Today: Proceedings*, 5, 2960–2964.

Machado, E. M. S. 2009. Reaproveitamento de resíduos da indústria do café como matéria-prima para a produção de etanol. *Dissertação de Mestrado em Engenharia Biológica, Universidade do Minho*, Portugal.

Moreira, M. M., Barroso, M. F., Boeykens, A., Withouck, H., Morais, S., Delerue–Matos, C. (2017). Valorization of apple tree wood residues by polyphenols extraction: Comparison between conventional and microwave–assisted extraction. *Industrial Crops & Products*, 104, 210–220.

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

Mussato, S. I., Carneiro, L. M., Silva, J. P. A., Roberto, I. C., Teixeira, J. A. 2011b. A study on chemical constituents and sugars extraction from spent coffee grounds. *Carbohydrate Polymers*, 83, 368–374.

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

Pandey, R., Shrivastava, S. L. (2018). Comparative evaluation of rice bran oil obtained with two–step microwave–assisted extraction and conventional solvent extraction. *Journal of Food Engineering*, 218, 106–114.

Passos, C. P., Coimbra, M. A. (2013). Microwave superheated water extraction of polysaccharides from spent coffee grounds. *Carbohydrate Polymers*, 94, 626–633.

Passos, C. P., Rudnitskaya, A., Neves, J. M. M. G. C., Lopes, G. R., Evtuguin, D. V., Coimbra, M. A. (2019). Structural features of spent coffee grounds water-soluble polysaccharides: Towards tailor-made microwave assisted extractions. *Carbohydrate Polymers*, 214, 54–61.

Pintathong, P., Kaewkhao, W., Sirichot, O., Chaiwut, P. 2011. Recovery of phenolic antioxidants from spent coffee grounds using microwave–assisted extraction. *1s International Conference on "Food and Applied Bioscience"*, Chiang Mai (Thailand).

Prieto, P., Pineda, M., Aguilar, M. 1999. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. *Analytical Biochemistry*, 269, 337–341.

Rodriguez–Jasso, R. M., Mussatto, S. I., Pastrana, L., Aguilar, C. N., Teixeira, J. A. (2011). Microwave–assisted extraction of sulfated polysaccharides (fucoidan) from *Brown seaweed*. *Carbohydrate Polymers*, 86, 1137–1144.

Singh, A., Sabally, K., Kubow, S., Donnelly, D. J., Gariepy, Y., Orsat, V., Raghavan, G. S. V. 2011. Microwave–assisted extraction of phenolic antioxidants from potato peels. *Molecules*, 16, 2218–2232.

Singleton, V. L., Rossi Jr, J. A. 1965. Colorimetry of total phenolics with phosphomolybdic–phosphotungstic acid reagents. *American Journal of Enology and Viticulture*, 16, 144–158.

Thirugnanasambandham, K., Sivakumar, V. (2017). Microwave assisted extraction process of betalain from dragon fruit and its antioxidant activities. *Journal of the Saudi Society of Agricultural Sciences*, 16, 41–48.

Upadhyay, R., Ramalakshmi, K., Rao, L. J. M. 2012. Microwave–assisted extraction of chlorogenic acids from green coffee beans. *Food Chemistry*, 130, 184–88.

CHAPTER 5

CHEMICAL COMPOSITION AND SENSORY PROFILE OF FERMENTED AND DISTILLED BEVERAGES FROM SPENT COFFEE GROUNDS

Abstract

This work describes a new process for the production of beverages from spent coffee grounds (SCG), as well as the chemical and sensory profiles. The process consisted of the extraction of antioxidant phenolic compounds of SGC, followed by the fermentation of this extract supplemented with sucrose and fermented broth distillation. Thus, two fermented (10.4 % and 10.0 % of ethanol, by volume) and two distilled (38.1 % and 40.2 % of ethanol, by volume) beverages were obtained. A total of 45 and 59 volatile compounds (alcohols, esters, aldehydes, terpenes, lactones, pyrazines, norisoprenoids, volatile phenols and acids) identified and quantified by GS-MS characterized the aroma and flavor of the fermented and distilled beverages, respectively. Twenty sensory descriptors define the sensory profile of the two beverages which corroborated the pleasant smell and taste of coffee in distillate beverage. Therefore, this work demonstrates that the fermented and distilled beverages obtained from spent coffee grounds have acceptable organoleptic qualities that make them suitable for human consumption.

Keywords: Spent coffee grounds, *Saccharomyces cerevisiae*, Fermented and distilled beverage, Sensory analysis, Volatile compounds.

5. Introduction

Coffee is the most consumed beverage in the world being served more than 400 billion cups per year. Large amount of wastes are generated in the coffee industry every year. Spent coffee ground (SCG) that are obtained during the process of raw coffee powder production to prepare instant coffee is a waste generated in large amounts in the coffee industry, being about over 6 000 000 tons. For each ton of processed green coffee, are generated 650 kg (dry matter) of spent coffee grounds with 70 % to 80 % humidity (Mussatto *et al.*, 2011a). This waste has a composition rich in compounds of industrial interest such as carbohydrates, proteins and high levels of phenolic compounds with significant antioxidant activity, which suggests the possibility of using the waste as an inexpensive source of natural antioxidants (Mussatto *et al.*, 2011b). This residue presents an extraordinary residual aroma of roasted coffee beans, being an interesting feedstock for the production of a new distilled beverage (Sampaio *et al.*, 2013).

The beverage industry has shown a great interest in the development of new products from different raw materials, so the development of products with flavor extracts and natural flavors has received great emphasis due to restrictions on the use of synthetic chemicals in foods and beverages (Coelho *et al.*, 2015). The production and consumption of alcohol by fermentation of cereal grains, is being done for more than 6000 years (Verdi *et al.*, 2006). The first distilled beverage, the vodka made from rye, came up around the year 1100, in Poland or Russia (Santos *et al.*, 2006). The distillation consists in concentrating the alcohol of a fermented liquid in order to get a new drink. Nowadays, these beverages have assumed such importance that became the official drinks in some of the countries, such as cachaça (sugar cane) to Brazil, vodka (cereals and tubers) to Russia, tequila (agave) to Mexico, rum (molasses) to Cuba, whiskey (cereals) to Scotland, cognac (wine) to France, soju (rice) to Korea, among others (Santos *et al.*, 2007). The use of fruit in the preparation of fermented alcoholic or distilled beverages is a form of exploitation in order to avoid waste when it is not possible to have an immediate consumption, as well as to generate new applications and technologies (Sampaio *et al.*, 2013).

Microwave-assisted extraction (MAE) is a technology of interest to the industry, which represents an alternative to conventional extraction processes (Moreira *et al.*, 2017). This technique has little impact on the environment, compared to traditional techniques, and MAE has aroused great interest for its application in the extraction of high-value compounds. As stated in the previous chapter, it was evaluated the extraction of sugars and antioxidant phenolic compounds from SCG through the

technique of MAE and determined the operating condition that maximizes the release of the compounds, at which the optimum point for extraction was achieved (microwave power at 71 %, extraction time 20 min, pressure 827.6 kPa, and using solvent/solid ratio of 20 mL/g SCG).

Fermented and distilled beverages are famous by containing a considerable amount of volatile compounds that arise during fermentation, distillation, and storage processes. The composition and concentration of such compounds may vary widely from beverage to beverage (Plutowska *et al.*, 2008). So, the identification of these compounds has a high importance, because it allows determination of the flavor characteristics of the beverage, in order to identify anomalies that may occur during the manufacturing process (Fitzgerald *et al.,* 2000). The sensory attributes are also one of the most important features to be considered when developing a new product, since they are the feature of the product and largely contribute to its acceptability in the market. Sensory evaluation methods are extensively used in wine, beer and distilled beverage characterization (Sampaio *et al.*, 2013). The sensory analyses are made using Quantitative Descriptive Analysis (QDA), which is the best method to identify and quantify the beverage's sensory attributes (Lawless *et al.*, 1998).

The aim of this work was to study the process for the elaboration of fermented and distilled beverages from SCG, as well as the chemical characterization of volatile compounds and determination of the sensory profile.

5.1. Material and methods

5.1.1. Raw material and chemicals

The raw material used was spent coffee grounds (SCG) which was supplied by a Portuguese company of reference in this sector NovaDelta – Comércio e Indústria de Cafés, Lda (Campo Maior, Portugal). The provided material was dried in an oven at 60 °C until approximately 10 % moisture content and stored afterwards for use in the following steps. The chemical composition of SCG was determined according to Sampaio *et al.* (2013), consisted of (g/100 g): glucan (8.6), arabinan (1.7), galactan (13.8), mannan (21.2), protein (13.6), lignin (32.1), ashes (1.6), acetyl groups (2.2), and extractives (5.2).

