Universidade de Aveiro Departamento de Química
2019

Guido Rocha Lopes

Estudo da composição de infusões de café tendo como alvo as propriedades do café expresso

Comprehensive study of coffee infusions composition targeting espresso properties

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Tese apresentada à Universidade de Aveiro para cumprimento dos requisitos necessários à obtenção do grau de Doutor em Ciência e Tecnologia Alimentar e Nutrição, realizada sob a orientação científica do Doutor Manuel António Coimbra Rodrigues da Silva, Professor Associado com Agregação do Departamento de Química da Universidade de Aveiro, do Doutor José António Couto Teixeira, Professor Catedrático do Departamento de Engenharia Biológica da Universidade do Minho e da Doutora Carla Isabel Igreja Rodrigues, Investigadora no Centro de Inovação do Grupo Nabeiro.

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“(…) a atitude científica é estar solidamente ancorado no presente, não esquecer nenhum dos passos do passado, nem esquecer nenhuma possibilidade do futuro.”

Agostinho da Silva

À minha FAMÍLIA

o júri

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palavras-chave café, processo de extração, infusão, polissacarídeos, cafeína, café expresso

resumo

Diferentes bebidas de café, como o café expresso, filtrado ou instantâneo, exibem propriedades físico-químicas distintas. Isto é devido às propriedades intrínsecas do café torrado e às condições usadas para preparar a bebida (*e.g.* quantidade de café a ser extraído, volume e temperatura de extração, assim como o grau de moagem). No entanto, todas as bebidas são preparadas com apenas dois ingredientes: café torrado moído e água. Assim as bebidas de café contêm carboidratos, proteínas, cafeína, ácidos clorogénicos e compostos voláteis, que variam numa determinada gama, constituindo uma mistura complexa de compostos. Estes compostos estão associados a propriedades que podem ser valorizadas em formulações, nomeadamente as organoléticas (*e.g.* corpo, aroma, cor ou textura) e de saúde (*e.g.* fibra dietética). Assim, pode colocar-se a hipótese de que a modulação do processo de extração de café pode dar origem a extratos semelhantes ao café instantâneo, filtrado ou expresso, de acordo com as características desejadas para cada bebida.

Para validar esta hipótese, foram realizados estudos de infusões de café tendo como objetivo perceber como é que as variáveis operacionais afetam a eficiência e/ou seletividade de extração dos compostos do café. A variabilidade relacionada com a espécie/origem do café e/ou grau de torra foi eliminada pela utilização do mesmo café torrado em todas as experiências. Foram utilizadas metodologias de superfície de resposta que visaram estudar e comparar dois métodos de extração distintos: a infusão convencional a pressão atmosférica (extração sólido-líquido) e a extração assistida por micro-ondas (MAE). O uso de um sistema fechado permite atingir temperaturas superiores às do ponto de ebulição da água. O rendimento de extração e a caracterização química dos extratos, designadamente, o conteúdo e a composição em carboidratos, cafeína e ácido clorogénico (5-CQA) e as propriedades de cor foram usados como respostas para descrever os sistemas.

O processo de infusão convencional foi delineado de acordo com um planeamento experimental compósito central com 4 fatores (tempo e temperatura de extração, razão massa/volume e grau de moagem) numa gama alargada de condições (10-360 min, 20-80 °C, 1-6 g/30 mL e três níveis de moagem). As condições aplicadas permitiram extrair até 30% dos compostos do café. A temperatura foi o fator mais determinante para a diferenciação dos extratos, tendo em conta o rendimento global e em carboidratos. Foi estimada a contribuição dos dois principais polissacarídeos do café: galactomananas (GM) e arabinogalactanas (AG). A composição dos extratos está mais relacionada com a extração das GM do que com as AG, já que a variação das AG no extrato (7,5-12,0%) é menor do que a das GM (5,4-18,3%), com um rácio GM/AG de 0,7-1,9. A viscosidade das soluções de café está positivamente correlacionada com o teor de GM no extrato.

Em condições de extração a frio é promovida essencialmente a extração das AG. No entanto, para tempos de extração longos há aumento das GM. A extração das GM aumenta também com o aumento da temperatura. As elevadas temperaturas e os reduzidos rácios massa/volume levam à extração de compostos mais castanhos. A cafeína e o 5-CQA são amplamente extraídos nas condições testadas, variando o seu conteúdo relativo no extrato devido à maior ou menor quantidade dos outros compostos.

A extração assistida por micro-ondas foi estudada através de um desenho experimental Box-Behnken com 3 fatores e 3 níveis: tempo (1-10 min), temperatura (120-180 °C) e razão massa/volume (2-6 g/60 mL) no rendimento de extração (24-47%), e em parâmetros como o conteúdo em carboidratos (18-43%), composição em açúcares, cafeína (4-7%) e 5-CQA (1-2%). Neste caso, a temperatura foi também o fator mais decisivo para a diferenciação dos extratos, permitindo obter através da MAE maiores rendimentos do que os obtidos com o sistema convencional, principalmente a 180 °C. O aumento está associado a um aumento da extração das AG em relação às GM, resultando em extratos com composição semelhante à composição do café instantâneo. Praticamente toda a arabinose e a galactose foram extraídas do café a 180°C, restando um resíduo rico em GM e celulose. Além disso, verificou-se um amarelamento dos extratos com o aumento da temperatura de extração. Assim, a MAE provou ser um método viável para produzir extratos de café com uma composição definida de ingredientes, como a de extratos de café instantâneo, gerando ainda um resíduo rico em GM e celulose, que pode ser valorizado como fonte de fibra dietética.

Como os polissacarídeos constituem uma grande fração das bebidas de café expresso e contribuem para as suas propriedades organoléticas (viscosidade e estabilidade da espuma), foram usados como alvo para se poder obter um extrato com propriedades semelhantes às do café expresso, usando o processo convencional de extração. As condições otimizadas de extração foram utilizadas para uma extração a maior escala. Os extratos foram concentrados por liofilização e por atomização, tendo sido comparados com amostras comerciais de café expresso e instantâneo tendo em conta a sua composição (conteúdo em açúcares, proteína, melanoidinas, lípidos, cafeína, 5-CQA e compostos voláteis) e propriedades (cor, dissolução em água, viscosidade, pH e capacidade de formação de espuma). Os extratos obtidos mostraram ter uma composição semelhante à do café expresso, com predominância de GM em relação às AG, contrariamente às amostras de café instantâneo, mesmo as referidas no rótulo como sendo “café expresso”. A análise de FTIR confirmou a semelhança geral dos extratos de café expresso e os obtidos por infusão, excetuando a menor presença de lípidos. Todos os extratos foram capazes de produzir espuma quando submetidos à injeção de CO_2 ou utilizando agentes efervescentes. O perfil volátil, incluindo aromas-chave, obtido para os extratos liofilizados de café expresso e obtido por infusão, foi semelhante. Embora haja uma perda da intensidade de compostos voláteis em comparação com café expresso acabado de preparar, o perfil volátil é semelhante ao obtido com o café expresso tirado na hora com uma máquina expresso. Apesar do menor teor de lípidos e concentração de compostos voláteis com impacto no aroma ser ainda um desafio para a aproximação à composição do café expresso, tal poderá ser ultrapassado pela adição da fração lipídica do café torrado, rica em compostos voláteis, numa futura formulação, melhorando as qualidades organoléticas do produto de café expresso-instantâneo.

keywords Coffee, extraction process, infusion, polysaccharides, caffeine, espresso coffee

abstract

The different coffee brews as espresso, filtered, or instant coffee exhibit distinct physico-chemical characteristics due to the intrinsic properties of roasted coffee as well as to the conditions used to prepare the brews (e*.g.* amount of coffee to extract, volume and temperature of extraction, and grinding degree). Nevertheless, all coffee brews are prepared with only two ingredients: roasted ground coffee and water. Therefore, all coffee brews contain carbohydrates, proteins, caffeine, chlorogenic acids, lipids and volatile compounds that vary in a certain range, constituting a complex mixture of compounds. They are related with properties that can be valued in formulations with defined organoleptic (*e.g.* body, flavour, colour or texture) and/or nutritional and health benefits (*e.g.* dietary fibre). Thus, it may be hypothesized that the modulation of a coffee extraction process may allow to obtain extracts that can be more related to instant, filtered or espresso coffee according to the desired brew characteristics.

To fulfil this hypothesis, coffee infusion experiments were conducted to infer how operational variables affect the efficiency and/or selectivity of coffee compounds. The use of the same roasted coffee in all experiments eliminated the variability related to coffee species/origin and/or roasting degree of coffee. Response surface methodology (RSM) was used to comprehensively study and compare two distinct extraction methods: a conventional coffee infusion process at atmospheric pressure (solid-liquid extraction) and a coffee microwave-assisted extraction (MAE). The use of closed-vessel systems allows to achieve, in MAE, temperatures higher than the water boiling point. The extraction yield and the extracts chemical composition, namely, carbohydrates content and composition, caffeine, and chlorogenic acid (5-CQA), as well as colour properties were used as responses to describe the systems.

The conventional infusion process was delineated according to a face-centered central composite design with 4 factors (time and temperature of extraction, mass-to-volume ratio (w/V) and grinding degree) in a wide range of conditions (10-360 min, 20-80 ºC, 1-6 g/30 mL, and three grinding levels). The conditions applied allowed to extract up to 30% of coffee compounds. Temperature was the major factor for coffee extracts differentiation, regarding both overall and carbohydrates yield of extraction and composition. It was estimated the contribution in the extract of the two main coffee carbohydrates: galactomannans (GM) and arabinogalactans (AG). The distinct extract composition across the design space was more related to GM extraction than AG, as the variation of AG content was lower (7.5-12.0%) when compared to GM (5.4-18.3%), with GM/AG ratio varying from 0.7 to 1.9. The viscosity of the coffee solutions was positively related to the GM content of the extract.

In cold brew conditions, mainly the extraction of AG was promoted. However, prolonged times promoted the increase of GM extraction. Moreover, the extraction of GM from coffee powder increased with the increasing of temperature. The higher temperature and lower w/V ratio lead to browner coffee extracts. Caffeine and 5-CQA were extensively extracted in the conditions tested. For this reason, their relative content in each extract varied due to the content of the other extracted compounds.

The effect of coffee MAE was studied by a Box-Behnken design with 3 factors and 3 levels: time (1-10 min), temperature (120-180 ºC) and w/V ratio (2-6 g/60 mL) on the overall extraction yield (24-47%w/w), and in parameters as carbohydrates content (18-43% w/w), sugars composition, caffeine (4-7% w/w) and 5-CQA (1-2% w/w). In this case, temperature was also the major factor for coffee extracts differentiation, with MAE allowing considerably higher overall extraction yields than those obtained with the conventional system, mainly when performed at 180 ºC. This was associated to a substantial increase in AG extraction over GM, resulting in extracts approaching instant coffee composition. Almost all arabinose and galactose presented in roasted coffee powder was extracted during the experiments at 180 °C, while the residue was still rich in GM and cellulose. Moreover, there was a yellowing of the extracts. Indeed, MAE prove to be a reliable method to produce extracts that can be used as defined food/brew ingredients, as instant coffee-like extracts, providing also a GM and cellulose rich residue that can also be valued as a source of dietary fibre under a circular economy concept.

As polysaccharides account for a considerable amount of espresso coffee brews and contribute to their organoleptic properties (viscosity and foam stability), they were used as target to resemble espresso properties under the milder conventional infusion process. The optimized conditions regarding espresso similarity were scaled up and concentrated via freeze- and spray-drying and compared to commercial espresso and instant coffee samples regarding content and composition in sugars, protein, melanoidins, lipids, caffeine, 5-CQA, and volatile compounds, and physical properties such as colour, dissolution in water, viscosity, pH, and foamability. The scaled-up extract exhibited a sugars composition similar to espresso coffee, with predominance of GM over AG, contrary to instant coffee, even the ones labelled as “espresso coffee”. FTIR analysis confirmed the overall resemblance of infusion and espresso coffee extracts composition, except on the lower amount of lipids present. All extracts were able to produce foam when submitted to CO_2 injection or when an effervescent agent was used. The volatile profile, including key odorants, for the infusion freeze-dried and the espresso coffee extracts was similar. Even though there was a loss of volatiles intensity in comparison with the ready-made espresso coffee sample, it resembled the fresh brewed espresso coffee. Although the lower amount of lipids and volatile compounds with impact on aroma is still a challenge to pursuit the target of approaching espresso composition by infusion methods, this can be overcome with the addition of roasted coffee oil, rich in volatile compounds, in a future formulation, aiming to improve the organoleptic quality of the instant-espresso coffee product.

List of publications/communications during the period of PhD

This thesis originated the following publications/communications:

Publications in international scientific journals with Referee:

1- **Lopes, G.R.,** Passos, C.P., Rodrigues, C., Teixeira, J.A., Coimbra, M.A., Modulation of infusion processes to obtain coffee-derived food ingredients with distinct composition, *European Food Research Technology*, **2019**, 245, 2133-2146. https://doi.org/10.1007/s00217-019-03318-9

2- **Lopes, G.R.,** Passos, C.P., Rodrigues, C., Teixeira, J.A., Coimbra, M.A., Impact of microwave-assisted extraction on roasted coffee carbohydrates, caffeine, chlorogenic acids and coloured compounds, **2019**, submitted.

3- **Lopes, G.R.,** Passos, C.P., Rodrigues, C., Teixeira, J.A., Coimbra, M.A., Coffee brews with espresso properties using quality by design approach targeting carbohydrates, **2019**, submitted.

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1- **Lopes, G.R.,** Passos, C.P., Rodrigues, C., Teixeira, J.A., Coimbra, M.A., Porque é que há bebidas de café com propriedades tão distintas, *"A Bioquímica do Dia-a-Dia - Saúde e alimentos"*, **2019**. (Book Chapter)

2- **Lopes, G.R.**, Coimbra, M.A., *Café: o maior aliado do aluno ao professor, do caloiro ao reitor*, *Matriz*, 3ª edição, **2018**. (Scientific Sharing)

Communications:

1- **Lopes, G.R.**, Rodrigues C., Teixeira, J.A. Coimbra, M.A., Espresso coffee: anytime, the convenience of innovation, *Research Summit*, **2019**, July 3-5, Aveiro, Portugal. (Pitch Presentation)

2- **Lopes, G.R.**, Rodrigues, C., Teixeira, J.A., Coimbra, M.A., Porque é que há bebidas de café com propriedades tão distintas?, *Biochemistry Day*, **2019**, May 19, Aveiro, Portugal. (Pitch Presentation)

3- **Lopes, G.R.,** Passos, C.P., Coimbra, M.A., Use of microwave-assisted extraction for the production of instant coffee-like extracts from roasted coffee, *5th CoCotea - Fourth International Congress on Cocoa, Coffee and Tea*, **2019**, June 26-28, Bremen, Germany. (Plenary Lecture)

4- **Lopes, G.R.,** Passos, C.P., Rodrigues, C., Teixeira, J.A., Coimbra, M.A., Coffee brews with espresso properties by modulation of the carbohydrate content of coffee extracts, *27th ASIC - Association for Science and Information on Coffee*, **2018**, September 16-20, Portland, EUA. (Oral Communication)

5- **Lopes, G.R.,** Passos, C.P., Rodrigues, C., Teixeira, J.A., Coimbra, M.A., Different extraction methodologies, different instant coffee properties?, *4th CoCotea - Fourth International Congress on Cocoa, Coffee and Tea*, **2017**, 25-28 June, Turim, Italy. (Oral Communication)

6- **Lopes, G.R.,** Passos, C.P., Rodrigues, C., Teixeira, J.A., Coimbra, M.A., Selective coffee carbohydrates extraction through different solid-liquid methodologies, *Glupor12 – 12ª Reunião do Grupo de Glúcidos*, **2017**, September 11-13, Aveiro, Portugal. (Poster Presentation)

Other publications:

1- Coreta-Gomes, F., **Lopes, G.R.**, Passos, C.P., Moreno, M.J., Coimbra, M.A., Hypocholesterolemic properties of chemical compounds present in coffee extracts, *Glupor12 - 12ª Reunião do Grupo de Glúcidos*, **2017**, September 11-13 setembro, Aveiro, Portugal (Poster Communication)

2- Coreta-Gomes, F., **Lopes, G.R.**, Passos, C.P., Geraldes, F.G., Moreno, M.J., Nyström, L., Coimbra, M.A., *5th CoCoTea - Fifth International Conference on Cocoa, Coffee and Tea*, **2019**, June 26-28, Bremen, Alemanha (Oral Communication)

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2- Ferreira, S.S., Passos, C.P., Cepeda, M.R., **Lopes, G.R.**, Teixeira-Coelho, M., Madureira, P., Nunes, F.M., Vilanova, M., Coimbra, M.A., Structural polymeric features that contribute to in vitro immunostimulatory activity of instant coffee, *Food Chemistry*, **2018**, 242, 548-554. DOI: 10.1016/j.foodchem.2017.09.059

3- Passos, C.P., Rudnitskaya, A., Neves, J.M.M.G.C., **Lopes, G.R.**, Coimbra, M.A., Data on yields, sugars and glycosidic-linkage analyses of coffee arabinogalactan and galactomannan mixtures and optimization of their microwave assisted extraction from spent coffee grounds, *Data in Brief*, **2019**, 24, 103931. https://doi.org/10.1016/j.dib.2019.103931

4- Passos, C.P., Rudnitskaya, A., Neves, J.M.M.G.C., **Lopes, G.R.**, Evtuguin, D.V., Coimbra, M.A., Structural features of spent coffee grounds water-soluble polysaccharides: Towards tailor-made microwave assisted extractions, *Carbohydrate Polymers*, 2019, 214, 53-61. https://doi.org/10.1016/j.carbpol.2019.02.094

Communications:

1- **Lopes, G.R.**, Ferreira, A.S., Pinto, M., Passos, C.P., Coelho, E., Rodrigues, C., Figueira, C., Rocha, S.M., Coimbra, M.A., GCxGC-ToFMS for the analysis of espresso coffee volatile compounds: from powder to the brew, from global to particular, *40th ISCC and 13th GCxGC Symposium*, **2016**, May 29-June 3, Riva del Garda, Italy. (Poster Presentation)

2- **Lopes, G.R.**, Ferreira, A.S., Pinto, M., Passos, C.P., Coelho, E., Rocha, S.M., Coimbra, M.A., GCxGC-TOFMS as a powerful tool for in-depth analysis of single-dose espresso coffee capsules powder volatile compounds, *XVI COLACRO (XVI Latin-American Congress on Chromatography) e 9 ENC (9thNational Meeting on Chromatography)*, **2016**, January 5-9, Lisboa, Portugal. (Poster Presentation)

3- **Lopes, G.R.**, Ferreira, A.S., Pinto, M., Passos, C.P., Coelho, E., Rocha, S.M., Nunes, F.M., Coimbra, M.A., Espresso Coffees from single-dose capsules: carbohydrates content and composition, *Glupor 11 - 11th International Meeting of the Portuguese Carbohydrate Chemistry Group, 6th Iberian Carbohydrate Meeting*, **2015**, September 6-10, Viseu, Portugal. (Poster Presentation)

4- **Lopes, G.R.**, Ferreira, A.S., Pinto, M., Passos, C.P., Coelho, E., Rocha, S.M., Nunes, F.M., Coimbra, M.A., Polysaccharides composition of single-dose espresso coffee capsules: from chemistry to nutrition, *3th CoCotea - Third International Congress on Cocoa, Coffee and Tea*, **2017**, June 22-24 June, Aveiro, Portugal. (Poster Presentation)

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III.1. INFUSION PROCESS: CONVENTIONAL EXTRACTION

Abbreviations

3D	Three dimensional
5-CQA	5-*O*-caffeoylquinic acid
a*	Red-green value
AG	Arabinogalactans
AGP	Arabinogalactan-protein
ANOVA	Analysis of variance
Ara	Arabinose
ATR	Attenuated total reflection
b*	Yellow-blue value
BBD	Box-Behnken design
C*	Chroma
DAD	Diode array detector
DB	Degree of branching
DMSO	Dimethylsulfoxide
DP	Degree of polymerization
DPPH	2,2-Diphenyl-1-picrylhydrazyl
DVB/CAR/PDMS	50/30 μm Divinylbenzene/carboxen™/poly(dimethylsiloxane) StableFlex™
EC	Espresso coffee
e.g.	*Exempli gratia*/for example
-f	Furanose
FCCD	Face-centered central composite design
FD	Freeze dried
FID	Flame ionization detector
FTIR	Fourier-transform infrared spectroscopy
Gal	Galactose
GC	Gas chromatography
GC-FID	Gas chromatography – flame ionization detection
GC-MS	Gas chromatography-mass spectrometry
GCqMS	Gas chromatography coupled to quadrupole mass spectrometry
Glc	Glucose
GM	Galactomannans
h_{ab}	Hue angle

HMWM	High molecular weight material
HMWMins	High molecular weight material insoluble in cold water
HMWMsol	High molecular weight material soluble in cold water
HPLC	High-performance liquid chromatography
HS-SPME	Headspace solid-phase microextration
IC	Instant coffee
ICO	International Coffee Organization
I.D.	Internal diameter
L*	Lightness
LMWM	Low molecular weight material
MAE	Microwave-assisted extraction
Man	Mannose
min	Minutes
w/V	Mass/Volume ratio
m/z	Mass-to-charge ratio
-p	Pyranose
PC1	Principal component 1
PC2	Principal component 2
PCA	Principal component analysis
PMAA	Partially methylated alditol acetates
Rha	Rhamnose
$\mathbf{RI_{calc}}$	Calculated retention index
$\mathbf{RI_{lit}}$	Retention index from literature of the same GC column or equivalents
SCG	Spent coffee grounds
SD	Spray dried
SNV	Standard normal variate
TFA	Trifluoroacetic acid
TCD	Thermal conductivity detector
tr	Traces

Chapter I - Introduction

I.1. ROASTED COFFEE: THE COMPOUNDS

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I.1.2. Polymeric nitrogenous compounds: protein and melanoidins

I.1.3. Caffeine and chlorogenic acids

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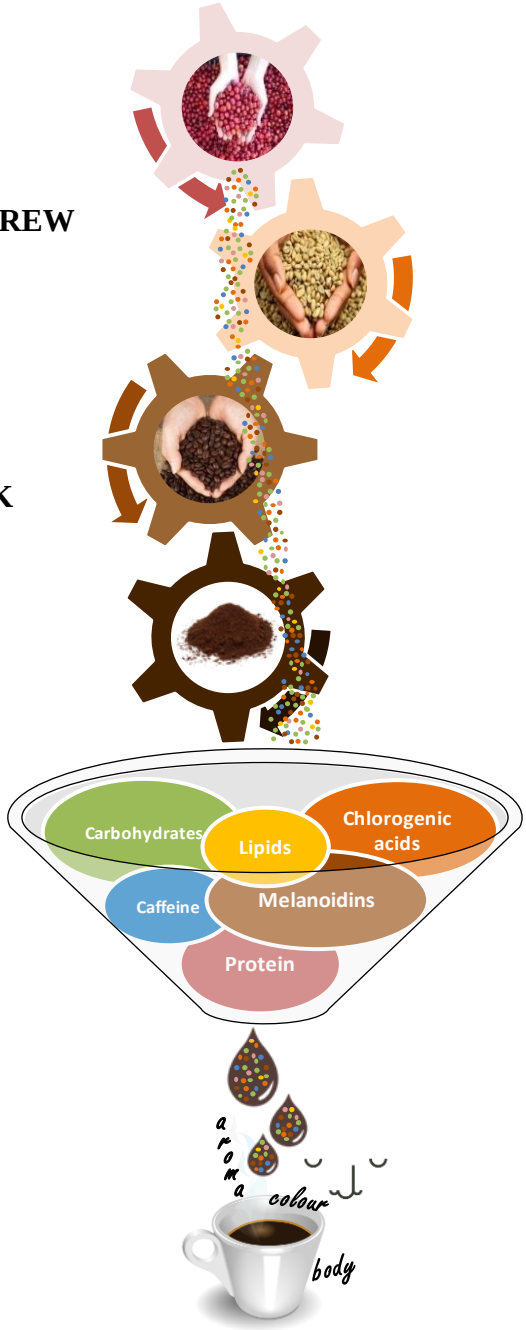
I.2. ESPRESSO COFFEE: A PARTICULAR BREW

I.2.1. Coffee brews: the extraction process

I.2.2. Composition of espresso coffee

I.3. MOTIVATION AND AIM OF THE WORK



Coffee is a term used to nominate the seeds of berries (beans) from *Coffea* plant, both green or roasted, and the brew prepared with them. The physico-chemical composition of a coffee cup depends on the coffee species, the stage of coffee fruit development, the harvesting method, the roasting process, the grinding degree and the method of brew preparation (Illy & Viani, 2005; Petracco, 2001). The cultivation of *Coffea* plants relies on environmental characteristics and varies due to agricultural (*e.g.* fertilizers costs) and climatic conditions (*e.g.* level of rainfall), which also affect coffee content and quality (Njoroge et al., 2005). A steady growth on coffee production over the recent years has been verified (Figure I.1), with the increment of Asian production, mainly due to Vietnam emergence, although South America continues to dominate the sector. Nevertheless, the major coffee consumption is observed in Europe and North America.

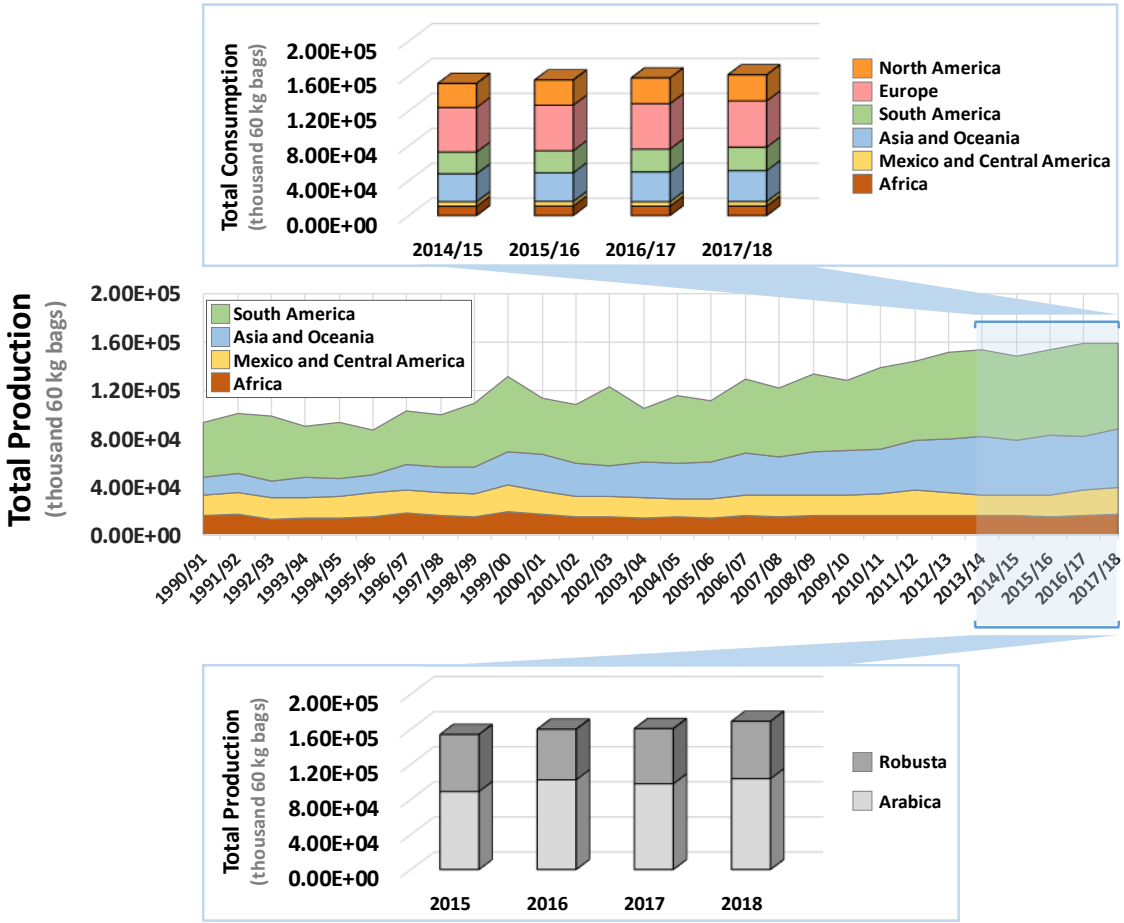


Figure I.1. Total production of coffee by regions from 1990, highlighting where it was consumed in the recent years and the type of coffee produced (ICO, 2019a; 2019b).

From all known coffee species (over 100) only two have commercial importance: *Coffea arabica* species (coffee Arabica) and *Coffea canephora* var. Robusta (coffee Robusta). While coffee Arabica has a larger overall production and higher price associated,

the coffee Robusta is easier and cheaper to produce (Farah, 2012; Toledo & Moguel, 2012). The composition of these two coffee species of *Coffea* genus (*Rubiaceae* family), differ in price, quality and consumer acceptance due to their distinct taste, coffee body performance and aroma (Illy & Viani, 2005). Although Arabica is still the major coffee species (60-70 %), the Robusta production clearly increased in the last years due to contribution of Vietnam coffee (ICO, 2014). Although coffee brews depend on the properties of the coffee species used, the commercially available coffees are usually blends of different coffees, either made from distinct species or different origins.

Several compounds are present in the green beans and survive throughout processing stages, while others are only formed due to the roasting process and some are destroyed/transformed along the process, ceasing their presence in the brews. Nevertheless, several hundred compounds are present in the brews, which turns coffee brews as one of the most complex food matrices, modulated by their coffee species, geographic origin, roasting process and brewing methodologies.

I.1. Roasted coffee: the compounds

Roasted coffee powder composition has been elucidated along the years to perceive their contribution for organoleptic properties of coffee brews, and also due to the health, nutritional and biological effects related to coffee consumption. Among the large amount of coffee compounds that are present in green beans, some are degraded and some are formed through processing, mainly during roasting, with only 50% of original green beans material remaining chemically unchanged (Kuhnert et al., 2013). Table I.1 summarizes the composition of green and roasted coffee beans. The extraction process plays a determinant role on compounds selectivity, hindering or facilitating the passage to the brew, which significantly affects the final brew composition. The compounds may be divided in two main groups: the non-volatile ones (*e.g* carbohydrates, melanoidins, lipids, proteins, among others) and the volatile ones, that in coffee may exceed one thousand compounds. However, the volatile ones derive from the reaction/transformation of the non-volatile compounds during the coffee processing. As an example, sucrose, an abundant compound in green beans, is almost absent in roasted coffee because during roasting it generates

carboxylic acids, furans, and aldehyde-containing compounds, acting as an aroma precursor (Ginz et al., 2000; Wei & Tanokura, 2015).

Table I.1. Composition (%w/w dry matter) of green and roasted beans of Arabica and Robusta coffee (based on Belitz et al., (2004); Illy & Viani, (2005); Perrone, et al., (2008); Wang & Lim, (2015); Wei and Tanokura, (2015)).

Compound	Arabica		Robusta	
	Green	Roasted	Green	Roasted
Polysaccharides	43.0-55.0	24.0-39.0	37.0-48.3	41.5
Sucrose	6.3-8.5	tr	0.9-4.9	tr
Lipids	12.0-18.0	14.5-20.0	8-13.0	11.0-16.0
Proteins	8.5-13.0	7.5-15.0	8.5-13.0	7.5-15.0
Free Amino acids	0.2-0.8	-	0.2-0.8	-
Aliphatic acids	1.7-2.9	2.4	1.3-2.2	2.5
Chlorogenic acids	6.5-9.2	2.7	7.1-12.1	3.1
5-CQA	3.0-5.6	0.1-2.7	4.4-6.6	0.2-3.1
Caffeine	0.8-1.4	1.3	1.6-4.0	2.4
Trigonelline	0.6-1.2	0.5-1.0	0.3-0.9	0.3-0.7
Minerals	3.0-4.2	3.5-4.5	3.6-4.8	4.6-5.6
Volatile compounds	Traces	0.1	Traces	0.1
Melanoidins	-	16.0-23.0	-	16.0-23.0

I.1.1. Polysaccharides

Polysaccharides comprise the major compounds present in green beans, but also in roasted coffee and the coffee brews. The green coffee polysaccharides exhibit low solubility, even in harsh conditions of extraction, difficulting the disclosure of their fully composition, with the whole bean composition being achieved by the characterization of small extractable fractions (Oosterveld et al., 2003a). Nevertheless, polysaccharides accounted for approximately half of the green coffee (37-55%, Table I.1), with mannose as major residue (43-47%mol), followed by galactose (23-26%mol) and glucose (15-18%mol), and minor amounts of arabinose, rhamnose and xylose (Bradbury & Halliday, 1990; Fischer et al., 2001; Oosterveld et al., 2003a). In coffee, these residues are structurally linked to form three polysaccharides: galactomannans (GM), type II arabinogalactans (AG) and cellulose, with variation in structural composition due to roasting process (Nunes & Coimbra, 2001; Redgwell et al., 2002a). Generally, GM consist mostly on a linear backbone of mannose residues (β1→4)-Man*p* branched at *O*-6 mainly with single (α1→6)-linked galactose side chains (Oosterveld et al., 2003a) (Figure I.2). Moreover, glucose residues are (β1→4)-linked in the main backbone of GM that have also

some of the mannose residues acetylated in *O*-2 and/or *O*-3 (single and doubly acetylated, occasionally in consecutive residues) (Nunes et al., 2005). Due to the low degree of branching (DB) with galactose in the structure, in the past GM were used to be named as mannans (Bradbury & Halliday, 1990). The degree of branching describes the proportion of the substituted mannose residues in the main backbone chain with polymers with different DB due to a different stage of bean development (Redgwell, et al., 2003). Another parameter used to describe polysaccharides is the polymerization degree which, in this case, describes the number of mannose residues in the main backbone. The variation of these parameters is related to the higher/lower extractability and solubility of the polysaccharides in water, with different values found for coffee polysaccharides due to their heterogeneity.

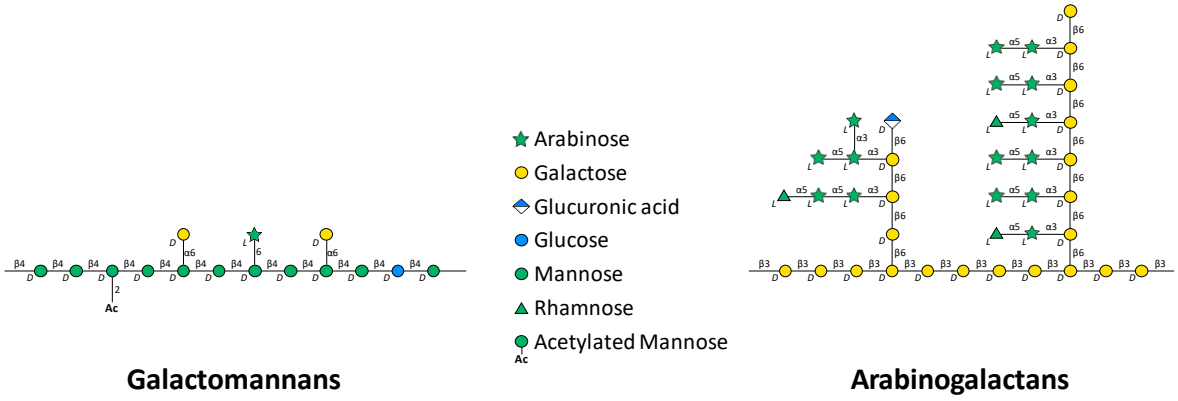


Figure I.2. Representation of galactomannans and arabinogalactans of green coffee (based on Moreira et al., 2012).

AG are composed predominantly by a backbone of (β1→3)-linked galactose residues with some of them branched at *O*-6 by (β1→6)-linked short galactose chains that are substituted with terminally (α1→5)-linked arabinose, with rhamnose and glucuronic acid (Bradbury & Halliday, 1990) (Figure I.2). The (1→3) and (1→3,6)-linked structure is indicative of type II structures, contrary to (1→4)-linked galactose associated to type I structures (Oosterveld et al., 2003a). In green coffee only nearly 7% of AG are hot water soluble (Nunes et al., 2008). Moreover, AG are also found covalently linked to proteins, with protein accounting for 0.4-4.2% of the structure, the reason for describing such structures as arabinogalactan-proteins (AGP) (Nunes et al., 2008; Redgwell et al., 2002b). AG are described as heterogeneous structures concerning the extension and type of residues present in the side chains as well as the degree of branching (Fischer et al., 2001; Redgwell et al., 2002b).

The small fraction of green coffee polysaccharides extractable with hot water is composed mainly by AG (62%) with lower level of GM (24%), predominantly insoluble in green coffee, although the higher amount of GM than AG present in the whole green bean compared to AG (Bradbury & Halliday, 1990; Nunes & Coimbra, 2001). This behaviour evidences the different extractability of coffee polysaccharides. On the other hand, the extraction of roasted coffee in hot water results in GM (69%) predominance over AG (28%), highlighting that roasting process induces a series of modifications that enhance their extraction (Nunes & Coimbra, 2001). Moreover, while only nearly 7% of polysaccharides are extractable from green coffee, nearly 30% may be obtained using roasted coffee (Oosterveld et al., 2003a). Depending on roasting conditions, 12-40% of bean polysaccharides are degraded, with AG being more affected than GM, and cellulose slightly affected by the processing (Redgwell et al., 2002a). Contrary to GM and AG, cellulose remains insoluble even after the roasting process, ending up in the residue left after brew extraction and not in the beverage (Navarini et al., 1999; Nunes & Coimbra, 2001). Moreover, the low molecular weight sugars that are present in the green beans are degraded during roasting, which turn insignificant their amount in the coffee brews obtained from ground roasted coffee. The structural modifications induced by roasting include the debranching and depolymerization of polysaccharides that increase polysaccharides water extractability, influencing the amount that ends up in the brew (Figure I.3). Moreover, transglycosylation reactions also occur explaining the presence of structures in roasted coffee with higher molecular weight compared to the ones found in green coffee (Moreira et al., 2011; 2016).

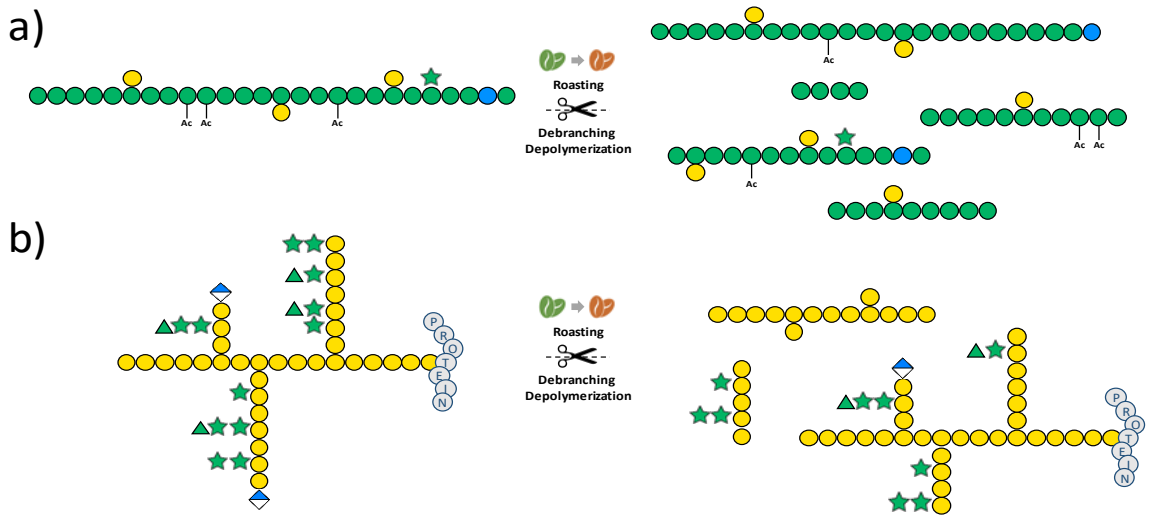


Figure I.3. Representation of modifications occurring during roasting processing to coffee polysaccharides: a) galactomannans; b) arabinogalactan-proteins (based on Moreira et al. (2012); Nunes & Coimbra (2010)).