5.1.2. Extraction process and fermentation medium

In the first step, the SCG was submitted to a microwave-assisted extraction process aiming to extract antioxidant phenolic compounds, using the optimum point previously obtained. Prior to extraction, SCG was mixed with water using water (mL) to material (g) ratio 20:1. The extraction conditions consisted were as follow microwave power 71 %, pressure 120 psi, time 20 min. In the end, the residual solid material was separated by vacuum filtration and the SCG extract obtained was stored at 5 °C. In the following step, for fermentation medium, SCG extract was supplemented with 135 g to 576 g of sucrose to a final concentration of 180 g/L sucrose and 0.13125 g to 0.55 g in the concentration of 175 mg/L potassium metabisulfite, for the two different methods of fermentations realized (Mussatto *et al.*, 2011c). The pH was adjusted between 5 and 5.5 by adding up calcium carbonate in order to proceed with the fermentation with the yeast *Saccharomyces cerevisiae*.

5.1.3. Microorganism and inoculum

The fermentations of SCG extract were performed with *saccharomyces cerevisiae* (RL–11), previously reported to be able to produce ethanol from this fermentation medium (Machado, 2009). Cultures of this yeast were maintained at 4 °C in Petri dishes containing malt extract agar prepared with the following composition (g/L): yeast extract (3.0), malt extract (3.0), peptone (5.0), glucose (10.0), and agar (20.0).

In order to obtain the inoculum, the yeast (*S. cerevisiae*) was cultured in a semisynthetic culture medium composed by (g/L): glucose (30.0), $(NH_4)_2HPO_4$ (3.0), $MgSO_4.7H_2O$ (1.0), and yeast extract (3.0). The concentrated solutions of each compound were prepared separately and sterilized in an autoclave at 121 °C for 20 min. Additionally, glucose and yeast extract were autoclaved at 112 °C for 15 min. The obtained solutions were mixed aseptically in a laminar flow hood in order to obtain the desired concentration of each nutrient in the culture medium. The inoculum was prepared by pitching cells from the Petri dishes that were inoculated in 500 mL Erlenmeyer flasks containing 200 mL of the medium of fermentation and incubated on a rotary shaker at 30 °C, 200 min^{-1}, 24 h.

5.1.4. Fermentation and distillation conditions

The fermentations assays were performed by two different methods, each in duplicate. The first method was conducted in a 6.5 L bioreactor (B. Braun Biotech International, Melsungen, Germany) containing 3 L of fermentation medium inoculated at an initial cell concentration of 1 g/L. Figure 5.1 shows the equipment used for this process. Fermentations were incubated at 30 °C with continuous stirring at 150 min^{-1}. During the fermentations, samples were collected from the fermentation broth, and immediately centrifuged (5000x *g*, 10 °C, 15 min) for separation and determination of the concentration of biomass. The obtained supernatant was filtered by sterile cellulose acetate membrane of 0.2 μm and used for determining the total of sugars concentration in order to determine the end of the fermentation. At the end of the fermentation, the fermented broth was centrifuged (5000x *g*, 10 °C, 15 min) to separate the biomass, and the liquid phase was stored at 4 °C for further distillation. This method was used for the production of distilled beverages.

Figure 5.1. Equipment used for the fermentations assays in the first method.

The second method was performed in 2 L Erlenmeyer flasks containing 75 mL of fermentation medium inoculated at an initial cell concentration of 1 g/L. The inoculated flasks were incubated at 30 °C on a rotary shaker at 150 min^{-1}. The remainder process was done similarly but, in this time, the final products were the fermented beverages.

The distillation of fermented broth was done using a system comprising a vigreux column (36 cm of length), a condenser, a heating mantle, and a 4 L flask filled with 1 L to 1.5 L of fermented broth. Figure 5.2 shows the equipment used in the distillation of fermented broth. After the fermentations by the first method, the fermentation medium was split in three similar shares to be distilled. The first fermentation medium was split in one share with a volume of 1.2 L and the two other ones with about 1.1 L. The second fermentation medium was split in one share with a volume of 1.2 L, another one with about 1 L and the last one with 1.25 L.

Figure 5.2. Equipment used for the distillation of fermented broth.

During the distillation were recovered samples of approximately 5 mL to 25 mL of different fractions of distilled product, at different temperatures (70 °C, 80 °C, and 90 °C) and the ethanol content in each one of them was determined by HPLC. In this process, there are three fractions according to their ethanol content: the foreshot or "head" (>70 mL/100 mL), the middle cut or "heart" (70 mL/100 mL to 40 mL/100 mL), and the feints or "tail" (<40 mL/100 mL). The fraction corresponding to the heart was corrected for an ethanol concentration to 40 mL/100 mL by adding SCG extract and was stored in glass bottles with caps and plastic coverings at room temperature for chemical and sensory analyses.

5.1.5. Analytical methods

The cell concentration was quantified by dry weight of a sample which was dried at 105 °C to constant weight. The biomass was determined by weight difference between the crucibles before and after the addition and in a further phase the samples were dried out. The cell concentration was expressed as dry weight per volume. The total amount of sugars concentration was determined by the anthrone method (Dreywood *et al.*, 1946). Standard glucose solutions were prepared with concentrations between 0.1 g/L and 0.7 g/L. Then, 0.5 mL of each solution (or water, for the blank) was transferred to test tubes where 1 mL of anthrone solution was added and then the tubes were placed on ice to cool. After cooling, the tubes were placed in a bath at 80 °C for 15 min and then allowed to cool in an ice bath. Finally the absorbance was read at 630 nm. The concentration of ethanol was determined by high-performance liquid chromatography (HPLC) on a Jasco chromatograph (Jasco, Tokio, Japan) that is equipped with a refractive index detector and a Varian Metacarb 67H column (300 mm x 6.5 mm). To operate, the conditions consisted in using a temperature of 60 °C, 5 mmol/L sulfuric acid as eluent at a flow rate of 0.7 mL/min and a sample volume of 20 µL. The ethanol content, expressed as volumetric percentage was obtained with the ratio between formed product, expressed as mass concentration and the density of ethanol (0.789 g/mL).

The major volatiles were evaluated after adding 100 µL of an ethanolic solution of 4.02 g/L of internal standard (4-nonanol, Merck ref. 818 773) to 5 mL of sample. The analysis was performed with the injection of 1 µL of the sample. The volatile compounds were studied in a Chrompack CP–9000 gas chromatograph (Chrompack, Middelburg, The Netherlands) equipped and a split/splitless injector and a flame ionization detector (FID) and a capillary column, coated with Meta–Wax (30 m x 0.25 mm, 0.2 µm film thickness). The injector and detector temperatures were both set to 250 °C at the split ratio of 15:1 mL/min. The oven temperature was held at 50 °C for 2 min, then programmed to rise from 50 °C to 177.5 °C, at 5 °C/min, and then programmed to rise again from 177.5 °C to 225 °C, at 10 °C/min, and finally maintained at 220 °C for 20 min. The carrier gas was helium 4x (Praxair) at an initial flow rate of 1 mL/min.

The minor volatile compounds were evaluated by addition of 100 µL of an ethanolic solution of 40.2 mg/L of internal standard (4-nonanol, Merck ref. 818 773) to 8 mL of sample. The extraction was performed through mixing the sample with 400 µL of dichloromethane (Merck, ref. 1.06054) for 15 min on a magnetic stir plate (Oliveira *et al.*, 2006). Then, after cooling at 0 °C for 10 min, the organic phase was separated by centrifugation (5118 g, 5 min, 4 °C). The volatile compounds were examined

by GC–MS (Varian 3800 GC gas chromatograph equipped with a 1079 injector, and a Varian Saturn 2000 ion–trap mass detector). Each 1 μL extract was injected in splitless mode (30 s), in a Sapiens–Wax MS column (30 m x 0.15 mm, 0.15 μm film thickness). The carrier gas was helium 4 x at a constant flow rate of 1.3 mL/min. The detector was used in electron impact mode with ionization energy of 70 eV and acquisition mass range (m/z) between 35 and 300, acquiring at intervals of 610 ms. The oven temperature was held at 60 °C for 2 min, then programmed to rise from 60 °C to 234 °C, at 3 °C/min, and then programmed to rise again from 234 °C to 260 °C, at 5 °C/min. Finally, was maintained at 260 °C during 5 min. The injector's and transfer line temperatures were maintained at 250 °C during the analysis time and a split flow rate of 30 mL/min.

The identification of volatiles was performed using the software Star-Chromatography Workstation version 6.9.3 (Varian, Walnut Creek, CA. USA) by comparing the mass spectra and retention indices with those of pure reference compounds (Oliveira *et al.*, 2006). All compounds were quantified as equivalents of 4-nonanol. The distillate samples were pre-diluted with water to 15/40 and fermented samples did not undergo any dilution. Each sample was extracted in triplicate.

5.1.6. Sensory analysis

Sensory analysis of beverages was carried out by five trained panellists from Apellation Orujo de Galicia (Galicia, Spain), with ages between 40 and 50 years old. All the judges were experienced spirit tasters and all of them have previously participated in similar studies.

The sensory analysis was performed in a professional–standard room in agreement with the ISO Norm 8589 (Norme ISO 8589, 1988). The evaluation was carried out in two sessions. In the first one, descriptors of the fermented and distillate samples were established by using the QDA methodology (Lawless *et al.*, 1998). Two training periods of 1 h were carried out, where judges generated descriptive terms in visual, olfactory, and gustatory phases to define the spirits. In the second session, a constant sample volume of 30 mL of each fermented and spirit beverage was evaluated in spirit–taster glasses at 12 °C. During the analysis, the judges smelled and tasted the samples, and the perceived descriptors were indicated. The panelists scored the intensity of each attribute using a 9–point scale, where 9 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.* $GM/\% = \sqrt{(I \times F)} \times 100$, where 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 beverage by using the Geometric Mean (*GM*) according to the International Organization for Standardization – ISO Norm 11035 (Norme ISO 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).

5.2. Results and discussion

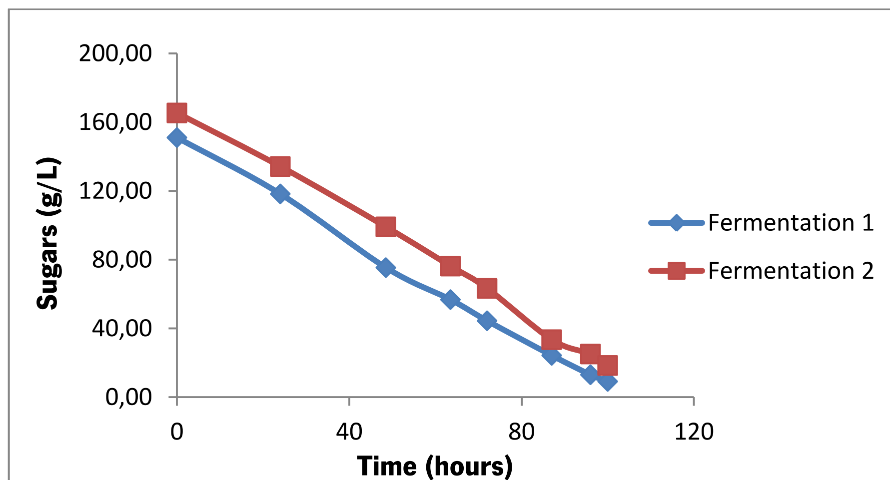
5.2.1. Beverage production

In the first step the SCG was submitting to a microwave-assisted extraction process aiming to extract antioxidant phenolic compounds, using the optimum point obtained in this process (microwave power at 71 %, extraction time 20 min, pressure 120 psi and using solvent/solid ratio of 20 mL/g). In the end of this process, the obtained extracts were used as the fermentation medium by two different fermentation methods. As this fermentation medium does not have a high concentration of sugars from this raw material (Mussatto *et al.*, 2011c), it provides an inefficient production of ethanol by fermentation with the yeast *S. cerevisiae*. It was used this medium as a source of sugars by adding a sucrose supplementation.

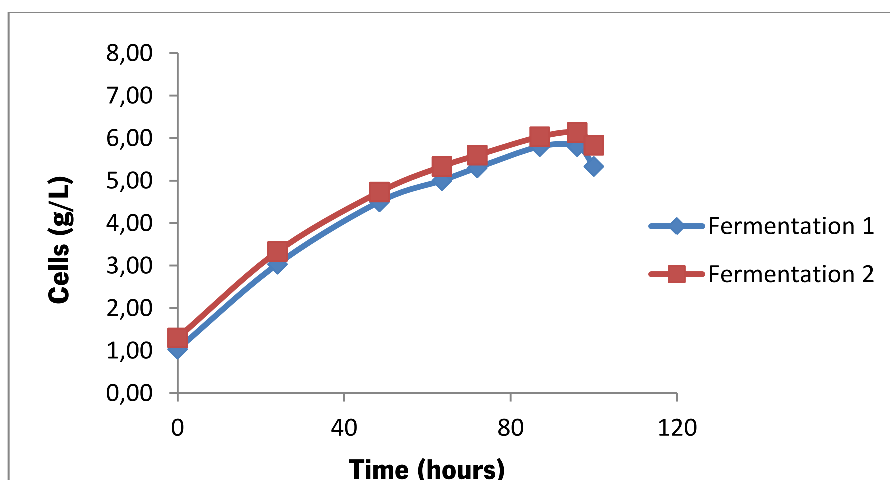
In the first method, two fermentations were performed that afterwards were submitted to a distillation process in order to obtain two distilled beverages: distilled beverage 1 (D1) and distilled beverage 2 (D2). The kinetic behaviour of sucrose consumption and cell growth of *S. cerevisiae* RL–11 cultivated in this medium is shown in Figure 5.3a and Figure 5.3b. During the two fermentations it was possible to verify that the yeast consumed practically all the sugar in the fermentation medium, in 100 h of processing (Figure 5.3a). Part of this carbon source that was consumed was employed for cellular growth and the rest was used for the production of ethanol. The cellular growth of the yeast during the fermentations can be observed in Figure 5.3b. The cellular concentration in these two media

increased in an equivalent way from 1 g/L until the maximum of 5.80 g/L for fermentation 1 and 6.13 g/L for fermentation 2, showing that the yeasts remained very active during the fermentation process.

In the second method, two more fermentations were performed, resulting in two fermented beverages: fermented beverage 1 (F1) and fermented beverage 2 (F2). The kinetic behaviour of sucrose consumption and cell growth of *S. cerevisiae* RL–11 cultivated in this medium for this method are shown in Figure 5.4a and Figure 5.4b. During the two fermentations, it was possible to verify that the yeast consumed practically all the existent sugar in the fermentation medium, in 112 h of processing (Figure 5.4a).



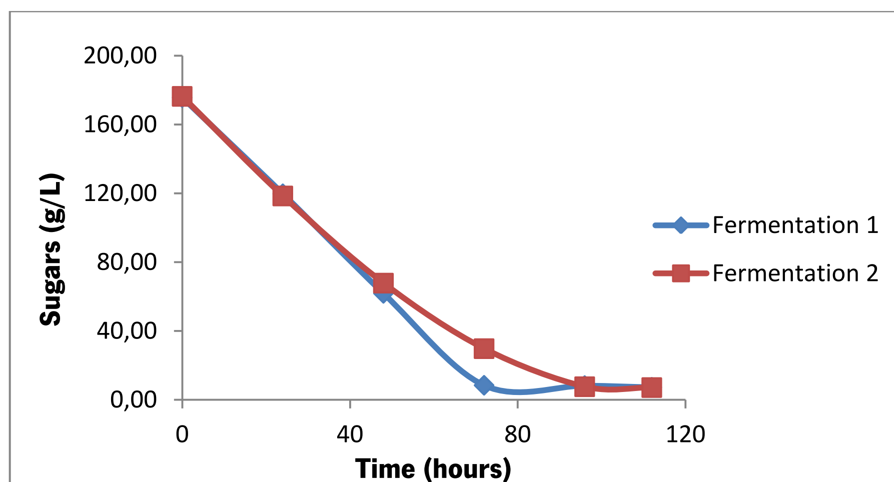
(a)



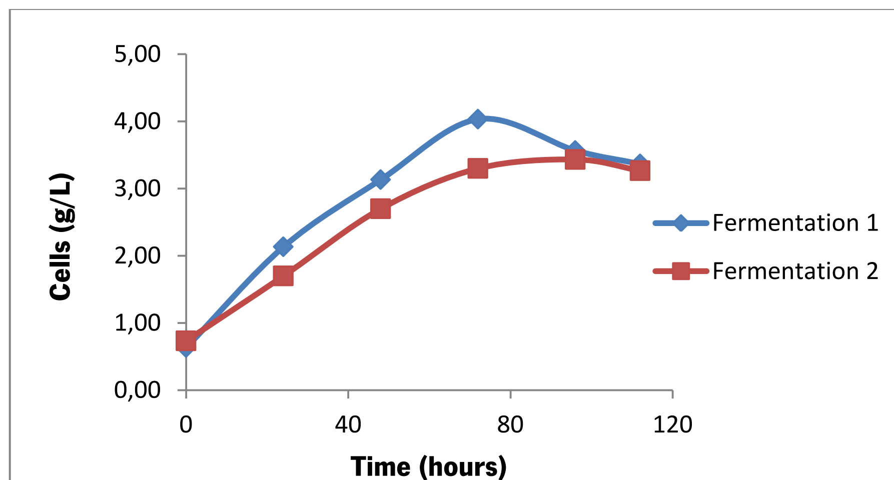
(b)

Figure 5.3. Sugars consumption (a) and cell growth (b) of *S. cerevisiae* (RL–11) from spent coffee ground extract using the first method of fermentation.