Furthermore, the GM structural features are of relevant interest for coffee industry. For instance, their solubility is a key factor in extraction yield during instant coffee processing. Indeed, GM confers high viscosity and tends to precipitate, which is associated to the formation of sediment during the process (Delgado et al., 2008). It has been studied the use of enzymes (mannanases) for the hydrolysis of the structures that, on overall, will reduce the costs associated to concentration steps (Chauhan et al., 2014; Delgado et al., 2008)

Considering the above-mentioned limited extractability of polysaccharides (30% from roasted coffee), special attention has been made, in the past few years, on the recovery of GM and AG from coffee residue (spent coffee grounds) (Ballesteros et al., 2017; Passos & Coimbra, 2013). For that purpose, methodologies that allow to achieve high temperatures and pressures are required to efficiently extract coffee polysaccharides from the coffee matrix. In this sense, microwave-assisted extraction (MAE) has proved to efficiently recover AG and GM from coffee residues (Passos & Coimbra, 2013; Passos et al., 2014a, 2019a). However, extraction conditions (as time and temperature) must be properly used because severe conditions (temperatures above 170 ºC) and successive extractions are associated to a decrease of polysaccharides polymerization, resulting in the formation of oligosaccharides and debranched structures (Passos et al., 2014a, 2019a). Nonetheless, to the best of our knowledge, MAE has never been used as a direct method of extraction of polysaccharides from coffee powder. In this sense, MAE may be seen as a fast and green methodology to obtain coffee formulations rich in GM and AG.

Polysaccharides are important for brew acceptability as they contribute to the creamy sensation, usually defined as body of the brew, the retention/modulation of aroma compounds release, and brew viscosity, associated with the stability of the foam in espresso (Illy & Viani, 2005; Nunes & Coimbra, 1998, 2001; Nunes et al., 1997). Indeed, the increase of polysaccharides content is associated with the increase of coffee extracts viscosity (Trugo, 1985). On the other hand, these structures are associated to important biological activities as immunostimulatory properties or contributing to the physiological effects associated with fibre fermentation (Gniechwitz et al., 2007a, 2007b; Simões et al., 2009).

I.1.2. Polymeric Nitrogenous compounds: Protein and Melanoidins

The protein content, approximately 10% in green coffee, is composed by a soluble (albumin) and insoluble-water fraction in similar amounts with non-clearly difference between coffee species (Illy & Viani, 2005; Macrae, 1985). Proteins account for nearly 40% of the polymeric material (>12 kDa) of green coffee infusions, with major coffee proteins having molecular weights of 38 kDa and 58 kDa (Nunes & Coimbra, 2001). Proteins suffer extensive changes during roasting with the green coffee proteins being denatured and degraded (≤ 14 kDa) or also polymerized (>200 kDa). This leads to a decrease of reactive amino acids as arginine, cystine, or serine, and relatively increasing of stable amino acids as glutamic acid and leucine (Belitz et al., 2004; Illy & Viani, 2005). Moreover, free amino acids are present only in traces amounts in the roasted coffee. The protein of roasted coffee is much less extractable, probably due to the insolubility of denatured proteins, as well as proteins degradation to small products may also occur (Nunes & Coimbra, 2001). The overall determination of protein in coffee is difficult as their estimation is obtained through nitrogen content of the samples, which leads to a clear overestimation due to interference of other nitrogenous components as caffeine. Thus, an amino acid's analysis is usually more accurate for protein content determination. However, after proteins interactions/reactions with carbohydrates and phenolic compounds yielding melanoidins, the analysis by hydrolysis to obtain amino acids content cannot accurately infer the original protein content of the sample (Macrae, 1985). The determination of protein in coffee brews is scarce, due to the difficulties of analysis still observed at present, as denatured proteins are quite insoluble, hindering their extraction through the brews.

The proteins (as well as peptides and free amino acids) participate during the roasting process in the Maillard and Strecker degradation reaction, taking a key role in the formation of volatile compounds (*e.g.* furans, pyridines, pyrazines, pyrroles or aldehydes) and also melanoidins, leading to the formation of the characteristic coffee colour and aroma (Farah, 2012). In fact, either carbohydrates (as galactomannans and arabinogalactans, 30%), phenolic compounds (as chlorogenic acids, 33-42%) and proteins (as well as peptides and free amino acids, 8-9%) are incorporated in melanoidins that represent up to 25%w/w of roasted coffee (Borrelli et al., 2002; D'Agostina et al., 2004). Melanoidins are polymers formed from various non-enzymatic reactions between

compounds with a free amino group and reducing sugars during heat processing of food products, or succinctly between proteins and carbohydrates via Maillard reaction (Moreira et al., 2012). These nitrogenous compounds that present a somewhat undefined composition are responsible for the brown colour of the roasted coffee and play a determinant role in biological activities associated to coffee, namely the antioxidant properties (Cämmerer & Kroh, 2006; Delgado-Andrade & Morales, 2005).

Melanoidins antioxidant action is associated to reactions involving radicals (*i.e.* scavenging of hydroxyl radicals, the ability to break radical chain mechanisms or by oxygen scavenging) (Cämmerer & Kroh, 2006). As the phenolic compounds can be noncovalently linked to coffee melanoidins, melanoidins may act as carriers of low molecular weight compounds, as well as the antioxidant activity may be associated in part to these small compounds (Delgado-Andrade & Morales, 2005; Rufián-Henares & Morales, 2007). Thus, these low molecular weight compounds may be released during gastrointestinal tract, acting as dietary fibre with antioxidant properties (Lopes et al., 2016; Moreira et al., 2012; Rufián-Henares & Morales, 2007). Melanoidins are reported to help in the prevention of cardiovascular disease and control of colorectal cancer reaching the colon in its integral form, increasing colon motility and being fermented by colonic microbiota (Vitaglione et al., 2012). Coffee melanoidins are also associated to the inhibition of metalloproteases, anti-inflammatory, anticariogenic, antihypertensive or antiglycative activity, highlighting their preponderance in human health (Moreira et al., 2012; Nunes & Coimbra, 2010). Melanoidins are also viewed as a foaming agent, which is important in espresso coffee brews (D'Agostina et al., 2004; Illy & Navarini, 2011). Despite their great importance, the quantification of melanoidins is quite difficult due to the structural diversity and complexity of these compounds, being estimated by difference from total amount of high molecular weight material and the amount of compounds determined as carbohydrates and proteins.

I.1.3. Caffeine and chlorogenic acids

Caffeine is the most studied coffee compound due to its health associated effects and, therefore, the most popular coffee molecule worldwide. On the other hand, chlorogenic acids constitute the most abundant family of phenolic compounds of coffee,

responsible for the astringency, bitterness and acidity of the brews, and also highlighted due to their functional properties (Farah, 2012). They have in common the fact that both types of compounds (caffeine and chlorogenic acids) depend on coffee species with Robusta containing higher contents than Arabica coffee, in green or roasted coffee beans (Table I.1) (Illy & Viani, 2005). Moreover, it was reported that these compounds form a complex with an equimolar ratio, ending up with 10% of caffeine and 6% of chlorogenic acid in such form in coffee brew (Belitz et al., 2004). A great difference is that chlorogenic acids content significantly decreases during roasting process, while caffeine levels only slightly diminishes. Indeed, it could be used a ratio between the more stable caffeine and chlorogenic acids, as a good marker of roasting process (Ludwig et al., 2014a).

Regarding caffeine, some wild species of coffee (non-commercially available) have remarkably little or none caffeine content but poor coffee quality, whereby the decaffeinated coffee commercially available is obtained after caffeine removal by industrial decaffeination processes (Macrae, 1985). Besides caffeine, other dimethylxanthines (theobromine and theophylline) are present in coffee and also in higher amount in Robusta than Arabica, although in quite lower content than caffeine in coffee brews (Illy & Viani, 2005; Rodrigues & Bragagnolo, 2013). Caffeine is higher in coffee comparing to all other dietary products, with their cup amount depending most on the brew preparation method and coffee species, and less on roasting degree (Tfouni et al., 2014). Indeed, caffeine content in both green and roasted coffee is similar, although some is lost due to sublimation (Wang & Lim, 2015). This alkaloid is recognized due its stimulatory effects, leading to enhanced perception and increased capacity to remain awake for longer periods (Fredholm et al., 1999; Ludwig et al., 2014b). Otherwise, the excessive consumption may result in a state of excitement and anxiety, headaches and sleep disturbances, related to individual dose response and tolerance to caffeine (Ludwig et al, 2014a, 2014b).

The esterification of *trans*-cinnamic phenolic acids (*e.g.* caffeoyl-, *p*-coumaroyl and feruloyl acids) with quinic acid results in the formation of water-soluble structures named chlorogenic acids (Figure I.4). The substitution occurs in the 3-, 4- and 5- position of quinic acid, with one or more substituents, with equal or different structures, which allows a great diversity of possible combinations (Farah & Donangelo, 2006). Along the years a great variety of compounds (more than 60) have already been identified in green coffee

(Clifford et al., 2006; Jaiswal et al., 2010). According to the cinnamic substituents (*i.e.* nature, number and position) different subclasses of compounds are obtained as caffeoylquinic (CQAs), the main class, feruolyquinic (FQAs), *p*-coumaroylquinic (*p*-CoQAs) and dicaffeoylquinic (di-CQAs). The 5-caffeoylquinic acid (5-CQA) is the most abundant chlorogenic acid in green, roasted and coffee brews (Farah, 2012; Ludwig et al., 2012). The roasting process leads to their degradation or to their incorporation in coffee melanoidins through noncovalent or covalent bounds (Bekedam et al., 2008a; Nunes & Coimbra, 2010). Thus, many of the structures present in the green beans do not reach the coffee brews. Indeed, the initial chlorogenic acids present in green coffee may be significantly reduced by roasting degree (Farah et al., 2005; Moon et al., 2009; Trugo & Macrae, 1984a). Due to their thermal instability, the roasting process may change chlorogenic acids via isomerization, epimerization or lactonization reactions, or degradation to lower molecular weight compounds (Farah, 2012). This behaviour is also important considering the brewing procedure, as coffee brews are prepared under hot temperatures (Dawidowicz & Typek, 2011; De Maria et al., 1998; Li et al., 2015). Moreover, phenols and catechols may be formed from chlorogenic acids which may give unpleasant sensory notes to coffee brews (Farah, 2012). Coffee variety seems to exhibit less effect comparing to roasting degree with Robusta with higher content than Arabica coffee (Farah et al., 2005).

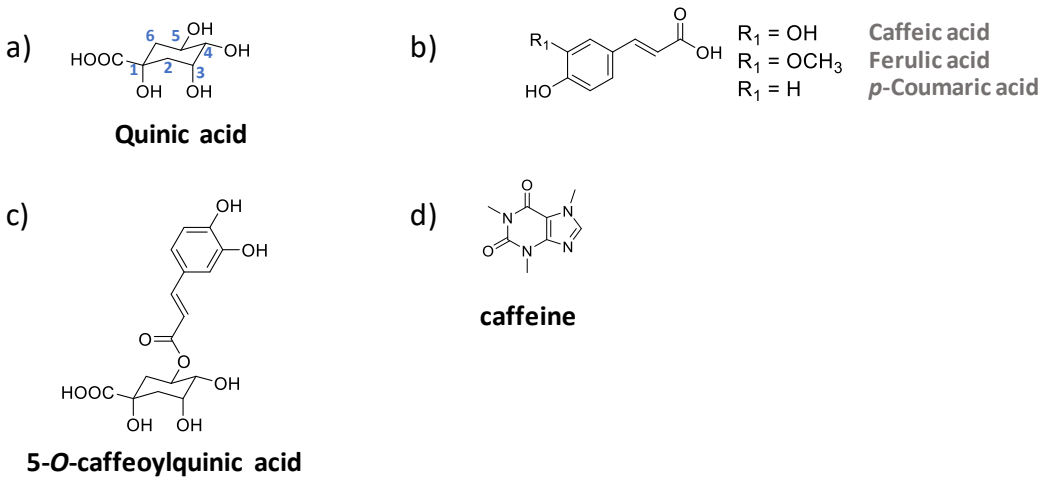


Figure I.4. Structure of a) quinic acid; b) caffeic, ferulic and *p*-coumaric acids; c) 5-*O*-caffeoylquinic acid and d) caffeine.

I.1.4. Lipids

The aqueous environment of coffee brews hinders the effective extraction of lipids due to their hydrophobic characteristics. The Arabica coffee contains higher amount of lipids than robusta (Table I.1) (Illy & Viani, 2005). On the other hand, the roasting process does not affect significantly the content and composition of coffee lipids. Regarding their composition, triglycerides are the major compounds representing nearly 75% of coffee lipids, while diterpenes (cafestol, kahweol and 16-*O*-methylcafestol) constitute approximately 20%, accounting sterols, tocopherols and free fatty acids for the remaining lipids fraction (Figure I.5) (Farah, 2012; Illy & Viani, 2005; Speer & Kölling-Speer, 2006). The composition regarding fatty acids found either in green and roasted coffee, as well as in the brews are quite similar, with linoleic (C18:2) and the palmitic (C16:0) acids as the major ones, followed by oleic (C18:1) and stearic (C18:0) acids, whereas linolenic (C18:3) and arachidic (C20:0) acids are found in smaller amounts (Gloess et al., 2013; Speer & Kölling-Speer, 2006). The diterpenes, which are mostly esterified with fatty acids, have been intensively studied due to their implications in coffee biological/physiological effects with controversial results concerning potential beneficial/harmful effects (*e.g.* antioxidant, hepatoprotective, anticarcinogenic or hypercholesterolemic properties) (Boekschoten et al., 2005; Cavin et al., 2002; de Roos & Katan, 1999; Lee et al., 2007; Terpstra et al., 2000).

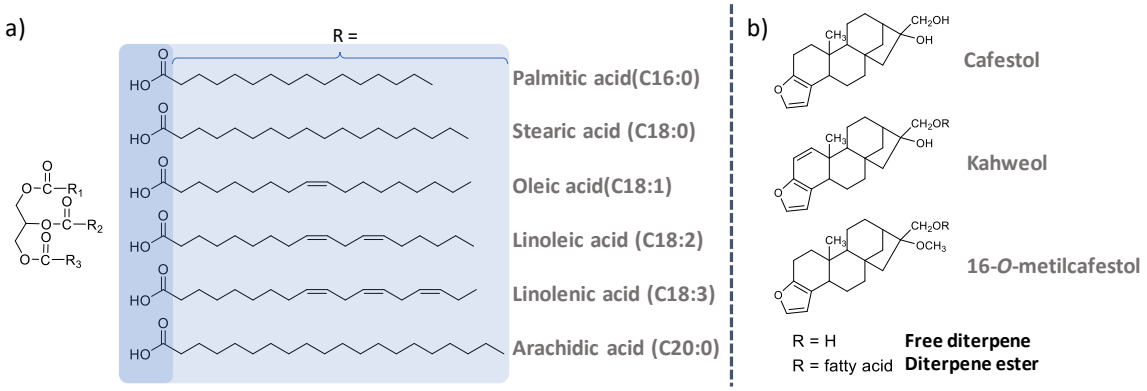


Figure I.5. Representation of major lipid structures in coffee: a) triglycerides (highlighting the main fatty acids present) and b) diterpenes.

I.1.5. Volatile compounds

The aroma, a very distinctive feature of roasted coffee, is clearly dependent on the conditions of the roasting process and the reactions involved (*i.e.* Maillard reaction, Strecker degradation and pyrolysis). Thus, the variety and concentration of volatile compounds in roasted coffee depends on the non-volatile composition of green beans and the conditions used for their production (*e.g.* soil, weather, and geographical origin) (Farah, 2012). Generally, Arabica variety is known to give a better aroma cup quality than Robusta. Approximately one thousand compounds were already identified in roasted coffee, within several chemical families of compounds as acids, alcohols, aldehydes, esters, furans, ketones, phenols, pyrazines, pyridines, pyrroles, thiophenes and sulfur compounds (Mondello et al., 2004), although only nearly 30 seem to have impact on coffee aroma (Rocha et al., 2004). Most of these compounds derive from Maillard reaction, Strecker degradation and interaction between intermediate decomposition products (Buffo & Cardelli-Freire, 2004; Caprioli et al., 2012; Toledo et al., 2016). Caffeic, quinic and chlorogenic acids can also generate a great fraction of coffee volatiles (Moon & Shibamoto, 2010). Taking into account these compounds and their concentration in the brews, aroma models (synthetic mixtures) with a coffee-like smell, both for ground coffee and brews have been developed, helping on the interpretation of coffee aroma (Czerny et al., 1999; Mayer et al., 2000; Semmelroch & Grosch, 1996).

The study of the volatile profile of a coffee powder is not directly related to the one that consumer will be perceived, the brew, due to the modulation by extraction process of the balance of volatiles ending up in the coffee cup. Therefore, some compounds may be present in low amount in the powder in relation to others, but their higher extraction, increases their importance in the brew. Indeed, there is a change in compounds concentration and not the formation of new odorants during the preparation of the brews (Blank et al., 1992; Rocha et al., 2004). Nonetheless, the coffee aroma depends on compounds concentration and the inherent odour threshold, *i.e.* the lowest concentration perceivable by human perception. Even those present in small concentration may be more clearly noted by the consumer compared to compounds in greater concentration due to the different aroma perception limits. Most of the coffee odorants are associated to sweet and caramel-like, earthy, smoky/phenolic and sulfurous/roasty notes, composing the aroma

profile of coffee powder (Belitz et al., 2004). The brews exhibit a weaker roasty note and more intensive phenolic, buttery, and caramel-like ones (Belitz et al., 2004).

I.2. Espresso coffee: a particular brew

I.2.1 Coffee brews: the extraction process

Coffee brews were primarily appreciated as stimulant for work, but shifted to be also seen as a means of rest and relax, making coffee more a circumstance than a substance, as a distinct example that *"food has a constant tendency to transform itself into situation"* (Barthes, 2008; Morris, 2013). The psychosociology of food consumption pointed out that this occurred mainly when basic nutritional requirements are satisfied, as in developed countries, with the context of food consumption determinant for their meaning and coffee as a great example. Coffee brews may be seen differently according to context: in the morning for waking up (namely diluted brews), with the spreading of espresso at breaks all over daily journey (especially in Latin countries) or in moments of relaxing (namely in Anglo-Saxon and northern European countries) (Illy & Viani, 2005). Every hour may be appropriate for brewing espresso, although it is necessary to be near a coffee machine to satisfy such desire. The growth rate regarding consumption of coffee increased 2.4% since 2000, thriving in a global perspective, explained by a significant boost verified recently in emerging countries (*e.g.* South Korea, Russia, or China) (ICO, 2014). There is a continuous growth of coffee consumption supported by the perception of coffee as a healthy product with an increase of research papers reporting the benefits of coffee to human health, as well as there seems to still exist opportunities in niches as the specialty and certified coffees (ICO, 2014). Knowing the market trends, consumer preferences at regional, national or worldwide level, the economic state of the industry and the processes is important when dealing with product development. Indeed, the susceptibility to the introduction of new products should be linked to different consumption habits and levels of consumption associated to each country. For instance, an International Coffee Organization (ICO) report from 21 countries in the period from 1997-2011 shows that the countries have substantial differences in consumption *per capita*, with Nordic European countries exhibiting the highest values (Figure I.6).

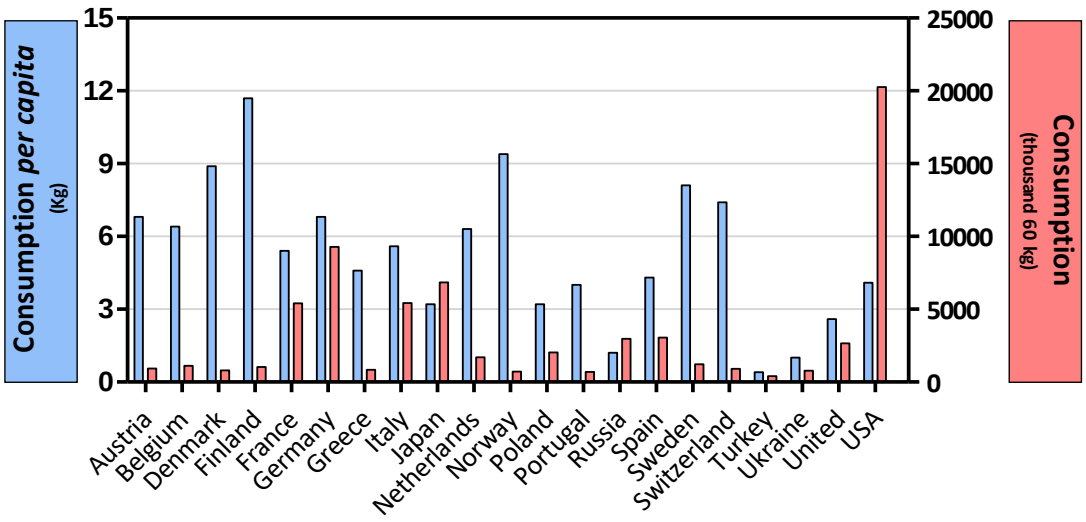


Figure I.6. Average annual coffee consumption (*per capita* and in total amount*)* in 21 importing countries in the period of 1997 to 2011 (ICO, 2012).

Another perspective shows that coffee exporting countries (the coffee producers) are associated to home-consumption, while in the importing ones the fraction of out-of-home consumption fraction is higher, namely in the Latin countries, related to cultural issues and Mediterranean habits (ICO, 2014) (Figure I.7). Portugal is the only country where out-of-consumption is the major fraction (53%), showing a trend of drinking coffee brew everywhere. The coffee industry, from coffee production to product consumption, generates several million euros of economic value with importing countries handling the major revenues, from out-of-home consumption of coffee.

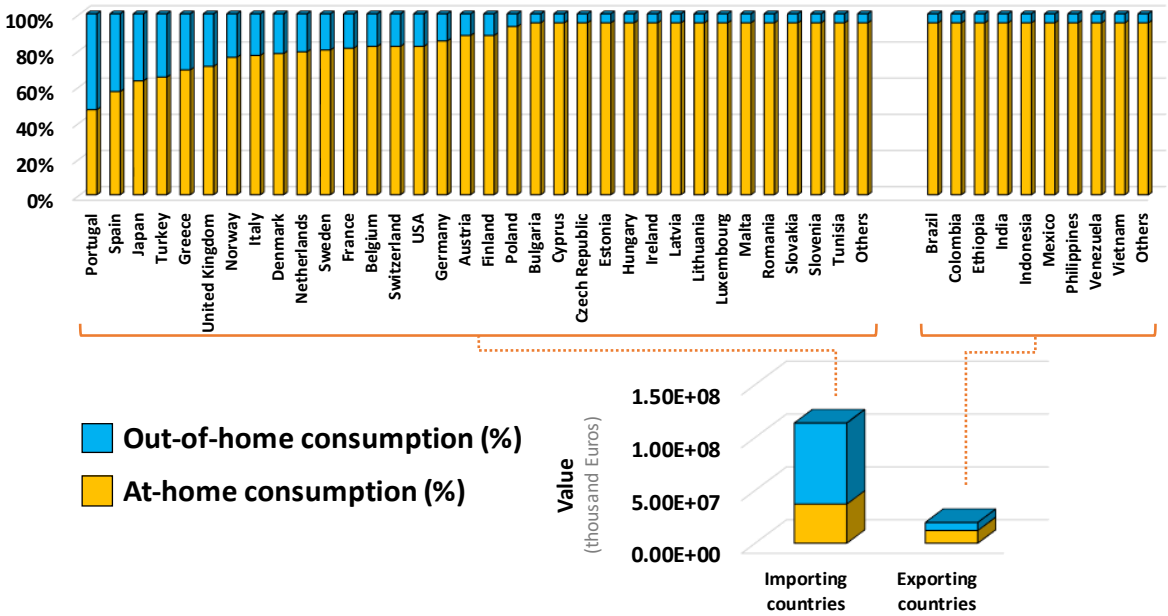


Figure I.7. Coffee consumption values regarding at-home or out-of-home consumption in importing and exporting countries (in 2012) (ICO, 2014).

The coffee brews available - filtered or drip, espresso, French press or Moka coffee among others - are made only with roasted coffee powder and water, applying decoction, infusion or percolation methods. The classification of coffee brews, namely concerning the decoction and infusion methods, is unclear (Petracco, 2001). An infusion is prepared when ground coffee is put in contact with boiling water during a short period of time and then filtrated or decanted (Clarke, 1987). A decoction occurs, when the suspension is heated during all process of contact, associated to boiling brews, as Turkish coffee. However, when the extraction is not performed with boiling water, the process is commonly referred also as infusion process, which is the base of the decision to describe the studies performed in this thesis as infusions, as referred in some related studies in the past (Nunes & Coimbra, 2001; Nunes et al., 2005). Nevertheless, in any method, using only two "ingredients", several coffee brews may be obtained. The different coffee brews depend on distinct devices and operational parameters as time and temperature of extraction, grinding level and the amount of coffee powder to the water used, the existence of a filter, and the pressure associated to the process (Gloess et al., 2013; Petracco, 2001) (Figure I.8). Moreover, although almost all coffee beverages are consumed warmed, they can also be served cold and/or with ice. Cold brews, extracted with cold water (and not cooled after hot water extraction), have become a new market trend nowadays. Furthermore, there is also other coffee brew whose extraction process is not performed in the moment of consumption: the instant or soluble coffee. This product is the soluble fraction remaining after the industrial extraction of compounds from roasted coffee, constituting a product that can be directly dissolved in a cup by adding water. The process consists of selection/blending of green coffee, roasting and grinding, extraction, drying and packing (Clarke, 2001). The industrial instant coffee production occurs at high temperatures (100-180 ºC) and pressures, and intends to maximize the extraction of coffee compounds, obtaining extracts with high concentration of coffee solids (25-30%) in battery column percolators working through the counter-current principle (Clarke, 2001). The liquor composed by coffee solids is concentrated and then dried through freeze or spray-drying methods. The conditions used to extract the maximum of compounds turn the product quite different from the homemade coffee brews.

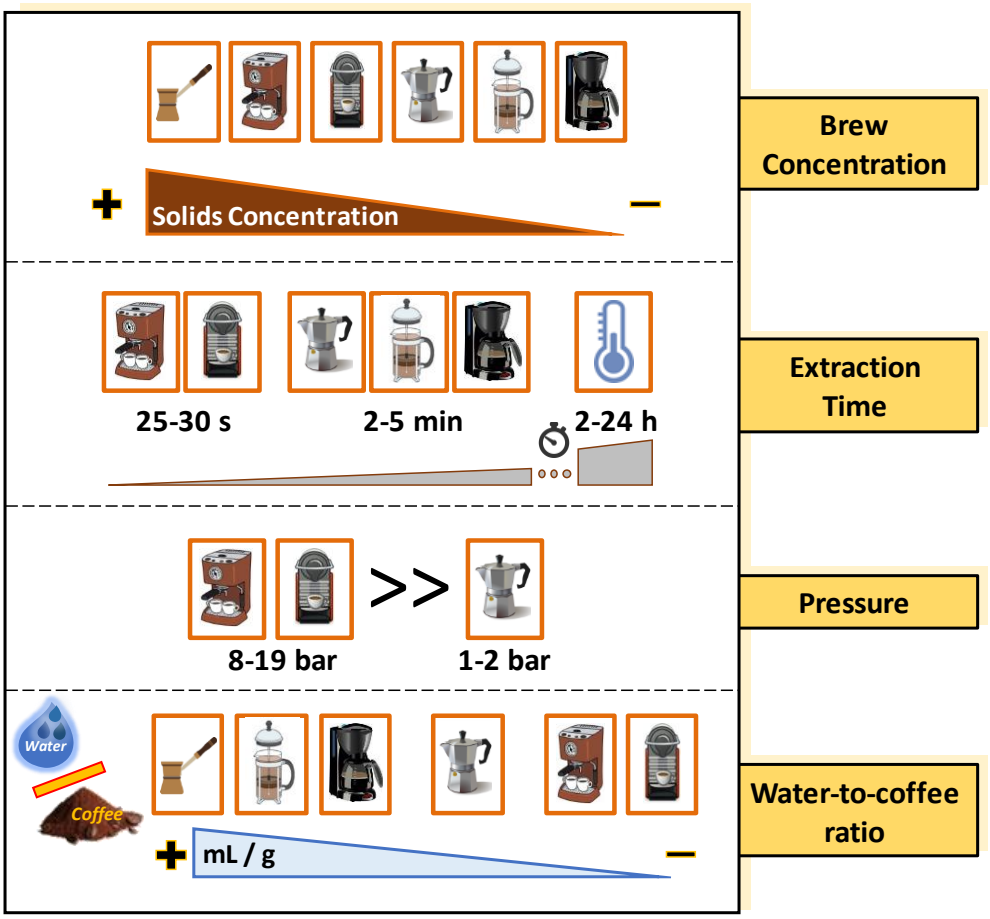


Figure I.8. Different coffee brew preparation methods: Turkish coffee, espresso coffee (conventional and capsules system), Moka, French Press and filtered coffee and grinding degree associated (based on Mestdagh et al. (2017); Petracco (2001)).

For brew preparation, the roasted beans need to be grinded to facilitate the extraction of coffee compounds that are entrapped in the bean. The grinding reduces the beans to small particles (up to 1000 µm) which assist the release of volatile compounds and contribute to the easier dissolution of non-volatile ones. Each coffee brew has a grinding degree associated, with a narrow distribution of particles size, having all particles equal dimension (von Blittersdorff & Klatt, 2017) (Figure I.9). However, this does not happen with espresso that requires a plurimodal particle size distribution for an optimal percolation (Illy & Viani, 2005). The coarser particles allow the flowing of water, while the finer ones enhance the exposed extraction surface. On the other hand, the small particles (*fines*) should contribute to a pressure build-up (von Blittersdorff & Klatt, 2017). These particles may even end up in the cup, ranging their preponderance in the total solids from 0.5% in filtered coffee to 15.7% in boiled coffee (Petracco, 2001).

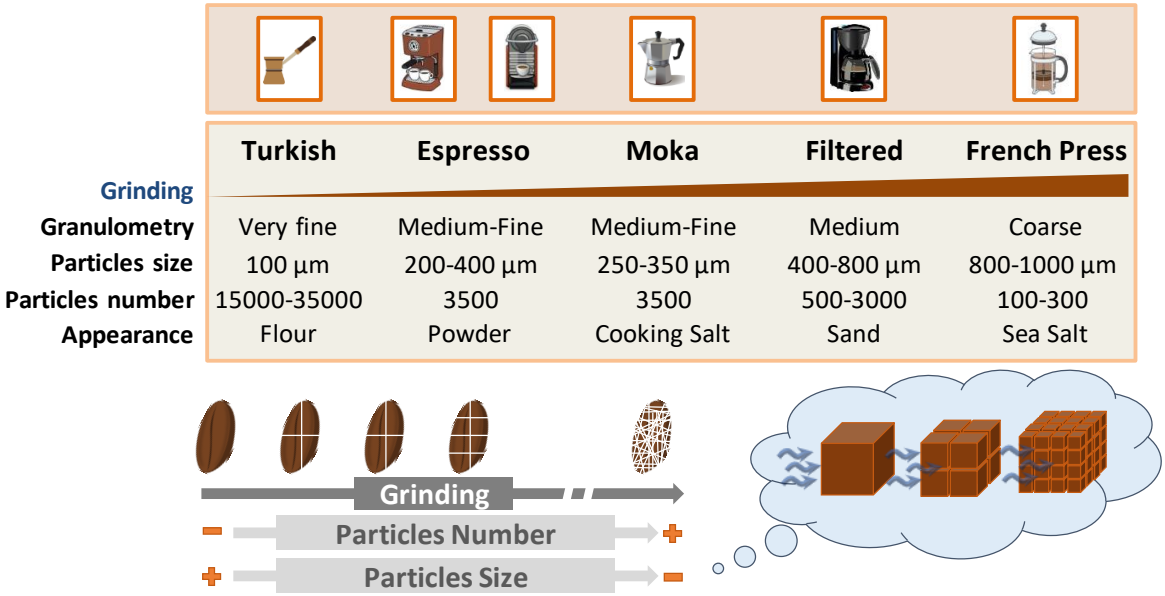


Figure I.9. Schematic representation of the grinding degree associated to different coffee brew preparation methods (based on von Blittersdorff and Klatt, 2017).

Coffee consumption and brew preparation are clearly dependent on the cultural and social habits inherent to each country. A clear example is the use of different volumes of brew of an espresso coffee in Portugal, Spain or Italy, the main consumer's countries of this beverage. The study of consumer trends of importing countries allowed to perceive the preponderance of instant coffee consumption in some countries (> 75% of total consumption in United Kingdom, Russia, Ukraine, and Turkey) or the prospering of coffee pods, used mainly in espresso coffee preparation (Figure I.10) (ICO, 2012). These systems were well received by the population as it brings convenience to the consumers. In Portugal, for instance, was observed an increase from 0.2% to 11.7% in the period studied (2004-2011) and an annual growth rate of 24% for all countries. These systems give to people the possibility of experience a varied set of coffee blends, with distinct aroma, body, colour and intensity. However, they generate a huge amount of garbage and wastes (capsules with the residue). The current environmental concerns may lead to the substitution/improvement of this brewing method. These systems involving coffee machines, pods and capsules (metal, plastic or paper) standardize the extraction process of espresso, with lower operational parameters to be controlled, making it easier to obtain an acceptable espresso brew. The quick growing of these systems attributed to the convenience of preparation (*i.e.* ease and clean use with individual dosing) is a good indicator of what people seek: readiness.

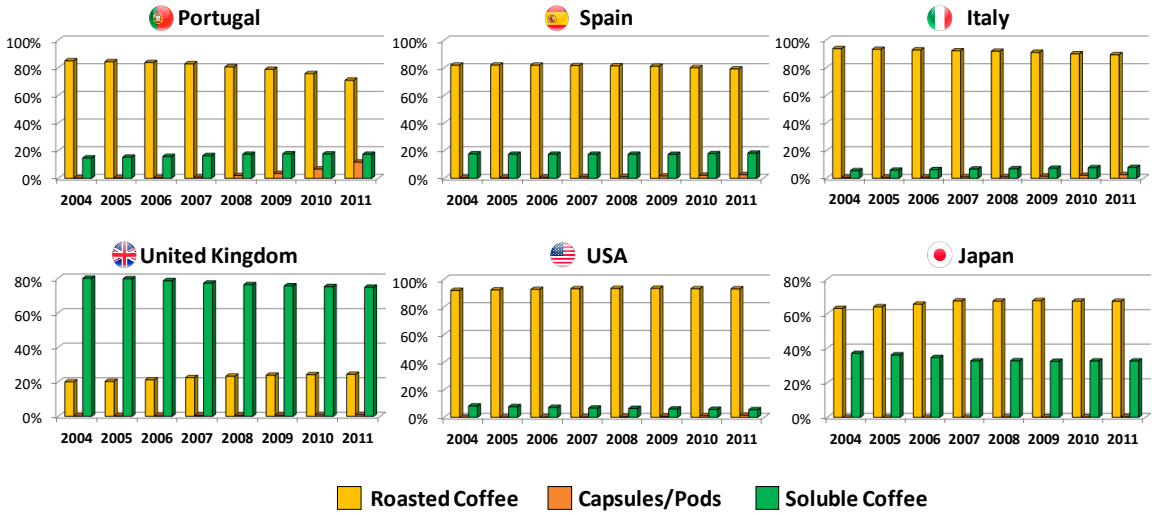


Figure I.10. Consumption habits in different countries (ICO, 2012).

I.2.2. Composition of espresso coffee

The Italian word *espresso* points out the idea of immediate moment, to be made and serve immediately, in an expeditiously way. However, it is also interpreted as related to under pressure due to the Latin etymology of the word espresso, which means *pressed out* (Illy & Viani, 2005). Therefore, espresso coffee is closely linked to the idea of quickness, with taste degradation if the consumers spent too much time to drink the brew, due to acidity increase and unbalanced saltiness over cooling and, mainly, the fading of the characteristic foam. The emerging of espresso machines in the HoReCa market (hotels, restaurants and coffeehouses) in Italy, espresso originated nation, and countries as Portugal or Spain, was due to their quickness in producing the brew serving an increasing urban working-class in 1950s and 1960s (Morris, 2013). The at-home espresso consumption owes much of its increase to single-dose capsules espresso machines. This allowed to everyone a premium espresso experience, with high convenience and variability of coffee sensations according to consumer preference, only experienced previously at an out-of-home level. Indeed, the success attributed to espresso was based on technology development which can change the culture of coffee for new brews styles and new consumption habits. Furthermore, from a homogeneous market where competition is based on price, such variation may lead to a segmented market, with offers related to quality and service, justifying a higher coffee product price, adapted to a fraction of consumers.

Espresso coffee may be defined as a concentrated coffee brew obtained by the percolation of a small quantity of hot water (25-50 mL) through ground roasted coffee powder during a brief period of time (Illy & Viani, 2005). In espresso brews, depending on the final volume, different names may be used to describe it: *ristretto* with up to 25 mL, regular with nearly 40 mL and *lungo* with higher volumes. The denominations have some differences according to the country habits of consumption. Espresso is considered one of the most difficult brewing methods with several parameters affecting the brew properties either the main factor associated to espresso (pressure), or other extraction conditions (*e.g.* temperature and time of extraction), the grinding degree of the beans or coffee itself (*e.g.* origin, variety, roasting degree) (Albanese et al., 2009; Andueza et al., 2002, 2003a). Brewing espresso, requiring so much skills to prepare such brew, constitutes an important part of World Barista Championship (https://worldbaristachampionship.org/) that takes place every year from 2000. Indeed, "*brewing espresso…unlike other methods of brewing coffee…is rocket science*" (Knox & Huffaker, 1996). On the other hand, such contest also promotes the development of new coffee brews, evidencing the constant demand for development in coffee brewing industry/market.

A vast amount of literature reports is devoted to coffee in general with increasing papers all over the last 40 years (Figure I.11), a trend also verified for espresso coffee that constitutes a small fraction (nearly 200 reports). Coffee publications covers mainly the research areas of agriculture, food science and chemistry, showing the interest in the study of its chemical composition on brew properties and related it to biological and nutritional effects to human health.

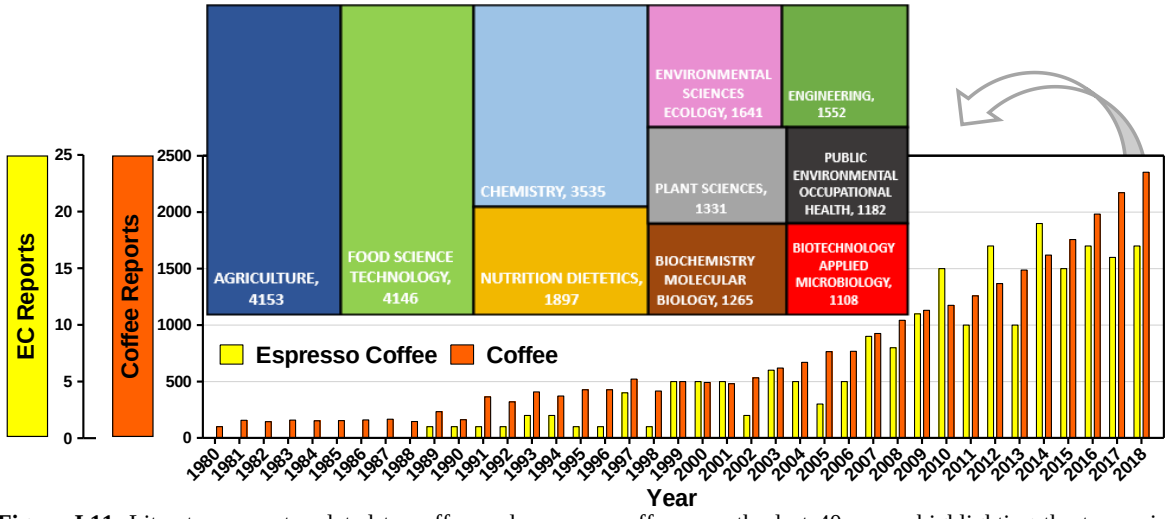
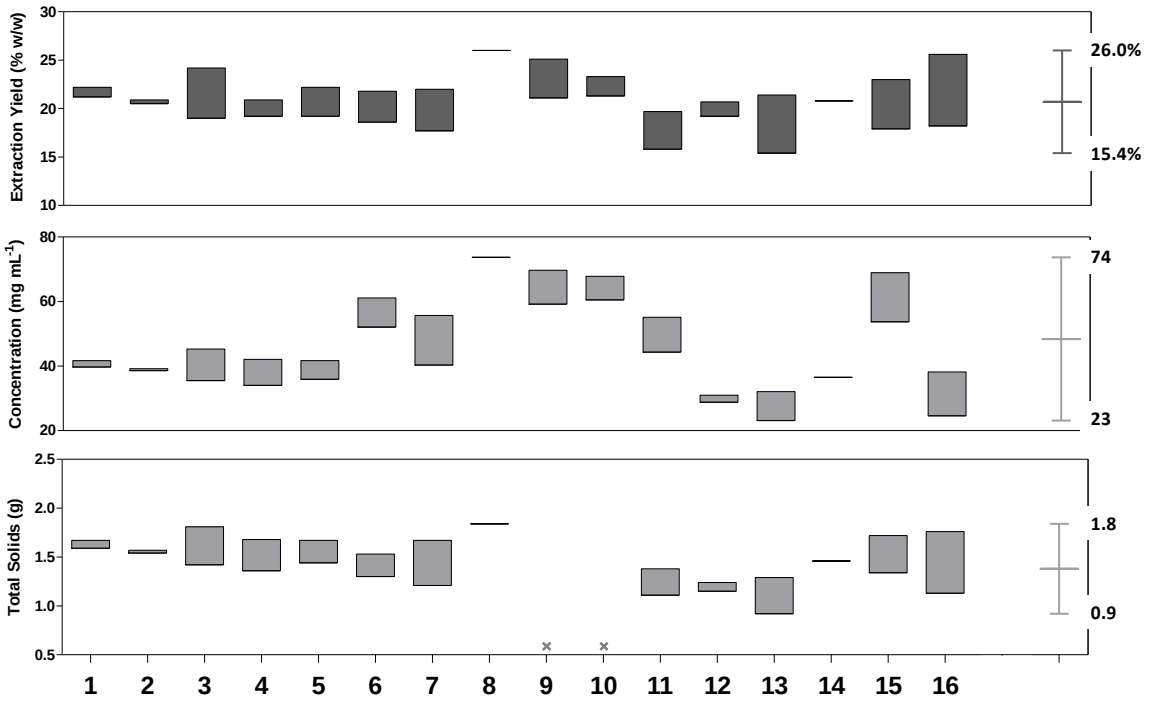


Figure I.11. Literature reports related to coffee and espresso coffee over the last 40 years, highlighting the ten main research areas covered. Database ISIWebofKnowledge: topic "coffee" or "espresso coffee" (accessed in August 2019).

Several features distinguish espresso coffee from the other coffee brews, as the typical aroma, intense colour, full bodied appearance, bitter/acid lingering taste, the persistent hazelnut foam constituted by a layer of small bubbles that cover the liquid or the opaque appearance (D'Agostina et al., 2004; Illy & Viani, 2005). The extraction process of espresso allows that a large amount and variability of compounds end up in the brew. Table I.2-I.7 present the ranges found in several espresso reports concerning some brew properties and main compounds. Although pure Arabica coffees are commercially common, pure Robusta coffee is not usual for espresso preparation and thus the data is scarce even because these may not represent brews available to consumers. The data collection of literature reports allows to better define the chemical properties of espresso coffee brews, although it also allowed to verify a wide range of values for some parameters related to different methods of analysis of the compounds and/or to inherent variability of the extraction method (*e.g.* coffee species, amount of coffee, volume of water, temperature, pressure). Most of the studies use commercially available samples, but some of them use samples that are laboratorial roasted beans, as well as pure Robusta samples in order to find differences/correlations in brew properties associated to such variations in roasting degree and coffee species. Thus, such samples may not precisely describe the usual consumption of espresso. Nevertheless, it allows to define a characteristic profile that can be hypothetically replicated by other coffee methods. During espresso extraction, 15.4-26.0% of roasted powder coffee compounds are extracted to the brew, yielding 0.9-1.8 g of total solids (Table I.2).

Table I.2. Literature data regarding the extraction yield, total solids concentration ($mg.mL^{-1}$) and content (mg *per* cup) for espresso coffee brews, with a schematic representation of the results.*the coffee species used for extraction is described when available and labelled commercial when the authors do not provide such information.

Col.	Coffee Species*	Coffee (g)	Volume (mL)	Temp. (ºC)	EC Machine	Extr. Yield (%w/w)	Conc. ($mg.mL^{-1}$)	Amount (g)	Ref.
1	Arabica	7.5	40	96	Conv.	22.2	41.7	1.67	Maeztu et al., (2001a)
	Blend (80R:20A)	7.5	40	96	Conv.	21.2	39.7	1.59	
2	Arabica	7.5	40	92	Conv.	20.5-20.9	38.6-39.2	1.54-1.57	Andueza et al., (2002)
3	Blend (80R:20A)	7.5	40	92	Conv.	19.0-24.2	35.5-45.3	1.42-1.81	Andueza et al., (2003b)
4	Arabica	6.5-8.5	40	92	Conv.	19.8-20.9	34.0-42.1	1.36-1.68	Andueza et al., (2007)
	Blend (80R:20A)	6.5-8.5	40	92	Conv.	19.2-20.7	35.6-40.8	1.42-1.63	
5	Arabica	7.5	40	88-98	Conv.	19.2-22.2	35.9-41.7	1.44-1.67	Andueza et al., (2003a)
	Blend (80R:20A)	7.5	40	88-98	Conv.	19.9-21.5	37.7-40.3	1.51-1.61	
6	Arabica	7	25	90-110	Pods	18.6-21.5	52.1-60.2	1.30-1.51	Albanese et al., (2009)
	Robusta	7	25	90-110	Pods	19.8-21.8	55.4-61.1	1.39-1.53	
	Blend (80-20R:20-80A)	7	25	90-110	Pods	18.8-21.7	52.6-60.9	1.32-1.52	
7	Commercial	8	30	90-92	Conv.	17.7-20.9	47.0-55.7	1.41-1.67	Gloess et al., (2013)
	Commercial	5.5	30	-	Capsules	22.0	40.3	1.21	
8	Arabica	7	25	93	Pods	26.0	73.7	1.84	Caporaso et al., (2014)
9	Commercial	7.25	-	92	Conv.	21.1	59.2	-	Parenti et al., (2014)
	Commercial	6.7-6.9	-	92	Capsules	24.4-25.1	66.1-69.7	-	
10	Commercial	7	-	75-85	New Method	21.3-23.3	60.5-67.8	-	Masella et al., (2015)
11	Commercial	7	25	92	Conv.	15.8-19.7	44.3-55.1	1.11-1.38	Severini et al., (2015)
12	Commercial	6	40	-	Capsules	19.2-20.7	28.8-31-0	1.15-1.24	Lopes et al., (2016)
13	Arabica	6	40	-	Conv.	15.8-20.9	23.6-31.3	0.95-1.25	Nunes et al., (1997)
13	Robusta	6	40	-	Conv.	15.4-21.4	23.1-32.1	0.92-1.29	
14	Commercial	7	40	90	Conv.	20.8	36.5	1.46	López-Galilea et al., (2007)
15	Arabica	7.5	25	88-93	Conv.	17.9-23.0	53.7-68.9	1.34-1.72	Salamanca et al., (2017)
	Robusta	7.5	25	88-93	Conv.	18.8-22.3	56.3-66.9	1.41-1.67	
16	Arabica	5.2-7.9	46	-	Capsules/Pods	18.2-25.6	28.2-38.2	1.30-1.76	Bartel et al., (2015)
	Blend (65R:35A)	5.2	46	-	Capsule	21.7	24.6	1.13	

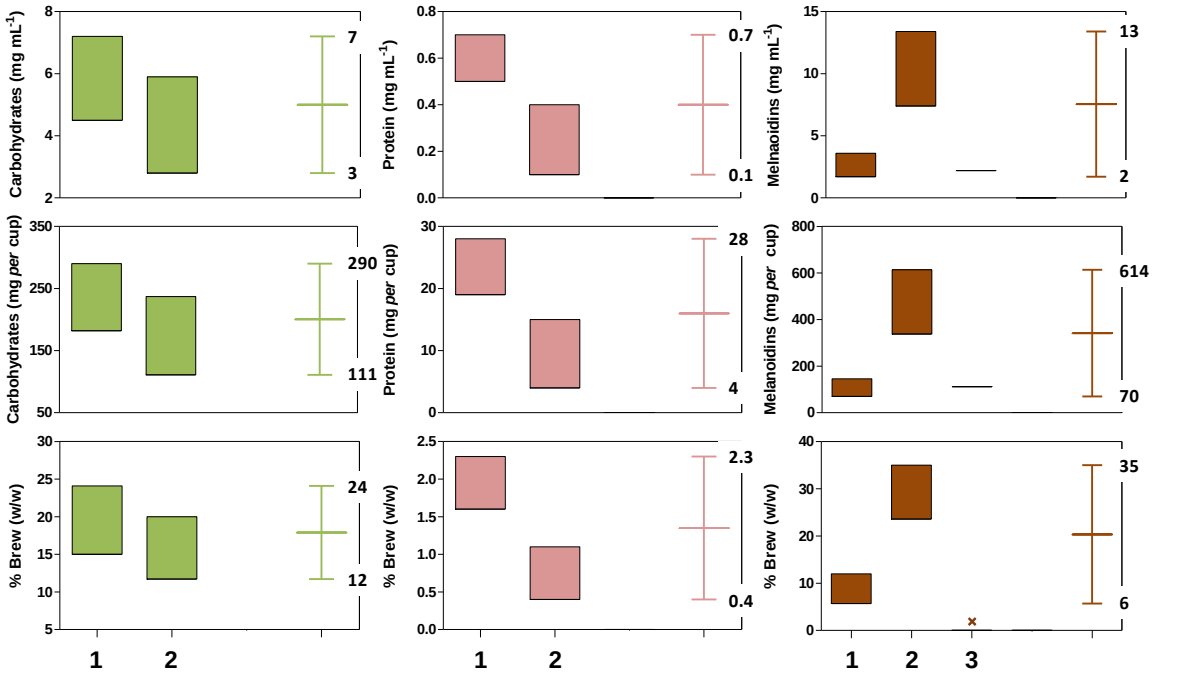


The different amounts of solids in espresso coffee are mainly due to different quantity of coffee powder used to the extraction (5.2-8.5 g) and the final volume of the brew (25-46 mL). This explains the wide variation in total solids concentration pattern (23-74 $mg.mL^{-1}$) and highlights that the modulation of conditions of extraction led to considerable variations in the total solids. This "*concentrated brew*" depends clearly on personal, local, and national preferences and preparation practices that led to different levels of concentrations. Indeed, the analysis of fractionated portions of the brew shows that during the extraction of espresso coffee, the concentration of total solids decreases along time (Caprioli et al., 2012). Nevertheless, espresso is pointed out as the more concentrated brew in different studies comparing some coffee brews, although the total amount of solids may be higher in other preparations due to substantial higher brew volume (Caporaso et al., 2014; Gloess et al., 2013).

The analysis of carbohydrates and melanoidins in espresso samples is scarce compared to the other compounds, although they account for a considerable fraction of the solids (Table I.3). The same occurs with proteins, which account for a small portion of the brew. The carbohydrates content in the cup increases until a certain roasting degree with a following decrease with darker roasts (Nunes et al., 1997). Moreover, galactomannans are the predominant structures over arabinogalactans (Lopes et al., 2016). The amount of carbohydrates is associated with espresso coffee foam stability (Nunes et al., 1997). Besides carbohydrates, proteins are also important in foam properties, as higher contents are correlated to higher foam formed (foamability) during brew preparation (Nunes et al., 1997). Nevertheless, proteins are present in low quantity in espresso coffee, representing 0.4-2.3 %w/w of total solids and up to nearly 30 mg *per* cup (Table I.3). As coffee brews have a low protein content and lack essential amino acids, it cannot be considered a good nutritional source of protein (Farah, 2012). For foam formation, the diffusion of soluble proteins to the air-water interface should occur followed by their denaturation and concentration (Nunes et al., 1997). Robusta seems to exhibit a higher protein content in espresso coffee than Arabica coffee (Caprioli et al., 2012; Nunes et al., 1997). Protein material is extracted at the beginning of the extraction process, with 31-39% of total content in the first 10 s of extraction (Caprioli et al., 2012). Then, protein content follows a logarithmic trend to decrease along the extraction process.

Table I.3. Literature data regarding the carbohydrates, proteins and melanoidins content (mg.mL^{-1}, amount *per* cup and %w/w of the brew solids) for espresso coffee brews, with a schematic representation of the results. *the coffee species used for extraction is described when available and labelled commercial when the authors do not provide such information.

Col.	Coffee Species*	Coffee (g)	Volume (mL)	Temp. (ºC)	EC Machine	Conc. (mg.mL^{-1})	Amount *per* cup (mg)	% Brew (%w/w)	Ref.
Carbohydrates									
1	Commercial	6	40	-	Capsules	4.5-7.2	182-290	15.0-24.1	Lopes et al., (2016)
2	Arabica	6	40	-	Conv.	2.8-5.9	111-237	11.7-20.0	Nunes et al., (1997)
	Robusta	6	40	-	Conv.	3.3-4.7	132-189	13.9-15.3	
Proteins									
1	Commercial	6	40	-	Capsules	0.5-0.7	19-28	1.6-2.3	Lopes et al., (2016)
2	Arabica	6	40	-	Conv.	0.1-0.3	4-11	0.4-0.9	Nunes et al., (1997)
	Robusta	6	40	-	Conv.	0.1-0.4	5-15	0.6-1.1	
Melanoidins									
1	Commercial	6	40	-	Capsules	1.7-3.6	70-146	5.7-12.0	Lopes et al., (2016)
2	Arabica	5.2-7.9	46		Capsules/Pods	7.4-13.4	338-614	23.6-35.0	Bartel et al., (2015)
	Blend (65R:35A)	5.2	46		Capsule	7.4	340	30.0	
3	Arabica		50		Conv.	2.2	112	-	Fogliano & Morales (2011)



Regarding melanoidins, as their formation are related to roasting degree, their amount in coffee brews increases with increasing roasting intensity of the coffee powder, while their molecular weight decreases (Borrelli et al., 2002). Moreover, it was reported that the intake of three espresso coffees may represent an intake of nearly 10% of the daily recommendation of dietary fibre, which is of nutritional relevance (Díaz-Rubio & Saura-Calixto, 2007; Lopes et al., 2016). The discrepancy of the values found in literature (70 to

614 mg *per* cup, Table I.3) is associated to the method of determination of their content. Melanoidins may be considered the total amount of polymeric material obtained after dialysis or ultrafiltration processes. In this case, the amount determined should be much higher than when carbohydrates and proteins content are excluded and only the polymeric unknown material is considered.

Lipids content in coffee brews clearly depend on preparation method (Figure I.12). The pressure associated to brew preparation increases the extraction of lipophilic compounds, which are missing/reduced in other coffee preparations (*e.g.* filtered brews) (Illy & Viani, 2005; Ratnayake et al., 1993; Speer & Kölling-Speer, 2006). In the filtered ones, a considerable amount of the lipid fraction is retained in the filter, containing the brew only 0.4% of lipids (Ratnayake et al., 1993). Indeed, the greater fraction of coffee lipids remained in the final residue after brew preparation regardless the method of preparation. Particularly, unfiltered methods (Scandinavian-boiled coffee and Turkish coffee) exhibited higher concentration of diterpenes than espresso brews and the latter higher contents compared to filtered and also instant coffee (Gross et al., 1997; Moeenfard et al., 2015b).

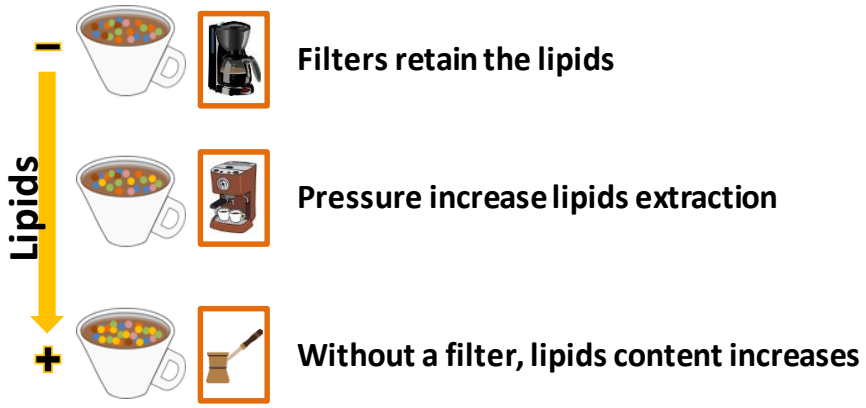


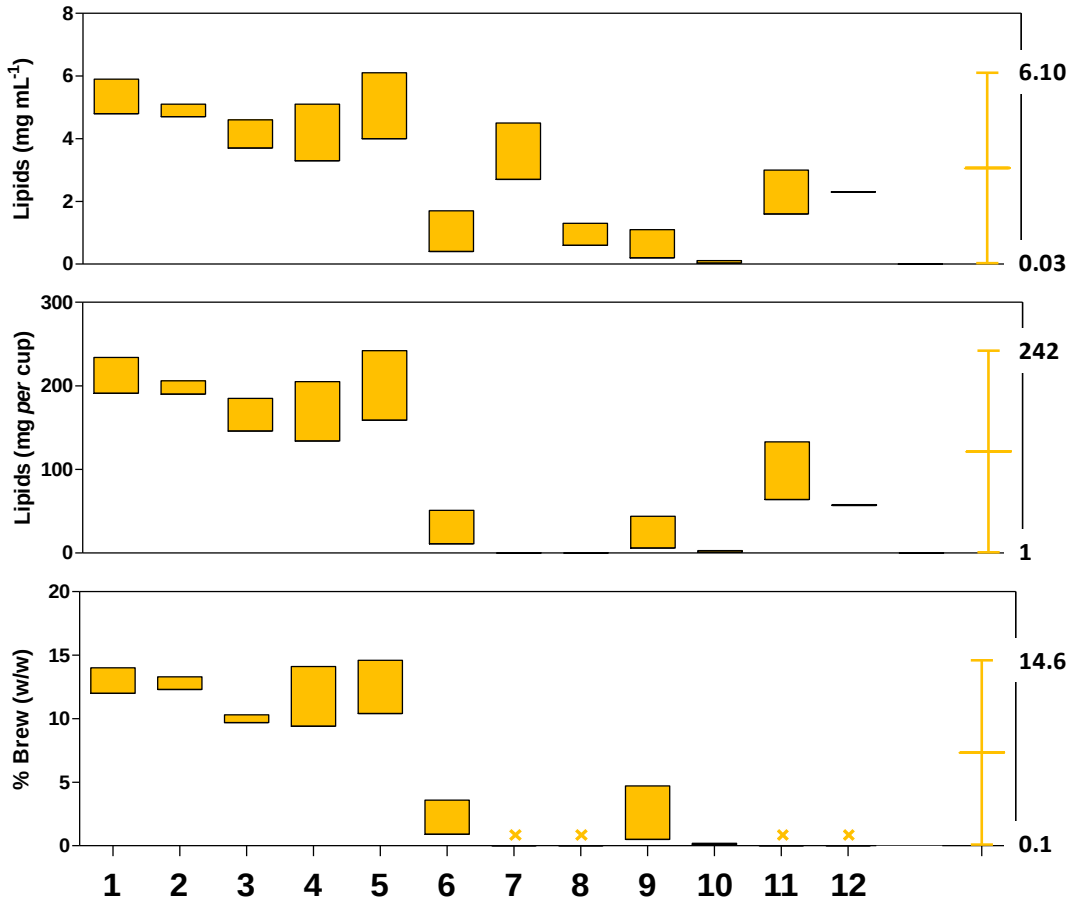
Figure I.12. Lipids content variation according to coffee brew preparation method.

The lipids content in espresso coffee is clearly dependent on the coffee species as Arabica coffee brews exhibit higher content than Robusta ones (Table I.4). Regarding roasting process, the increase in roasting degree is associated to a decrease in the lipids found in espresso cup (Nunes et al., 1997). Lipids content also increase inversely with particle size used for espresso preparation (Andueza et al., 2003b; De Vivo et al., 2019). A relevant feature for coffee quality is the fact that lipid content has a strong negative correlation with espresso foam formation, with foam destabilization by lipids also

documented for other beverages (Dickie et al., 2001; Illy & Navarini, 2011; Nunes et al., 1997). On the other hand, they are important as aroma carriers (Czerny et al., 1999; De Vivo et al., 2019; Illy & Viani, 2005). Table I.4 shows a great variability of lipids content in the brew (0.1-14.6 %w/w of total solids) which may be related to different methods of analysis (*e.g.* Soxhlet extraction, liquid-liquid extraction, and direct transesterification of coffee samples), although several parameters affect espresso cup lipids content. Some values may be overestimated due to the co-extraction of other compounds, as caffeine, with some authors performing a clean-up step to purify the lipid fraction (Nunes et al., 1997). Lipids extraction has been reported to occur mainly in the first 10 seconds of extraction (Caprioli et al., 2012). Furthermore, there is no consensus in the higher or lower amount of lipids in EC regarding the use of capsules/pods systems (Gloess et al., 2013; Parenti et al., 2014). However, the extraction conditions used in the studies for conventional and capsules systems are different which hinders an accurate comparison.

Table I.4. Literature data regarding the lipids content (mg.mL^{-1}, amount *per* cup and %w/w of the brew solids) for espresso coffee brews, with a schematic representation of the results. *the coffee species used for extraction is described when available and labelled commercial when the authors do not provide such information.

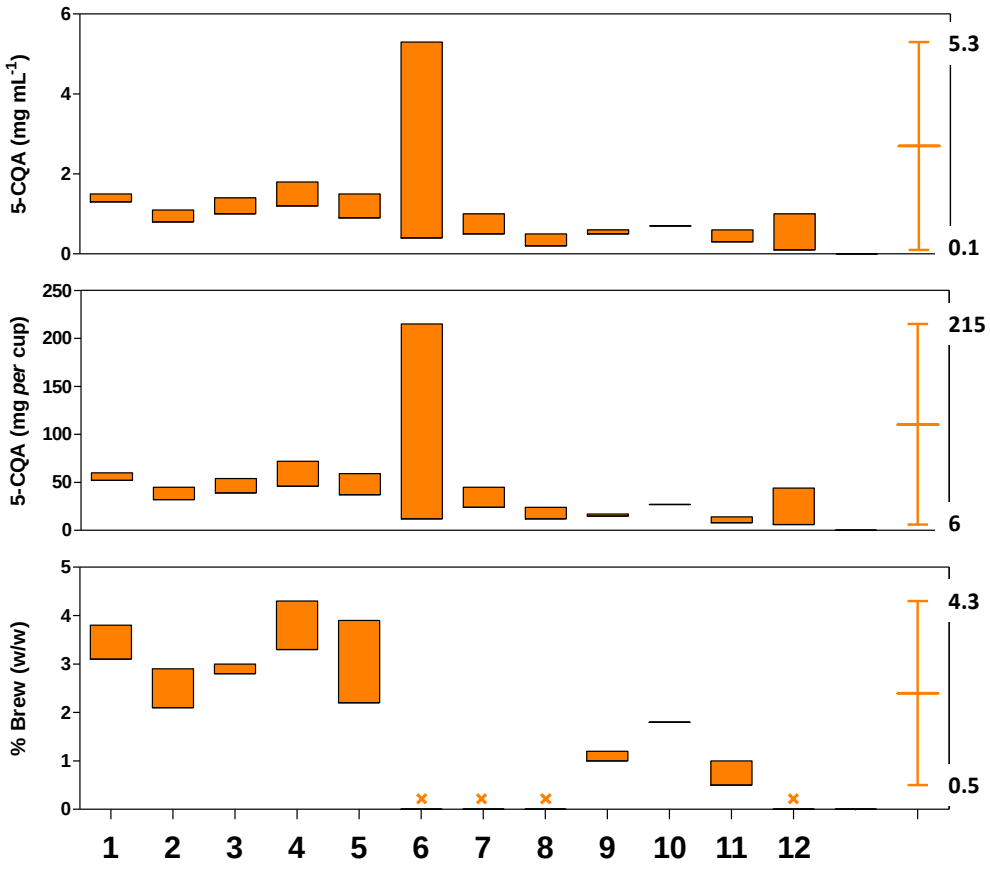
Col.	Coffee Species*	Coffee (g)	Volume (mL)	Temp. (ºC)	EC Machine	Conc. (mg.mL^{-1})	Amount *per* cup (mg)	% Brew (%w/w)	Ref.
1	Arabica	7.5	40	96	Conv.	5.9	234	14.0	Maeztu et al., (2001a)
	Blend (80R:20A)	7.5	40	96	Conv.	4.8	191	12.0	
2	Arabica	7.5	40	92	Conv.	4.7-5.1	190-206	12.3-13.3	Andueza et al., (2002)
3	Blend (80R:20A)	7.5	40	92	Conv.	3.7-4.6	146-185	9.7-10.3	Andueza et al., (2003b)
4	Arabica	6.5-8.5	40	92	Conv.	4.8-5.1	192-205	12.2-14.1	Andueza et al., (2007)
	Blend (80R:20A)	6.5-8.5	40	92	Conv.	3.3-3.9	134-157	9.4-10.5	
5	Arabica	7.5	40	88-98	Conv.	4.9-6.1	197-242	13.1-14.6	Andueza et al., (2003a)
	Blend (80R:20A)	7.5	40	88-98	Conv.	4.0-5.0	159-198	10.4-12.3	
6	Commercial	8	30	90-92	Conv.	1.3-1.7	40-51	2.4-3.6	Gloess et al., (2013)
	Commercial	5.5	30	-	Capsules	0.4	11	0.9	
7	Commercial	7.25	-	92	Conv.	2.7	-	-	Parenti et al., (2014)
	Commercial	6.7-6.9	-	92	Capsules	3.9-4.5	-	-	
8	Commercial	7	-	75-85	New Method	0.6-1.3	-	-	Masella et al., (2015)
9	Arabica	6	40	-	Conv.	0.4-1.1	16-44	1.3-4.7	Nunes et al., (1997)
	Robusta	6	40	-	Conv.	0.2-0.6	6-23	0.5-2.5	
10	Arabica	7.5	25	88-93	Conv.	0.06-0.11	2-3	0.1-0.2	Salamanca et al., (2017)
	Robusta	7.5	25	88-93	Conv.	0.03-0.09	1-2	0.1	
11	Arabica	6.5-9-5	30-60	70-90	Conv.	1.6-3.0	64-133	-	Moeenfard et al., (2015a)
12	Arabica	4	25	-	Conv.	2.3	57	-	Ratnayake et al., (1993)
	Robusta	4	25	-	Conv.	2.3	58	-	



The amount of chlorogenic acids present in an espresso coffee brew is clearly dependent on the roasting process, with decreased amount with more intense roasting degree (Alves et al., 2010; Ludwig et al., 2014a). Furthermore, coffee/water ratio, grinding degree or the pressure applied in espresso preparation are factors that affect the chlorogenic acids content in cup (Andueza et al., 2002, 2003b, 2007). These factors explain the variability of values found in Table I.5, where it is possible to find a 17-fold variation in chlorogenic acids content (and 5-CQA), among 20 commercial espresso coffees prepared with different volumes of brew (Crozier et al., 2012). Espresso is pointed out as the coffee preparation method that allows a higher cup concentration of chlorogenic compounds, comparing with other techniques (*e.g.* boiled or filtered coffee) (Moeenfard et al., 2014).

Table I.5. Literature data regarding the 5-CQA content (mg.mL^{-1}, amount *per* cup and %w/w of the brew solids) for espresso coffee brews, with a schematic representation of the results. * the coffee species used for extraction is described when available and labelled commercial when the authors do not provide such information.

Col	Coffee Species*	Coffee (g)	Volume (mL)	Temp. (ºC)	EC Machine	Conc. (mg.mL^{-1})	Amount *per* cup (mg)	% Brew (%w/w)	Ref.
1	Arabica	7.5	40	96	Conv.	1.3	52	3.1	Maeztu et al., (2001a)
	Blend (80R:20A)	7.5	40	96	Conv.	1.5	60	3.8	
2	Arabica	7.5	40	92	Conv.	0.8-1.1	32-45	2.1-2.9	Andueza et al., (2002)
3	Blend (80R:20A)	7.5	40	92	Conv.	1.0-1.4	39-54	2.8-3.0	Andueza et al., (2003b)
4	Arabica	6.5-8.5	40	92	Conv.	1.2-1.8	46-72	3.4-4.3	Andueza et al., (2007)
	Blend (80R:20A)	6.5-8.5	40	92	Conv.	1.2-1.5	47-61	3.3-4.0	
5	Arabica	7.5	40	88-98	Conv.	0.9-1.1	37-45	2.2-2.9	Andueza et al., (2003a)
	Blend (80R:20A)	7.5	40	88-98	Conv.	1.3-1.5	51-59	3.3-3.9	
6	Commercial	-	23-70	-	-	0.4-5.3	12-215	-	Crozier et al., (2012)
7	Arabica	7	45	-	Conv.	1.0	45	-	Ludwig et al., (2012)
	Robusta	7	45	-	Conv.	0.5	24	-	
8	Arabica	7	50	95-97	Conv.	0.4	21	-	Niseteo et al., (2012)
	Commercial	7	50	95-97	Conv.	0.3-0.5	13-24	-	
	Commercial	7	50	95-97	Single-Dose	0.2	12	-	
9	Commercial	8	30	90-92	Conv.	0.5-0.6	15-17	1.0-1.1	Gloess et al., (2013)
	Commercial	5.5	30	-	Capsules	0.5	15	1.2	
10	Commercial	7	40	90	Conv.	0.7	27	1.8	López-Galilea et al., (2007)
11	Arabica	7.5	25	88-93	Conv.	0.5-0.6	12-14	0.7-1.0	Salamanca et al., (2017)
	Robusta	7.5	25	88-93	Conv.	0.3-0.4	8-11	0.5-0.7	
12	Commercial	5-8	22-51	-	Capsules	0.2-1.0	6-28	-	Angelino et al., (2018)
	Commercial	5.5-10	83-206	-	Capsules	0.1-0.4	13-44	-	



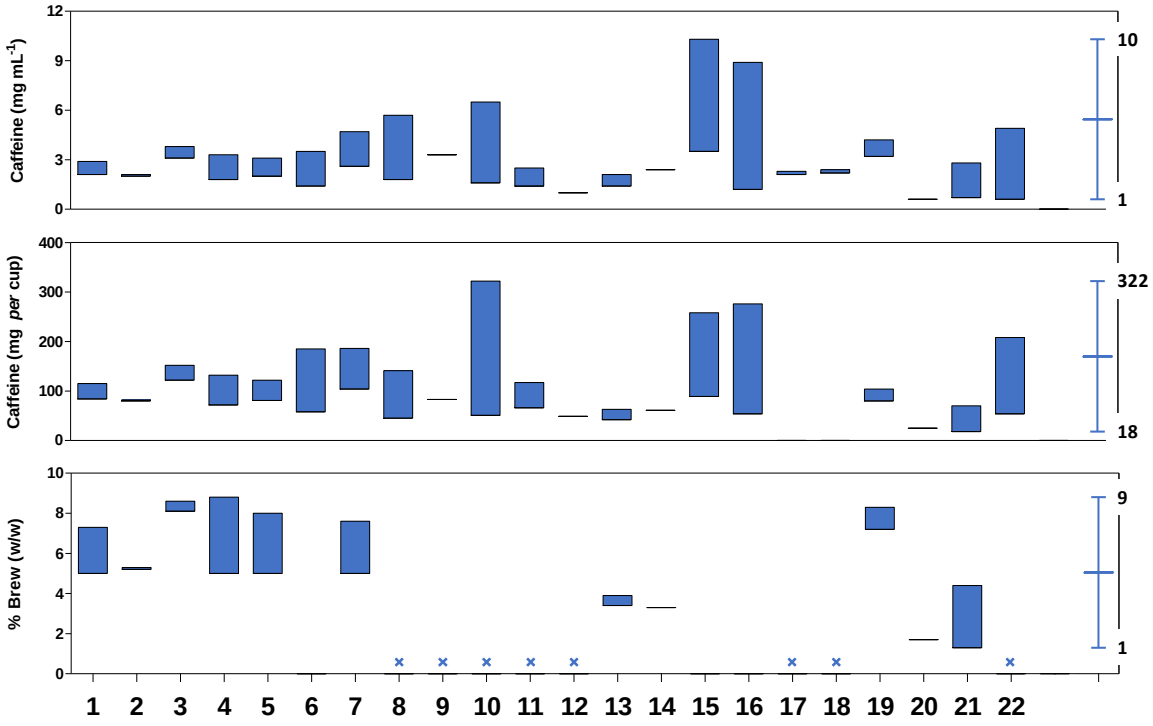
Regarding caffeine, brews prepared with Robusta coffee exhibit higher contents than Arabica, while the roasting degree seems not to affect caffeine content at a great extent (Illy & Viani, 2005; Ludwig et al., 2014a; Maeztu et al., 2001a). Literature reported discrepant values for caffeine *per* cup (18-322 mg), corresponding to 1-9% of brew total solids (Table I.6). However, some studies seem to underestimate the caffeine, as at nearly 75-100 mg are expected to be found in espresso brews (dePaula & Farah, 2019; Nehlig, 1999). The higher values are associated to studies performed in stores without the control of extraction conditions and amount of coffee powder to be extracted (Crozier et al., 2012). Nevertheless, the variation in caffeine cup content is evident.

Table I.6. Literature data regarding the caffeine content ($mg.mL^{-1}$, amount *per* cup and %w/w of the brew solids) for espresso coffee brews, with a schematic representation of the results. *the coffee species used for extraction is described when available and labelled commercial when the authors do not provide such information.

Col	Coffee Species*	Coffee (g)	Volume (mL)	Temp. (ºC)	EC Machine	Conc. ($mg.mL^{-1}$)	Amount *per* cup (mg)	% Brew (%w/w)	Ref.
1	Arabica	7.5	40	96	Conv.	2.1	84	5.0	Maeztu et al., (2001)
	Blend (80R:20A)	7.5	40	96	Conv.	2.9	115	7.3	
2	Arabica	7.5	40	92	Conv.	2.0-2.1	80-82	5.2-5.3	Andueza et al., (2002)
3	Blend (80R:20A)	7.5	40	92	Conv.	3.1-3.8	122-152	8.1-8.6	Andueza et al., (2003b)
4	Arabica	6.5-8.5	40	92	Conv.	1.8-2.2	72-88	5.0-5.3	Andueza et al., (2007)
	Blend (80R:20A)	6.5-8.5	40	92	Conv.	3.0-3.3	120-132	8.1-8.8	
5	Arabica	7.5	40	88-98	Conv.	2.0-2.3	81-92	5.0-5.6	Andueza et al., (2003)
	Blend (80R:20A)	7.5	40	88-98	Conv.	2.9-3.1	118-122	7.4-8.0	
6	Commercial	-	40-84	-	-	1.4-3.5	58-185	-	McCusker et al., (2003)
7	Arabica	7	25	90-110	Pods	2.6-3.3	104-132	5.0-5.5	Albanese et al., (2009)
	Robusta	7	25	90-110	Pods	3.6-4.7	142-186	6.4-7.6	
	Blend (80-20R:20-80A)	7	25	90-110	Pods	2.8-4.4	111-175	5.3-7.2	
8	Commercial	9.3	25	-	Conv.	2.2-5.7	54-141	-	Candeias et al., (2008)
	Commercial	5.3	25	-	Capsules	1.8-2.4	45-61	-	
9	Arabica	10	25	-	Conv.	3.3	83	-	Santini et al., (2011)
10	Commercial	-	23-70	-	-	1.6-6.5	51-322	-	Crozier et al., (2012)
11	Arabica	7	45	-	Conv.	1.4	66	-	Ludwig et al., (2012)
	Robusta	7	45	-	Conv.	2.5	117	-	
12	Commercial	7	50	95-97	Single-Dose	1.0	49	-	Niseteo et al., (2012)
13	Commercial	8	30	90-92	Conv.	1.8-2.1	55-63	3.8-3.9	Gloess et al., (2013)
	Commercial	5.5	30	-	Capsules	1.4	42	3.4	
14	Arabica	7	25	93	Pods	2.4	61	3.3	Caporaso et al., (2014)
15	Arabica	7.5	25	88-92	Conv.	3.5-5.8	89-144	-	Caprioli et al., (2014)
	Blend (95R:5A)	7.5	25	88-92	Conv.	4.4-10.3	111-258	-	
	Commercial	7.5	25	92	Conv.	4.7-8.0	117-200	-	
16	Arabica	18.1-20.4	22-55	92	Conv.	3.7-7.9	152-232	-	Ludwig et al., (2014a)
	Commercial	5-18	13-104	-	Conv.	1.2-8.9	54-276	-	
17	Commercial	7.25	-	92	Conv.	2.2	-	-	Parenti et al., (2014)
	Commercial	6.7-6.9	-	92	Capsules	2.1-2.3	-	-	
18	Commercial	7	-	75-85	New Method	2.2-2.4	-	-	Masella et al., (2015)

(continued)

Col	Coffee Species*	Coffee (g)	Volume (mL)	Temp. (ºC)	EC Machine	Conc. (mg.mL^{-1})	Amount *per* cup (mg)	% Brew (%w/w)	Ref.
19	Commercial	7	25	92	Conv.	3.2-4.2	80-104	7.2-8.3	Severini et al., (2015)
20	Commercial	7	40	90	Conv.	0.6	25	1.7	López-Galilea et al., (2007)
21	Arabica	7.5	25	88-93	Conv.	0.7-2.6	18-64	1.3-4.3	Salamanca et al., (2017)
	Robusta	7.5	25	88-93	Conv.	1.2-2.8	30-70	2.1-4.4	
22	Commercial	5-8	22-51	-	Capsules	1.5-4.9	54-208	-	Angelino et al., (2018)
	Commercial	5.5-10	83-206	-	Capsules	0.6-1.9	75-190	-	



Operational parameters as volume and temperature of extraction, grinding degree and coffee/water ratio affects espresso caffeine cup content (Albanese et al., 2009; Andueza et al., 2003a; 2003b; 2007) Furthermore, literature refers that in espresso percolation, caffeine extraction is incomplete (75-85%) due to the short time to remove caffeine from cellular structure (Illy & Viani, 2005) (Figure I.13). Thus, there is a considerable effect of espresso coffee volume on caffeine cup content. In the case of other brews (filtered, Moka or percolator) the caffeine may achieve higher extraction values and be fully extracted (Petracco, 2001). Espresso may have higher caffeine concentration in the cup, but the higher volume and amount of coffee used for preparation of other brews allows the higher amount of caffeine *per* cup (Caporaso et al., 2014)

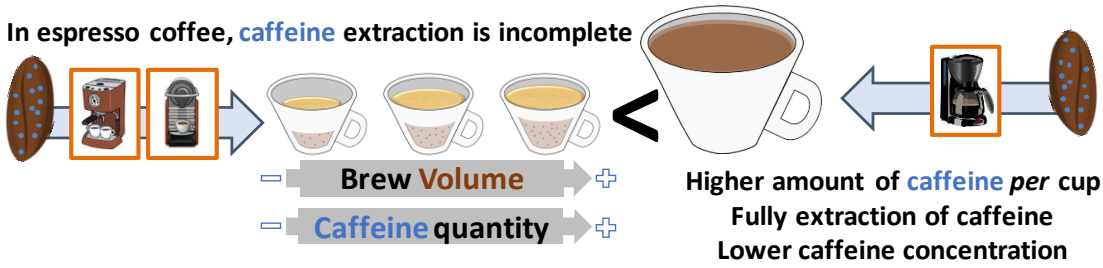


Figure I.13. Effect of espresso volume on caffeine content in the cup.