In comparison with the first method it was able to observe a 12 h disparity for the end of the process. The cellular growth of the yeast during the fermentations can be verified in Figure 5.4b. The cellular concentration in these two mediums has increased in a very equivalent way, from 0.63 g/L until the maximum of 3.37 g/L for fermentation 1 and 3.27 g/L for fermentation 2, but it comparison with the first method, these results were lower. At the end of these fermentations, we had obtained two fermented beverages with a volumetric percentage of 10.4 % and 10.0 % of ethanol, fermented beverage 1 (F1) and fermented beverage 2 (F2), respectively.



(a)



(b)

Figure 5.4. Sugars consumption (a) and cell growth (b) of *S. cerevisiae* (RL–11) from spent coffee ground extract using the second method of fermentation.

An efficient conversion of sugars to ethanol by the yeast is advantageous for the process, as the greater the ethanol content in the fermented broth, the greater the volume of spirit that can be achieved. This yeast strain is reported to have great capacity to convert sugars to ethanol, so it is recommended for the production of alcoholic beverages (Sampaio *et al.*, 2013).

The different fractions of distillate collected accordingly with the conditions previously optimized by Dragone *et al.* (2009a) were characterized for their ethanol content (Table 5.1 and Table 5.2).

Table 5.1. Portions collected during the distillations of the first fermented SCG extract (D1)

Distillation 1 (1.2 L)		**Distillation 2 (1.1 L)**		**Distillation 3 (1.1 L)**	
Volume collected (mL)	**Ethanol content (%)**	**Volume collected (mL)**	**Ethanol content (%)**	**Volume collected (mL)**	**Ethanol content (%)**
15	79.1	15	75.2	15	73.3
10	80.2	10	85.1	7.5	83.1
15	79.7	15	83.1	15	80.5
10	82.4	10	78.6	10	82.2
10	80.2	10	73.7	10	75.4
20	82.8	20	76.1	22.5	73.0
27.5	74.6	25	72.8	25	71.8
22.5	66.2	20	70.8	15	59.9
15	39.9	10	54.4	10	47.4
22.5	16.3	7.5	50.5	7.5	22.9
7.5	5.3	-	-	-	-

The fractions with a volumetric percentage between 40 % and 70 % were mixed to form the distillate heart. After mixing the collected fractions near these values, we obtained two distilled beverages: the first one (D1) with a volumetric percentage of 66.1 % of ethanol and the second one (D2) with a volumetric percentage of 58.8 % of ethanol. These drinks were afterwards diluted with an extract collected by microwave–assisted extraction, which was previously filtrated, instead of water, to have a more intense flavor of coffee and thus getting a final volumetric percentage of 38.1 % and 40.2 % of ethanol, distilled beverage 1 (D1) and distilled beverage 2 (D2), respectively.

Table 5.2. Portions collected during the distillations of the second fermented SCG extract (D2)

Distillation 1 (1.2 L)		Distillation 2 (1 L)		Distillation 3 (1.25 L)	
Volume collected (mL)	Ethanol content (%)	Volume collected (mL)	Ethanol content (%)	Volume collected (mL)	Ethanol content (%)
10	77.0	10	77.8	10	70.9
10	86.1	10	81.5	10	82.2
10	79.6	10	81.5	10	81.0
10	77.4	10	78.2	10	71.7
10	74.5	10	75.3	10	71.4
13	71.6	13	72.9	13	71.4
15	70.5	15	68.7	15	68.5
25	66.9	25	58.5	25	53.6
20	50.5	10	49.4	20	49.4
15	43.1	5	41.0	15	45.1
15	29.7	-	-	13	33.3

5.2.2. Volatile composition of fermented and spirit beverages

Major and minor compounds were analyzed in the fermented and distillate beverages. The major volatile compounds are usually formed during the fermentation process, with their formation influenced by the conditions used. On the other hand, the minor volatile compounds are largely from the raw material used, so they are responsible for the distinctive aroma in the produced beverage. The improvement of the aroma was attributed to the modification of the composition of aroma precursors in green coffee beans observed following fermentation (Lee *et al.*, 2015). On the other hand, a coffee with a distinctive aroma of fruits could be produced using the starter cultures in coffee. The selection of yeast strains has great potential for use as starter cultures and to help standardize the fermentation process and produce coffee beverages with novel and desirable flavor profiles (De Melo Pereira *et al.*, 2014).

In fermented beverage 1 (F1) the most present compound was acetaldehyde (339.6 mg/L), followed by two higher alcohols, i. e., 2–methyl–1–propanol (152.1 mg/L) and 3–methyl–1–butanol (106.8 mg/L) (Table 5.3). Ethyl acetate was also detected in fermented beverage F1 at a concentration of 73.5 mg/L. Ethyl acetate contents between 50 mg/L to 80 mg/L contribute positively to the

beverage aroma (Steger *et al.*, 2000), while values above 150 mg/L provide deterioration characteristics (Apostolopoulou *et al.*, 2005). In fermented beverage 2 (F2), an increase of isobutanol and a decrease of acetaldehyde was observed. This can be explained by the fact that after the production of fermented beverages, they weren't properly stored at a temperature of –20 °C, but rather at a temperature of 5 °C in a cold room. In these circumstances, some change of fermented beverages 1 and 2 may have occurred.

Table 5.3. Concentration (*C*) and standard deviation (*SD*) of major volatile compounds identified and quantified in fermented and distillate samples

Compound	F1		F2		D1		D2	
	C/(mg/L)	*SD*	*C*/(mg/L)	*SD*	*C*/(mg/L)	*SD*	*C*/(mg/L)	*SD*
acetaldehyde	339.6	22.2	192.5	2.1	6.3	0.5	19.6	1.0
ethyl acetate	73.5	1.7	72.1	11.4	7.8	1.3	18.7	2.6
methanol	30.1	1.3	44.1	1.6	14.0	1.3	7.6	1.0
1–propanol	15.5	0.5	17.1	0.9	23.2	1.5	35.7	1.2
2–methyl–1–propanol	152.1	1.4	231.7	6.3	49.4	2.2	222.0	6.6
2–methyl–1–butanol	40.9	0.4	36.0	0.7	31.5	0.3	137.1	4.0
3–methyl–1–butanol	106.8	2.0	97.1	2.5	191.1	4.2	633.2	19.2
2–phenylethanol	34.7	4.6	38.2	4.4	35.6	3.8	21.5	2.1

Fermented beverage 1 (F1) and 2 (F2); Distilled beverage 1 (D1) and 2 (D2).

The majority of volatile compounds largely presented in the distilled beverages from SCG were higher alcohols as shown in Table 5.3. In distilled beverage 1 (D1), isoamyl alcohol (3–methyl–1–butanol), isobutyl alcohol (2–methyl–1–propanol), 2–phenylethanol and active amyl alcohol (2–methyl–1–butanol) were found with highest quantities (191.1 mg/L, 49.4 mg/L, 35.6 mg/L and 31.5 mg/L, respectively). On the other hand, for the distilled beverage 2 (D2) the volatile compounds that obtained highest concentrations were 3–methyl–1–butanol, 2–methyl–1–propanol, and 2–methyl–1–butanol (633.2 mg/L, 222.0 mg/L and 137.1 mg/L, respectively), increasing the beverage's aroma compounds concentration.