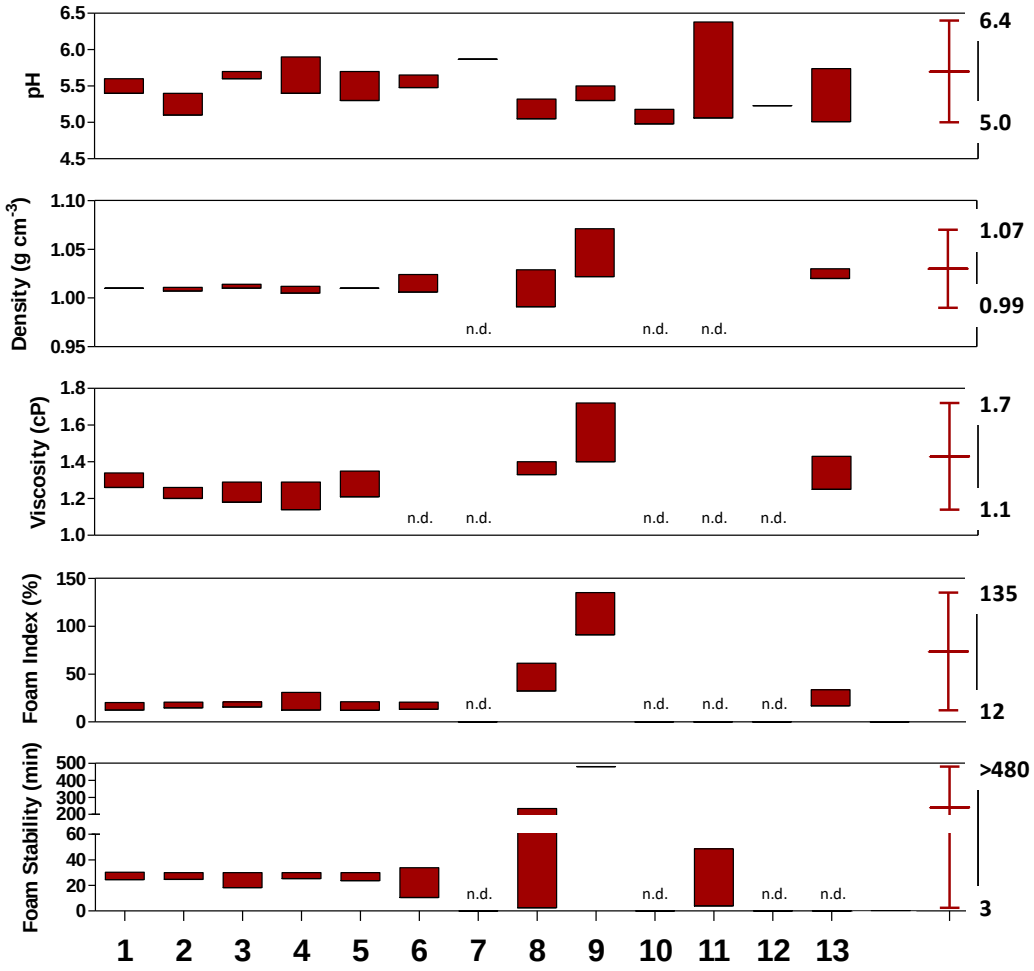
The analysis of different commercial brands shows that the content of caffeine *per* cup widely varies (up to six-fold variation) and that even within the same brand (in the same outlet), the consumption at different times of the day results in different amounts of caffeine ingested (Candeias et al., 2008; Crozier et al., 2012; Ludwig et al., 2014a). Such differences arise from the lack of standardization in the extraction conditions, which is diminished by the use of capsules systems (Candeias et al., 2008). These wide differences are important once the nature and magnitude of the effects of caffeine, a psychoactive substance, depends on the dose administered and may be problematic regarding the safe consumption limits of caffeine (400 mg/day), which is even more dangerous to vulnerable population groups (300 mg/day) (Candeias et al., 2008; Mejia & Ramirez-Mares, 2014).

Other physical properties are usually described for espresso brews, as the pH, density, viscosity, and foam properties (Table I.7) The pH of espresso varies from 5.0 to 6.4, with increasing values associated to darker roasted coffees, related to the degradation of chlorogenic acids. Moreover, it was also verified a correlation between pH and foamability (Nunes et al., 1997). The density of espresso coffee was not much different from the water density (0.99-1.07 g cm^{-3}), as the coffee brew was composed mainly by water (*ca.* 97%) (Table I.7). The differences may arise from variations in total solids, and mainly in lipids content that should lower the density values. Regarding viscosity, espresso is considered one of the most viscous coffee brews exhibiting values ranging from 1.1 to 1.7 cP (Table I.7). This parameter, as well as the total solids and density are related to the body of the cup. The development of new espresso brewing methods, such as the use of sealed chamber and pressurized air, allows to achieve higher viscosities in comparison to conventional espresso brews, affected by pressure and temperature applied (Masella et al., 2015). The foam, a distinctive characteristic of espresso coffee, is usually evaluated by its index (the ratio of foam and liquid volumes) and its stability (the time that the liquid below the foam took to appear after brew preparation) (Andueza et al., 2002; Nunes et al., 1997).

Regarding the foam index (12-35%, Table I.7) it is described that it should be at least 10% to have a well-prepared brew (Illy & Viani, 2005). The sealed chamber and pressurized air method of extraction of espresso coffee is described to have significantly higher values (>100%), meaning that the brew has more foam than liquid, which is not usual in conventional methods (Masella et al., 2015). This foam is also highly stable (more than 8 h of foam stability), which is considerable different from the usual values of 10-30 min of foam stability.

Table I.7. Literature data regarding the pH, density, viscosity, foam index and foam stability for espresso coffee brews, with a schematic representation of the results. *the coffee species used for extraction is described when available and labelled commercial when the authors do not provide such information.

Col	Coffee Species*	Coffee (g)	Volume (mL)	Temp. (ºC)	EC Machine	pH	Density (g cm^{-1})	Viscosity (Cp)	Foam Index (%)	Foam Stability (min.)	Ref.
1	Arabica	7.5	40	96	Conv.	5.40	1.010	1.34	12.3	30.3	Maeztu et al. (2001a)
	Blend (80R:20A)	7.5	40	96	Conv.	5.60	1.010	1.26	20.3	24.5	
2	Arabica	7.5	40	92	Conv.	5.10-5.40	1.007-1.011	1.20-1.26	14.7-20.7	24.7-30.0	Andueza et al., (2002)
3	Blend (80R:20A)	7.5	40	92	Conv.	5.60-5.70	1.010-1.014	1.18-1.29	15.5-20.8	18.3-30.0	Andueza et al., (2003b)
4	Arabica	6.5-8.5	40	92	Conv.	5.40-5.50	1.005-1.010	1.23-1.29	12.3-17.7	25.3-30.0	Andueza et al., (2007)
	Blend (80R:20A)	6.5-8.5	40	92	Conv.	5.80-5.90	1.010-1.012	1.14-1.17	15.8-30.8	30.0	
5	Arabica	7.5	40	88-98	Conv.	5.30-5.40	1.010	1.25-1.35	12.1-12.8	26.2-30	Andueza et al. (2003a)
	Blend (80R:20A)	7.5	40	88-98	Conv.	5.60-5.70	1.010	1.21-1.26	17.4-21.0	23.7-25.8	
6	Arabica	7	25	90-110	Pods	5.48-5.52	1.006-1.019	n.d.	13.4-19.4	24.0-33.8	Albanese et al., (2009)
	Robusta	7	25	90-110	Pods	5.58-5.65	1.020-1.024	n.d.	14.0-20.7	10.5-18.7	
	Blend (80-20R:20-80A)	7	25	90-110	Pods	5.50-5.64	1.015-1.021	n.d.	13.2-20.2	13.5-28.3	
7	Arabica	7	25	93	Pods	5.87	n.d.	n.d.	n.d.	n.d.	Caporaso et al., (2014)
8	Commercial	7.25	-	92	Conv.	5.32	1.029	1.36	32.4	3.9	Parenti et al., (2014)
	Commercial	6.7-6.9	-	92	Capsules	5.05-5.15	0.991-1.012	1.33-1.40	39.7-61.3	2.5-234.3	
9	Commercial	7	-	75-85	New Method	5.30-5.50	1.022-1.071	1.40-1.72	91.1-135.0	>480	Masella et al., (2015)
10	Commercial	7	25	92	Conv.	4.98-5.18	n.d.	n.d.	n.d.	n.d.	Severini et al., (2015)
11	Arabica	6	40	-	Conv.	5.06-6.38	n.d.	n.d.	n.d.	4.2-33.9	Nunes et al., (1997)
	Robusta	6	40	-	Conv.	5.20-6.23	n.d.	n.d.	n.d.	4.0-48.6	
12	Commercial	7	40	90	Conv.	5.23	n.d.	n.d.	n.d.	n.d.	López-Galilea et al., (2007)
13	Arabica	7.5	25	88-93	Conv.	5.14-5.74	1.020-1.030	1.26-1.43	16.8-23.8	n.d.	Salamanca et al., (2017)
	Robusta	7.5	25	88-93	Conv.	5.01-5.71	1.026-1.030	1.25-1.36	23.2-33.5	n.d.	



In Figure I.14 is represented the ranges found for the different compounds and properties described.

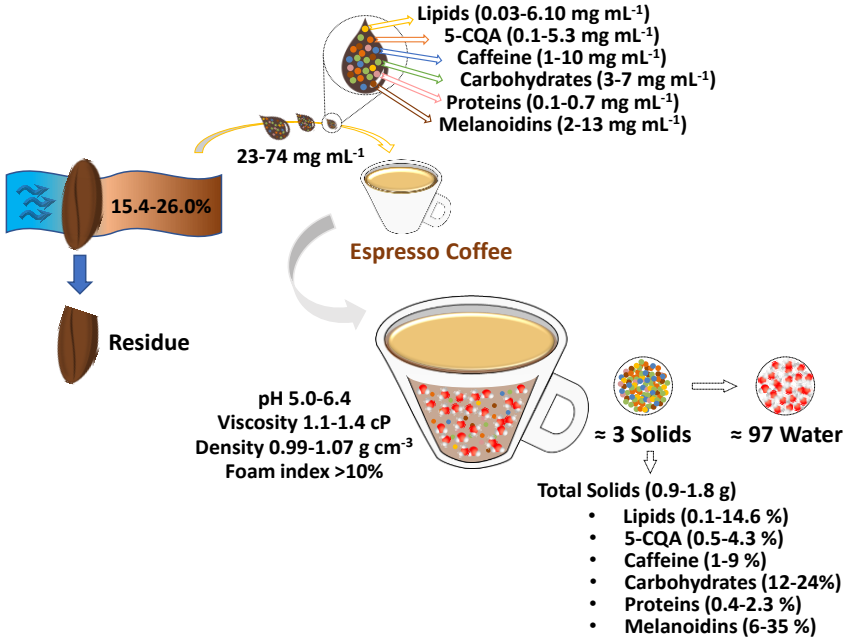


Figure I.14. Representation of espresso coffee extraction showing the ranges for different compounds and properties.

I.3. Motivation and aim of the work

Based on the knowledge achieved so far about espresso coffee composition and properties, this brew is particular considering the persistent hazelnut foam, the intense colour, the full-bodied appearance, and the intense aroma. These characteristics are defined by the chemical compounds that end up in the cup. On the other hand, all coffee brews are prepared with only two ingredients: roasted coffee powder and water. As in any other extraction method, the higher/lower extraction and selectivity of compounds ending up in the extract/brew is dependent on the extraction conditions. Thus, it may be hypothesized that the modulation of an infusion process could originate an extract (or coffee brew) with the same chemical characteristics of espresso coffee if some conditions are satisfied. Espresso coffee is also associated to a higher price-to-volume and expensive equipment required when comparing to other coffee brews. Single-dose machines, starting as a premium product, become nowadays more and more economical. However, the necessity of the machine is still there. Thus, the exemption of coffee machine will make this "expensive" brew more affordable either in time, place and price. Portugal seems to be a good location to introduce new products/concepts of espresso coffee brews, as present some particular features as the preference for espresso, out-of-home coffee consumption, and good acceptance of new coffee related products (*e.g.* single-dose coffee machines).

To fulfil this hypothesis, the state-of-the-art describing what occurs to compounds from the green beans to the coffee cup was presented in this **Chapter I**, with emphasis to the discussion of the espresso coffee composition, and characteristics of this brew. In **Chapter II** are described the *material and methods* used throughout the studies performed.

The results and discussion of the studies performed are presented in **Chapter III:**

- In Chapter III.1. (***Infusion process: conventional extraction***) is discussed the effect of the variation of operational parameters in the chemical characteristics of extracts using a coffee infusion process;
- In Chapter III.2. (***Microwave-assisted extraction of coffee***) is presented the impact of microwave-assisted extraction in the extraction of coffee compounds as an alternative to quickly produce coffee extracts.
- In Chapter III.3. (***Modulation of infusion process targeting espresso properties***) the infusion conditions that better resemble espresso coffee were used

to produce extracts that were compared with commercial samples, highlighting the similarities/discrepancies between both group of samples.

In **Chapter IV** are presented the general concluding remarks of this study, including a discussion about future perspectives risen by the work carried out and the results obtained.

The references cited along the thesis are presented in **Chapter V**, while statistical data of the models developed are available at the end of the text in the section **Annexes**.

Chapter II

Materials and Methods

II.1. Coffee samples

A commercial blend of roasted coffee beans (Delta® Lote Chávena) was used to perform the coffee extraction experiments (espresso and solid-liquid extractions). The moisture content was below 5% all over the experiments (ISO 11817:1994). For comparison purposes, different commercial single-dose coffee capsules Delta Q® were used to prepare espresso coffee brews. Different blends were used: Qonvictus (labelled intensity 5, EC Blend 2), Qalidus (labelled intensity 10, EC Blend 3), Qontrast Light Roast (EC Blend 4) and Qontrast Dark Roast (EC Blend 5), the latter two from the same coffee blends but prepared with different roasting degrees. An instant commercial coffee powder (Delta® Café Solúvel - IC1) was used as reference of an instant coffee sample (IC). Three other commercial instant coffee powders from distinct brands, referred as "espresso" in the label, were also analysed (IC2 - IC4).

II.1.1. Grinding of roasted coffee samples

A coffee grinder (Flama - 1231) was used to grind the roasted coffee beans. This equipment allows the selection of different grinding levels from finer (level 1) to coarser (level 3) particles. The particle size profile of the grounded samples was determined with a Malvern Mastersizer 2000 particle size analyser equipped with a Scirocco 2000 accessory.

II.2. Coffee brews

II.2.1. Espresso coffee extractions

Espresso coffee extractions were performed in a home brewing device (Flama 10, 15 bar) with distilled water (40 ± 2 mL) with freshly grounded coffee (6.0 g). The extraction with commercial single-dose coffee capsules Delta Q® (6.0 g) were performed on a Delta Q® QOSMO machine (19 bar) with distilled water (40 ± 2 mL).

II.2.2. Solid-liquid coffee extractions

II.2.2.1. Infusion extractions

Solid-liquid extractions were performed by using freshly grounded coffee according to the desired level of grinding and 30 mL of distilled water in a 100 mL Erlenmeyer flask covered and maintained in a water-bath with magnetic agitation during the intended time and temperature. After the experiments, the flasks were cooled to room temperature and their content was vacuum filtrated (1.2 μm glass microfiber filter) with the retained material washed with an additional 30 mL of water. The filtrates were all frozen and freeze-dried. The extraction yields were determined by weight and the extracts were stored under an anhydrous atmosphere until characterization analyses. The condition that better resemble expresso coffee composition was performed at a larger scale in the conditions established: 10 min of extraction at 50 ºC, using 175 g of coffee powder (grinding level 3), and 1.5 L of distilled water using the same coffee product (3 independent extractions). The infusion was then filtrated and frozen. In each infusion method, half of the filtrate was processed by freeze-drying and the remaining by spray-drying. For the spray-drying process the conditions were settled as follows: inlet temperature (150 ºC), outlet temperature (80 ºC), spray-gas flow (6 mL min^{-1}), pump (20%), and aspirator (95%), using a low solids content solution with 0.03 g mL^{-1}, similar to the one used for freeze-drying.

II.2.2.2. Coffee microwave-assisted extraction (MAE)

The MAE experiments were conducted in 100 mL reactors able to endure high temperatures and pressures (up to 250 ºC and 55 bar, respectively) placed on a multimode microwave oven - MicroSYNTH Labstation (maximum output: 1 kW, 2.45 GHz; Milestone Srl, Sorisole (BG), Italy). The device allows to monitor and control the vessel inner temperature in real-time as described in Passos and Coimbra (2013), with the content stirred continuously with a magnetic bar. Roasted coffee powder beans were grounded before the experiments and the powder placed on the reactor with 60 mL of distilled water. The experiments were performed in duplicate (two reactors in the apparatus). Microwave

power was continuously adjusted by the system to attain the desired temperature in 2 min and maintained it during the experiment according to defined conditions. After the MAE, the reactors were cooled down to room temperature and their content was vacuum filtrated (1.2 µm glass microfiber filter), frozen and freeze-dried, giving the overall extraction yield, and the insoluble residue oven dried (105 °C, 24 h). It was also performed a MAE at 80 ºC (2 min, 2 g/60 mL).

II.3. Design of experiments

Regarding the responses of interest, the analysis of coffee samples is usually performed to a certain class of compounds, as chlorogenic acids and caffeine, or sugars, and not studied simultaneously. The broad perspective of analysis should allow to comprehensively study how the different structures react to extraction processes, as all coffee compounds should not behave equally in extraction processes. Response surface methodologies are already used in the coffee field for the development and optimization of analysis procedures of coffee compounds or to the valorisation and reuse of the coffee residue, the spent coffee grounds, or other coffee industry by-products as coffee silverskin (Ballesteros et al., 2014, 2017; Barbosa et al., 2014; Ribeiro et al., 2010). The hypothesis studied in this thesis is that some variables are able to modulate extraction process originating extracts with different composition. The development of models of response allows to describe the system by equations that fit the experimental data and would allow to predict characteristics within the range of parameters studied. Single-factor studies may allow to preliminary define the region of interest (levels of factors) by varying one parameter and fixing the remaining. However, such experiments do not allow the study of interaction effects between variables and are time consuming. Comprehensive studies may be conducted through factorial, central composite or Box-Behnken designs, depending on the purpose of the research (*e.g.* screening vs optimization) varying in the number of experiments to be conducted, which is usually determinant for the choice of experimental design based on economic reasons, for instance. Moreover, the different designs do not test the same experimental points of the design. For instance, Box-Behnken design prevents the occurrence of problems in the system associated to the extreme values, once the factors are not maintained at their highest or lowest levels simultaneously.

II.3.1. Single-factor design

Infusion experiments were performed varying only one factor (time, temperature, mass-to-volume ratio, and grinding level) while the other three were maintained constant. The constant values for the different factors were: 30 min for time, 80 ºC for temperature, 1 g *per* 30 mL for w/V ratio, and the level 3 for the grinding level (Table II.1).

Table II.1. Conditions used in the study of coffee infusion process through single-factor design.

Time (min)	Temperature (°C)	w/V ratio (g / 30 mL)	Grinding Degree (Level)
5 15 30 120 360	80	1	3
30	10 23 (RT) 40 80 100	1	3
30	80	0.5 1.0 2.0 4.0 6.0	3
30	80	1	1 2 3

II.3.2. Central composite design

The conditions used to study the main effects and interactions between the independent variables were settled based both on the results from single-factor results, as well as experimental limitations (avoiding high temperatures, as boiling water, and prolonged times) and/or new coffee trends as cold brews. An unreplicated 2^4 full-factorial design to evaluate the effect of time (X_1, min), temperature (X_2, ºC), mass to volume (w/V) ratio (X_3, g *per* 30 mL), and grinding level (X_4) was used. Each factor has 2 levels - X_1 (10 and 360 min), X_2 (20 and 80 ºC), X_3 (1 and 6 g *per* 30 mL), and X_4 (level 1 and 3) - coded (+1) and (-1) for the higher and lower limit of each one, respectively (Table II.2). In a two-levels full factorial design 2^k runs are required, where k represents the number of factors to be analysed, resulting in 16 experiments performed. Additionally, a center point with four

replicates was tested to detect the presence of curvature in the responses, to measure the process variability/stability, and to estimate the pure error needed for the analysis of variance. The design was composed of a total of 20 runs that were performed randomly to avoid bias, minimizing the effect of uncontrolled variables. The initial 2^4 full factorial design was augmented with axial experiments to a face-centered central composite design (FCCD, $\alpha = 1$) allowing to estimate higher order terms, namely quadratic terms once an inflation point related to curvature is detected in the design space. Thus, the additional axial (8 runs) and center points (2 runs) were performed to obtain models that better describe the system analysed. The experimental data was fitted to a second-order response surface model represented by a quadratic polynomial equation given as

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_i x_i + \sum_{i=1}^{k} \beta_{ii} {x_i}^2 + \sum_{i=1}^{k} \sum_{j=i+1}^{k} \beta_{ij} x_i x_j \quad (1)$$

where Y is the response observed in the dependent factor of interest, β_0, β_i, β_{ii} and β_{ij} terms represent the constant, linear, quadratic, and two-factor interaction regression coefficients, respectively, while x_i represents the independent variables in a dimensionless coded form. The dependent variables studies were: extraction yield (%w/w), sugars content in the extract (%w/w), galactomannans content in the extract (%w/w), arabinose, mannose, and galactose (%mol) and $K_{mix,\ 405\ nm}$ (mL mg^{-1} cm^{-1}). The different coefficients (main effects, interactions, and high-order effects) were found by analysis of variance (ANOVA) using 95% significance level (p-value). The Pearson's correlation coefficient (r), with confidence level $p<0.05$, was used to describe the relationships among compounds. The experimental data were statistically analysed using Statistica v12 and Minitab v17 software.

Table II.2. Conditions settled for the study of coffee infusion process.

Factor	Lower Level (-1)	Intermediate Level (0)	Higher Level (+1)
Time (X_1, min)	10	185	360
Temperature (X_2, °C)	20	50	80
w/V ratio (X_3, g / 30 mL)	1.0	3.5	6.0
Grinding Degree (X_4, Level)	1	2	3

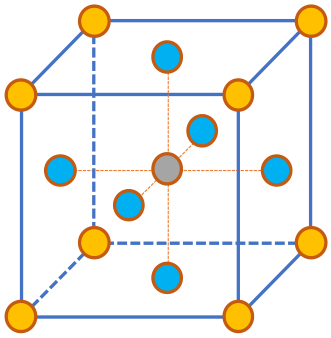


Figure II.1. Representation of the runs performed in the central composite design for conventional infusion method.

II.3.3. Box-Behnken designs

The delimitation of the experimental design space is important as certain conditions are reported to cause the overpressure of the system which would compromise the applicability of the experimental data (Passos & Coimbra, 2013). As preliminary tests showed that overpressure occurs using high w/V ratios (>6 g/60 mL) during prolonged times (10 min), these conditions were used to delimit the extreme levels in the experimental design. The conditions used in each experiment were established according to a Box-Behnken design (BBD) with three independent factors - time (X_1, min), temperature (X_2, ºC) and w/V ratio (X_3, g/ 60 mL) - and 3 levels - low, intermediate, and high coded (-1), (0) and (+1), respectively (Table II.3). All experiments were performed in duplicate (two microwave reactors) comprising the design a 30-run experiment with six replicates of the centre point. The data obtained were adjusted to a second-order surfaced model represented by Eq. (1). In this case, the dependent variables were: extraction yield (%w/w), sugars content (%w/w), galactomannans and arabinogalactans (%w/w), arabinose, mannose, and galactose (%w/w), caffeine and 5-CQA (%w/w) content in the extract. The different coefficients (main effects, interactions, and high-order effects) were found by analysis of variance using 95% significance level (*p*-value). The experimental data were statistically analysed using Statistica v12 and Minitab v17 software.

Table II.3. Conditions settled for the study of microwave-assisted coffee extraction.

Factor	Lower Level (-1)	Intermediate Level (0)	Higher Level (+1)
Time (X_1, min)	1.0	5.5	10
Temperature (X_2, °C)	120	150	180
w/V ratio (X_3, g / 60 mL)	2.0	4.0	6.0

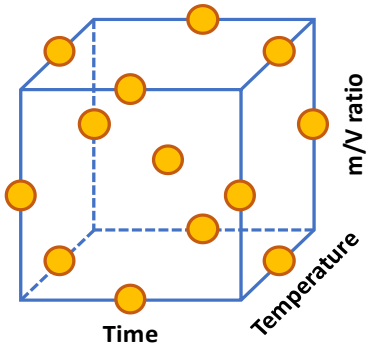


Figure II.2. Representation of the runs performed in the Box-Behnken design for the coffee MAE experiments.

II.4. Extracts characterization

II.4.1. Carbohydrate analysis

II.4.1.1. Neutral sugar analysis

The carbohydrates content of the freeze-dried and spray-dried extracts (from espresso, infusion and microwave-assisted extraction experiments), as well as the instant coffees, the residues of MAE experiments and the initial ground roasted coffee were evaluated through the sum of the amount of the individual sugars achieved after acid hydrolysis and derivatization to alditol acetates, as described in Lopes et al. (2016). Coffee samples (1-2 mg) were incubated with 72% (H_2SO_4) during 1.5 h at 30 ºC with occasional stirring and hydrolysed for 1 h at 120 ºC with 2 M H_2SO_4. $NaBH_4$ (15 % in NH_3 3M,1 h, 30 ºC) was used to reduce the sugars released that were then acetylated with acetic anhydride (3 mL) in the presence of 1-methylimidazole (450 µL) at 30 ºC during 30 min. Alditol acetate derivatives were separated by washes with dichlorometane. GC-FID analysis was performed in a Perkin Elmer Clarus 400 gas chromatograph equipped with a flame ionization detector (FID) and a DB-225 column (30 m x 0.25 mm and 0.15 µm of film thickness, J&W Scientific). The use of 2-deoxyglucose (1 $mg.mL^{-1}$) as internal

standard allowed the quantification of each sugar residue. The temperature of the injector was 220 ºC while the detector operated at 230 ºC. The following oven temperature programme was used: the initial temperature was set at 200 ºC and then rises to 220 ºC at 40 ºC min^{-1}, standing for 7 min, reaching 230 ºC with a 20 ºC min^{-1} rate maintaining this temperature for 1 min. The flow rate of the carrier gas (H_2) was set at 1.7 $mL.min^{-1}$.

II.4.1.2 Glycosidic-linkage analysis

The glycosidic-linkages of polysaccharides were determined through a methylation procedure. The coffee extracts (2 mg) were dissolved in anhydrous dimethyl sulfoxide (1 mL, 24 h). Powdered NaOH (40 mg) was added under an argon atmosphere and the samples were methylated with CH_3I (80 µL) during 20 min with stirring. Then, distilled water was added (2 mL) and the solution neutralized with HCl 1M. Dichloromethane was added (3 mL) and the organic phase was collected and washed twice with distilled water (2 mL). After evaporation to dryness, the sample was remethylated as described previously. Then, the samples were hydrolyzed (TFA 2M, 1h, 121 ºC), and the resultant monosaccharides reduced ($NaBD_4$) and acetylated, as described previously for neutral sugars. The partially methylated alditol acetates (PMAA) were separated and analyzed by a gas chromatography-mass spectrometer (GC-qMS, Shimadzu GCMS-QP2010 Ultra), equipped with a capillary column DB-1 (30 m length, 0.25 mm of internal diameter and 0.10 µm of film thickness, J&W Scientific) as described in Oliveira et al. (2017). The injector temperature was 250 ºC and the following temperature program used: the initial temperature was set at 80 ºC and then rises to 140 ºC at 7.5 ºC min^{-1}, standing for 5 min, reaching 143.2 ºC with a 0.2 ºC min^{-1} rate and then 200 ºC at 12 ºC min^{-1} and finally increasing 50 ºC min^{-1} until 250 ºC maintaining this temperature for 5 min. Helium was the carrier gas at 8.5 mL min^{-1}. The mass quadrupole selective detector operated with an electron impact mode at 70 eV, scanning the range *m*/*z* 50-700 in a 1s cycle in a full scan mode acquisition. The peak area was used to determine the relative amount of each PMAA in the sample.

II.4.2. Caffeine and chlorogenic acid (5-CQA) analysis

The determination of caffeine and 5-caffeoylquinic acid (5-CQA) was performed using the freeze-dried and spray-dried coffee extracts by the preparation of aliquots of 10 $mg.mL^{-1}$ that were passed through 0.22 µm filters prior HPLC injection (2 replicates). The runs were performed on a HPLC with a photodiode array detector (DAD) apparatus equipped with a C18 column (Waters Sherisorb S10 ODS2, 4.6 mm x 250 mm, 10 µm) equilibrated with formic acid 5% (eluent A). The samples were eluted at a flow rate of 0.8 $mL\ min^{-1}$ with a gradient composed by 95% of eluent A and 5% eluent B (methanol) in the first 5 min. Then the eluent B increased to 40% from 5 to 45 min, from 45 to 65 min increased from 40% to 70 % and from 65 to 75 min the eluent B decreased to the initial 5% as described by Nunes et al. (2012). The caffeine peak was detected at 280 nm and for 5-CQA at 325 nm. For quantification purposes, calibration curves of caffeine (R^2 = 0.997) and 5-CQA (R^2 = 0.993) were prepared.

II.4.3. Lipids

Soxhlet methodology with glass fiber cartridges (4 h, *n*-hexane, 80 ºC) was used to extract the total lipids from 1 g of coffee extracts and initial roasted coffee (3 replicates). The hexane extract was rotary evaporated to dryness (< 40 ºC). A clean-up step was then performed for elimination of co-extracted compounds (*e.g.* caffeine) with liquid-liquid extractions (5 mL) with hexane/water (1:1) and the amount of lipids quantified by weight after solvent evaporation of hexane fraction.

II.4.4. Fractionation of coffee extracts

Defatted coffee samples (3 replicates) were dissolved in distilled water (8 mL) and dialysed (MW cutoff 12-14 kDa, Visking size 8, Medicell International Ltd., London, UK) against distilled water (120 mL) at 4 ºC with constant stirring. After the first 6 h, the dialysate was collected, frozen and freeze-dried, giving the low molecular weight material (LMWM). The dialysis procedure continued with 3-4 distilled water renewals until conductivity reached values lower than 5 $\mu S\ cm^{-1}$. The content of the dialysis bag volume

was made up to 30 mL with distilled water and a fraction (1 mL) was frozen and freeze-dried, giving the high molecular weight material (HMWM). Then, the retentate was centrifuged (24,400 g, 15 min) and the precipitate and supernatant obtained were frozen and freeze-dried, giving the high molecular weight material soluble (HMWMsol) and insoluble (HMWMins) in cold water, respectively.

II.4.5. Protein

The polymeric fractions (HMWM, HMWMsol and HMWMins) were used to determine the nitrogen content by elemental analysis in a Truspec 630-200-200 elemental analyser with a TCD detector. The nitrogen content was converted to protein content (%w/w) using the 5.5 factor, as described by Bekedam et al. (2006).

II.4.6. Density, viscosity, pH and electrical conductivity measurements

A helium pycnometer was used to determine the true density of the extract solid samples, and the density of coffee solutions (30 $mg.mL^{-1}$) was determined by weighing the mass of the sample on an analytical balance with a defined volume at 20 ºC (6 replicates). A Cannon-Fenske routine viscometer (Size 50) was used to perform the viscosity measurements in distilled water (30 $mg.mL^{-1}$), kept in a thermostatic water bath at 25.00 ± 0.01 ºC. It was recorded the efflux time in triplicate for each independent extraction with an electronic digital stopwatch (0.01 s) that was used to determine the kinematic viscosity of the samples, multiplying the efflux time by the constant value provided by the manufacturer for that viscometer. The samples were then used to determine the pH and electrical conductivity measurements with a Crison pH-meter at 25 ºC (3 replicates).

II.4.7. Colour measurements

Colour of the samples (solid and in solution - 30 $mg.mL^{-1}$) was assessed with Konica Minolta CM 2300d spectrophotometer and computed through SpectraMagicTM NX software, obtaining the CIELab coordinates L^* (lightness), a^* (red/green), and b^* (yellow/blue). Chroma (C^*) was calculated through $C^* = (a^{*2} + b^{*2})^{1/2}$ and hue angle (h_{ab})

as h_{ab} = $\tan^{-1}$ (b^*/a^*). The brown colour of the extracts was also spectrophotometrically evaluated through the specific extinction coefficient at 405 nm ($K_{mix,405nm}$) determined in a microplate reader using several dilutions (0-1 $mg.mL^{-1}$ in distilled water) prepared with the freeze-dried coffee extracts (Bekedam et al., 2006; Lopes, et al., 2016). Simultaneously, the measure was performed at 280 and 325 nm allowing to determine the $Kmix_{,280nm}$ and $K_{mix,325nm}$.

II.4.8. Antioxidant activity

The antioxidant activity of the MAE coffee extracts was determined through DPPH assay. The reaction mixture consists of 50 µL (0.1 $mg.mL^{-1}$) and 250 µL of DPPH solution (8.6×10^{-5} M in ethanol) placed in a 96-well microplate that stands for 30 min in darkness at 30 ºC prior to the spectrophotometric measurement (517 nm). Water with DPPH was used as a control solution. DPPH scavenging activity was calculated according to Eq. (2):

$$\%\text{Inhibition} = \left[\frac{A_0 - (A - A_b)}{A_0}\right] \times 100 \quad (2)$$

representing A_0 the control (DPPH without sample), A the absorbance of the sample with DPPH and A_b the sample without DPPH (blank sample) (Souza et al., 2012).

II.4.9. FTIR analysis

Fourier-transform infrared spectroscopy (FTIR) analysis was performed in an infrared spectrometer (Bruker Alpha Platinum-ATR) in the mid-infrared region (4000-400 cm^{-1}) with a resolution of 4 cm^{-1} and 32 scans, operated in a room with controlled temperature (25 %) and humidity (35 %). Extract samples were placed on the crystal of the attenuated total reflectance accessory (ATR), cleaned with ethanol (70 %) between each measurement. The spray-dried extract samples were also dissolved in water (30 $mg.mL^{-1}$) and freeze-dried prior to FTIR analysis, giving SDFD. Five replicates spectra were obtained for each sample in a random order. The FTIR spectra were baseline and SNV (standard normal deviate) corrected before PCA analysis that were performed using MetaboAnalyst 4.0 (web interface).

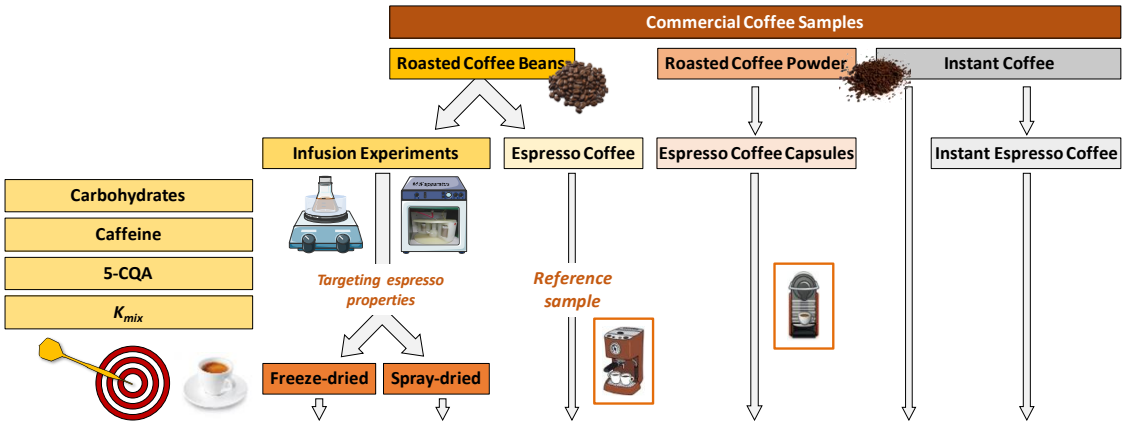
II.4.10. Volatile composition

A HS-SPME methodology was used to study the composition of coffee extracts and espresso coffee. The SPME device included a fused silica fibre coating partially cross-linked with 50/30 μm DVB/CAR/PDMS (1 cm stable-flex^{TM} fused silica fibre, coated with partially cross-linked 50/30 μm divinylbenzene/Carboxen^{TM}/ polydimethylsiloxane). The fibre was conditioned before use, according to the specific manufacturer's recommendations. For each HS-SPME assay, 1.2 g of coffee extract was dissolved in 40 mL of distilled water, kept at 70 ºC, and placed into a 120 mL glass vial, corresponding to a ratio of the volume of the liquid phase to the headspace volume (1/β) of 0.5. Each glass vial was previously placed in a thermostated bath adjusted to 60.0 ± 0.1°C, during 5 min. Then, the sample was introduced in the vial and the SPME fibre was manually inserted into the sample headspace vial for 3 min, at constant stirring and 400 rpm. Sampling was performed in triplicate. The SPME coating fibre containing the headspace volatile compounds was manually inserted into the GC injection port at 250 ºC and kept for 3 min for desorption. The injection port was lined with a 0.75 mm (I.D.) splitless glass liner. The desorbed volatile compounds were separated in an Agilent Technologies 6890 N Network gas chromatograph, equipped with a 30 m×0.32 mm (I.D.), 0.25 μm film thickness DB-FFAP fused silica capillary column (J&W Scientific), connected to an Agilent 5973 quadrupole mass selective detector. Spitless injections were used. The oven temperature program used was as follow: initial temperature was 40 ºC with a linear increase of 5 ºC min^{-1} up to 220 ºC, followed by linear increase of 10 °C min^{-1} until 250 °C, remaining thus until the end of the run (42 min). The transfer line was heated at 255 ºC. The helium carrier gas had a flow of 1.7 mL min^{-1} and a column head pressure of 2.61 psi. The mass spectrometer was operated in the electron impact mode (EI) at 70 eV scanning the range of 33-300 *m/z* in 3 cycles s^{-1}, in a full scan acquisition mode. In order to identify the various volatile compounds, the respective mass spectra were compared with those of the software-included library (Wiley 275). Identification was also possible by comparing the calculated retention indexes (RI_{calc}) with those reported in the literature (RI_{Lit}), for DB-FFAP columns or other columns with similar phases. A series of C8-C20 *n*-alkanes was used to calculate the experimental retention indexes, according to the equation of van Den Dool & Kratz

(1963). Heatmaps and PCA were used to present the results after statistical analysis using MetaboAnalyst 4.0 (web interface) and the peak area of each compound.

II.4.11. Foam and effervescent experiments

Foamability of coffee extracts was tested using an adaptation of the Bikerman method (Mosalux device), (Pueyo et al., 1995) as described in Coelho et al. (2011). CO_2 of analytical grade from a cylinder was injected through the bottom of a column equipped with a glass-frit fitted where the coffee solution (7 mL, 30 $mg.mL^{-1}$) was placed. The CO_2 flow rate (1.2 L/h) and pressure (1 bar) were maintained constant for 50 seconds and then detached. Foamability was evaluated by the measure of the foam height increase on the top of coffee solution (in cm) and converted to mL by measuring the volume and height of coffee solutions. The time of foam stability formed was measured until the liquid below the foam appeared. Foamability was also evaluated with an effervescent formulation approach: sodium bicarbonate (72 mg), citric acid (60 mg) and the extracts (1.2 g, 3 replicates) were weighed and mixed before the addition of water at 70 ºC, after preliminary tests with different quantities of the compounds. The foamability was evaluated measuring the foam volume in the cup (determined by height increase in cm and converted in mL), and the foam stability by measuring the appearance of the halo of coffee solution beneath the foam (in seconds). The variation in pH was evaluated with a Crison pH-meter when the solution cooled down to 25 ºC.