The concentration of these compounds in D2 is comparable to the values found by Sampaio *et al.* (2013) and Dragone *et al.* (2009b). Sampaio *et al.* (2013) showed contents of 810 mg/L, 269 mg/L, and 185 mg/L of 3–methyl–1–butanol, 2–methyl–1–propanol, and 2–methyl–1–butanol, respectively, for a distillate prepared from the spent coffee grounds hydrolysate. Dragone *et al.* (2009b) prepared a distillate from cheese whey, which contained 887 mg/L of 3–methyl–1–butanol, 542 mg/L

of 2–methyl–1–propanol and 176 mg/L of 2–methyl–1–butanol. Additionally, the relations 3–methyl–1–butanol/2–methyl–1–propanol, and 2–methyl–1–propanol/1–propanol, are considered indicative of the quality of the drink and must be greater than one unit (Sampaio *et al.*, 2013). In our study, distillates D1 and D2 showed this relation >1.

Among the identified and quantified esters, ethyl acetate was the most abundant (7.8 mg/L and 18.7 mg/L for the D1 and D2, respectively), as well as acetaldehyde (6.3 mg/L and 19.6 mg/L for the D1 and D2, respectively). The concentration of this compound in the distillate was less than the amount reported for other spirits, such as spent coffee grounds spirit (80 mg/L) (Sampaio *et al.*, 2013), cheese whey spirit (36.7 mg/L) (Dragone *et al.*, 2009a), bagaceiras (600 mg/L) (Silva *et al.*, 1996), and orujo (262 mg/L) (Cortés *et al.*, 2005). Ethyl acetate and acetaldehyde are the major compounds responsible for the flavor of alcoholic beverages and their amounts determine the quality of the distillate (Apostolopoulou *et al.*, 2005; Dragone *et al.*, 2009a). Ethyl acetate has a significant effect on the organoleptic characteristics of distillates. The presence of this ester in low concentrations results in a pleasant aroma with fruity properties, which turns vinegary at levels above 150 mg/L, providing features of deterioration to the beverage (Apostolopoulou *et al.*, 2005). On the other hand, low concentrations of acetaldehyde in SCG spirit are interesting once it gives an aroma of walnuts, sherry, and ripe apples. Higher concentrations than 125 mg/L for this compound negatively affect the organoleptic properties of the beverage (Sampaio *et al.*, 2013).

In SCG distillate, other major volatile compounds were identified and quantified, such as 1–propanol, 2–phenylethanol, and methanol, but in a lower concentration (Table 5.3). Low concentrations of 1–propanol promote a pleasant, sweet odor, but very high concentrations of this compound exhale an odor of "solvent" that does not allow one to detect the positive odors of the distillate (Fundira *et al.*, 2002). The concentration obtained in distillates D1 and D2 was 23.2 mg/L and 35.7 mg/L, respectively; low values that did not impair the odor of the beverages. For 2–phenylethanol, values of 35.6 mg/L and 21.5 mg/L, respectively, were obtained for distillates D1 and D2. Low concentrations of this compound provide a sweet and rose-like aroma to the distillate (Falqué *et al.*, 2001).

The presence of methanol in the distilled of SCG was also confirmed at a very low concentration (14.0 mg/L and 7.6 mg/L for D1 and D2, respectively). Many distillates contain this compound at low concentration, which is a positive aspect due to the toxicity of this compound. Methanol can be harmful to the human health when present in high concentrations (>4000 mg/L). According to Regulation (EC)

No 110/2008 of the European Parliament and of the Council, the legal limit for this compound in this kind of beverages is 1000 mg/hL in 100 % volume of ethanol (EUR–Lex, 2008).

The difference in the values between the two distilled beverages can be explained by the fact that in the first distilled beverage the collected fractions were not made in the most effective way, since they were performed according to Sampaio *et al.* (2013). It was verified that for this work the same mode of collection could not be followed, once higher values of alcoholic degree were obtained from the fractions collected by this author, which led to only two fractions collected between 40 % and 70 % ethanol for the "heart of the distillate". However, in the second distilled beverage, the quantities of fractions to be collected for a certain temperature were modified, and thus more than two fractions could be added. Considering this aspect, the distilled beverage produced from SCG may be considered as having organoleptic quality acceptable for human consumption.

Table 5.4 shows the minor volatile compounds concentrations, as 4-nonanol equivalents, identified in fermented and distillate SCG. Fermented beverages (F1 and F2) were characterized mainly by volatile acids with the highest concentration for hexanoic, octanoic, and 2-methylpropanoic acids. The most abundant compound in fermented beverages was 2-furanmethanol (308.4 μg/L for F1 and 329.5 μg/L for F2). This compound was found in the headspace of the oil obtained from the coffee residue and was identified as being responsible for the coffee-like aroma (Turek, 2010). Among terpenes, nerol was quantified in fermented beverages but at a low concentration (2.7 μg/L and 1.0 μg/L for F1 and F2) below its odor threshold (400 μg/L; (Marais, 1983)).

Table 5.4. Concentration (*C*) and standard deviation (*SD*) of minor volatile compounds identified and quantified in fermented and distillate samples

Compound		F1		F2		D1		D2	
	LRI	*C*/(μg/L)	*SD*	*C*/(μg/L)	*SD*	*C*/(μg/L)	*SD*	*C*/(μg/L)	*SD*
ethyl butyrate	995	10.3	0.8	3.9	0.8	tr	-	tr	-
ethyl 2–methylbutyrate	1052	-	-	-	-	17.0	2.4	33.7	3.9
ethyl 3–methylbutyrate	1070	-	-	-	-	6.5	2.8	28.6	3.6
3–methylbutyl acetate	1119	38.1	1.9	33.8	2.8	-	-	35.2	3.6
ethyl hexanoate	1229	42.9	1.9	20.2	1.6	57.9	1.3	156.9	10.1
1–pentanol	1239	21.0	1.2	16.9	0.7	19.6	2.4	37.9	1.7
2–methylpyrazine	1255	-	-	-	-	13.8	0.6	13.0	0.8
2,6–dimethylpyrazine	1318	-	-	-	-	36.5	3.9	42.9	2.0
2–ethylpyrazine	1324	-	-	-	-	6.6	1.2	7.2	0.3
2,3–dimethylpyrazine	1334	-	-	-	-	8.3	2.1	4.7	0.1
ethyl lactate	1335	21.6	0.3	103.1	9.4	54.1	3.6	45.9	5.6
1–hexanol	1344	23.9	0.9	19.3	2.6	10.5	1.5	44.1	4.5

Table 5.4. (Conclusion) Concentration (*C*) and standard deviation (*SD*) of minor volatile compounds identified and quantified in fermented and distillate samples