Samples codes / Compound/Property	FD	SD	EC1	EC2	EC3	EC4	EC5	IC1	IC2	IC3	IC4
Carbohydrates	✓	✓	✓	✓				✓	✓		
Glycosidic linkages	✓	×	✓	×				×	×		
Caffeine	✓	✓	✓	×				✓	×		
5-CQA	✓	✓	✓	×				✓	×		
Lipids	✓	✓	✓	×				✓	×		
pH	✓	✓	✓	×				✓	×		
Viscosity	✓	✓	✓	×				✓	×		
Density	✓	✓	✓	×				✓	×		
Electrical Conductivity	✓	✓	✓	×				✓	×		
Colour Parameters (Cielab)	✓	✓	✓	×				✓	×		
K_{mix}	✓	✓	✓	×				✓	×		
HMWM	✓	×	✓	×				✓	×		
LMWM	✓	×	✓	×				✓	×		
Melanoidins	✓	×	✓	×				✓	×		
Protein	✓	×	✓	×				✓	×		
Foam Studies	✓	✓	✓	×				✓	×		
Volatiles Compounds	✓	✓	✓	✓				✓	✓ / × / ×		
FTIR analysis	✓	✓	✓	✓				✓	✓		

Figure II.3. Scheme of the samples and analyses performed in this thesis. The symbol ✓ indicates that the analysis was performed for that sample, while × means that it was not performed.

Chapter III

Results and Discussion

III.1. INFUSION PROCESS: CONVENTIONAL EXTRACTION

III.2. MICROWAVE-ASSISTED EXTRACTION OF COFFEE

III.3. MODULATION OF INFUSION PROCESS TARGETTING ESPRESSO PROPERTIES

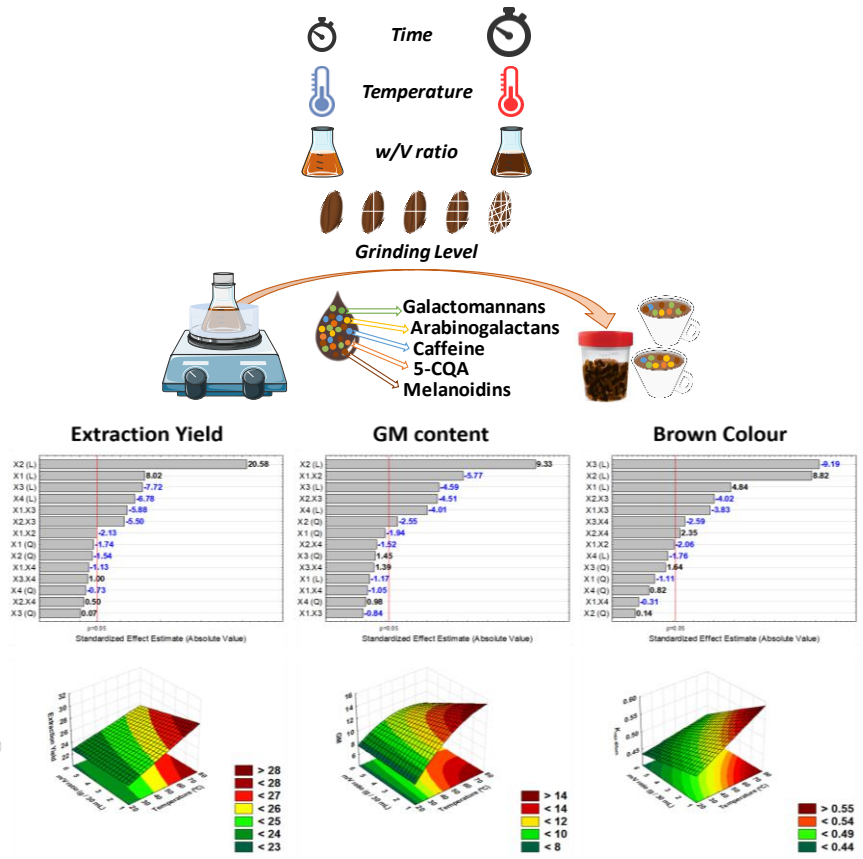
III.1. INFUSION PROCESS: CONVENCIONAL EXTRACTION

The content of this chapter was published in the following publication:

Lopes, G. R., Passos, C. P., Rodrigues, C., Teixeira, J. A., & Coimbra, M. A. (2019). Modulation of infusion processes to obtain coffee-derived food ingredients with distinct composition. *European Food Research and Technology*, *245* (10), 2133-2146.

III.1.1. Abstract

Coffee infusion experiments were conducted to infer how operational variables (time, temperature, mass to volume ratio, and grinding) might affect the efficiency and/or selectivity of compounds extraction. Although the different variables have extensively being reported independently, to the best of our knowledge no experimental design was yet delineated to study the simultaneous effect of variables in coffee composition. This study fulfils this gap by constructing surface models that reflect the responses in a wide-ranging design space. The freeze-dried extracts were compared regarding the overall yield of extraction, carbohydrate content and composition, caffeine, chlorogenic acid (5-CQA) content, colour, and viscosity. Temperature was the major factor for coffee extracts differentiation, regarding both overall and carbohydrates yield and composition. The extraction process efficiency is more related to galactomannans extraction than arabinogalactans. Varying operational conditions, coffee extracts with distinct chemical properties are obtained from the same roasted coffee, broadening their applications in food formulations.



III.1.2. Aim of the study

In this study, fulfilling the hypothesis that different operational variables provides extracts with different compositions and properties from the same roasted coffee beans, the effect of time and temperature of extraction, mass of coffee to volume of water ratio, and coffee grinding level were tested on the chemical composition of the brew (viewed as a freeze-dried coffee extract) obtained after extraction. The use of the same coffee for all experiments eliminated the variability related to roasting degree or coffee species (Chapter I). The intent was to understand how different extraction conditions affect the properties of coffee extracts in a fixed water volume to understand the variability that may be achieved regarding coffee brew compounds. Moreover, a statistical and holistic approach was used to infer the factors that affect the coffee extraction system, constructing significant models for modulation of the coffee extract characteristics. This approach will allow to obtain coffee extracts with the desired chemical properties regarding their use in new food formulations.

III.1.3. Results and discussion

III.1.3.1. Grinding results

In Figure III.1.1 is presented the profile of coffee particles size for the three levels tested. For the extraction of compounds from roasted coffee beans, they must be grounded, breaking the matrix, and making the compounds accessible for extraction when in contact with water. The degree of grinding used, from coarser to finer particles, should affect the extraction rate due to the different surface area available or the porosity between the coffee particles which defines the water flow through coffee cake during coffee percolation processes (Illy & Viani, 2005; von Blittersdorff & Klatt, 2017). The grinding level may influence the percolation of water or the extraction of compounds due to the variation of surface area of the particle. Once in this experiment the particles were in contact with the water, without the formation of a coffee cake, the effect regarding grinding is related to the extraction surface area of contact and the diffusion phenomena. The increase of fines, damaged cells, and smaller particles increases the extraction of solids through the reduction of the mass transfer resistance (Moroney et al., 2015).

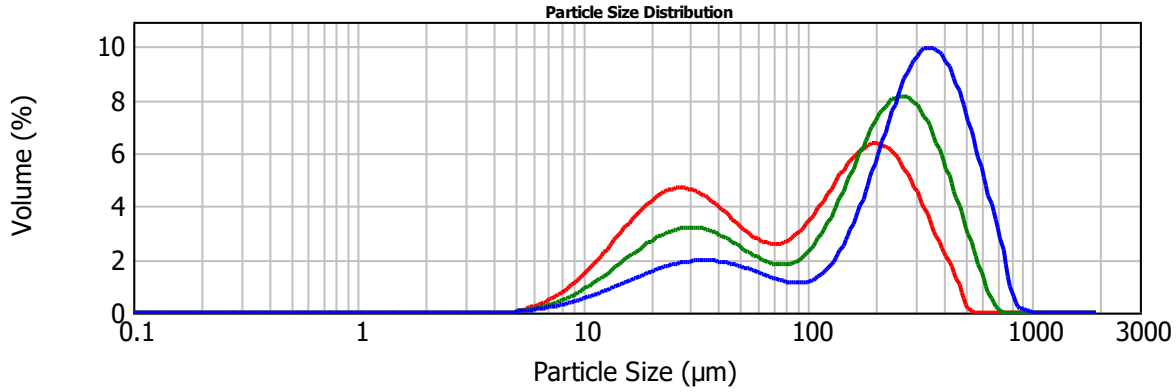


Figure III.1.1. Profile of the particle size distribution of the grinding levels used in the coffee extraction experiments: level 1 (**red line**), level 2 (**green line**), and level 3 (**blue line**).

III.1.3.2. Extraction yield

The single-factor experiments of coffee extraction process showed that when varying the temperature, the range of values (7.2%, between the minimum and maximum) was higher than for the other factors studied (2.1, 1.9, and 1.6% for time, w/V ratio or grinding level, respectively) at a defined temperature (80 ºC) (Table III.1.1) Longer extraction times and temperatures increase the extraction of coffee compounds, which also occurs with lower w/V ratio and finer particles associated to the lower level of grinding. These results agree with several studies regarding infusion type extraction methods, as well as brews as espresso coffee, that deals with shorter extraction times (Andueza et al., 2003b, 2007; Merritt & Proctor, 1959; Petracco, 2001; Voilley & Simatos, 1979). Several reports have been trying to find the influence of operational parameters, one-by-one, on different coffee brews properties and/or compare the composition of the coffee brews prepared with different methods (Andueza et al., 2003b, 2007; Gloess et al., 2013; Merritt & Proctor, 1959; Parenti et al., 2014). However, there are so many variables that the uniformity of conditions and results is difficult to achieve in this way. Mathematical modelling through physical-engineering approaches has been useful for the understanding of the coffee extraction processes (Espinoza-Pérez et al., 2007; Melrose et al., 2018; Moroney et al., 2015; Voilley & Simatos, 1979). Nevertheless, these models do not reflect the overall composition obtained through the variation of the different extraction conditions.

Table III.1.1. Extraction yield results from the single-factor experiments of roasted coffee extractions.

Process Variables				Extraction Yield[a]
Time (min)	**Temperature** (°C)	**w/V ratio** (g/ 30 mL)	**Grinding Level**	(% w/w)
5				25.9±0.6
15				26.2±0.6
30	80	1	3	26.6±0.3
120				27.2±0.3
360				28.0±0.4
	10			20.5±0.0
	23 (RT)			22.4±0.1
30	40	1	3	23.8±0.4
	80			26.6±0.3
	100			27.7±0.1
		0.5		26.7±0.0
		1.0		26.6±0.3
30	80	2.0	3	26.2±0.2
		4.0		25.7±0.0
		6.0		24.8±0.2
			1	27.8±0.5
30	80	1	2	27.0±0.8
			3	26.6±0.3

[a] : mass of freeze-dried coffee compared to the mass of roasted coffee prior to the extraction process.

As the single-factor experiments do not allow the determination of the combined effects for the factors or independent variables, a wide range of conditions were used. The idea was to infer the main effects of this process and the interactions between them, which is normally not studied in coffee extraction processes. The higher level of temperature (80 ºC) was selected to avoid the loss of solvent and pressure problems, related to a closed system when using higher extraction times. The higher level of time was selected (6 h) according to the literature, as the increase of the extraction time for more than 5-10 h is not reported to increase the extracted solids for coffee brew (Moroney et al., 2015). Moreover, coffee cold brews extraction methods were associated to prolonged extraction times (6 or more h) (Angeloni Guerrini, Masella, Innocenti et al., 2019; Mestdagh et al., 2017). Indeed, the lower level of temperature was chosen considering the lack of characterization, namely concerning carbohydrates content and composition, related to the coffee cold brews, that gained attention in the last years. The extraction yield ranged from 20.4 to 29.9 %w/w among all conditions studied (Table III.1.2). These results are in accordance to literature, where extraction yield ranges from 18 to 32%, depending on the method of coffee extraction used (Clarke, 1987; Gloess et al., 2013; Petracco, 2001).

Table III.1.2. Roasted coffee extractions settled according to a face-centered central composite design.

Run Order	Process Variables[a]				Extr. Yield[b]	Sugars[c]	Rha	Ara	Man	Gal	Glc	$K_{mix,\ 405nm}$	Caffeine[c]	5-CQA[c]	Kinematic Viscosity
	Time	Temperature	w/V ratio	Grinding Level											
	(X_1, min)	(X_2, °C)	(X_3, g/ 30 mL)	(X_4)	(Y_1, %w/w)	(Y_2, %w/w)			%mol			(Y_4, mL mg^{-1} cm^{-1})	(%w/w)	(%w/w)	cSt
1*	185 (0)	50 (0)	3.5 (0)	2 (0)	25.2	21.5±0.9	3.8±0.0	16.2±0.2	47.3±0.4	28.2±0.2	4.5±0.5	0.48±0.02	9.2±0.3	2.5±0.1	-
2	10 (-1)	20 (-1)	6.0 (+1)	1 (-1)	22.6	16.4±0.5	5.3±0.0	20.1±0.0	35.6±0.2	33.9±0.1	5.1±0.1	0.44±0.00	9.5±0.3	2.7±0.1	1.04±0.02
3	360 (+1)	80 (+1)	6.0 (+1)	1 (-1)	25.8	21.8±2.6	4.3±0.4	18.7±1.6	33.4±5.2	39.4±2.8	4.2±0.4	0.49±0.01	8.6±0.0	2.2±0.1	1.06±0.00
4	10 (-1)	20 (-1)	1.0 (-1)	3 (+1)	20.4	14.8±1.0	5.7±0.3	21.3±0.2	32.9±0.7	33.5±1.3	6.5±0.6	0.41±0.02	9.4±0.1	2.7±0.0	-
5	360 (+1)	20 (-1)	1.0 (-1)	3 (+1)	23.2	18.3±2.1	4.2±0.3	17.4±0.6	41.2±0.8	31.7±0.1	5.5±0.5	0.52±0.03	8.9±0.3	2.6±0.0	-
6	10 (-1)	20 (-1)	1.0 (-1)	1 (-1)	22.1	17.6±0.1	5.2±0.0	19.1±0.6	37.0±1.0	33.2±0.2	5.5±0.5	0.47±0.02	9.7±0.0	2.7±0.0	-
7*	185 (0)	50 (0)	3.5 (0)	2 (0)	26.2	23.0±2.3	3.7±0.1	15.7±0.6	48.9±0.5	27.7±0.3	4.1±0.4	0.51±0.01	8.8±0.1	2.3±0.0	-
8	10 (-1)	80 (+1)	1.0 (-1)	1 (-1)	26.9	29.6±4.0	2.8±0.2	11.8±0.6	57.0±3.4	24.4±2.4	4.0±0.7	0.53±0.03	7.1±0.2	2.3±0.0	-
9	10 (-1)	80 (+1)	6.0 (+1)	3 (+1)	24.7	21.6±0.2	3.7±0.0	15.4±0.3	49.6±0.7	27.4±0.4	3.9±0.0	0.48±0.03	8.6±0.2	2.5±0.0	1.09±0.00
10	360 (+1)	80 (+1)	1.0 (-1)	3 (+1)	27.9	19.3±0.8	3.7±0.2	16.1±0.7	48.8±1.3	26.8±0.1	4.5±0.2	0.64±0.06	8.4±1.2	2.2±0.2	-
11	10 (-1)	80 (+1)	1.0 (-1)	3 (+1)	26.4	26.5±3.6	3.4±0.0	13.1±1.3	56.3±5.6	23.5±3.5	3.6±0.7	0.59±0.00	8.5±0.0	2.3±0.1	-
12	360 (+1)	20 (-1)	6.0 (+1)	3 (+1)	22.3	18.4±0.4	4.6±0.0	18.6±0.4	38.4±0.9	32.5±0.6	5.9±0.1	0.44±0.02	8.8±0.1	2.6±0.1	1.05±0.01
13	360 (+1)	20 (-1)	6.0 (+1)	1 (-1)	23.3	21.5±1.6	3.7±0.1	20.1±5.1	41.4±3.3	29.7±1.5	5.0±0.2	0.49±0.02	9.0±0.1	2.7±0.1	1.05±0.01
14*	185 (0)	50 (0)	3.5 (0)	2 (0)	25.8	22.5±1.4	3.4±0.2	14.7±0.0	47.6±1.1	29.9±0.4	4.4±0.4	0.49±0.03	8.9±0.0	2.5±0.1	-
15	360 (+1)	80 (+1)	1.0 (-1)	1 (-1)	29.9	29.7±1.8	2.9±0.2	12.4±0.1	55.0±1.4	26.6±1.5	3.2±0.0	0.62±0.04	7.4±0.7	1.9±0.1	-
16	360 (+1)	20 (-1)	1.0 (-1)	1 (-1)	25.3	20.1±2.8	4.2±0.1	16.6±0.2	43.3±0.4	31.1±0.9	4.8±0.2	0.54±0.01	9.2±0.3	2.4±0.1	-
17	10 (-1)	80 (+1)	6.0 (+1)	1 (-1)	26.0	24.8±0.8	3.3±0.2	13.5±0.4	52.0±1.5	27.0±0.2	4.2±0.7	0.51±0.03	8.7±0.1	2.4±0.1	1.12±0.00
18	360 (+1)	80 (+1)	6.0 (+1)	3 (+1)	24.5	18.9±1.4	4.3±0.1	18.4±0.6	34.0±0.1	38.8±0.7	4.6±0.1	0.45±0.01	8.4±0.1	2.3±0.2	1.05±0.01
19	10 (-1)	20 (-1)	6.0 (+1)	3 (+1)	21.5	16.1±1.2	5.5±0.3	20.5±1.5	33.3±0.7	34.4±1.2	6.3±0.1	0.40±0.01	9.7±0.1	2.8±0.0	1.02±0.00
20*	185 (0)	50 (0)	3.5 (0)	2 (0)	25.1	24.4±0.9	3.3±0.1	14.3±0.8	51.0±2.8	27.7±2.0	3.8±0.0	0.49±0.05	8.8±0.0	2.5±0.0	-
21*	185 (0)	50 (0)	3.5 (0)	2 (0)	25.0	22.2±0.7	3.0±0.0	13.4±0.2	50.0±0.2	29.6±0.2	3.9±0.2	0.47±0.04	8.7±0.0	2.6±0.0	-
22	185 (0)	50 (0)	1.0 (-1)	2 (0)	26.5	21.8±1.3	2.5±0.1	11.6±0.6	53.2±0.6	28.1±0.2	4.5±0.2	0.54±0.00	8.3±0.1	2.3±0.0	-
23	185 (0)	50 (0)	3.5 (0)	1 (-1)	25.7	23.3±2.2	3.2±0.3	13.0±0.4	50.7±1.5	28.1±0.8	5.0±1.2	0.48±0.02	8.3±0.3	2.5±0.0	-
24	185 (0)	50 (0)	3.5 (0)	3 (+1)	25.2	20.8±3.8	3.5±0.1	15.1±1.2	47.6±2.3	29.4±1.3	4.4±0.3	0.51±0.04	8.9±0.1	2.4±0.1	-
25	185 (0)	50 (0)	6.0 (+1)	2 (0)	24.8	22.9±0.4	2.9±0.1	14.5±0.4	47.7±1.1	31.2±0.8	3.7±0.2	0.46±0.01	8.5±0.1	2.4±0.1	-
26	185 (0)	80 (+1)	3.5 (0)	2 (0)	27.0	21.6±2.0	3.2±0.3	13.7±0.6	48.0±0.3	30.7±0.4	4.4±0.5	0.52±0.00	8.2±0.4	2.4±0.0	-
27	10 (-1)	50 (0)	3.5 (0)	2 (0)	24.2	18.1±1.5	3.5±0.0	14.7±0.2	46.7±1.5	30.3±1.3	4.7±0.3	0.46±0.03	9.3±0.0	2.9±0.0	-
28	360 (+1)	50 (0)	3.5 (0)	2 (0)	26.2	19.3±1.5	3.5±0.2	14.3±0.6	48.3±2.5	29.6±0.7	4.3±1.1	0.48±0.01	8.8±0.4	2.4±0.0	-
29	185 (0)	20 (-1)	3.5 (0)	2 (0)	23.5	17.6±2.9	4.8±0.3	18.0±0.1	37.4±1.6	34.2±0.7	5.6±0.7	0.45±0.02	9.4±0.3	2.7±0.1	-
30*	185 (0)	50 (0)	3.5 (0)	2 (0)	25.4	22.6±0.9	3.6±0.1	13.1±0.4	52.7±2.9	26.4±2.0	4.1±0.5	0.47±0.01	8.6±0.1	2.4±0.2	-
				Roasted Coffee Powder		48.8±3.7	0.5±0.2	6.5±0.6	45.5±0.8	28.4±0.9	19.1±0.6		1.9±0.2	0.7±0.1	

[a] : The process variables are shown in real and (coded) values; [b] : mass of freeze-dried coffee compared to the mass of roasted coffee prior to the extraction process; [c] : mass of compound present in the freeze-dried extract; *: center points of the design.

Considering the runs of the central composite design, the mass of freeze-dried extract and the volume of water used in the experiments (30 mL), the coffee solution after filtration process had 6.8-52.0 g L^{-1} of total solids (204-1560 mg *per* cup). The range of values for the different brewing methods (10.3-55.7 g L^{-1}) found in literature (Angeloni et al., 2019; Gloess et al., 2013; Illy & Viani, 2005; Lopes et al., 2016; Maeztu et al., 2001a; Petracco, 2001) is in accordance with the results obtained in the present study. The solids concentration in the brew or filtrate obtained (0.7-4.9 %w/w) are relatable to diluted home brewing processes and are quite far away from high concentration of soluble solids (15-25 %w/w) obtained in the final extracts of industrial coffee extraction processes (Clarke, 1987). These results suggest that applying an infusion extraction procedure and modulating the extraction parameters, different extraction yields and total solids concentrations can be achieved, with comparable results to the different homemade coffee brews. Pareto chart (Figure III.1.2) represents the effects on extraction yield that are statistically significant (at 95% confidence level) as the ones beyond the vertical plotted line with the length of the bars proportional to the standardized effect in the model.

Figure III.1.2 shows that the linear terms of all variables (X_1-X_4) exhibit significant effect on coffee extraction yield, which does not happen when considering the quadratic terms, assuming this process a predominant linear behaviour all over the design space. However, Pareto chart evidences that linear temperature term exerts clearly the most preponderant (explained 59% of the variability observed) and positive effect, with time, w/V ratio, and grinding level linear terms with a quite similar influence. Time had a positive effect, meaning that the longer the extraction the higher the extraction yield, while the w/V ratio and grinding level exhibit a negative effect (*i.e.* higher ratio leads to lower extraction yield and the coarser the coffee particles the lower the extraction yield). The w/V ratio effect is elucidated once water becomes more saturated and with decreasing ability to extract more compounds.

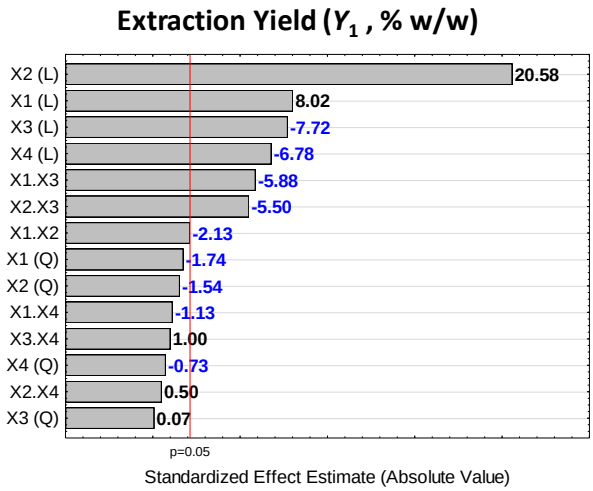


Figure III.1.2. Pareto chart for the effects of time (X_1), temperature (X_2), w/V ratio (X_3), and grinding level (X_4) during the coffee extraction experiments on the extraction yield (Y_1, %w/w). The negative and positive effects are highlighted in blue and black, respectively. L and Q represent the linear and quadratic effects, respectively. The region (right side) of statistical significance (95% confidence level) is defined by the vertical line.

The grouping of all runs regarding the temperature effect shows a clear distinction between the levels studied: 20.4-25.3 %w/w (20 ºC), 24.2-26.5 %w/w (50 ºC), and 24.5-29.9 (80 ºC). However, it also shows similar extraction yields regardless the temperature used, *i.e.* extraction at 20 ºC with higher extraction yield than some experiments performed at 50 ºC or 80 ºC. Thus, with all temperatures may occur equal extraction yields. However, this is based only on the extraction yield, *i.e.* considering that the extract composition obtained is barely the same in all conditions. For the remaining variables, a greater dispersion was verified for the grouped values due to the greater influence of temperature over the other factors. Pareto chart (Figure III.1.2) also shows as significant two 2-way interactions: the terms $X_1.X_3$ and $X_2.X_3$ (p<0.05); and marginally the term $X_1.X_2$ (p=0.0505) (Table 1 in Annex A.III.1.). When an interaction is significant it means that the effect of a term on the response is distinct at different levels of another independent variable. Thus, the analysis of significant interactions indicates that the effect of w/V ratio is dependent on the level of time and temperature, being more significant when using longer times and higher temperatures (Figure III.1.3). Moreover, the strict analysis of the full factorial of the design space allows to infer that 3- and 4-way interactions are non-significant in this system. The experimental data for coffee extraction yield fits a full quadratic model able to explain nearly 98% of the variability occurred in the system (R^2 = 0.98) with a non-significant lack-of-fit, meaning a close agreement between the experimental data and the results predicted by the model. The non-significant terms were removed in order to

simplify/reduce the model (Table 1 in Annex A.III.1.). Thus, the data fit a reduced but significant (p<0.0001) linear model for extraction yield with a high determination coefficient (R^2 = 0.92) and high predictive ability (R^2 = 0.85), as presented in Table III.1.3.

Table III.1.3. Models developed for the description of the variation in dependent variables (Y_1 - extraction yield, Y_2 - sugars, Y_3 - galactomannans and Y_4 - $K_{mix,\ 405nm}$) as function of the parameters studied (X_1 - time, X_2 - temperature, X_3 - w/V ratio, and X_4 - grinding level) with the corresponding coefficients of determination (R^2). The models are expressed in terms of coded values ((-1), (0), (+1)).

Response	Model Equation	R^2
Extr. Yield	$Y_1 = 24.95 + 0.76\ X_1 + 1.94\ X_2 - 0.73\ X_3 - 0.64\ X_4 - 0.59\ X_1.X_3 - 0.55\ X_2.X_3$	0.92
Sugars	$Y_2 = 22.02 + 0.10\ X_1 + 2.94\ X_2 - 0.85\ X_3 - 1.67\ X_4 - 1.31\ X_1^2 - 1.64\ X_1.X_2 - 1.22\ X_2.X_3$	0.82
GM	$Y_3 = 11.68 - 0.33\ X_1 + 2.61\ X_2 - 1.28\ X_3 - 1.12\ X_4 - 1.62\ X_2^2 - 1.71\ X_1.X_2 - 1.34\ X_2.X_3$	0.88
Kmix$_{405nm}$	$Y_4 = 0.494 + 0.021\ X_1 + 0.038\ X_2 - 0.040\ X_3 - 0.008\ X_4 - 0.017\ X_1.X_3 - 0.018\ X_2.X_3 + 0.011\ X_2.X_4 - 0.012\ X_3.X_4$	0.89

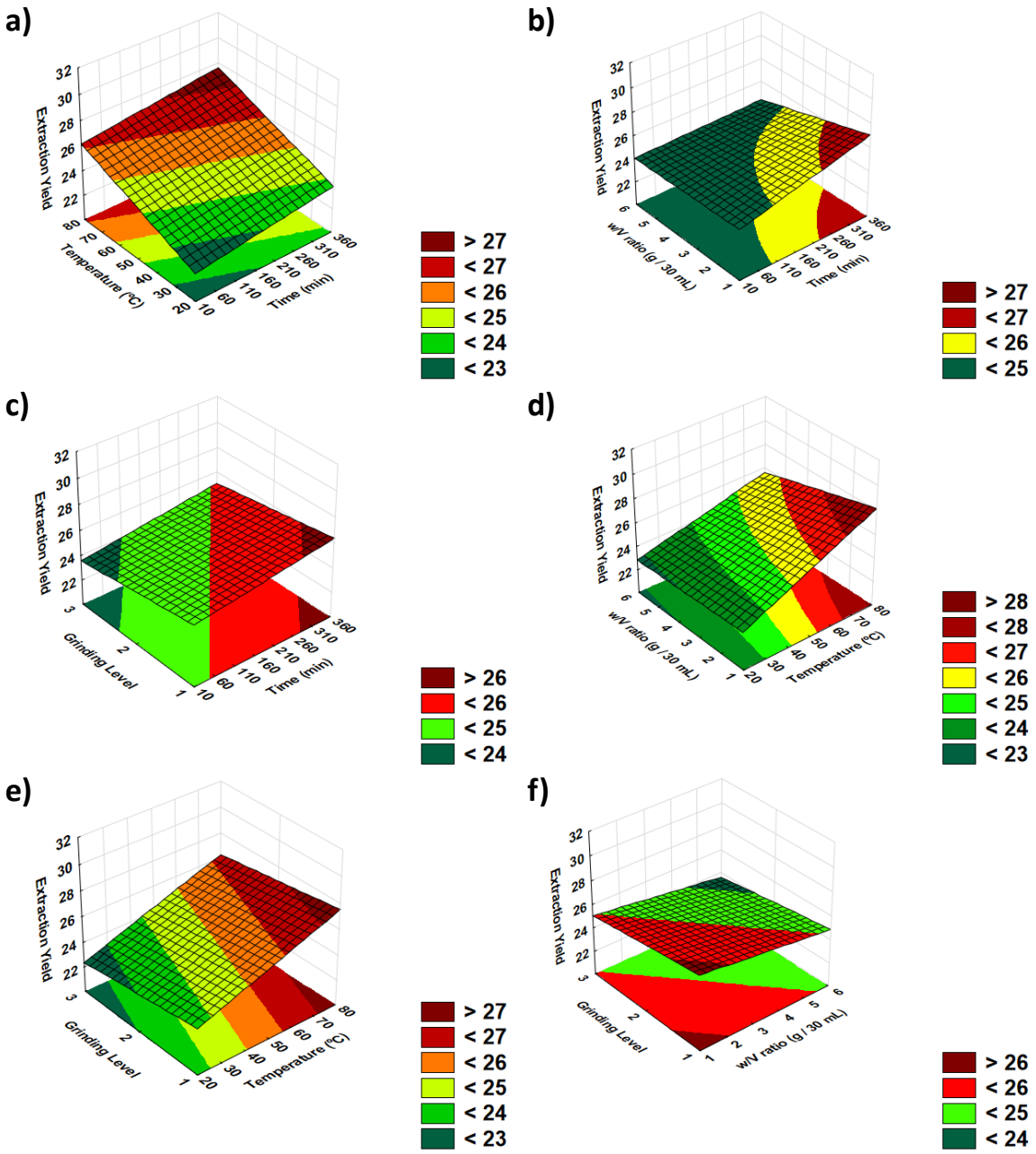


Figure III.1.3. Response surface plots (3D) of the model developed representing the extraction yield (Y_1, %w/w) showing the effect of the different extraction parameters. In each plot two of the independent variables were maintained at their intermediate level: *time* (X_1) - 185 min; *temperature* (X_2) - 50 ºC; *w/V ratio* (X_3) - 3.5 g / 30 mL; (X_4) - *grinding level* – level 2. a) time (X_1) × temperature (X_2); b) time (X_1) × w/V ratio (X_3); c) time (X_1) × grinding level (X_4); d) temperature (X_2) × w/V ratio (X_3); e) temperature (X_2) × grinding level (X_4); f) w/V ratio (X_3) × grinding level (X_4).

III.1.3.3. Sugars content

Polysaccharides constitute a considerable fraction of roasted coffee beans and coffee brews (Nunes & Coimbra, 2001; Oosterveld et al., 2003b). Only the drastic extraction conditions used in industrial soluble coffee processing are able to degrade the

polysaccharides (Clarke, 1987). Thus, the analysis performed is a valid estimation of carbohydrate content of the coffee brews, once the extraction conditions used in this experiment are presumably unable to degrade coffee polysaccharides. The single-factor experiments (Table III.1.4) point out that the factor that seems to exhibit higher preponderance for carbohydrates content in coffee extracts is temperature, with a range of variation of 12% when compared to 3-7% from the other experiments.

Table III.1.4. Sugars results from the single-factor experiments of roasted coffee extractions.

Process Variables				Sugars[a]	Rha	Ara	Man	Gal	Glc
Time	**Temperature**	**w/V ratio**	**Grinding**						
(min)	(°C)	(g/ 30 mL)	**Level**	(% w/w)			(%mol)		
5				19.2±2.2	3.5±0.1	14.9±0.9	50.6±2.6	26.4±1.3	4.5±0.3
15				23.5±4.4	3.0±0.0	12.2±0.1	54.4±1.6	26.4±1.3	4.1±0.2
30	80	1	3	23.7±3.8	3.1±0.8	11.7±1.0	53.3±3.8	27.0±0.4	4.9±2.1
120				21.1±1.3	3.2±0.0	15.2±2.7	50.4±2.3	26.9±0.5	4.3±0.1
360				24.0±2.9	2.9±0.2	12.6±0.6	53.5±1.0	27.6±0.5	3.5±0.3
	10			12.5±4.0	5.2±0.1	22.3±4.0	30.9±4.0	36.2±0.5	5.4±0.6
	23 (RT)			13.8±0.3	3.9±0.4	17.9±1.1	39.3±0.3	34.1±0.0	4.9±1.1
30	40	1	3	17.2±0.8	3.7±0.1	15.1±0.4	43.1±1.7	32.3±2.4	5.9±1.2
	80			23.7±3.8	3.1±0.8	11.7±1.0	53.3±3.8	27.0±0.4	4.9±2.1
	100			24.5±2.9	3.2±0.1	12.8±0.4	49.9±4.0	30.2±4.8	3.9±0.2
		0.5		16.1±1.3	3.8±0.2	14.4±0.8	50.4±1.5	27.5±0.4	3.9±0.2
		1.0		23.7±3.8	3.1±0.8	11.7±1.0	53.3±3.8	27.0±0.4	4.9±2.1
30	80	2.0	3	19.4±0.1	3.1±0.0	12.7±0.3	52.6±0.1	28.0±0.1	3.6±0.1
		4.0		20.8±0.2	3.3±0.0	13.1±0.1	52.1±1.3	27.9±1.1	3.7±0.1
		6.0		23.1±1.0	3.2±0.2	14.3±0.5	49.8±0.2	27.7±0.5	5.0±0.4
			1	22.3±1.5	2.5±0.1	10.8±0.1	55.4±0.9	28.1±0.8	3.2±0.1
30	80	1	2	22.8±1.2	3.1±0.2	12.9±0.5	53.5±0.8	26.5±0.1	4.0±0.1
			3	23.7±3.8	3.1±0.8	11.7±1.0	53.3±3.8	27.0±0.4	4.9±2.1

[a] : mass of compound present in the freeze-dried extract.

The study of the central composite design shows that the sugars content of coffee freeze-dried extracts varied from 14.8 to 29.7 %w/w, a 2-fold increase from minimum to maximum value (Table III.1.2). Pareto chart (Figure III.1.4) shows that temperature exerts a fundamental role regarding the extraction of carbohydrates from coffee matrix, through the increase of polysaccharide solubilisation with increasing temperatures. Regarding temperature, the linear term is positive and significant (p<0.0001, accounting for 43% of the data variability), as well as the interaction of this variable with time (X_1) and w/V ratio (X_3) (p<0.05) (Figure III.1.5). The linear term of the parameter time is non-significant (p>0.05), suggesting that in this system prolonged time does not always result in an increase of the carbohydrate content in the extracts. However, at low temperature (20 ºC) a considerable difference is observed between the extreme levels, *i.e.* longer extraction time (360 min, 18.3-21.5 %w/w) favours the carbohydrate extraction, comparing to shorter

extraction time (10 min, 14.8-17.6 %w/w). This did not happen with the highest temperature level (80 ºC, 10 min - 21.6-29.6 %w/w; 360 min – 18.9-29.7 %w/w), suggesting that with low temperatures a prolonged time of extraction must be necessary to increase the sugars content.

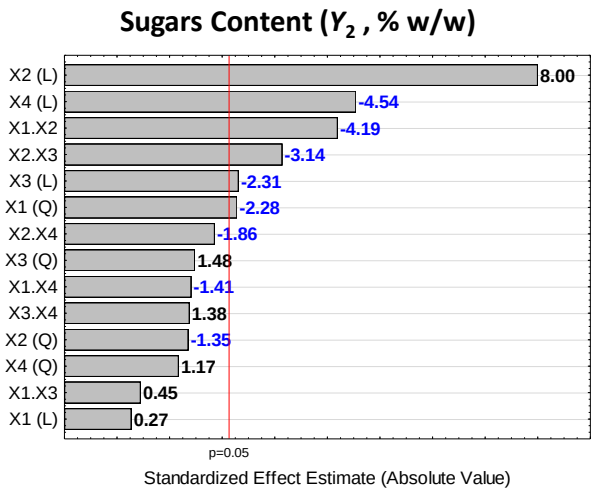


Figure III.1.4. Pareto chart for the effects of time (X_1), temperature (X_2), w/V ratio (X_3), and grinding level (X_4) during the coffee extraction experiments on the sugars content (Y_2, %w/w). The negative and positive effects are highlighted in blue and black, respectively. L and Q represent the linear and quadratic effects, respectively. The region (right side) of statistical significance (95% confidence level) is defined by the vertical line.

The quadratic term is significant (p<0.05), suggesting a U-shape extraction profile for the sugars extraction in the conditions tested. Both w/v ratio (X_3) and grinding level (X_4) linear terms were significant and have a negative impact, meaning that sugars content in the extract are lower with higher w/V ratios and coarser particles. After removing the non-significant terms (p>0.05), the full quadratic model (R^2 = 0.82, p<0.0001) for the carbohydrate data fits a second order polynomial equation, with non-significant lack-of-fit (Table III.1.3). Moreover, the analysis of the initial extract shows that the roasted coffee has 48.8±3.7 %w/w of sugars in its composition, in accordance with literature (Bekedam et al., 2006; Oosterveld et al., 2003a) (Table III.1.2**)**. Considering the overall yield and sugars extraction, it was observed that 6.2-18.2% of coffee carbohydrates end up in the extract, with a clear distinction in lower (20 ºC, 6.2-10.4 %w/w) and higher temperature levels (80 ºC, 9.5-18.2 %w/w). Moreover, it was verified a positive linear correlation (r=0.77, p<0.0001) between extraction yield and sugars content within central composite design results, associating a higher extraction yield to an increase in the carbohydrates extraction.

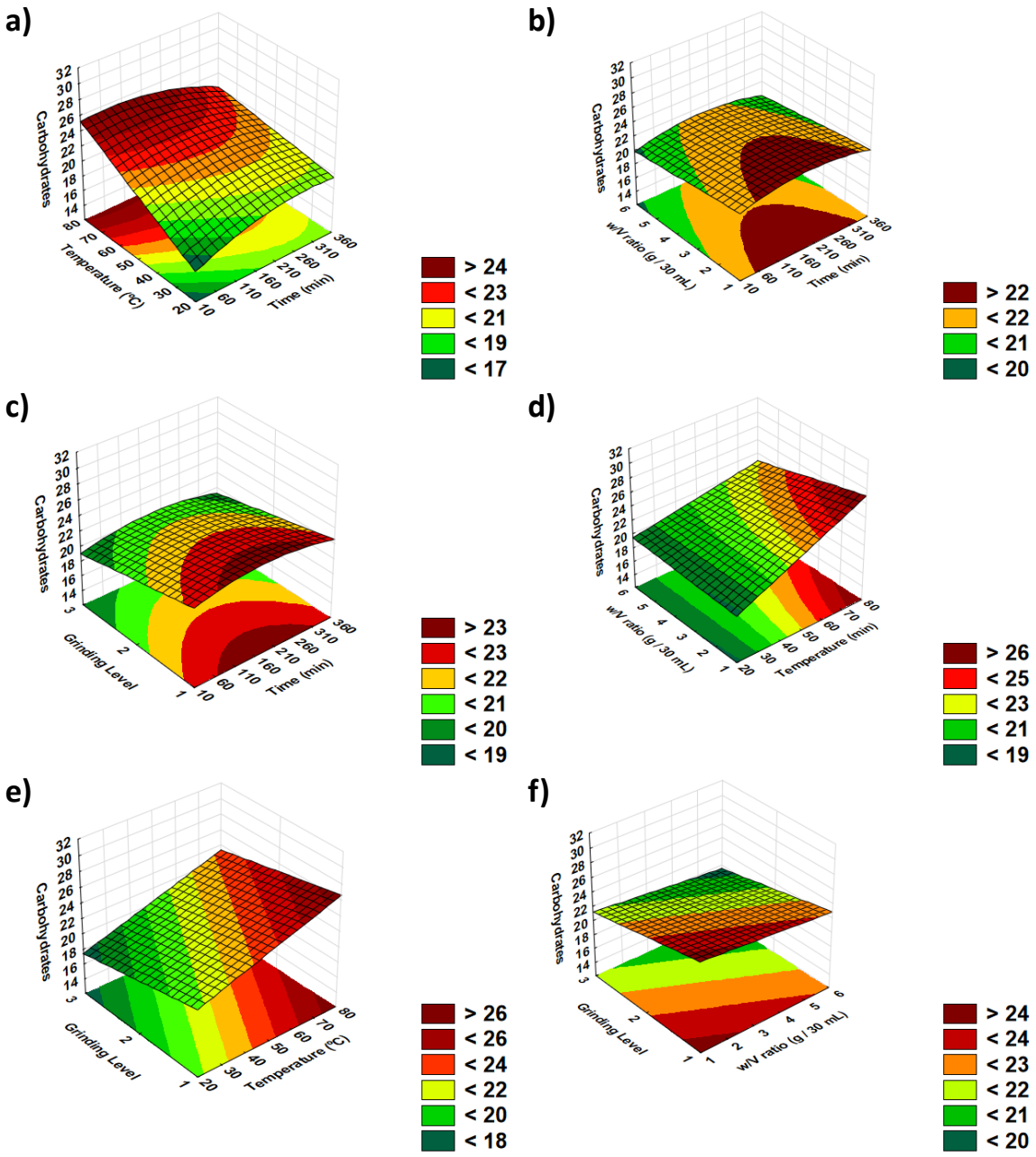


Figure III.1.5. Response surface plots (3D) of the model developed representing the carbohydrates content in the extract (Y_2, %w/w) showing the effect of the different extraction parameters. In each plot two of the independent variables were maintained at their intermediate level: *time* (X_1) - 185 min; *temperature* (X_2) - 50 ºC; *w/V ratio* (X_3) - 3.5 g / 30 mL; (X_4) - *grinding level* – level 2. a) time (X_1) × temperature (X_2); b) time (X_1) × w/V ratio (X_3); c) time (X_1) × grinding level (X_4); d) temperature (X_2) × w/V ratio (X_3); e) temperature (X_2) × grinding level (X_4); f) w/V ratio (X_3) × grinding level (X_4).

III.1.3.3.1. Sugars composition

Regarding the sugar composition of coffee extracts, the single-factor experiments shows that mannose, galactose, and arabinose were the sugar residues most abundant in the coffee extracts and the most affected by the operational parameters, once the range of

variation among the conditions tested for rhamnose and glucose was quite low (1-3%, expressed as relative %mol). The range of variation observed when tested different temperature for arabinose (10 %), mannose (21 %), and galactose (9 %) was much greater than when considering the variation observed with the other parameters (1-5 %, for the three sugar residues), in analyses performed at 80 ºC. The study of central composite design shows that a 1.7-1.8 fold variation may be observed for the three residues among all runs that represented 88-94 % of sugar residues in all conditions tested (Figure III.1.6).

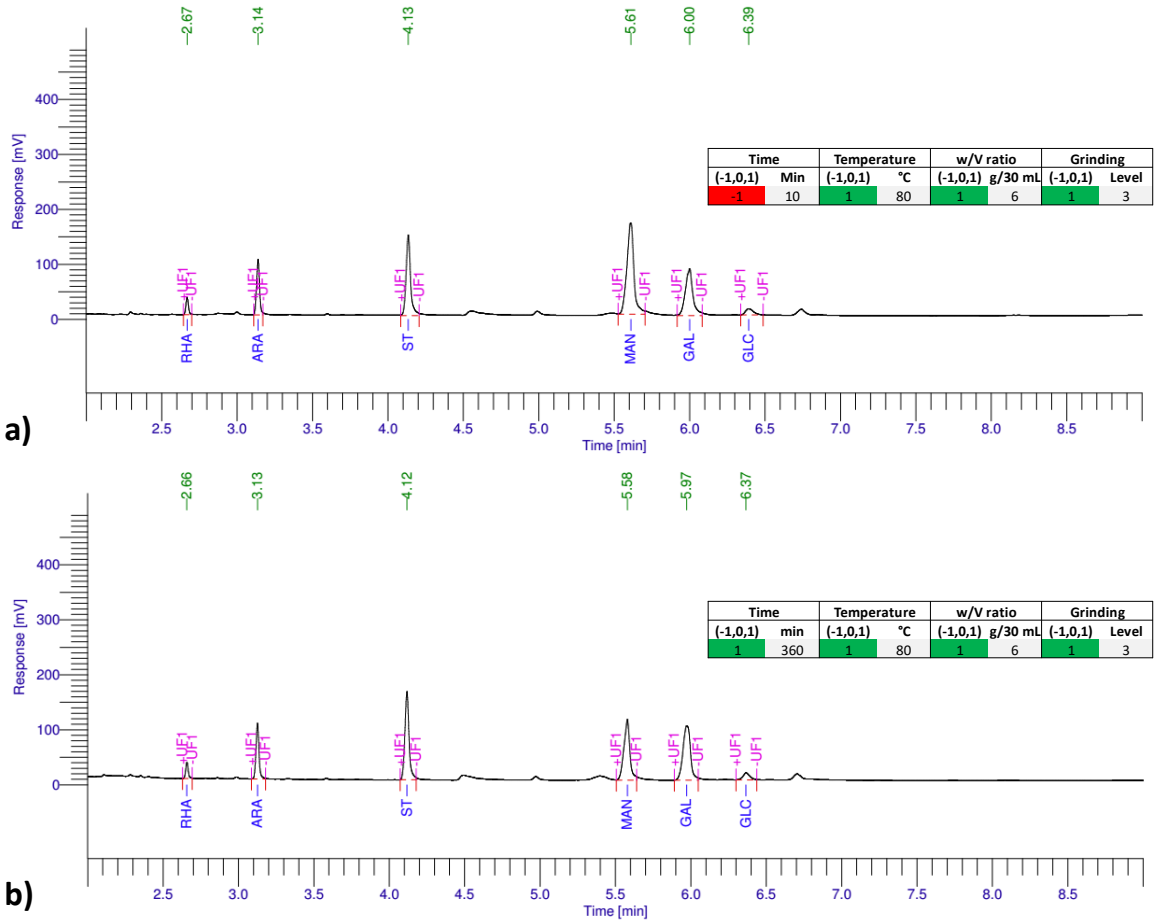


Figure III.1.6. Examples of GC-FID chromatograms of the neutral sugar carbohydrates analysis of coffee conventional infusion experiments using two different conditions (Rha - ramnose; Ara - arabinose; ST - internal standard; Man - mannose; Gal - galactose; Glc - glucose).

Among all polysaccharide structures present in roasted coffee matrix, cellulose is insoluble in the tested conditions, remaining in the coffee residue after extraction and filtration. Galactomannans (GM), composed by mannose and a small fraction of the galactose content, and type II arabinogalactans (AG), constituted mainly by galactose and arabinose, are the main polysaccharide present in the different coffee brews (Lopes et al.,

2016; Nunes & Coimbra, 2001). The estimation of galactomannans (GM) can be performed by considering the mannose content plus the addition of 5% of the total amount of mannose that accounts for galactose content, assuming a degree of substitution of 1:20, meaning a substitution with single residues of galactose for every twenty residues of mannose in the main chain, *i.e.* a 5% degree of branching. This is in accordance with the degree of branching of GM (3-6%) obtained from roasted coffee infusions prepared with different roasting degrees from distinct coffee species and also GM extracted from spent coffee grounds (Nunes & Coimbra, 2002a, 2002b; Oosterveld et al., 2003a; Passos et al., 2019a). For the estimation of the content of arabinogalactans (AG), it was accounted the amount of galactose from which was subtracted the galactose content that is part of GM structure and added the content of arabinose. Thus, it is assumed that all galactose was component of the AG (except an amount corresponding to 5% of total mannose) and that all arabinose in the extracts was from AG. GM (68-69%) are the predominant polysaccharides in roasted coffee infusions, followed by AG (25-30%) and with a much lower glucans content (2-6%) (Nunes & Coimbra, 2001). As in this study the coffee sample was always the same in all runs, the differences could only be due to the operational parameters used. Figure III.1.7 shows the distribution of the GM and AG in relation to the carbohydrate content in each run. It is evident that AG content variation among all runs are lower (7.5-12.0 %w/w) when compared to GM (5.4-18.3 %w/w). Thus, the GM extraction is more affected by operational parameters over the extraction conditions tested. This also suggests that roasted coffee powder, when subjected to water extractions, has AG that are readily solubilized and extracted quite independently of the conditions used. The wide range of conditions tested, namely higher temperatures and longer extraction times, did not promote a significant increase of AG content.

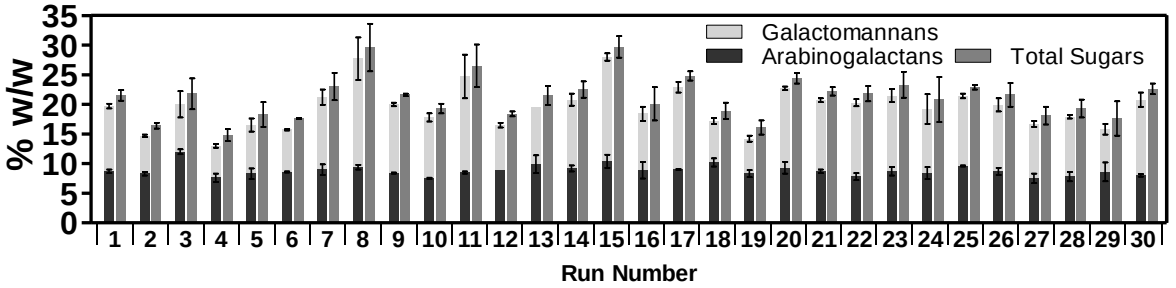


Figure III.1.7. Representation of the distribution of the coffee polysaccharides along the experiments: arabinogalactans (AG, %w/w), galactomannans (GM, Y_3, %w/w) as part of the total sugars determined through the central composite design results presented in Table III.1.2.

Moreover, the variation of GM content can be linked to the observed increase in the carbohydrates extracted, once there is a positive and strong linear relationship between the content of GM and the sugars content in the extracts (r=0.96, p<0.0001), evidencing that the variation of sugars content among the extracts is related to the higher or lower extraction of GM (Figure III.1.8).

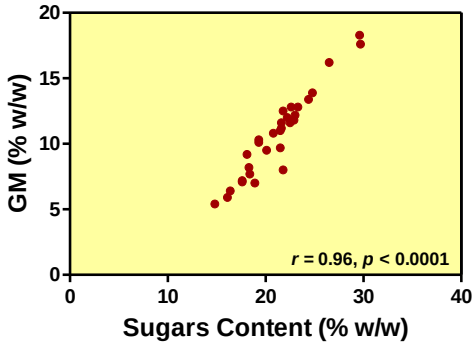


Figure III.1.8. Representation of estimated galactomannans content in the extract (GM, Y_3, %w/w) in function of carbohydrates in the extract (Y_2, %w/w).

Figure III.1.9 shows that, as occurred in the extraction of carbohydrates, the linear term of temperature (X_2) is the variable with clearly the main preponderance for higher relative GM content in the extracts.

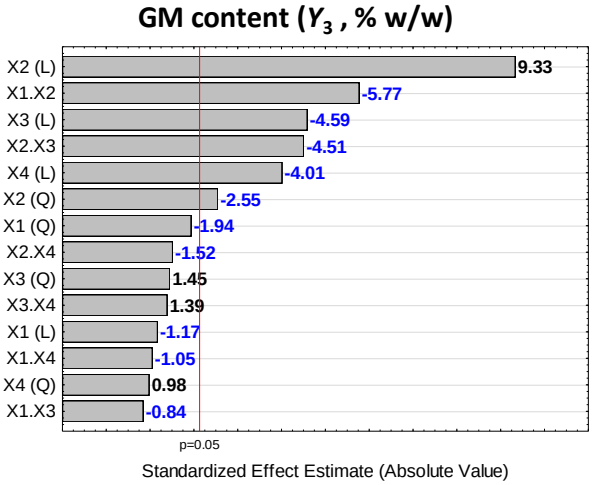


Figure III.1.9. Pareto chart for the effects of time (X_1), temperature (X_2), w/V ratio (X_3), and grinding level (X_4) during the coffee extraction experiments on the GM content (Y_3, %w/w). The negative and positive effects are highlighted in blue and black, respectively. L and Q represent the linear and quadratic effects, respectively. The region (right side) of statistical significance (95% confidence level) is defined by the vertical line.

A significant model (p<0.0001) fits the data for GM content (R^2 = 0.88) after elimination of non-significant terms, exhibiting a non-significant lack-of-fit (Table III.1.3)

and the response surface plots presented in Figure III.1.10 show how the factors studied affect the content of GM across the experimental design. The graphs are presented keeping constant the remaining two independent variables of each plot in the intermediate level of the design.

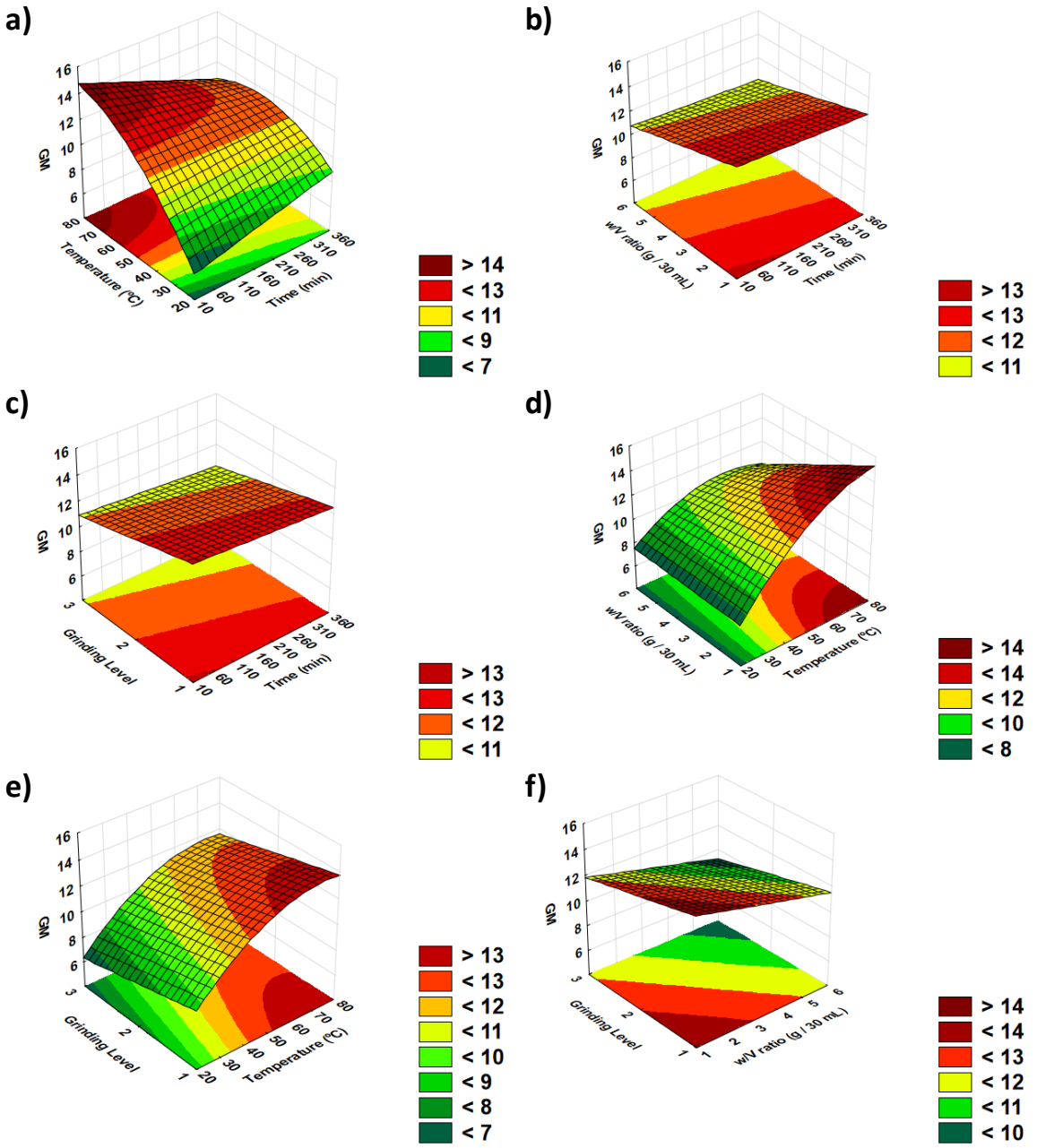


Figure III.1.10. Response surface plots (3D) of the model developed representing the content of galactomannans (Y_3, %w/w) in the coffee extracts showing the effect of the different extraction parameters. In each plot two of the independent variables were maintained at their intermediate level: *time* (X_1) - 185 min; *temperature* (X_2) - 50 ºC; *w/V ratio* (X_3) - 3.5 g / 30 mL; (X_4) - *grinding level* – level 2. a) time (X_1) × temperature (X_2); b) time (X_1) × w/V ratio (X_3); c) time (X_1) × grinding level (X_4); d) temperature (X_2) × w/V ratio (X_3); e) temperature (X_2) × grinding level (X_4); f) w/V ratio (X_3) × grinding level (X_4).

At shorter extraction temperatures (20 ºC), there is a clear difference on the relative GM content in the extract between using shorter extraction times (10 min, 5.4-7.1 %w/w) and longer extraction times (360 min, 7.7-9.7 %w/w). Thus, considering shorter extraction times, the GM represented only 36.5-40.3% of the extract carbohydrates, with a predominance of AG, while with longer extraction times, the GM content is similar or even slightly higher than AG in coffee brew extract. It can be concluded that lower temperatures promote mainly the extraction of AG, minimizing the amount of GM extracted. At lower temperatures, the amount of GM can be increased if prolonged extraction times are used. This allows to infer that the AG are more accessible in roasted coffee matrix than the GM, that require prolonged times at lower temperature for their extraction. At higher temperatures the extraction of GM (80 ºC, 7.0-18.3 %w/w) was higher when comparing to lower temperatures (20 ºC, 5.4-9.7 %w/w), even at shorter times (Figure III.1.10a). At the highest temperature and during short times, the GM represented the major polysaccharide of the extract (53.7-61.8 %w/w). The effect of mass ratio and grinding level over time (Figure III.1.10 an 10c) shows that once temperature was constant, the differences in GM extraction were slight. Nevertheless, when extraction was performed during prolonged times, the relative content of GM decreased, becoming the AG the predominant polysaccharide, only when high w/V ratio is used (Figure III.1.10d). While with lower temperatures a prolonged time allows to extract more GM, it is possible that at higher temperatures and high w/V ratio a GM solvent saturation could occur not enabling the extraction of GM. It is also probable the occurrence of interaction/adsorption of the GM with the cellulose present in the matrix (Newman & Hemmingson, 1998; Wang et al., 2017), preventing their extraction. Thus, higher temperatures and prolonged times, especially using higher w/V ratio, should be avoided for GM extraction. The extraction of GM may be reduced if coarser particles were used when fixing the time and w/V ratio, while varying the temperature (Figure III.1.10e). When the temperature and time were constant, at lower w/V ratio and grinding level the GM extraction was slightly enhanced (Figure III.1.10f).

The results obtained show that the extraction of coffee powder at high temperatures (in conventional systems, not comparable to industrial extraction performed at extreme temperatures way over 100 ºC) may produce a coffee extract with low amount of GM. According to literature high temperatures originates extracts and brews with GM as the

main polymer present (Nunes & Coimbra, 2001; Oosterveld et al., 2003a). However, this occurs only for short periods of time and low mass-to-volume ratios, as used to prepare home coffee brews. Performing the extraction at 80 ºC during prolonged extraction times and using a high mass-to-volume ratio, the extracts have a predominance of AG over GM. The different GM/AG ratio verified all over the design space may also be associated to the beverage properties, such as the viscosity of coffee solutions. The higher amount of GM in the coffee extracts was shown to be associated to the increase in kinematic viscosity (Table III.1.2 and Figure III.1.11**)** through a strong and positive linear relationship (r=0.95, p<0.001). The carbohydrates in coffee brews and mainly GM act as viscosity improvers, stabilizing the foam in espresso coffee (Nunes & Coimbra, 1998). On the other hand, using low temperatures, there is a predominance of AG in the coffee extracts, in accordance with the composition of cold coffee brews, promoting the occurrence of polysaccharide structures with immune-modulating activity (Shin, 2017). Indeed, AG have immunostimulatory activity dependent on structural features, as terminally linked arabinose residues, as shown in instant coffee fractions (Ferreira et al., 2018), highlighting the influence of the different technological parameters to obtain extracts rich in polysaccharides with different properties suitable for distinct applications.

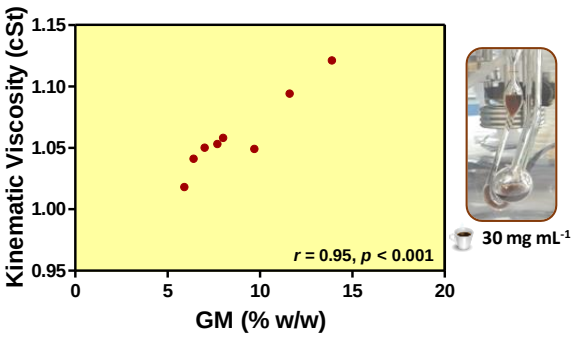


Figure III.1.11. Variation of kinematic viscosity of coffee solutions (30 mg.mL^{-1}) in function of GM in the extract (Y_3, %w/w).

The roasted coffee used is composed by 45.5, 28.4, 19.1 and 6.5 %mol of mannose, galactose, glucose, and arabinose, in accordance with literature (Oosterveld et al., 2003b). This allows to estimate a content of 23.6±1.6 %w/w for GM and 15.5±1.6 %w/w for AG. Thus, 5-22 %w/w of GM and 10-20 %w/w of AG are extracted to the coffee extracts with the conditions used, corresponding to nearly only 20 %w/w of the coffee powder polysaccharides extracted in the conditions tested. Based on the data obtained, higher temperatures are needed for a complete extraction of the coffee polysaccharides. This is in

accordance with the use of microwave-assisted extraction at temperatures reaching 200 ºC for a complete extraction of coffee polysaccharides from coffee matrix (Passos et al., 2014a).

III.1.3.4. Extract brown colour ($K_{mix,\ 405\ nm}$)

The single-factor experiments show that the range of variation for $K_{mix,\ 405\ nm}$ values varying temperature (0.15, the difference between maximum and minimum value) was slightly higher than the occurred with mass-to-volume ratio (0.12), following the observed with time (0.08) and with grinding level (0.03) (Table III.1.5).

Table III.1.5. $K_{mix,\ 405nm}$ results from the single-factor experiments of roasted coffee extractions.

Process Variables				$K_{mix,\ 405nm}$
Time (min)	**Temperature** (°C)	**w/V ratio** (g/ 30 mL)	**Grinding Level**	(mL mg^{-1} cm^{-1})
5				0.57±0.00
15				0.57±0.03
30	80	1	3	0.59±0.04
120				0.58±0.02
360				0.65±0.01
	10			0.44±0.01
	23 (RT)			0.44±0.01
30	40	1	3	0.51±0.02
	80			0.59±0.04
	100			0.56±0.01
		0.5		0.56±0.03
		1.0		0.59±0.04
30	80	2.0	3	0.61±0.02
		4.0		0.53±0.01
		6.0		0.49±0.00
			1	0.58±0.04
30	80	1	2	0.59±0.00
			3	0.59±0.04

This suggests that temperature and mass-to-volume ratio can greatly influence the brown colour of the extracts obtained due to the increase of brown compounds (melanoidins) or due to the dilution of such compounds with increasing extraction of colourless structures while maintaining the content of the brown ones. Melanoidins are brown compounds formed during the roasting process through Maillard reaction involving carbohydrates, proteins and phenolic compounds. The brown colour dilution factor measured at 405 nm is indicative of the melanoidins content, ranging from 0.40 to 0.64 mL mg^{-1} cm^{-1}, values comparable with literature reports for coffee brews (Bekedam et al., 2006, 2008b). The ANOVA and Pareto graph (Figure III.1.12) show that the linear terms

of w/V ratio (X_3) and temperature (X_2) were the main effects influencing $K_{mix,\ 405\ nm}$ at a similar level, explaining 33 and 30% of the variability observed. The higher temperatures and lower w/V ratios lead to browner coffee extracts, suggesting higher melanoidins extraction. The linear term linked to time (X_1) also significantly affects the $K_{mix,\ 405\ nm}$, but to a lower extent, meaning browner with longer extraction times. The browning of the extracts over the conditions studied may be linearly described by a significant model (p<0.0001), with high determination coefficient (R^2 = 0.89) and non-significant lack-of-fit (Table III.1.3 and response surface plot of Figure III.1.13).

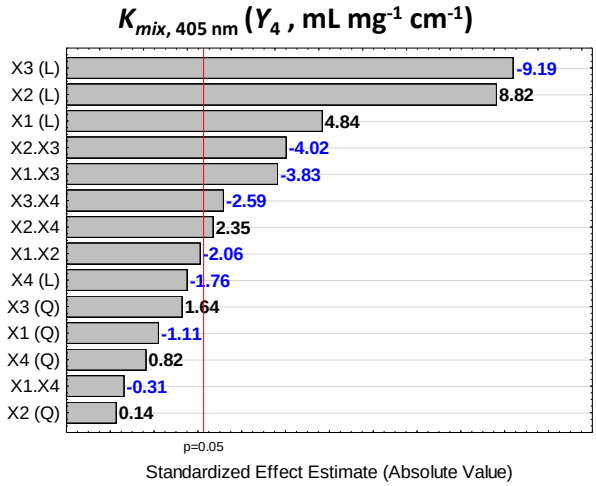


Figure III.1.12. Pareto chart for the effects of time (X_1), temperature (X_2), w/V ratio (X_3), and grinding level (X_4) during the coffee extraction experiments on the $K_{mix,\ 405nm}$(Y_4, %w/w). The negative and positive effects are highlighted in blue and black, respectively. L and Q represent the linear and quadratic effects, respectively. The region (right side) of statistical significance (95% confidence level) is defined by the vertical line.

Neither the linear nor the quadratic terms of the variable grinding level (X_4) exert a significant effect on $K_{mix,\ 405\ nm}$, explaining the slight variation observed when the grinding degree varied (Figure III.1.13c,e,f). Longer extraction times (Figure III.1.13a-c) and temperatures (Figure III.1.13a,d,e) increase the browning of the extracts obtained, in accordance with literature (Merritt & Proctor, 1959). Moreover, it seems that the effect of w/V ratio is more pronounced when longer extraction times (Figure III.1.13b) or higher extraction temperatures (Figure III.1.13d) were applied, explaining the significance of such interactions ($X_1.X_3$, $X_2.X_3$). Thus, maintaining constant the temperature of extraction, the extract become browner if longer extraction times and lower mass-to-volume ratio are applied, as well as higher $K_{mix,\ 405\ nm}$ values are obtained when higher temperature accompanied a lower mass-to-volume ratio fixing the time of extraction. The fact that a higher mass-to-volume ratio is associated with a lower $K_{mix,\ 405\ nm}$ seems to be explained by

the preferential extraction of colourless compounds, as occurred with AG, hindering the extraction of the more browned ones. It is also perceived a positive correlation between the extraction yield and the $K_{mix,\ 405\ nm}$ values of central composite design (r=0.81, p<0.0001), showing that the increase of extracted compounds from the coffee powder have a preferential contribution of brown compounds.

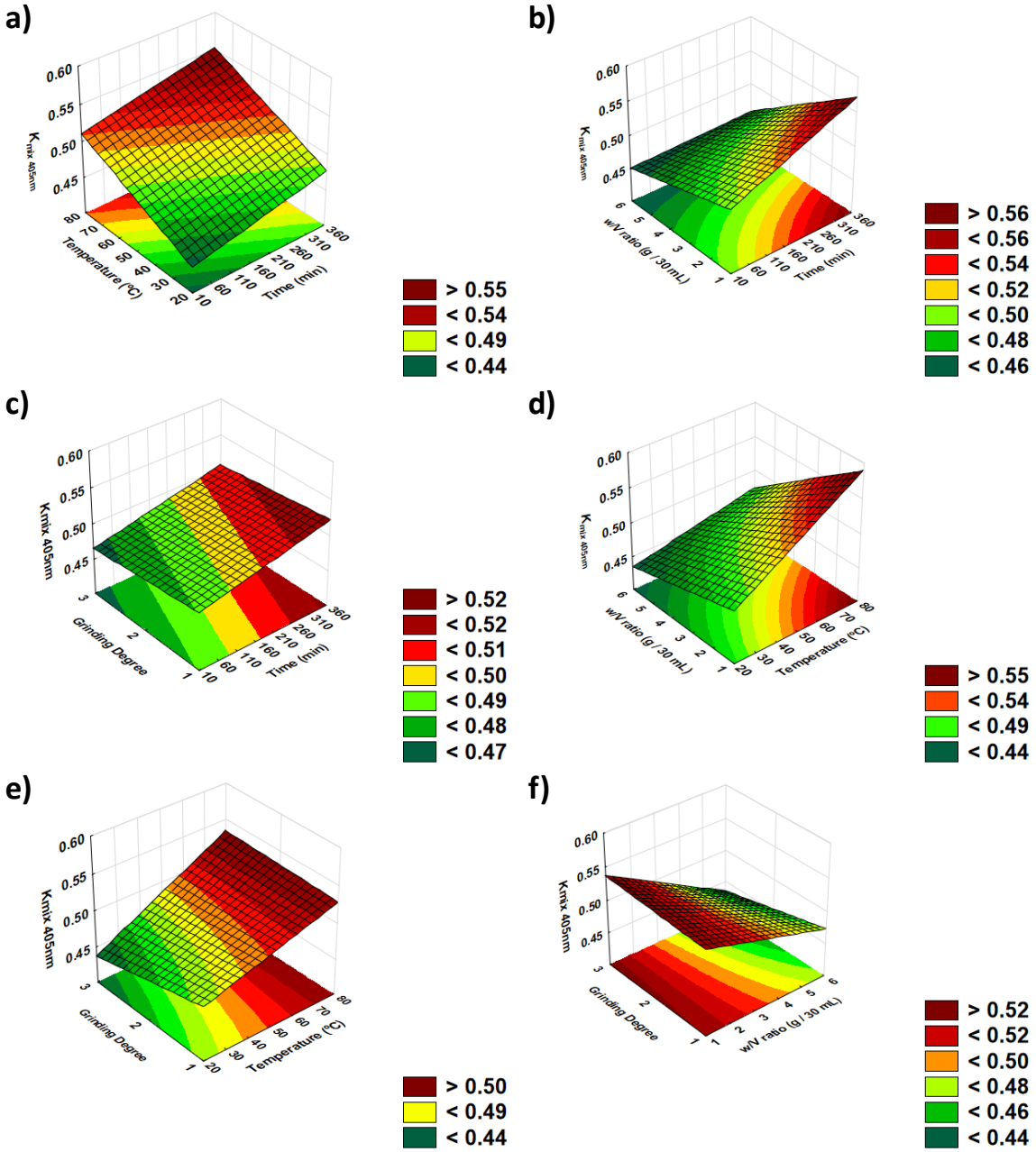


Figure III.1.13. Response surface plots (3D) of the model developed representing the variation of $K_{mix,\ 405\ nm}$ values (Y_4, mL mg^{-1} cm^{-1}) in the coffee extracts showing the effect of the different extraction parameters. In each plot two of the independent variables were maintained at their intermediate level: *time* (X_1) - 185 min; *temperature* (X_2) – 50 ºC; *w/V ratio* (X_3) – 3.5 g / 30 mL; (X_4) - *grinding level* – level 2. a) time (X_1) × temperature (X_2); b) time (X_1) × w/V ratio (X_3); c) time (X_1) × grinding level (X_4); d) temperature (X_2) × w/V ratio (X_3); e) temperature (X_2) × grinding level (X_4); f) w/V ratio (X_3) × grinding level (X_4).

III.1.3.5. Caffeine and 5-CQA

The analysis of caffeine content (Figure III.1.14) in the single-factor experiments shows that caffeine content in the extract has the higher variation when testing temperature (2.1 %) compared to all the other variables (0.5-1.1 %) (Table III.1.6).

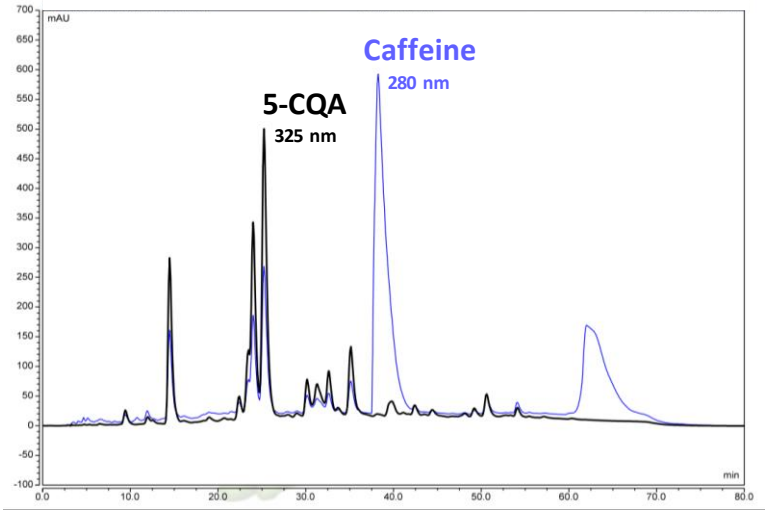


Figure III.1.14. Example of a HPLC chromatogram of coffee samples analysis showing the peak of caffeine detected at 280 nm and of 5-CQA detected at 325 nm.

Table III.1.6. Caffeine and 5-CQA results from the single-factor experiments of roasted coffee extractions.

Process Variables				Caffeine[a]	5-CQA[a]
Time (min)	**Temperature** (°C)	**w/V ratio** (g/ 30 mL)	**Grinding Level**	(% w/w)	(% w/w)
5				8.2±0.0	2.4±0.1
15				7.7±0.9	2.4±0.1
30	80	1	3	7.7±0.5	2.3±0.2
120				7.7±0.3	2.2±0.1
360				7.6±0.7	2.1±0.0
	10			9.8±0.6	2.8±0.0
	23 (RT)			9.3±0.3	2.5±0.1
30	40	1	3	8.8±0.4	2.6±0.2
	80			7.7±0.5	2.3±0.2
	100			7.8±0.0	2.2±0.1
		0.5		8.3±0.1	2.1±0.0
		1.0		7.7±0.5	2.3±0.2
30	80	2.0	3	8.8±0.1	2.2±0.0
		4.0		8.7±0.1	2.4±0.2
		6.0		8.1±0.8	2.5±0.0
			1	7.8±0.4	2.2±0.1
30	80	1	2	8.0±0.3	2.3±0.1
			3	7.7±0.5	2.3±0.2

[a] : mass of compound present in the freeze-dried extract.

From the analysis through the central composite design, the caffeine proportion in the extract varied from 7.1 to 9.7 %w/w (Table III.1.2), in accordance with the relative

content of caffeine in coffee brews (Andueza et al., 2003b, 2007; Maeztu, et al., 2001a). The statistical analysis showed that it is possible to significantly model the percentage of caffeine in the coffee extract (p<0.0001, R^2 = 0.89 and non-significant lack-of-fit), with the temperature exerting the most significant, and negative effect. When crossing the data from extraction yield and the caffeine content in the extract, it was observed a value (2.2±0.1 %w/w) correspondent to the amount of caffeine found in the roasted coffee in the initial sample (1.9±0.2 %w/w), which is in accordance with literature reports (0.8-2.6 %w/w) for different species and roasting degrees (Fujioka & Shibamoto, 2008; Hečimović et al., 2011; Moreira et al., 2017). Nevertheless, it should be noted that the analysis of the content in the coffee powder presupposes an extraction step, a coffee brew itself, that may influence the higher or lower quantification of caffeine in the roasted coffee. However, it can be concluded that in the conditions tested, the caffeine was almost or totally extracted. Literature reports that the solubility of caffeine increased widely with temperature ranging from 1.46 to 19.23 g *per* 100 g water at 20 and 80 ºC, respectively, the extreme values used in this study, becoming even more soluble at 100 ºC (66.6 %w/w) (Macrae, 1985). Considering the caffeine content present in the roasted coffee and the volume of water used (30 mL), all extraction experiments took place in conditions below these limits. Indeed, in several conventional extraction methods the extraction of caffeine reaches 81-100% (Illy & Viani, 2005; Petracco, 2001). The extraction times performed in these experiments should allow the dissolution of all caffeine from the coffee matrix, even considering the shorter extraction time (10 min). Hence, the variations observed in the relative mass content of caffeine in the extract should be related to the differences observed with the contribution of other compounds in the extract than with the content of caffeine itself. Thus, a higher or lower proportion of caffeine in the extract is not related to an increase in caffeine extraction, but with the dilution or concentration of such quantity of caffeine in the overall mass of the extract. In fact, the caffeine content may be negatively correlated to the sugars content in the extract (r=-0.80, p<0.0001), suggesting that higher/lower sugars content leads to a dilution/concentration effect of caffeine in the extract. On the other hand, due to the different mass-to-volume ratio, it can be observed also a large range of concentration of caffeine, from 0.6 to 4.5 $mg.mL^{-1}$, representing from 19 to 136 mg in the 30 mL used in all the experiments due to different mass of coffee powder from where the caffeine was extracted. This wide variability is also reported in the different homemade coffee brews

(1.4-7.9 $mg.mL^{-1}$), even within the same method (Crozier et al., 2012; Ludwig et al., 2014a). Thus, this concept of the higher extractability of caffeine from coffee powder, when compared to the other components, should be taken into consideration when determining the caffeine content in coffee brews.