Compound		F1		F2		D1		D2	
	LRI	*C*/(μg/L)	*SD*	*C*/(μg/L)	*SD*	*C*/(μg/L)	*SD*	*C*/(μg/L)	*SD*
ethyl octanoate	1429	13.2	1.5	-	-	239.4	11.1	698.0	88.7
furan linalool oxide, *trans-*	1434	-	-	-	-	tr	-	4.8	0.6
1–heptanol	1448	4.2	0.8	4.0	0.5	1.3	0.6	11.7	0.5
furfural	1457	58.4	3.0	54.7	3.2	3054.9	187.5	2853.8	262.5
2–ethyl–1–hexanol	1483	11.6	1.6	15.7	1.2	-	-	6.0	0.5
benzaldehyde	1511	7.2	6.3	11.1	0.9	80.3	6.1	315.3	21.8
furfuryl acetate	1532	9.1	1.3	5.2	0.4	-	-	tr	-
linalool	1542	-	-	-	-	tr	-	22.3	0.9
propanoic acid	1545	6.1	0.7	6.4	0.8	-	-	tr	-
5–methylfurfural	1564	5.4	0.7	4.7	0.9	732.1	36.0	474.3	41.9
2–methylpropanoic acid	1574	277.5	32.9	364.2	19.5	122.5	7.9	182.5	22.0
ethyl decanoate	1632	-	-	-	-	17.8	1.9	324.1	47.6
2–furanmethanol	1653	308.4	42.3	329.5	9.8	156.0	9.8	89.3	14.6
diethyl succinate	1668	16.7	1.8	19.9	1.7	146.8	9.9	722.8	76.0
2–methylbutyric + 3–methyl–butyric acids	1675	187.0	8.1	175.0	6.7	534.6	34.4	564.3	76.4
γ–caprolactone	1685	17.5	1.4	18.5	0.7	9.0	0.5	3.3	0.8
methionol	1705	26.0	2.0	19.3	0.5	-	-	-	-
citronellol	1759	8.1	0.6	2.4	0.2	-	-	12.8	1.3
ethyl phenylacetate	1774	3.1	0.9	7.1	1.0	8.4	2.0	16.3	2.4
nerol	1790	2.7	0.5	1.0	0.1	-	-	tr	-
2–phenylethyl acetate	1801	-	-	-	-	104.7	5.6	126.4	14.3
β–damascenone	1804	-	-	-	-	-	-	tr	-
hexanoic acid	1850	239.2	23.1	203.0	2.7	453.0	23.6	425.4	32.9
guaiacol	1851	31.5	3.1	33.5	1.4	138.8	6.2	89.9	9.8
benzyl alcohol	1862	9.7	0.1	19.2	1.8	7.0	0.4	4.1	0.2
γ–nonalactone	2009	67.0	2.8	62.0	3.3	168.7	9.6	106.2	11.1
4–ethylguaiacol	2017	5.1	1.3	4.9	0.5	174.5	6.1	106.5	8.6
nerolidol, *trans-*	2034	-	-	-	-	86.3	9.7	138.6	21.7
octanoic acid	2065	246.0	6.5	168.0	5.1	5614.9	207.8	4179.0	348.5
γ–decalactona	2122	6.9	0.1	3.7	0.1	28.7	1.0	27.8	3.4
4–vinylguaiacol	2181	42.9	3.5	24.6	1.9	224.1	6.3	343.5	37.9
γ–undecalactona	2237	-	-	-	-	9.2	1.1	6.8	0.9
decanoic acid	2279	7.1	1.6	2.1	0.5	2995.4	211.8	2535.4	193.9
E,E–farnesol	2344	-	-	-	-	54.2	9.4	133.3	15.8
dodecanoic acid	2492	-	-	-	-	39.3	4.4	27.9	3.9
5–hydroxymethylfurfural	2494	-	-	-	-	26.7	1.8	80.3	9.2
3–hydroxyl–β–damascone	2513	6.3	0.5	4.5	0.3	-	-	4.3	0.1
vanillin	2543	12.8	2.4	11.6	1.3	25.4	2.2	48.4	5.1
acetovanillone	2615	41.8	4.3	45.0	1.9	26.5	0.7	20.3	3.6
tyrosol	2989	18.4	5.0	16.9	2.1	-	-	-	-

Fermented beverage 1 (F1) and 2 (F2); Distilled beverage 1 (D1) and 2 (D2).
LRI, linear retention index: -, not detected; tr, traces.

Although the minor compounds are found in low concentration in distilled beverages, they are of great importance to their aroma. In fact, compounds appearing in trace quantities in alcoholic beverages quite frequently have a greater influence on their sensory properties than those compounds that appear in high concentrations (Plutowska *et al.*, 2008). Among the minor volatile compounds identified in SCG spirit, the volatile acids were the most abundant, followed by esters. Among volatile acids, hexanoic, octanoic, and decanoic acids were in high concentration in SCG distillate D1 and D2, but these acids are reported to have low flavor effect in the distillates (Soufleros *et al.*, 2004).

The most abundant esters were ethyl octanoate (239.4 μg/L and 698.0 μg/L for D1 and D2), ethyl hexanoate (57.9 μg/L and 156.9 μg/L for D1 and D2), and 2–phenylethyl acetate (104.7 μg/L and 126.4 μg/L for D1 and D2). Sampaio *et al.* (2013) showed higher values for SCG spirit (842 μg/L of ethyl octanoate, 337 μg/L of ethyl hexanoate and 130 μg/L of 2–phenylethyl acetate). These compounds contribute a pleasant fruity flavor and floral aroma to the drink (Escudero *et al.,* 2004). On the other hand, 4–vinylguaiacol was the most abundant phenol volatile in D1 and D2 SCG distillates. 4–Vinylguaiacol and 4–vinylphenol identified in the steam volatile concentrate were considered to be produced from ferulic and *p*–coumaric acids during steam–distillation of rice bran (Fujimaki *et al.*, 1977). 4–vinylguaiacol had the greatest impact on the flavor of ground coffee (Mayer *et al.*, 2000).

Pyrazines also are present in SCG distillates D1 and D2 but in low concentrations. Pyrazines are heterocyclic aromatic compounds containing a six–membered ring with two nitrogen atoms in positions 1 and 4, and they occur naturally in vegetables and insects. Pyrazines are the products of primary and secondary metabolic processes that take place in some microorganisms. In the case of agricultural distillates, pyrazines are the products of the Maillard reaction, which occurs when thermal processing is not optimal (Wiśniewska *et al.*, 2016).

Among the aldehydes, furfural was identified at a high level in the distillates D1 and D2. This compound is formed during processes that involve heating or roasting, e.g., roasting of coffee beans and/or distillation, due to degradation of fermentable pentose sugars, caused by heating in acid conditions, and/or Maillard reaction (Chaichia *et al.*, 2015; Mangas *et al.*, 1996; Vignoli *et al.*, 2014). Thus, high amounts of furfural might be attributed to the presence of high quantities of residual pentose sugars due to unfavourable fermentation conditions of the substrate. Its odour is reminiscent of bitter almond and cinnamon (Apostolopoulou *et al.*, 2005).

5.2.3. Sensory analysis of fermented and spirit beverages

In the sensory analysis, the duplicates of the fermented and distilled beverages were added, *i.e.*, the fermented beverage 1 (F1) and the fermented beverage 2 (F2) were added in the same volumetric proportion, obtaining a final fermented beverage (F), as well as the distilled beverage 1 (D1) and the distilled beverage 2 (D2) in order to obtain a final distilled beverage (D).

Table 5.5 shows visual, olfactory, and gustatory sensory descriptors identified in spirits and their correspondent means of frequency (*F*) and intensity (*I*) obtained by the tasting panels. Spirits were characterized with 17 sensory descriptors, one by visual analysis, eight by olfactory analysis, eight by gustatory analysis, and by a global value.

In the visual analysis, the clarity descriptor showed medium intensity in fermented (F) and distillated (D) samples (53 % and 51 %, respectively) and the highest frequency (100 %) in both. Therefore, the Geometric Mean (*GM*) was slightly higher for fermented (F) than distillates (D) samples in visual analysis.

In olfactory analysis, the quality and intensity of distillate (D) were higher than in the fermented beverage (F) with *GM* > 70 % in spirit beverage. Among the descriptors defining the beverages' aroma, all descriptors showed the highest *GM* value for Spirit, with an exception of apple (*GM* = 13 % in both beverages). Toasted was described for the fermented beverage, however caramel, vanilla, and coffee characterized the distillate beverage. Coffee was the most representative aroma descriptor by olfactory analysis in a novel spirit developed from spent coffee (Sampaio *et al.*, 2013). Caramel, vanilla, and coffee were not detected in the F sample by the tasting panel. Toasted was not detected in the D sample. Similar descriptors to caramel and toasted have been used in other studies to describe the flavor properties of coffee products (Czerny *et al.*, 1999; Mayer *et al.*, 2000).

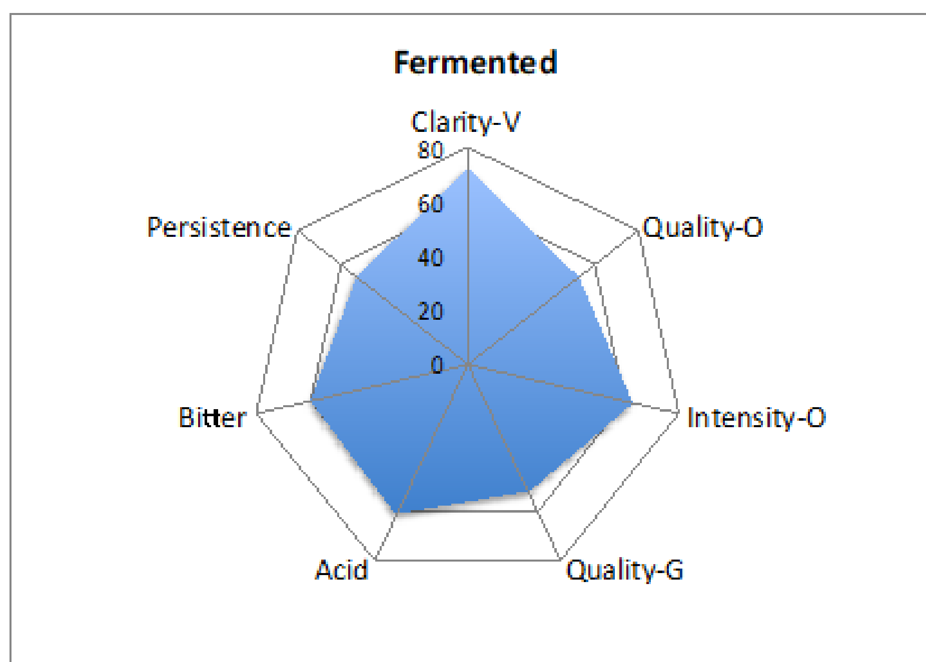
Quality and bitter were the most important descriptors in the D sample (*GM* = 67 %). However, acidity and bitter were the most representative descriptors in the gustatory analysis of fermented beverages (61 % and 60 % of *GM* respectively). The flavor profile of Turkish coffee brews showed as roasted/burnt, spicy, bitter, acidic, sweet, salty, astringent (dry), woody, fermented, earthy, and tobacco-like flavor characteristics (Kıvançlı, *et al.*, 2016). On the other hand, the global value of samples was higher for distillate (D) in intensity and frequency than for the fermented sample (F).