The chlorogenic acid present at the highest level in roasted coffee samples and coffee brews is the 5-CQA isomer (Fujioka & Shibamoto, 2008). The analysis of 5-CQA revealed a comparable pattern of extraction with that observed for caffeine. The proportion of this compound in the overall extracts varied from 1.9 to 2.9 %w/w, in accordance with the verified in coffee infusion brews (2.9 %w/w) or even different methods (1.8-2.4 %w/w) (Bekedam et al., 2008a; López-Galilea et al., 2007). However, considering the extraction yield, the content of 5-CQA extracted represented 0.55-0.69%, a value comparable to the initial content in the powder (0.7±0.1 %w/w), meaning that the compound was fully extractable. Literature also shows similar values for roasted coffee samples (0.7-1.5 %w/w) (Bekedam et al., 2008a; Moreira et al., 2017). During the experiments, it was possible to obtain coffee brews with 0.2 to 1.3 $mg.mL^{-1}$ of 5-CQA but such differences arose from the different amount of coffee used for the extraction. Nevertheless, it is possible to develop a significant model ($p<0.001$, $R^2 = 0.88$, data not shown) based on 5-CQA content in the extract where temperature exerted a significant and negative effect due to the higher relative extraction of other compounds. Therefore, its applicability is limited once the modulation should be more based on the other compounds than properly with 5-CQA.

III.1.4. Concluding remarks

This study analysed the effect of several operational factors in the roasted coffee extraction process highlighting the main effects and enabling the development of statistically significant models that describe the system. It was showed that temperature is the most significant effect regarding the overall extraction yield and sugars content and composition. A maximum of 29.9 %w/w of the compounds present in the roasted coffee may be extracted. The estimation of the two main polysaccharides in coffee (galactomannans (GM) and arabinogalactans (AG)), showed that, while the variation of AG content across the experimental design was low (7.5-12.0 %w/w), the variation concerning GM was substantial (5.4-18.3 %w/w). The extraction of GM from coffee

powder increases with increasing temperatures, while the extraction of AG is not so dependent on temperature. Indeed, AG are extracted quite regardless the conditions used. When using high temperatures, the AG are the main polysaccharides in the extract if longer times and high w/V ratio are used. The increase in GM content is reflected in a higher viscosity associated to brews prepared with such extracts. The overall mass extraction yield seems to be related to the brown colour of the coffee extracts (or the solutions prepared with them), exerting in such case w/V ratio and temperature the main significant effects. Caffeine and 5-CQA are extensively extracted in the conditions tested, while their content in each extract varied due to the concentration/dilution in relation to other extracted compounds. Although, temperature is the main factor affecting the system, the other parameters should also be considered as there are some interactions between coffee extraction variables. Thus, when studying extraction processes, it should be performed a comprehensive study of how operational variables affect the system. Regarding coffee extraction, namely, infusion methods, this study shows that is possible to modulate the conditions of the coffee extraction in order to obtain preferentially a desired compound/group of compounds, obtaining extracts with different compositions and, consequently, properties, either biological or sensorial.

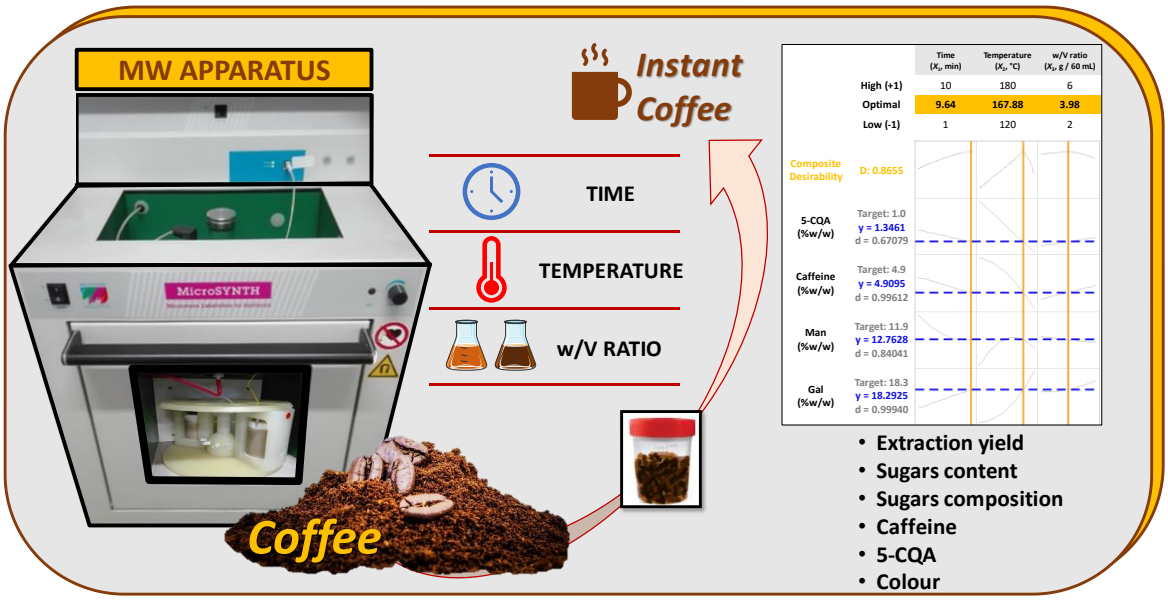
III.2. MICROWAVE-ASSISTED EXTRACTION OF COFFEE

The content of this chapter was submitted for publication:

Lopes, G. R., Passos, C. P., Rodrigues, C., Teixeira, J. A., & Coimbra, M. A. (2019). Impact of microwave-assisted extraction on roasted coffee carbohydrates, caffeine, chlorogenic acids and coloured compounds.

III.2.1. Abstract

Microwave-assisted extraction (MAE) allows to quickly achieve soluble compounds from solid matrices due to the promotion of temperatures higher than the solvent boiling point at atmospheric pressure, once a closed-vessel system is used for operating at high pressure. In this study, the feasibility of MAE for producing high yield coffee extracts with properties that allow their commercial application was tested through a quality by design approach. It was studied the influence of time of extraction (1, 5.5, 10 min), temperature (120, 150, 180 ºC) and the mass-to-volume ratio (2, 4, 6 g/60 mL) in the overall extraction yield (24-47 %w/w), carbohydrates content (18-43% w/w), sugars composition, caffeine (4-7% w/w), 5-caffeoylquinic acid (1-2% w/w), colour and antioxidant activity of the extracts. FTIR analysis was used to study the resemblance of coffee extracts and commercial instant coffee. MAE allowed overall extraction yields considerably higher than the home brewing methods, mainly when performed at 180 ºC, with a substantial increase in AG extraction associated to higher temperatures. Temperature exerted a crucial role in coffee extracts differentiation, although time and w/V ratio also lead to different values in the responses. Under a circular economy concept, MAE was able to produce extracts that can be used as defined food/brew ingredients and also provides a galactomannan and cellulose rich residue that can also be valued as a source of dietary fibre.



III.2.2. Aim of the study

The study under conventional conditions is limited to atmospheric pressure, although considerable variability of extracts could be obtained. In order to improve yields and/or obtain extracts with different composition, it was studied the extraction in a system that allowed temperatures higher than 100 ºC, the water boiling point. Microwave-assisted extraction (MAE) presents advantages compared to conventional methods as the reduction of extraction time, improved yield and/or better accuracy (Ameer et al., 2017; Delazar et al., 2012). The MAE may be conducted in open or closed-vessel systems. The later ones allow higher temperatures and pressures, while avoiding the loss of volatile substances and extraction solvent. In these systems, the temperature and pressure of extraction are controlled, while radiated microwaves interact with the sample, with turntables (multimode system) used to ensure an equal energy distribution (Ameer et al., 2017; Delazar et al., 2012). In MAE occurs the penetration of microwave energy through the sample resulting in the heating of the solution due to the molecular friction between the rotating molecules through dipole reorientation under the influence of the changing or alternating electric field or via the conductive migration of dissolved ions (Lam & Chase, 2012; Rodriguez-Jasso, et al., 2011). MAE was already used to extract chlorogenic acids from green coffee beans selectively produce chlorogenic acid-rich extracts to use in functional foods, modulating the MAE process (Upadhyay et al., 2012). Moreover, MAE was already tested for the reuse of antioxidants and carbohydrates from the coffee residues, the spent coffee grounds (SCG), that remain after coffee brews preparation (Passos & Coimbra, 2013; Passos et al., 2014a, 2019a; Pettinato et al., 2019; Ranic et al., 2014). However, SCG comprise a different and organoleptically poorer matrix when compared to roasted coffee, as some compounds were already greatly extracted to the brew. To the best of our knowledge, MAE application directly to the roasted coffee matrix has not been reported. Response surface methodology (RSM) was used as it allows to measure the influence of each parameter in extraction processes. The models developed by RSM should allow to modulate the coffee compounds extraction in order to obtain coffee extracts with desirable properties.

III.2.3. Results and discussion

III.2.3.1. Extraction yield

The coffee MAE shows a 2-fold variation (24.2-47.0%w/w) throughout the conditions tested: 1, 5.5, and 10 min of extraction, 120, 150, and 180 ºC, and 2, 4, and 6 g/60 mL (Table III.2.2). Usually, the temperatures achieved in coffee brew methods are limited to the atmospheric water boiling point (1 atm or 1.01325 bar, 100 ºC) or little more, when Moka pot is used (Illy & Viani, 2005; Navarini et al., 2009; Petracco, 2001). An experiment testing an infusion methodology and MAE with milder extraction conditions (80 ºC) related to conventional coffee brews were performed (Table III.2.1), for comparison to the more drastic conditions defined in Box-Behnken design (Chapter II).

Table III.2.1. Results obtained for extractions performed at 80 ºC with conventional (Conv.) and microwave-assisted extraction (MAE) methods and from an instant coffee product (IC).

Method.	Extr. Yield[a]	Sugars[b]	Rha	Ara	Man	Gal	Glc	$K_{mix,\ 405nm}$	Caffeine[b]	5-CQA[b]
	(% w/w)	(% w/w)			%mol			(mL mg^{-1} cm^{-1})	(% w/w)	(% w/w)
Conv.	26.6±0.3	23.7±3.8	3.1±0.8	11.7±1.0	53.3±3.8	27.0±0.4	4.9±2.1	0.59±0.04	7.7±0.5	2.3±0.2
MAE	26.0±0.2	23.8±3.4	3.8±0.3	13.3±1.1	49.0±4.8	26.6±2.4	7.3±1.6	0.60±0.03	8.4±0.1	2.4±0.0
IC	-	34.5±1.1	1.5±0.1	9.4±0.6	33.9±1.0	52.1±1.5	3.1±0.4	0.68±0.08	4.9±0.4	1.0±0.2

[a] : mass of freeze-dried coffee compared to the mass of roasted coffee prior to the extraction process; [b] : mass of compound present in the freeze-dried extract; Conventional methodology (Conv.): 30 min, 80 ºC, 1 g / 30 mL**;** MAE: 2 min, 80 ºC, 2 g / 60 mL.

The extraction performed at 80 ºC showed that the yield for both infusion (26.6±0.3 %w/w) and MAE method (26.0±0.2 %w/w) were similar. These values are within the values reported for household coffee brewing methods (<32 %) even promoting a wide variation in operational parameters as shown in literature and Chapter III.1 (Gloess et al., 2013; Petracco, 2001). It seems that the radiation *per se* does not induce substantial differences compared to conventional coffee brew methods, or that there is no clear difference between conductive and microwave heating considering overall extraction yield, as already reported (Damm & Kappe, 2011). On the other hand, the granulometry analysis of the residue left after the experiments indicated that the structural integrity of the coffee powder was maintained at a microscopic level, although a slight shift was observed suggesting a potential decrease in bigger particles size (Fig. III.2.1.)

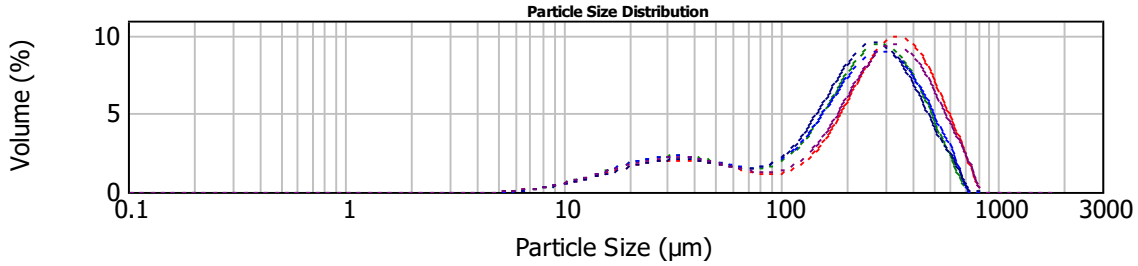


Figure III.2.1. Profile of the particle size distribution of the grinding levels used in the coffee MAE measured before the experiments (**violet line**, *run 1* and **red line**, *run 5*), and after the experiments showing examples of three different temperatures tested and equal time of extraction: 120 ºC (**green line**, *run 10*), 150 ºC (**blue line**, *run 6*) and 180 ºC (**dark blue line**, *run 2*).

Table III.2.2 shows that MAE was able to clearly improve the extraction yield in some of the conditions tested comparing to household methods. The closed vessels used promoted a fast increase of the water temperature to above the atmospheric boiling point. The MAE system used allowed to check in real-time the temperature of the sample inside the extraction vessel, with the desired temperatures achieved within the intended short times (2 min), which was maintained along the experiments (Figure III.2.2).

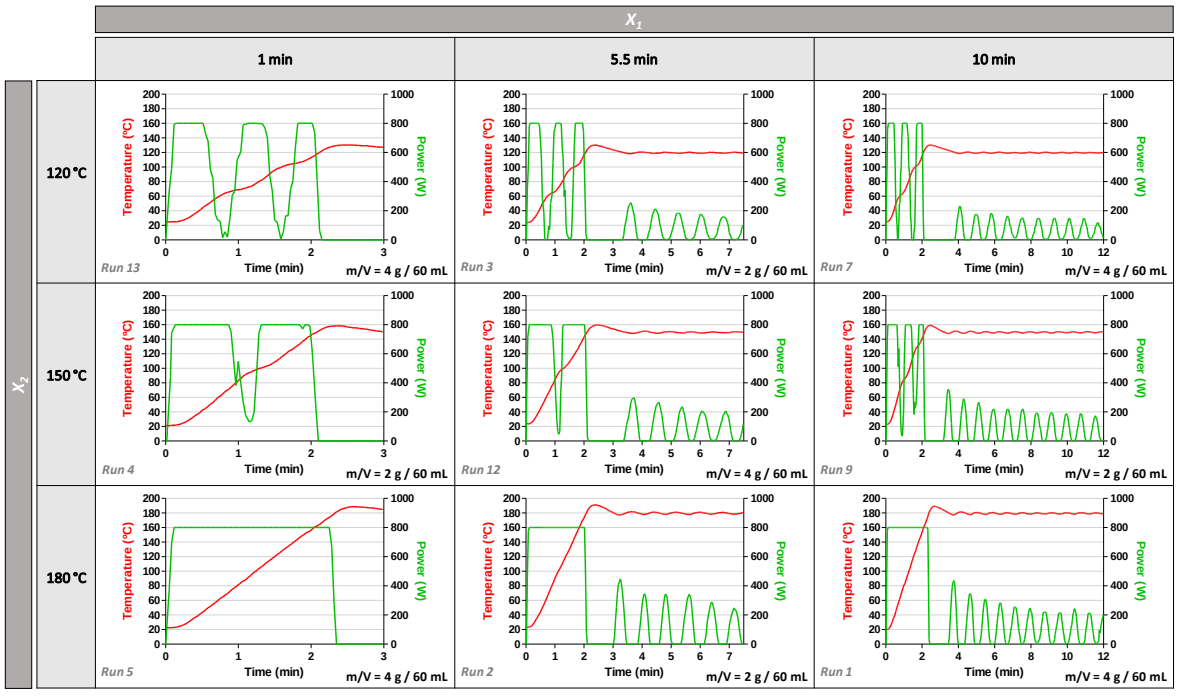


Figure III.2.2. Examples of the MAE experiment profile showing the measured temperature (—, ºC) and power (—, W), according to two of the independent variables tested: time (X_1, min) and temperature (X_2, ºC).

Table III.2.2. Resume of coffee MAE settled according to a Box-Behnken design with three levels and three independent factors.

Run Order	Process Variables[a]			Extr. Yield[b]	Total Sugars[c]	Rha	Ara	Man	Gal	Glc	Caffeine[c]	5-CQA[c]	$K_{mix,\ 405nm}$	Antiox. Act.
	Time	Temperature	w/V ratio											
	(X_1, min)	(X_2, °C)	(X_3, g/ 60 mL)	(Y_1, %w/w)	(Y_2, %w/w)	%mol					(%w/w)	(%w/w)	(mL mg^{-1} cm^{-1})	(% inib.)
1-a	10.0 (+1)	180 (+1)	4 (0)	47.0	43.1±3.2	1.1±0.1	12.1±0.2	26.3±0.6	58.6±0.6	1.8±0.1	3.8	1.1	0.3974	48.1
1-b	10.0 (+1)	180 (+1)	4 (0)	45.5	42.3±2.2	1.1±0.0	11.9±0.1	25.1±0.5	60.2±0.7	1.7±0.0	4.9	1.4	0.3600	49.6
2-a	5.5 (0)	180 (+1)	6 (+1)	42.5	42.7±4.0	1.3±0.0	12.4±0.4	33.1±1.2	51.1±0.9	2.1±0.1	4.1	1.1	0.3300	43.9
2-b	5.5 (0)	180 (+1)	6 (+1)	39.3	38.0±4.0	1.5±0.4	12.0±0.1	33.3±0.4	51.3±0.6	1.9±0.3	4.8	1.4	0.3574	47.1
3-a	5.5 (0)	120 (-1)	2 (-1)	29.1	26.2±3.2	3.2±0.1	12.1±1.0	55.7±2.6	25.6±0.9	3.3±0.7	6.5	1.8	0.5705	54.7
3-b	5.5 (0)	120 (-1)	2 (-1)	29.0	25.8±0.4	3.5±0.1	12.7±0.2	54.4±1.2	25.7±1.6	3.5±0.0	6.6	1.8	0.5957	57.8
4-a	1.0 (-1)	150 (0)	2 (-1)	31.5	29.7±1.6	2.6±0.1	11.7±0.8	56.8±0.4	25.8±0.4	3.2±0.2	5.9	1.7	0.5587	52.3
4-b	1.0 (-1)	150 (0)	2 (-1)	30.8	29.7±2.9	2.4±0.0	11.1±0.5	57.0±2.3	25.9±1.1	3.5±0.6	5.9	1.7	0.6435	52.7
5-a	1.0 (-1)	180 (+1)	4 (0)	37.3	36.4±1.2	1.9±0.1	13.7±0.4	41.1±0.5	40.8±0.4	2.5±0.2	5.0	1.4	0.4385	51.9
5-b	1.0 (-1)	180 (+1)	4 (0)	39.3	34.9±0.3	1.9±0.1	14.2±0.3	37.3±1.1	44.5±0.7	2.2±0.1	5.9	1.6	0.4679	47.4
6-a	5.5 (0)	150 (0)	4 (0)	30.0	25.3±2.0	3.1±0.1	13.7±0.1	48.5±0.3	31.3±0.9	3.5±0.3	6.2	1.7	0.4893	-
6-b	5.5 (0)	150 (0)	4 (0)	31.9	29.1±1.1	2.8±0.1	14.0±0.1	47.3±0.7	32.5±0.8	3.3±0.2	6.0	1.6	0.4891	51.6
7-a	10.0 (+1)	120 (-1)	4 (0)	27.9	20.7±3.1	3.5±0.7	15.1±2.1	44.5±7.4	32.7±4.0	4.3±0.9	6.2	1.8	0.4819	56.8
7-b	10.0 (+1)	120 (-1)	4 (0)	28.1	24.6±1.0	3.2±0.3	13.2±0.5	51.9±1.8	27.8±1.2	3.9±0.3	6.4	1.9	0.5242	57.3
8-a	5.5 (0)	150 (0)	4 (0)	29.3	25.3±0.6	3.1±0.3	12.7±0.1	53.3±1.4	27.2±1.1	3.8±0.1	6.7	1.7	0.5201	52.2
8-b	5.5 (0)	150 (0)	4 (0)	32.6	28.5±0.4	2.5±0.1	12.6±0.4	51.3±2.6	30.5±1.9	3.2±0.1	6.1	1.6	0.5093	52.3
9-a	10.0 (+1)	150 (0)	2 (-1)	30.4	27.2±1.8	3.1±0.1	12.7±0.1	54.5±2.4	26.3±1.8	3.5±0.6	5.8	1.6	0.5644	52.1
9-b	10.0 (+1)	150 (0)	2 (-1)	33.4	28.9±5.6	2.2±0.2	13.3±1.3	48.2±1.5	33.0±0.5	3.2±0.6	5.4	1.5	0.5394	50.1
10-a	5.5 (0)	120 (-1)	6 (+1)	24.2	17.8±1.1	4.4±0.1	18.6±1.0	33.4±1.5	38.5±0.7	5.1±0.3	7.3	1.9	0.5320	52.4
10-b	5.5 (0)	120 (-1)	6 (+1)	24.9	18.8±1.2	4.5±0.1	17.4±0.2	38.7±0.2	35.0±0.0	4.4±0.1	7.3	2.1	0.5495	68.3
11-a	10.0 (+1)	150 (0)	6 (+1)	35.1	35.8±4.5	1.7±0.2	15.3±0.9	27.4±4.6	53.5±3.4	2.0±0.4	5.3	1.5	0.3554	45.9
11-b	10.0 (+1)	150 (0)	6 (+1)	28.5	28.3±4.1	2.6±0.7	15.8±4.1	38.6±10.2	39.8±5.7	3.3±0.6	6.2	1.7	0.4595	48.8
12-a	5.5 (0)	150 (0)	4 (0)	33.9	26.3±0.8	2.7±0.1	15.6±0.5	43.0±0.5	36.0±0.4	2.8±0.4	5.8	1.6	0.4348	53.0
12-b	5.5 (0)	150 (0)	4 (0)	30.1	23.3±1.0	3.3±0.2	15.7±0.2	43.8±0.4	33.3±0.6	3.9±0.2	5.9	1.7	0.4849	55.1
13-a	1.0 (-1)	120 (-1)	4 (0)	27.9	21.9±0.6	3.3±0.0	12.3±0.3	55.0±1.0	25.8±0.8	3.6±0.0	6.6	1.9	0.4834	52.8
13-b	1.0 (-1)	120 (-1)	4 (0)	26.4	21.7±2.0	3.8±0.1	14.9±0.9	50.2±0.0	27.0±0.6	4.1±0.4	6.9	2.1	0.5515	52.6
14-a	1.0 (-1)	150 (0)	6 (+1)	33.5	29.9±2.3	2.3±0.1	13.8±0.9	45.3±4.5	35.8±3.4	2.7±0.0	6.3	1.7	0.4613	49.5
14-b	1.0 (-1)	150 (0)	6 (+1)	28.5	25.1±1.0	3.1±0.1	13.5±0.1	49.8±0.2	30.2±0.0	3.4±0.4	7.0	1.8	0.5769	50.4
15-a	5.5 (0)	180 (+1)	2 (-1)	46.9	39.8±0.4	1.3±0.2	13.6±0.2	26.5±0.0	56.7±0.5	1.8±0.1	3.7	1.1	0.4130	43.0
15-b	5.5 (0)	180 (+1)	2 (-1)	43.8	43.4±1.3	1.1±0.0	12.1±0.9	29.0±0.8	56.0±1.7	1.7±0.0	3.8	1.1	0.4390	42.8

[a] : The process variables are shown in real and (coded) values; [b] : mass of freeze-dried coffee compared to the mass of roasted coffee prior to the extraction process; [c] : mass of compound present in the freeze-dried extract;

The box plots in Figure III.2.3 highlight the temperature as the most preponderant effect for the differentiation in coffee overall extraction yield, when compared with time and w/V ratio, evidencing a non-linear behaviour. The grouping of the results by temperature showed distinct ranges for the three levels tested: 24.2-29.1 (120 ºC), 28.5-35.1 (150 ºC), and 37.3-47.0 %w/w (180 ºC). Thus, using 120 ºC does not result in an increment comparing to conventional system, while at 150 ºC similar or higher values were attained depending on conditions tested and, at 180 ºC, the extraction yield was always considerably higher than home brewing methods. The use of more drastic conditions (>150 ºC) should lead to the occurrence of solubilisation of the formerly unextractable compounds, namely with conventional brewing methods. It may be highlighted the fact that using just 3 min (2 min for heating, plus 1 min at 180 ºC) of extraction, 38.3±1.3 %w/w may be achieved, considerably higher than the obtained through conventional methods.

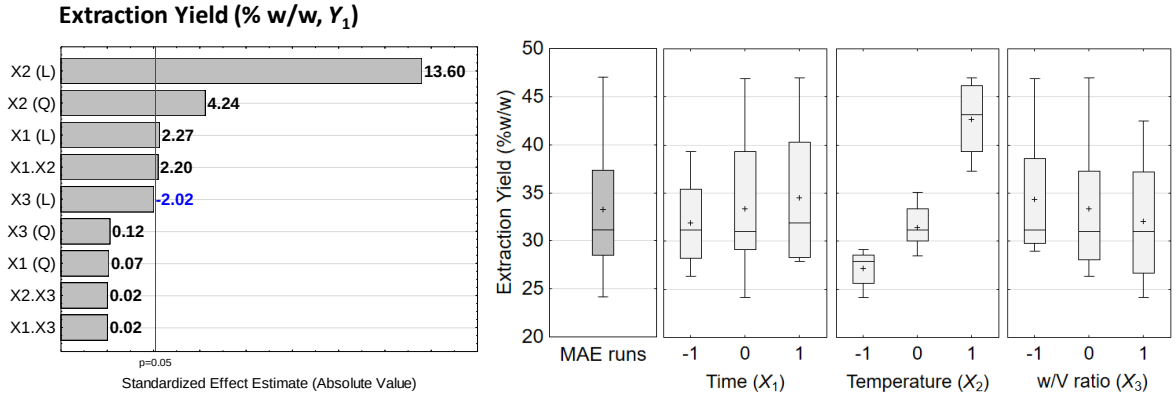


Figure III.2.3. Pareto chart (left) and box plot (right) showing the effects of time (X_1), temperature (X_2), and w/V ratio (X_3) during the MAE coffee experiments on the extraction yield (Y_1, %w/w). In the Pareto chart, the negative and positive effects are highlighted in blue and black, respectively. L and Q represent the linear and quadratic effects, respectively. The region (right side) of statistical significance (95% confidence level) is defined by the vertical line. In the box plot is represented the distribution of the raw data, containing the box the interquartile range and limiting the whisker the non-outlier range, with the median represented as (—) and the mean as ($^+$), while outliers (coeff. 1.5) are represented as (^{o}) and extremes (coeff. 3) as (*).

The grouping of results by the variables time and w/V ratio showed a wide variation in the three levels tested but suggested that longer time of extraction and lower w/V ratio slightly increased the extraction yield, as observed with infusion methods (Chapter III.1). The temperature (X_2) exerted clearly the main preponderance with both linear (L) and quadratic (Q) terms exhibiting significant influence (78.0 % and 7.6% of the results variability, respectively). The linear term for time is also significant, while the w/V

ratio presented marginal significance (p=0.0574), while the quadratic terms were non-significant. After removing the non-significant effects, the data fitted a reduced but significant (p<0.0001) model, with high determination coefficient (R^2 = 0.92) and non-significant lack-of-fit (Table III.2.3), represented by response surface plots (Figure III.2.4a-c). The interaction between time and temperature ($X_1.X_2$) was also significant, with the effect of temperature more noticeable when extraction time was longer (Figure III.2.4a).

Table II.2.3. Models developed for the description of the variation in dependent variables (Y_1 - extraction yield, Y_2 - sugars, Y_3 - galactomannans and Y_4 - arabinogalactans) as function of the parameters studied (X_1 - time, X_2 - temperature, X_3 - w/V ratio) with the corresponding coefficients of determination (R^2). The models are expressed in terms of coded values ((-1), (0), (+1)).

Response	Model Equation	R^2
Extr. Yield	$Y_1 = 31.39 + 1.29\ X_1 + 7.76\ X_2 - 1.15\ X_3 + 3.55\ X_2^2 + 1.78\ X_1.X_2$	0.92
Sugars	$Y_2 = 26.97 + 1.35\ X_1 + 8.94\ X_2 - 0.89\ X_3 + 3.24\ X_2^2 + 1.86\ X_3^2 - 1.61\ X_2.X_3$	0.89
GM	$Y_3 = 13.63 - 1.12\ X_1 + 0.83\ X_2 - 1.80\ X_3 + 0.81\ X_1^2 - 1.62\ X_2^2 + 0.37\ X_3^2 - 0.72\ X_1.X_2 + 2.57\ X_2.X_3$	0.83
AG	$Y_4 = 11.23 + 2.52\ X_1 + 8.28\ X_2 + 0.94\ X_3 + 5.04\ X_2^2 + 1.57\ X_3^2 + 2.36\ X_1.X_2 + 1.57\ X_1.X_3$	0.92

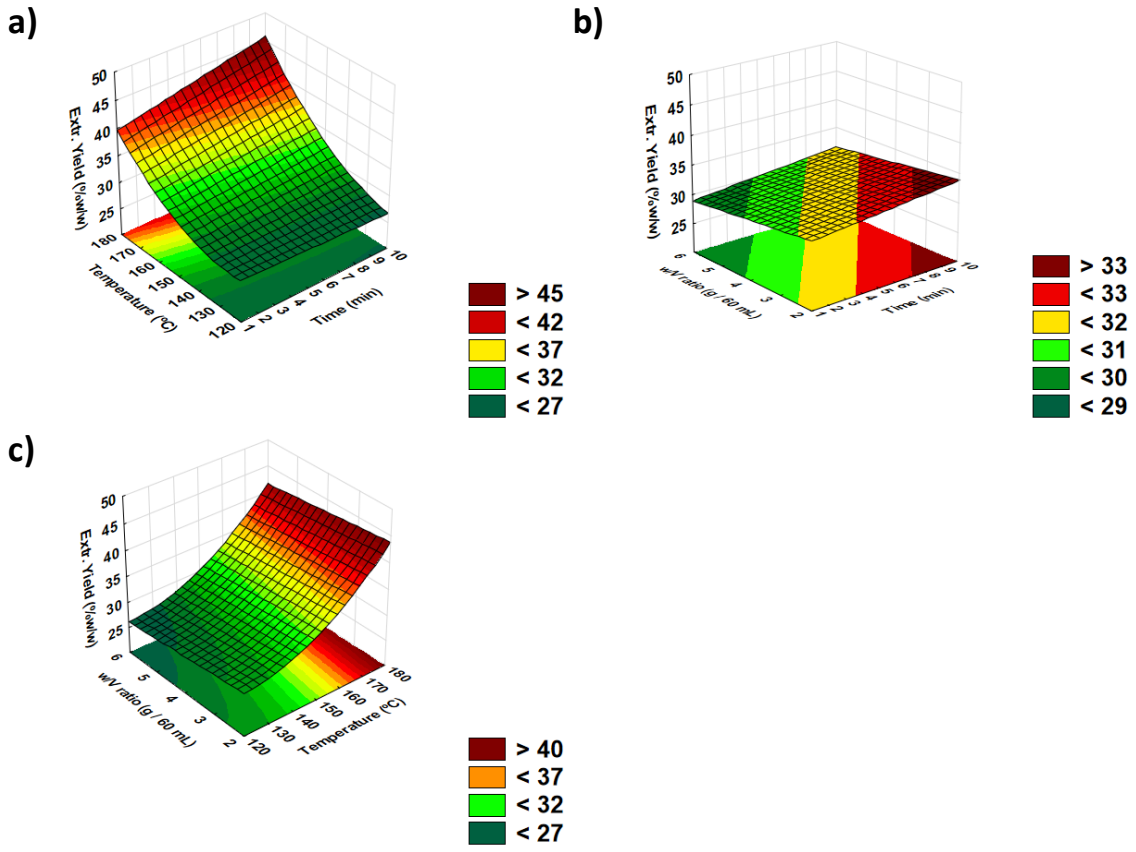


Figure III.2.4. Response surface plots (3D) of the model developed representing the variation of the overall MAE extraction yield (Y_1, %w/w) showing the effect of the different extraction parameters. In each plot, the remaining independent variable was maintained at their intermediate level: *time* (X_1) - 5.5 min; *temperature* (X_2) – 150 ºC; w/*V ratio* (X_3) – 4 g/60 mL. a) time (X_1) × temperature (X_2); b) time (X_1) × w/V ratio (X_3); c) temperature (X_2) × w/V ratio (X_3).

Considering the total solids obtained after freeze-drying process, and assuming 60 mL as the volume after coffee extraction, it may be inferred that the coffee solutions contain 10-42 g L^{-1} of extracted total solids, values in accordance with different household coffee brewing methods (Lopes et al., 2016; Maeztu et al., 2001a; Petracco, 2001). In such case, the greatest variability was observed when grouping the data by their w/V ratio, once the higher the w/V ratio, the higher the amount of soluble solids extracted (Figure III.2.5). Considering the mass of water in relation to the mass of solids obtained, it can be estimated that the coffee solution exhibited 1-4 %w/w of total solids. This range of values was distinct from those obtained during the industrial process of instant coffee. The industrial coffee production occurs at high temperatures (100-180 ºC) and pressures to maximize the extraction of coffee compounds, obtaining extracts with high concentration of coffee solids (25-30%) in battery column percolators working through the counter-current principle (Clarke, 2001).

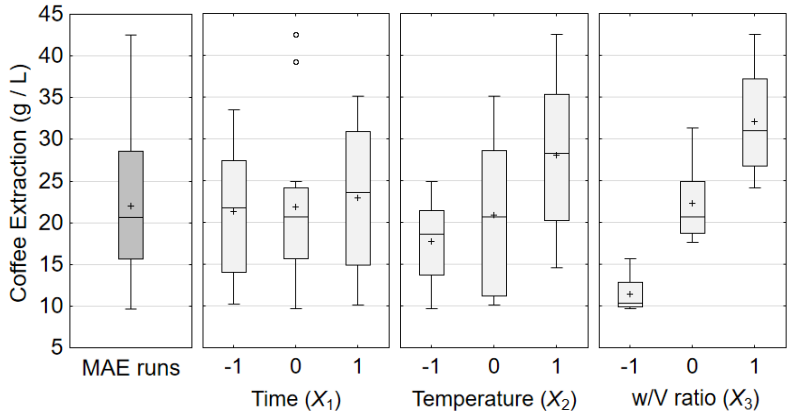


Figure III.2.5. Box plot showing the effects of time (X_1), temperature (X_2), and w/V ratio (X_3) during the MAE experiments on the coffee concentration after the extraction process. In the box plot is represented the distribution of the raw data, containing the box the interquartile range and limiting the whisker the non-outlier range, with the median represented as (—) and the mean as ($^+$), while outliers (coeff. 1.5) are represented as (^{o}) and extremes (coeff. 3) as (*).

III.2.3.2. Sugars content

The carbohydrate content of the extracts ranged from 17.8 to 43.4%w/w, a 2.4-fold variation. This range includes the value obtained for the commercial soluble coffee used as reference in this study (34.5±1.1%w/w, Table III.2.1), in line with literature reports of instant coffee products (35-39 %w/w) obtained either through a simulation of industrial processes or pure soluble coffee samples (Blanc et al., 1989; Capek et al., 2014; Leloup 2006). To reach instant coffee-like carbohydrates content using MAE at at least 180 ºC

should be used (>34.9 %). MAE allowed to obtain a range of 23.3-43.4 %w/w performing the extraction at 150 and 180 ºC. This range is close to the one obtained by Blanc et al. (1989) (32-39 %), but the MAE process was much faster comparing to the autoclave system used (first stage at 100 ºC, for 30 min, and a followed stage during 30-240 min, between 150 and 190 ºC). Beyond the importance of the energetic balance of the process, shorter extraction times is also preferential to keep the carbohydrates structural integrity, avoiding a continuous destruction of total carbohydrates (Blanc et al., 1989). Pareto chart (Figure III.2.6) points out that temperature is the main parameter for the different sugars content in the extracts, with higher temperatures associated to an increase in their content - 120 ºC (17.8-26.2 %w/w), 150 ºC (23.3-35.8 %w/w) and 180 ºC (34.9-43.4 %w/w), being this effect the only one with a clear distinction among the levels tested (Figure III.2.6). In this study, the carbohydrates present in the MAE (23.8±3.4) at 80 ºC, as well as in the infusion extract (23.7±3.8) are compared to home-extraction methods (15-30 %) (Bekedam et al., 2006; Illy & Viani, 2005; Petracco, 2001; Villalón-López et al., 2018), and Chapter III.1, and considerable different comparing with instant coffee (up to 40%). The ANOVA analysis (Table 3 in Annex A.III.2) revealed that temperature is responsible for 84.7% of data variability, distributed by the significant linear (79.1 %) and quadratic (4.6 %) terms.

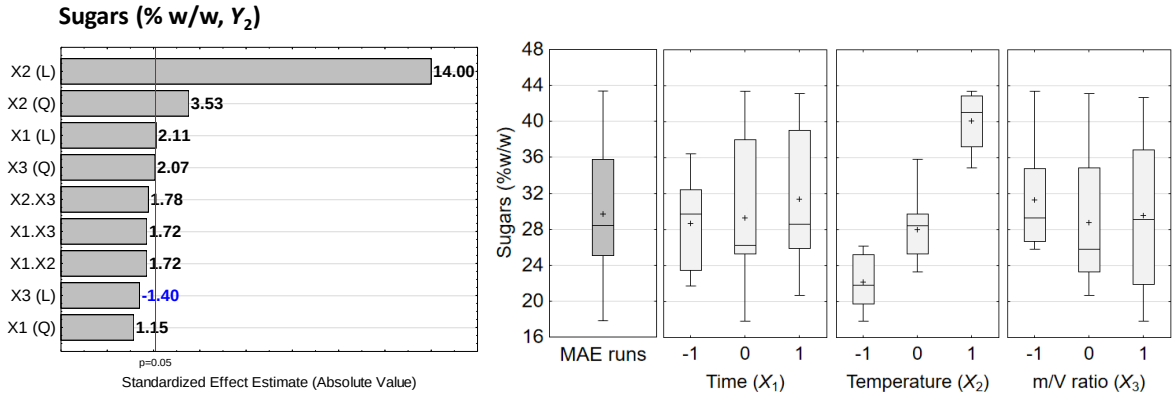


Figure III.2.6. Pareto chart (left) and box plot (right) showing the effects of time (X_1), temperature (X_2), and w/V ratio (X_3) during the MAE coffee experiments on the sugars content (Y_2, %w/w) in the extract. In the Pareto chart, the negative and positive effects are highlighted in blue and black, respectively. L and Q represent the linear and quadratic effects, respectively. The region (right side) of statistical significance (95% confidence level) is defined by the vertical line. In the box plot is represented the distribution of the raw data, containing the box the interquartile range and limiting the whisker the non-outlier range, with the median represented as (–) and the mean as (+), while outliers (coeff. 1.5) are represented as (º) and extremes (coeff. 3) as (*).

The response surface plots in Figure III.2.7 reinforces the preponderance of temperature for different carbohydrates relative content in the coffee extracts (Figure III.2.7a,c), as equal extraction temperatures result in more similar carbohydrates content

(Figure III.2.7b). Moreover, it was found a positive and strong linear relationship (r=0.95, p<0.0001, Figure III.2.7d) between the extraction yield and extracts carbohydrate content. This means that the variation in the extraction yield should be related to the more or less carbohydrates extracted, highlighted by surface plots similarity (Figure III.2.7a-c and Figure III.2.4). Moreover, Figure III.2.7e highlights the higher extraction verified in MAE comparing to the infusion method tested in the previous Chapter III.3.1.