Table 5.5. Intensity (*I*), frequency (*F*), and geometric mean (*GM*) to each descriptor of spent coffee grounds (SCG) fermented (F) and distillated (D)

	Descriptor	Fermented (F)			Distillated (D)		
		I (%)	*F* (%)	*GM* (%)	*I* (%)	*F* (%)	*GM* (%)
Visual	Clarity	53	100	73	51	100	71
Olfactory	Quality	27	100	52	53	100	73
	Intensity	40	100	63	51	100	71
	Toasted	13	20	16	0	0	0
	Caramel	0	0	0	44	80	60
	Vanilla	0	0	0	20	40	28
	Strawberry	9	20	13	13	20	16
	Coffee	0	0	0	20	40	28
	Apple	9	20	13	9	20	13
Gustatory	Quality	27	100	52	44	100	67
	Sweet	13	60	28	18	80	38
	Salt	16	60	31	22	80	42
	Acid	38	100	61	31	100	56
	Bitter	44	80	60	44	100	67
	Body	9	40	19	38	80	55
	Persistence	36	80	53	36	60	46
	Astringent	18	60	33	29	60	42

For descriptors, the *GM* obtained through the values of intensity and frequency of each attribute in fermented and spirit beverages was represented in Figure 5.5. Descriptors with *GM* greater than 50 % were considered the descriptors with the highest contribution in this study. Thus, seven descriptors (with *GM* > 50 %) defined the sensory characteristics of the fermented sample (Figure 5.5a), including clarity (visual analysis), quality and intensity (olfactory analysis), and quality, acid, bitter, and persistence (gustatory analysis). However, nine descriptors (*GM* > 50 %) defined the sensory characteristics of distillated sample (Figure 5.5b), clarity in visual analysis, quality, intensity, and caramel in olfactory analysis and quality, acid, bitter, and body in gustatory analysis. Global Value also showed *GM* > 50 % in D sample. Figure 5.5 shows the characteristic profiles of the fermented and distillated samples.

a)



b)

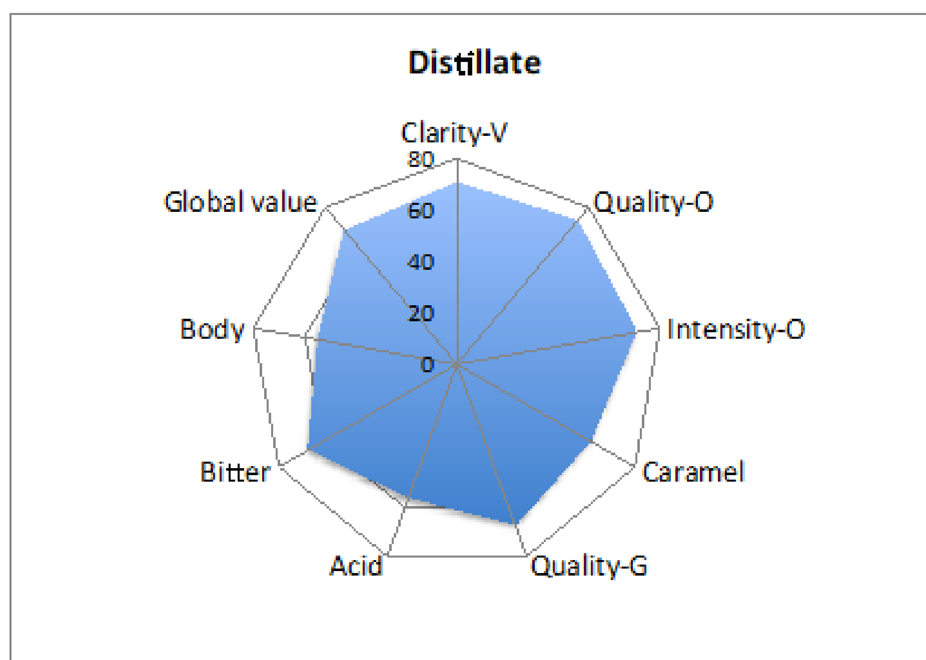


Figure 5.5. Sensory profile (*GM* > 50 %) of fermented and distillate samples. (V – visual analysis; O – olfactory analysis; G – gustatory analysis).

5.3. Conclusions

Fermented and distilled beverages from spent coffee grounds were characterized by chemical and sensory analysis. In fermented samples, an efficient conversion of sugars to ethanol by the yeast was achieved with a volumetric percentage of 10.4 % and 10.0 % of ethanol, and the distilled beverages reached a volumetric percentage of 38.1 % and 40.2 % of ethanol. The fermented beverages were characterized by higher alcohols, such as isobutanol and isoamylic, and esters contributing positively to the beverage aroma. Alcohols as major compounds and volatile acids and esters as minor were the most abundant on the distillate beverages, contributing to a pleasant fruity flavor and floral aroma to the drink. Olfactory quality and intensity showed a geometric mean (*GM*) > 50 % for fermented beverages and geometric mean (*GM*) > 70 % for distillates. The global value was major for distillates beverages. This work demonstrates that the fermented and distilled beverages have acceptable organoleptic qualities for human consumption, thus adding value to spent coffee grounds and increasing the sustainability of the coffee agro-industry.

Acknowledgements

This study was supported by the Portuguese Foundation for Science and Technology – FCT (grant SFRH/BD/75195/2010) under the scope of the strategic funding of UID/BIO/04469/2013 unit and COMPETE 2020 (POCI-01-0145-FEDER-006684) and BioTecNorte operation (NORTE-01-0145-FEDER-000004) funded by European Regional Development Fund under the scope of Norte2020— Programa Operacional Regional do Norte. Thanks to Appellation Orujo de Galicia (Galicia, Spain).

5.4. References

Apostolopoulou, A. A., Flouros, A. I., Demertzis, P. G., Akrida–Demertzi, K. (2005). Differences in concentration of principal volatile constituents in traditional Greek distillates. *Food Control*, 16, 157–164.

Chaichia, M., Ghasemzadeh-Mohammadia, V., Hashemib, M, Mohammadia, A. (2015). Furanic compounds and furfural in different coffee products by headspace liquid-phase micro-extraction followed by gas chromatography–mass spectrometry: Survey and effect of brewing procedures. *Food Additives & Contaminants Part B*, 8, 73–80.

Coelho, E., Vilanova, M., Genisheva, Z., Oliveira, J. M., Teixeira, J. A., Domingues, L. (2015). Systematic approach for the development of fruit wines from industrially processed fruit concentrates, including optimization of fermentation parameters, chemical characterization and sensory evaluation. *Food Science and Technology (LWT)*, 62, 1043–1052.

Cortés, S., Gil, M. L., Fernández, E. (2005). Volatile composition of traditional and industrial orujo spirits. *Food Control*, 16, 383–388.

Czerny, M., Mayer, F., Grosch, W. (1999). Sensory study on the character impact odorants of roasted Arabica coffee. *Journal of Agricultural and Food Chemistry*, 47, 695–699.

De Melo Pereira, G.V., Soccol, V.T., Pandey, A., Medeiros, A.B., Andrade Lara, J.M., Gollo, A.L., Soccol, C.R. (2014). Isolation, selection and evaluation of yeasts for use in fermentation of coffee beans by the wet process. *International Journal of Food Microbiology*, 188, 60–66.

Dragone, G., Mussato, S. I., Oliveira, J., Teixeira, J. A., (2009a). Characterization of volatile compounds in an alcoholic beverage produced by whey fermentation. *Food Chemistry*, 112, 929–935.

Dragone, G., Mussato, S. I., Vilanova, M., Oliveira, J., Teixeira, J. A., Silva, J. (2009b). Obtenção e caracterização de bebida destilada a partir da fermentação do soro de queijo. *Brazilian Journal of Food Technology*, 120–124.

Dravnieks, A. F. C. (1978). Bock, Comparison of odours directly and through profiling, *Chemical Senses*, 191–225.

Dreywood, R. (1946). Qualitative test for carbohydrate material. *Industry and Engineering Chemistry Analytical,* 18, 499.

Escudero, A., Gogorza, B., Melús, M. A., Ortín, N., Cacho, J., Ferreira, V. (2004). Characterization of the aroma of a wine from Maccabeo. Key role played by compounds with low odor activity values. *Journal of Agricultural and Food Chemistry*, 52, 3516–3524.

EUR–Lex. (2008). Available at: http://eur–lex.europa.eu/. Accessed 21 March 2018.

Falqué, E., Fernandez, E., Dubourdieu, D. (2001). Differentiation of white wines by their aromatic index. *Talanta*, 54, 271–281.

Fitzgerald, G., James, K. J., MacNamara, K., Stack, M. A. (2000). Characterization of whiskeys using solid–phase microextraction with gas chromatography–mass spectrometry. *Journal of Chromatography A*, 896, 351–359.

Fujimaki, M., Tsugita, T., Kurata, T. (1977). Fractionation and identification of volatile acids and phenols in the steam distillate of rice bran. *Journal Agricultural and Biological Chemistry*, 41(9), 1721–1725.

Fundira, M., Blom, M., Pretorius, I., van Rensburg, P. (2002). Selection of yeast starter culture strains for the production of marula fruit wines and distillates. *Journal of Agricultural and Food Chemistry*, 50, 1535–1542.

Kıvançlı, J., Elmacı, Y. (2016). Characterization of Turkish-Style Boiled Coffee Aroma by Gas Chromatography and Mass Spectrometry and Descriptive Analysis Techniques. International *Journal of Food Properties*, 19, 1681–1686.

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

Lee, L.W., Cheong, M.W., Curran, P., Yu, B., Liu, S.Q. (2015). Coffee fermentation and flavor. An intricate and delicate relationship. *Food Chemistry*, 15, 182–91.

Machado, E. M. S. (2009). Reaproveitamento de resíduos da indústria do café como matéria-prima para a produção de etanol. *Dissertação de Mestrado em Engenharia Biológica, Universidade do Minho*, Portugal.

Mangas, J., Rodríguez, R., Moreno, J., Blanco, D. (1996). Changes in the major volatile compounds of cider distillates during maturation. *Lebensmittel Wissenschaft und–Technologie*, 29, 357–364.

Marais, I. (1983). Terpenes in the aroma of grapes and wines: A review. *South African Journal for Enology and Viticulture*, 4, 49–60.

Mayer, F., Czerny, M., Grosch, W. (2000). Sensory study of the character impact aroma compounds of a coffee beverage. *European Food Research and Technology*, 211, 272–276.

Moreira, M.M., Barroso, M.F., Boeykens, A., Withouck, H., Morais, S., Delerue-Matos, C. (2017). Valorization of apple tree wood residues by polyphenols extraction: Comparison between conventional and microwave–assisted extraction. *Industrial Crops and Products*, 104, 210–220.

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

Mussatto, S. I., Carneiro, L. M., Silva, J. P. A., Roberto, I. C., Teixeira, J. A. (2011b). A study on chemical constituents and sugars extraction from spent coffee grounds. *Carbohydrate Polymers*, 83, 368–374.

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

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

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

Oliveira, J. M., Faria, M., Sá, F., Barros, F., Araújo, I. M. (2006). C_6–alcohols as varietal markers for assessment of wine origin. *Analytica Chimica Acta*, 563, 300–309.

Plutowska, B., Wardencki, W. (2008). Application of gas chromatography–olfactometry (GC–O) in analysis and quality assessment of alcoholic beverages – a review. *Food Chemistry*, 107, 449–463.

Sampaio, A., Dragone, G., Vilanova, M., Oliveira, J. M., Teixeira, J. A., Mussatto, S. I. (2013). Production, chemical characterization, and sensory profile of novel spirit elaborated from spent coffee ground. *Food Science and Technology (LWT)*, 54, 557–563.

Santos, J., Dinham, R. (2006). *O Essencial em cervejas e destilados*. Senac, São Paulo.

Santos, S. (2007). *Pequeno livro de destilados: Guia para toda a hora*. Verus Editora, Brasil, Campinas.

Silva, M. L., Malcata, F. X., de Revel, G. (1996). Volatile contents of grape marcs in Portugal. *Journal of Food Composition and Analysis*, 9, 72–80.

Soufleros, E. H., Mygdalia, A. S., Natskoulis, P. (2004). Characterization and safety evaluation of the traditional Greek fruit distillate "Mouro" by flavor compounds and mineral analysis. *Food Chemistry*, 86, 625–636.

Steger, C. L. C., Lambrechts, M. G. (2000). The selection of yeast strains for the production of premium quality South African brandy base products. *Journal of Industrial Microbiology & Biotechnology*, 24, 431–440.

Turek, I.A. (2010). The Effect of Microwave Extraction on the Chemical Composition and the Antioxidant Properties of the Coffee Residue. *Master's Thesis*, *Universidade de Aveiro*, Aveiro, Portugal.

Verdi, A. R. (2006). Dinâmicas e perspectivas do mercado da cachaça. *Informações Económicas*, 36(2), 93–98.

Vignoli, J.A., Viegas, M.C., Bassoli, D.G., Benassi, M.T. (2014). Roasting process affects differently the bioactive compounds and the antioxidant activity of arabica and robusta coffees. *Food Research International*, 61, 279–285.

Wiśniewska, P., Śliwińska, M., Dymerski, T., Wardencki, W., Namieśnik, J. (2016). The analysis of raw spirits – a review of methodology. *Journal of Institute of Brewing*, 122, 5–10.

CHAPTER 6

CONCLUSIONS AND SUGGESTIONS FOR FUTURE WORK

6. General conclusions and future perspectives

6.1. Conclusions

The main objective of this thesis was to extract phenolic antioxidant compounds from spent coffee grounds (SCG) and evaluate its use as a raw material for the production of fermented and distilled beverages. To successfully accomplish the purpose of the thesis, several steps had been taken into account. In the first stage were performed extractions of the antioxidant phenolic compounds using CO_2 supercritical extraction and microwave–assisted extraction. Subsequently, the extracted phenolic compounds were used for the elaboration of a fermented and a distilled beverage. At the end, the beverages were analyzed to determine their chemical composition and sensory profile. With this thesis it was possible to draw the following conclusions:

- Demonstrate that spent coffee grounds (SCG) are an excellent raw material, with very interesting properties for application in the beverage industry.

- Supercritical CO_2 extraction demonstrated not to be one of the most efficient techniques for recovering phenolic antioxidant compounds from SCG. It was possible to obtain an extract with a content of phenolic compounds, flavonoids and sugars of 0.36 mg GAE/g SCG, 1.55 mg QE/g SCG and 0.0006 mg Glucose/g SCG, respectively.

- Microwave–assisted extraction showed to be an efficient technology for extracting phenolic antioxidant compounds from SCG, using water as the solvent. It was possible to obtain an extract with a content of phenolic compounds, flavonoids and sugars of 32.33 mg GAE/g SCG, 4.86 mg QE/g SCG and 49.78 mg Glucose/g SCG, respectively.

- The fermented beverage produced from the extracts obtained by microwave–assisted extraction showed good concentration levels of higher alcohols, which allows this beverage to have a pleasant aroma.

- The distilled beverage produced with extracts from microwave–assisted extraction showed good levels of higher alcohols as major compounds, with volatile acids and esters being the most abundant minor compounds. This composition made the distillate to have a pleasant fruity flavor and floral aroma.

- In general, the results showed the excellent potential of SCG as a raw material in the beverage industry, not only because of its high content of phenolic antioxidant compounds, but also because it is a low–cost raw material with enormous availability.

6.2. Guidelines for future work

Although the objectives that were proposed for this thesis were mostly achieved, the future recommendations regarding the fermented and distilled beverage of spent coffee grounds are as follows:

- Evaluate the characteristics (chemical and sensorial) of the distilled beverage after aging in a wooden barrel.

- Analyze economically the developed bioprocesses and in parallel study the possibility of its scale up, aiming at industrial production.

Evaluate market and commercial potential in restaurants, bars and commercial areas, taking into account: competition, market position, associated costs, advertising, and launch strategy.