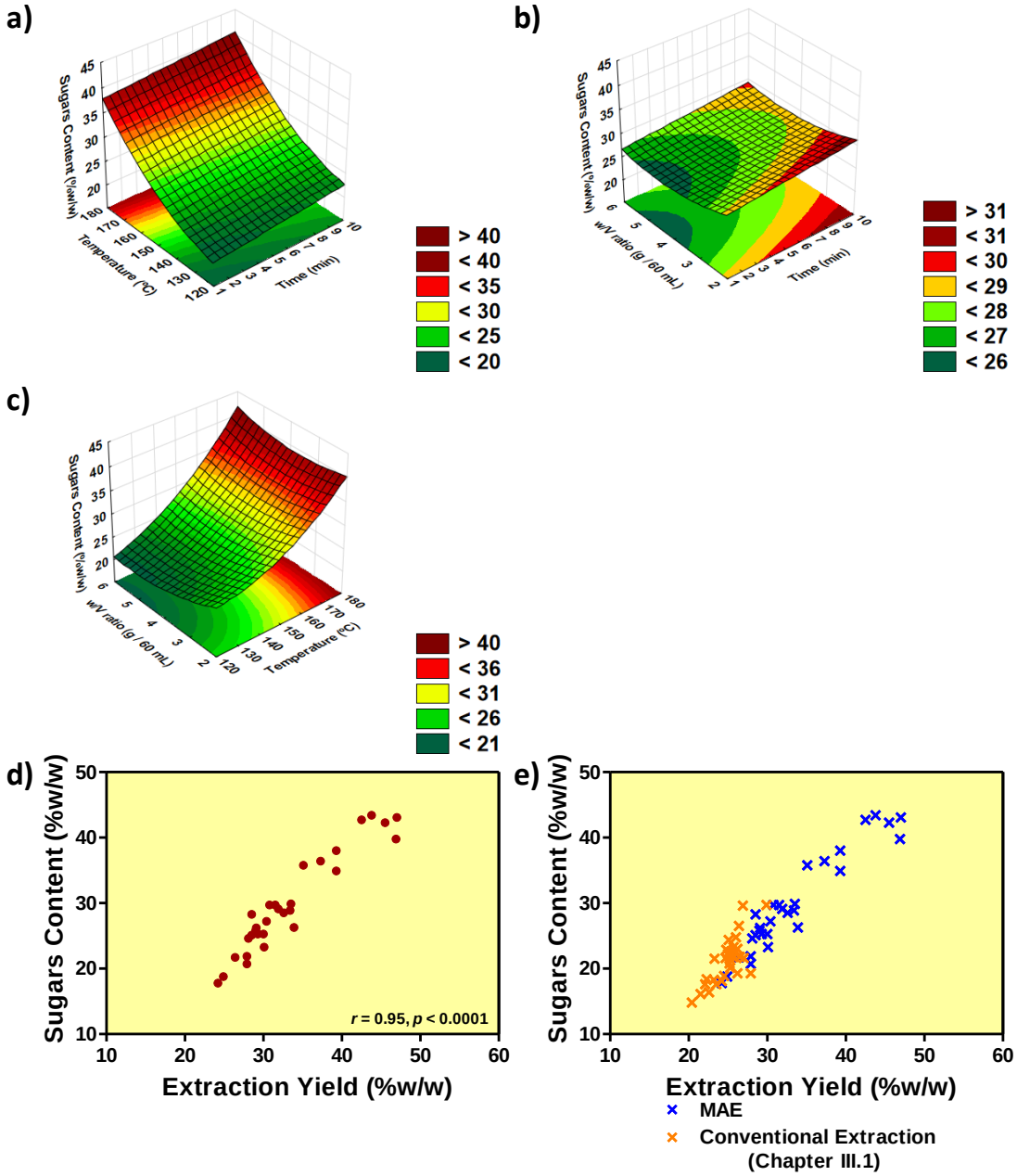


Figure III.2.7. Response surface plots (3D) of the model developed representing the variation of the overall MAE sugars content (Y_2, %w/w) showing the effect of the different extraction parameters (a-c). In each plot, the remaining independent variable was maintained at their intermediate level: *time* (X_1) - 5.5 min; *temperature* (X_2) – 150 ºC; w/*V ratio* (X_3) – 4 g/60 mL. a) time (X_1) × temperature (X_2); b) time (X_1) × w/V ratio (X_3); c) temperature (X_2) × w/V ratio (X_3). Representation of sugars content in the extract (Y_2, %w/w) in function of d) extraction yield (Y_1, %w/w) and e) comparison of conventional and MAE results.

The analysis of the initial roasted coffee sample showed a carbohydrate content of 48.8±3.7 %w/w, in accordance with literature (Bekedam, et al., 2006; Oosterveld, Harmsen, et al., 2003). Thus, 8.9-41.5 % of the carbohydrates present in the roasted coffee were extracted through MAE, with a clear differentiation between the levels of temperature tested: 120 ºC (8.9-15.7 %w/w), 150 ºC (14.4-25.8 %w/w), and 180 ºC (27.8-41.5 %w/w). The advantage of extracting carbohydrates from powder becomes evident when using 180 ºC (Fig. III.2.8.), namely when comparing to the range of values obtained using milder extraction conditions, as observed with the experiments at 80 ºC (13 %).

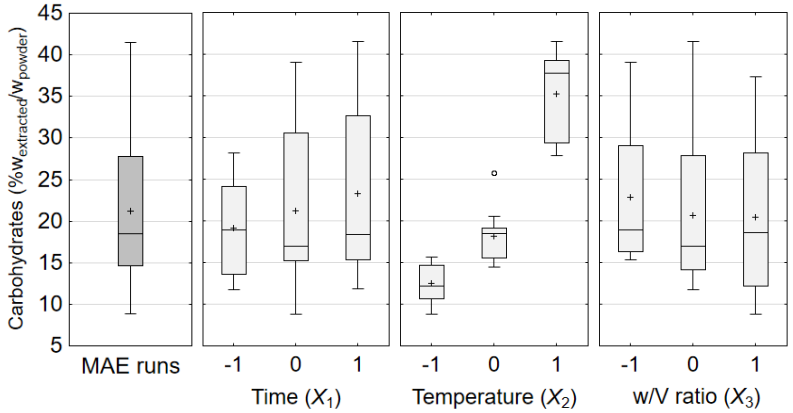


Figure III.2.8. Box plot showing the effects of time (X_1), temperature (X_2), and w/V ratio (X_3) during the MAE experiments on the carbohydrates extracted in relation to the content present in the powder (%w/w). In the box plot is represented the distribution of the raw data, containing the box the interquartile range and limiting the whisker the non-outlier range, with the median represented as (–) and the mean as ($^+$), while outliers (coeff. 1.5) are represented as (^{o}) and extremes (coeff. 3) as (*).

III.2.3.2.1. Sugars composition

The MAE coffee extracts exhibited mannose (25-57 %mol) and galactose (26-60 %mol) as the two main residues, with a dependent linear variation between them, once an increase in one residue followed the decrease of the other (r=-0.96, p<0.0001). The arabinose molar content varied from 11 to 19 %, while the rhamnose and glucose contents were kept at residual levels in all extracts (1-5 and 2-5 %mol, respectively). The commercial instant coffee (IC) product showed galactose as the main sugar residue (52.1 %mol), followed by mannose (33.9 %mol) and arabinose (9.4 %mol), and residual amounts of glucose (3.1 %mol) and rhamnose (1.5 %mol). According to the sugars content, the IC sample was composed by 18.3%w/w of galactose and 11.9 %w/w of mannose, in accordance with literature range for both residues: mannose (10.2-19.7 %w/w)

and galactose (13.0-24.7 %w/w), with a preponderance of galactose usually verified in instant coffee products (Blanc et al., 1989; Leloup, 2006). At 180 ºC, mannose and galactose accounted for 10.8-15.4 %w/w and 15.3-26.1 %w/w, respectively, in accordance with literature for instant coffee. At the other temperatures tested, the extracts lacked the galactose content. Contrarily to instant coffee products, the MAE performed at 80 ºC exhibited mannose as the main sugar residue (49.0 %mol, 12.1 %w/w), followed by galactose (26.6 %mol, 6.5 %w/w), and arabinose (13.3 %mol, 2.6 %w/w), a pattern relatable with the milder homebrewing coffee extraction methods, in this study tested by the infusion method (Table III.2.1).

Mannose residues in a linear chain and branched with unique residues of galactose form the galactomannans (GM). Galactose is also component, together with arabinose, of the highly branched polysaccharide named type II arabinogalactans (AG) (Nunes & Coimbra, 2002a, 2002b). Based on coffee polysaccharides structural features, it may be estimated the content of GM and AG (as described in Chapter III.1) in the MAE extracts, the commercial IC sample, and in the experiments made at 80 ºC through MAE and infusion method (Figure III.2.9).

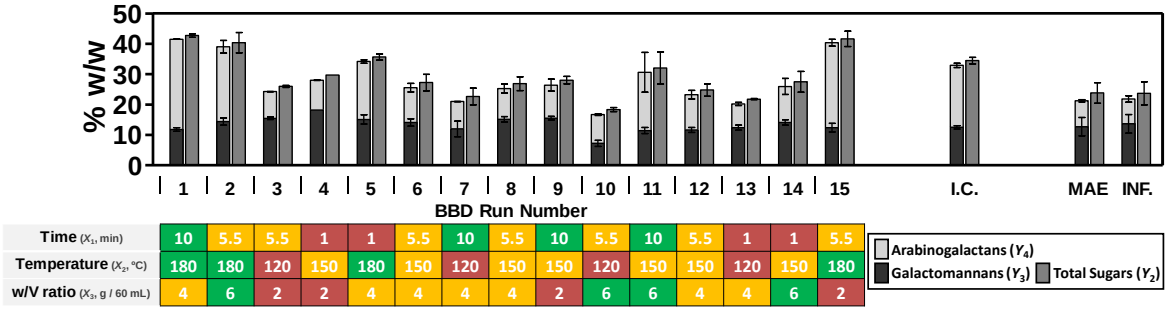


Figure III.2.9. Representation of estimation of the content of the galactomannans (Y_3, %w/w, darker bars) and arabinogalactans (Y_4, %w/w, lighter bars) as part of the total sugars determined (bars at the right) in the BBD runs, instant coffee sample (IC) and the experiments performed at 80 ºC, through MAE and infusion method. For MAE runs, the results from the two reactors for the same condition were plotted together and the conditions used in each run are displayed in **red** for the lower level (-1), **orange** for the intermediate level (0) and **green** for the higher level (+1) in the three factors tested.

The IC sample showed a predominance of AG over GM, while the extractions at 80 ºC showed GM as the main polysaccharide present (53-58 %), in accordance with analyses of roasted coffee infusions of Chapter III.1 and literature (Nunes & Coimbra, 2001; Oosterveld et al., 2003a). Indeed, in hot roasted coffee brews, from infusion to espresso coffee, the GM are the predominant carbohydrate structures, while AG are present as major polysaccharides in cold coffee brews, once such structures are easily extracted and

GM are more dependent on extraction conditions (Lopes et al., 2016; Nunes & Coimbra, 2001; Shin, 2017, and Chapter III.1). Figure III.2.9 shows that even performing the extraction at, at least 120 ºC, some of the MAE runs were more related to the coffee infusions, while others resembled the IC sample pattern. The GM ranged from 6.5 to 18.2 %w/w (a 2.8-fold variation), while the AG content varied from 7.5 to 29.7 % (a 4.0-fold variation). The Pareto chart (Figure III.2.10a) showed that the amount of GM in the extracts was more dependent on w/V ratio, however time and temperature also exerted considerable influence.

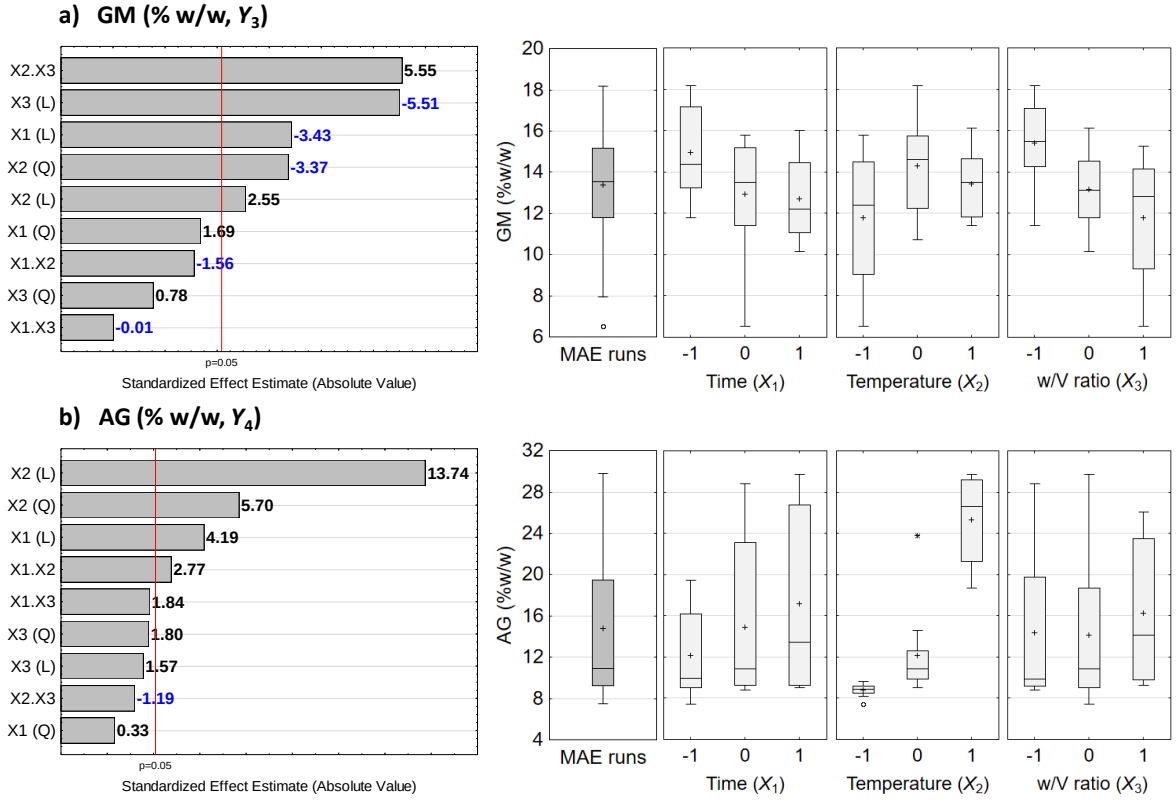


Figure III.2.10. Pareto chart (left) and box plot (right) showing the effects of time (X_1), temperature (X_2), and w/V ratio (X_3) during the MAE coffee experiments on the (a) GM (Y_3, %w/w) and (b) AG (Y_4, %w/w) content in the extract. In the Pareto chart, the negative and positive effects are highlighted in blue and black, respectively. L and Q represent the linear and quadratic effects, respectively. The region (right side) of statistical significance (95% confidence level) is defined by the vertical line. In the box plot is represented the distribution of the raw data, containing the box the interquartile range and limiting the whisker the non-outlier range, with the median represented as (—) and the mean as (+), while outliers (coeff. 1.5) are represented as (º) and extremes (coeff. 3) as (*).

Increasing time and incrementing the w/V ratio led to the production of extracts with lower relative content of GM (Figure III.2.11a-c). Moreover, the interaction $X_2.X_3$ appeared as significant, meaning that the w/V ratio effect was more significant at lower temperatures, with lower w/V ratio associated to higher GM presence in the extracts, while at higher temperatures the content was more similar (Figure III.2.11c).

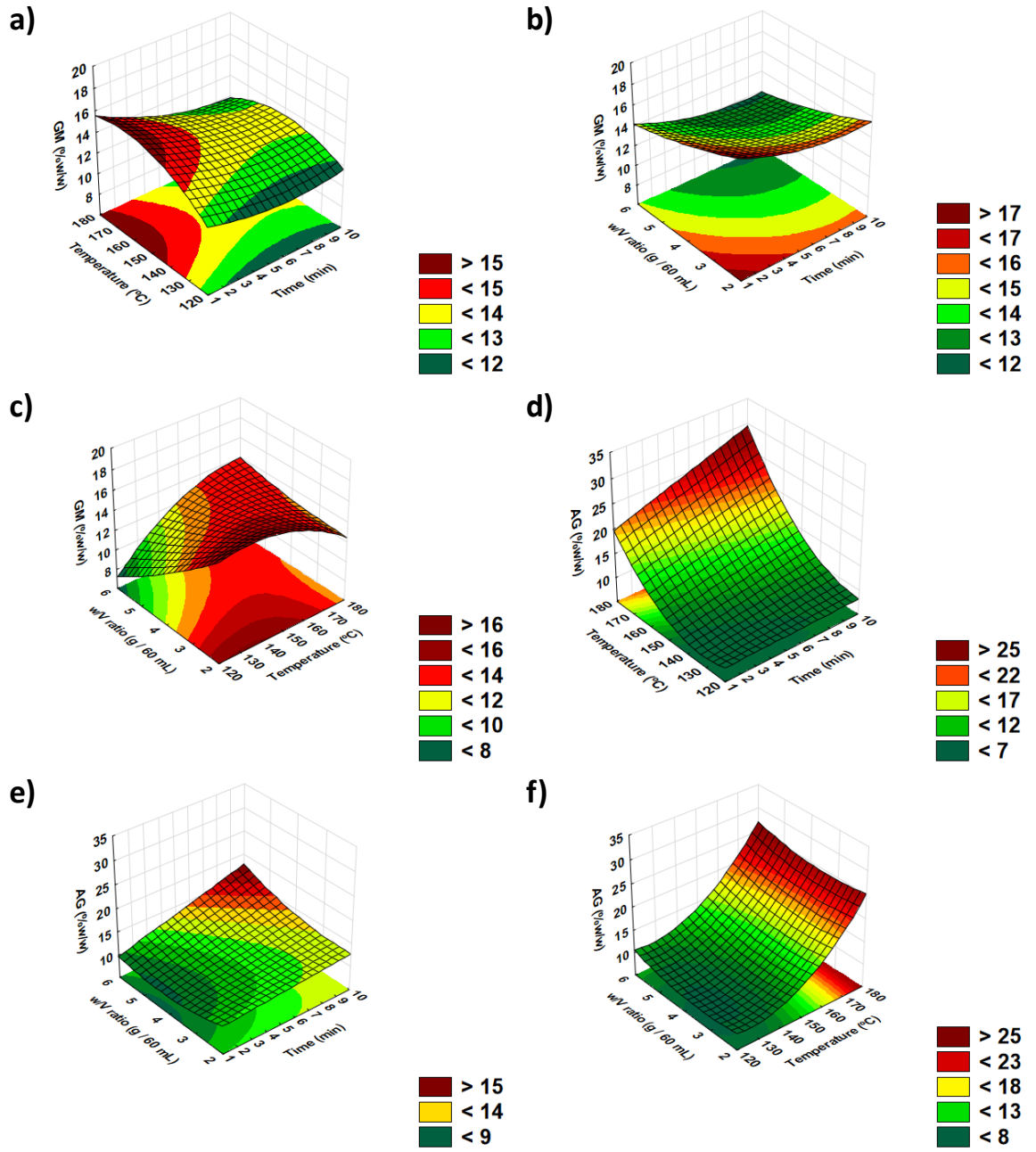


Figure III.2.11. Response surface plots (3D) of the model developed representing the variation of the galactomannans (a-c) and arabinogalactans (d-f) content in the extract showing the effect of the different MAE parameters. In each plot, the remaining independent variables was maintained at their intermediate level: *time* (X_1) - 5.5 min; *temperature* (X_2) – 150 ºC; w/*V ratio* (X_3) – 4 g/60 mL. a/d) time (X_1) × temperature (X_2); b/e) time (X_1) × w/V ratio (X_3); c/f) temperature (X_2) × w/V ratio (X_3).

Pareto chart (Figure III.2.10b) highlighted that the temperature was the main effect responsible for the variation of AG content, which was also illustrated by the response surface models in Figure III.2.11 (significant model (p<0.0001), with non-lack-of-fit and high determination coefficient (R^2= 0.92), Table II.2.3). The increase in the extraction time resulted in higher relative amount of AG (Figure III.2.11d,e). However, the significance of

the interaction $X_1.X_2$ highlights that the effect of time was much more remarkable when the temperature of extraction was higher, leading to considerable differences (as AG content increased) between performing the extraction during 1 or 10 min in MW device (Figure III.2.11d). There was a linear and strong positive correlation between the increase in AG (Y_4) and the total sugars in the extract (Y_2, r=0.94, p<0.0001, Figure III.2.12) and also extraction yield (Y_1, r=0.94, p<0.0001), meaning that the increase in the relative amount of carbohydrates in the extract or compounds extracted was accompanied by an increase in the preponderance of AG.

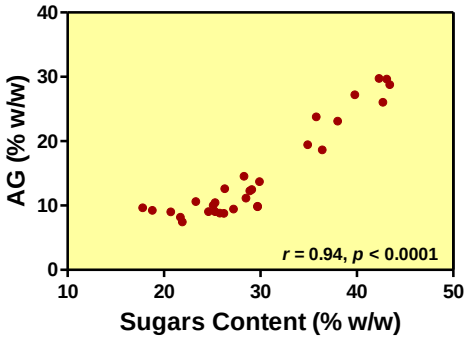


Figure III.2.12. Representation of estimated arabinogalactans content in the extract (AG, Y_4, %w/w) in function of carbohydrates in the extract (Y_2, %w/w).

In conventional methods (Chapter III.3.1) the same trend was observed for GM instead (Figure III.1.8 in Chapter III.3.1). The relative content of AG in the extract does not exceed 12 %w/w, while the maximum of GM (18 %w/w) is similar to the occurred in the present study, meaning that increasing the temperature did not result in an increase of GM presence in the extract, increasing the preponderance of AG instead.

III.2.3.3. Colour of the extracts ($K_{mix,\ 405\ nm}$)

$K_{mix,\ 405\ nm}$, a colour dilution factor, allows to measure the intensity of the brown colour of the coffee solutions and may also be seen as an indicative of the melanoidins content in the extract, the brown colour compounds with undefined structure formed during the roasting of the coffee beans (Bekedam et al., 2006; Lopes et al., 2016; Nunes et al., 2012). The higher the $K_{mix,\ 405\ nm}$, the browner the solutions and the extracts. The $K_{mix,\ 405\ nm}$ values ranged from 0.33 to 0.64 mL mg^{-1} cm^{-1}, a 2-fold variation along MAE conditions. The IC sample exhibited a higher value (0.68 mL mg^{-1} cm^{-1}), while the runs performed at 80 ºC exhibited similar values: 0.59 and 0.60 for infusion and MAE

experiment, respectively, in accordance with other infusion experiments (Chapter III.1). Box plots of the data (Figure III.2.13) shows that longer extraction times, higher temperatures, and w/V ratios in the MAE experiments led to a decrease in $K_{mix,\ 405\ nm}$ values, indicative of a decrease in brown colour of the extracts as illustrated by Figure III.2.13c. Pareto chart shows that all the parameters studied had significant effect on $K_{mix,\ 405\ nm}$ with higher preponderance for temperature, through significant linear and quadratic terms (41.9 and 5.9 %, respectively), and a negative effect.

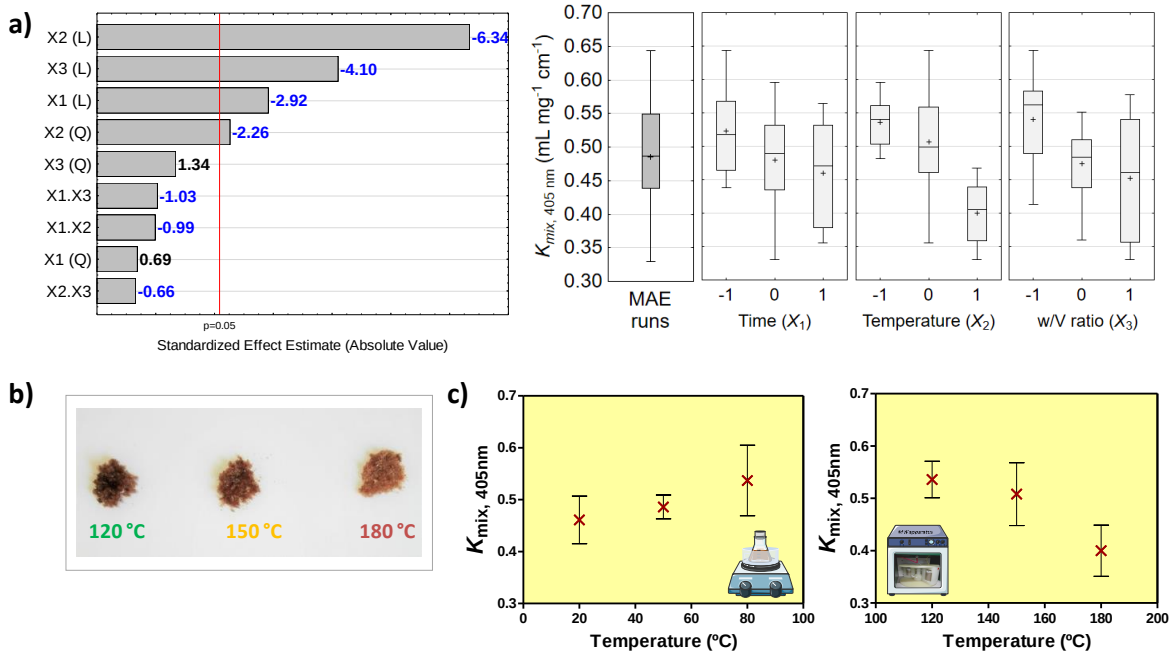


Figure III.2.13. (a) Pareto chart and box plot showing the effects of time (X_1), temperature (X_2), and w/V ratio (X_3) during the MAE coffee experiments on the $K_{mix,\ 405\ nm}$ (mL mg^{-1} cm^{-1}) values of the extracts. In the Pareto chart, the negative and positive effects are highlighted in blue and black, respectively. L and Q represent the linear and quadratic effects, respectively. The region (right side) of statistical significance (95% confidence level) is defined by the vertical line. In the box plot is represented the distribution of the raw data, containing the box the interquartile range and limiting the whisker the non-outlier range, with the median represented as (—) and the mean as ($^+$), while outliers (coeff. 1.5) are represented as (^{o}) and extremes (coeff. 3) as (*). (b) Representation of the colour of the extracts at the three MAE temperatures tested. (c) Comparison of $K_{mix,\ 405nm}$ values for the conventional system (left, Chapter III.1.) and MAE (right) grouped by the temperatures tested.

As the extract became lesser brown with increasing temperatures of extraction, associated to higher extraction yields, the compounds additionally extracted (at higher temperatures) should be colourless when compared to the ones more easily extracted. Alternatively, these compounds may derive from the degradation of the browner compounds, with a consequent decreasing in the browning intensity. In the conventional extraction the opposite occurred with increasing values with higher temperatures. It was

found a negative and strong correlation (r=-0.81, p<0.0001) between the content of AG in the extract and $K_{mix,\ 405nm}$ values, meaning that the extracted AG were less coloured than the compounds already present in the extract without the use of drastic conditions (180 ºC). Thus, it may be obtained an extract rich in AG, comparable to instant coffee, but with a yellowish appearance, as observed in some commercial brands of instant coffee. The clearer appearance than the brown coffee may be attractive for food applications, where the intense colouring could be a disadvantage.

III.2.3.4. Caffeine and 5-CQA

The caffeine content in each extract after MAE experiment ranged from 3.7 to 7.3 %w/w, a 2.0-fold variation, while the analysis of IC sample revealed a value of 4.9 %w/w, in accordance with literature reports (2.4-5.2 %w/w) (Capek et al., 2014; Gant et al., 2015; Leloup, 2006; Ludwig et al., 2014a; Moreira et al., 2005; Rodrigues & Bragagnolo, 2013). The extraction performed by infusion method and by MAE at 80 ºC showed higher values (7.7-8.4 %w/w), in accordance with home brewing coffee methods and Chapter III.3.1 (7.2-9.7 %w/w) (Petracco, 2001; Severini et al., 2015). Literature shows that instant coffee exhibit lower content *per* serving compared to home brewing coffee methods (Ludwig et al., 2014a; Moreira et al., 2005; Rodrigues & Bragagnolo, 2013; Villalón-López et al., 2018). MAE already proved to efficiently extract caffeine from green beans, achieving higher yields than conventional methods (Upadhyay et al., 2012). It was possible to establish a negative and strong correlation between the relative content of caffeine in the extracts and the extraction yield (r=-0.93, p<0.0001) and carbohydrates content (r=-0.92, p<0.0001) (Figure III.2.14). This suggests that the relative content of caffeine in the extract is more dependent on the overall extraction of carbohydrates than properly to caffeine itself, *i.e.* increasing the relative content of other compounds (as carbohydrates during instant coffee production) diminishes the preponderance of caffeine in the extract.

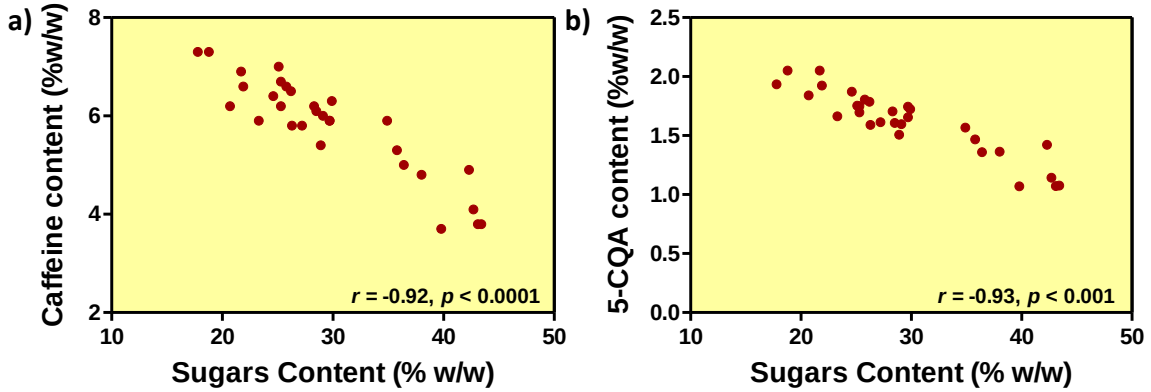


Figure III.2.14. Representation of a) caffeine and b) 5-CQA in the extract in function of carbohydrates in the extract (%w/w).

For 5-caffeoylquinic acid (5-CQA), the major chlorogenic acid present in coffee samples, a similar trend was observed. The amount of 5-CQA in the MAE extracts (1.1-2.1 %) was in accordance with literature reports for the content present in instant coffee samples (0.4-3.5 %w/w) and with the IC sample content (1.0 %w/w) (Moreira et al., 2005; Trugo & Macrae, 1984b). As caffeine, it was reported in literature the impoverishment in chlorogenic acids in instant coffee when compared to other beverages (Rodrigues & Bragagnolo, 2013), associated in the present study to the increment in extraction yield of carbohydrates (r=-0.93, p<0.0001, Figure III.2.14). In fact, the experiments at 80 ºC (MAE and infusion) revealed a greater content (2.3-2.4 %w/w) than the remaining extracts, although arising from the same coffee, with extraction process as the unique source of differentiation, and values in accordance with conventional infusion procedures (1.9-2.9 %w/w) (Chapter III.1). Efforts have been made in the enrichment of instant coffee products with chlorogenic acids to take advantage of the biological activities associated to these molecules (greatly destroyed by roasting process), namely using mixtures of green and roasted extracts (Corso et al., 2016; Gómez-Juaristi et al., 2018; Hoelzl et al., 2010; Sarriá et al., 2016).

5-CQA, as well as caffeine, can also be estimated through the measure of absorbance at the characteristic wavelengths of diluted solutions. It was found a strong positive correlation between 5-CQA content and $K_{mix,\ 325\ nm}$ (r=0.88, p<0.0001, Table 1 in Annex A.III.2) and caffeine content and $K_{mix,\ 280\ nm}$ (r=0.89, p<0.0001), and a negative correlation between these parameters and sugars content (r=-0.93, p<0.0001 for $K_{mix,\ 280\ nm}$ and r=-0.94, p<0.0001 for $K_{mix,\ 325\ nm}$), highlighting that a decrease of preponderance of

smaller molecules as caffeine and 5-CQA is accompanied by an increase in carbohydrates content.

The antioxidant activity of the coffee extracts obtained via MAE was studied by DPPH method. This procedure relies on measuring spectrophotometrically (517 nm) the disappearance of the purple colour of the DPPH radical that was scavenged by antioxidants in solution, presenting the percentage of inhibition as the result. The results (Table III.2.2) showed that inhibition ranged from 43 to 68 % among the coffee MAE runs, with extracts prepared with 0.1 $mg.mL^{-1}$. It was possible to split the values according to the temperatures analysed, with a decreasing inhibition percentage when extracts were obtained at higher temperatures. The reason for such pattern should be linked to the higher extraction of carbohydrates associated to higher temperatures, with a consequent decreasing in the preponderance of smaller molecules with higher antioxidant capacity than carbohydrates, as chlorogenic acids. This is illustrated by the negative correlation between carbohydrates content in the extracts (Y_2, %w/w) and the inhibition percentage (r=-0.76, p<0.0001) or the positive correlation between the latter and 5-CQA content (%w/w) in the extract (r=0.76, p<0.0001). However, many other compounds present in coffee must also play important roles in the antioxidant activity of the coffee extracts, as highlighted the correlations with $K_{mix,\ 280\ nm}$ (r=0.79, p<0.0001) and $K_{mix,\ 325\ nm}$ (r=0.78, p<0.0001), that account with all compounds absorbing at 280 and 325 nm, respectively. Indeed, the analysis of 5-CQA solutions at different concentrations showed that the range of inhibition percentages obtained with MAE runs was obtained with 0.02-0.04 $mg.mL^{-1}$ of 5-CQA. As the amount estimated in each coffee sample was approximately 0.001 $mg.mL^{-1}$ (1 % of the 0.1 $mg.mL^{-1}$ extract prepared), the results suggest that other molecules than 5-CQA should have higher impact for antioxidant capacity.

III.2.3.5. Application of MAE to obtain an instant coffee-like product

The desirability function has been used for optimization purposes, when the goal was to define the conditions that better satisfied the response of interest (Derringer & Suich, 1980; Vera Candioti et al., 2014). To test the feasibility of MAE to quickly obtain a composition similar to a commercial IC product a desirability approach was used. The objective was to obtain an extract with an amount of mannose of 11.9 %w/w, and 18.3

%w/w of galactose, as well as 1.0 %w/w of 5-CQA and 4.9 %w/w of caffeine, attributing equal importance to these parameters, using the developed significant models (p<0.0001), with high determination coefficients (Table III.2.4 and Tables 7-10 in Annex A.III.2). The analysis of single residues was preferred as different populations of carbohydrates must be found in coffee products, although previously the estimation of AG and GM was performed to investigate their preponderance in the coffee extract.

Table III.2.4. Models developed for the description of the variation in dependent variables: mannose (%w/w), galactose (%w/w), arabinose (%w/w), 5-CQA (%w/w), caffeine (%w/w) and $K_{mix,\ 405\ nm}$ (mL mg^{-1} cm^{-1}), as function of the parameters studied (X_1 - time, X_2 - temperature, X_3 - w/V ratio) with the corresponding coefficients of determination (R^2). The models are expressed in terms of coded values ((-1), (0), (+1)).

Response	Model Equation	R^2
Arabinose	$Y_{Ara} = 3.20 + 0.19\ X_1 + 0.82\ X_2 + 0.12\ X_3 + 0.27\ X_2^2 + 0.20\ X_1.X_3$	0.81
Galactose	$Y_{Gal} = 8.81 + 2.28\ X_1 + 7.50\ X_2 + 0.74\ X_3 + 4.67\ X_2^2 + 1.40\ X_3^2 + 2.33\ X_1.X_2 + 1.37\ X_1.X_3$	0.93
Mannose	$Y_{Man} = 13.20 - 1.07\ X_1 + 0.79\ X_2 - 1.71\ X_3 + 0.75\ X_1^2 - 1.57\ X_2^2 + 2.44\ X_2.X_3$	0.80
Caffeine	$Y_{Caffeine} = 6.03 - 0.34\ X_1 - 1.10\ X_2 + 0.30\ X_3 - 0.41\ X_2^2$	0.83
5-CQA	$Y_{5\text{-}CQA} = 1.61 - 0.08\ X_1 - 0.32\ X_2 + 0.06\ X_3$	0.85

Thus, setting the experimental conditions to 9.6 min of extraction time, 168 ºC, and 4.0 g/60 mL as the w/V ratio, allowed to obtain high overall desirability (D=0.8655), corresponding to an extract with 12.8, 18.3, 1.3, and 4.9 %w/w of mannose, galactose, 5-CQA, and caffeine, respectively (Figure III.2.15). This extract would contain a slightly higher amount of mannose and 5-CQA than the proportion verified in the IC sample. In fact, the level of 5-CQA in MAE samples was always slightly higher than in the IC sample used as example. In addition, the use of these conditions was associated to a 40 % extraction yield, which is close to the maximum of 47% achieved in this study.

	Time (X_1, min)	Temperature (X_2, °C)	w/V ratio (X_3, g / 60 mL)
High (+1)	10	180	6
Optimal	**9.64**	**167.88**	**3.98**
Low (-1)	1	120	2

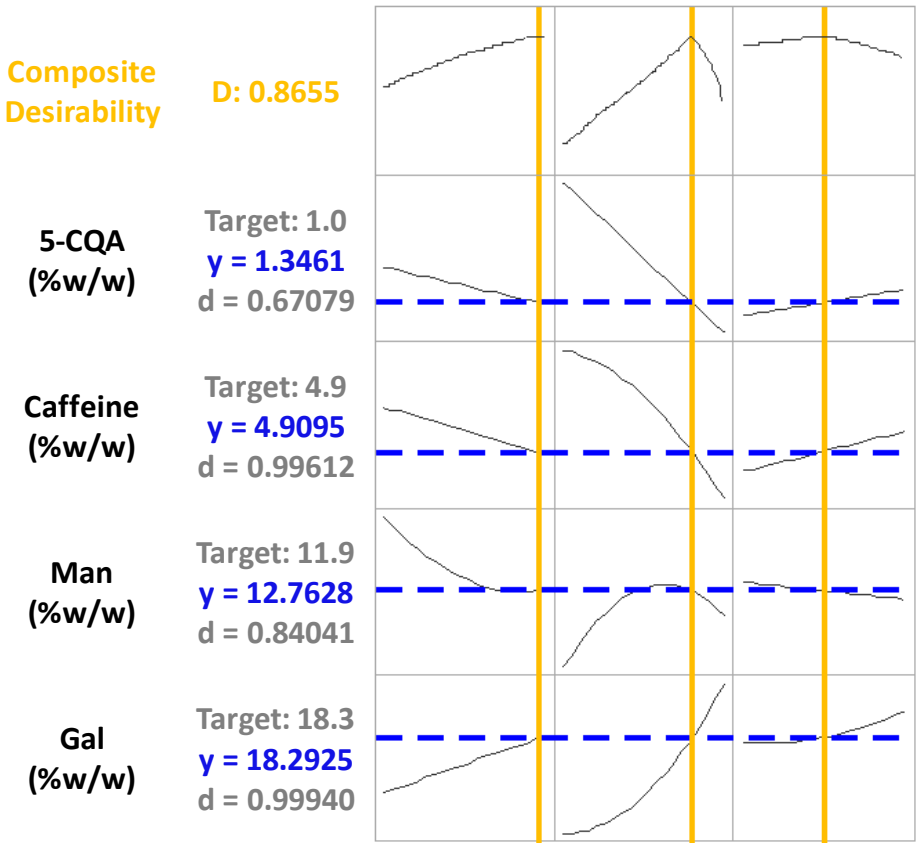


Figure III.2.15. Representation of desirability approach using instant coffee as a hypothetical goal for MAE conditions aiming to equal its composition through four parameters studied.

When 5-CQA was removed from the desirability approach, the overall desirability increased to D=0.9457, with 7.8 min of extraction time, 170 ºC, and 4.2 g/60 mL as w/V ratio, allowing to obtain great similarity with the IC sample (Figure III.2.16). On the other hand, as it was possible to modulate the arabinose content in the extract (p<0.0001, R^2=0.81), the same strategy was used attempting to obtain similar content of this sugar residue. However, the region where MAE coffee extracts exhibited arabinose content related to the one found in IC sample (lower temperatures, Figure 1 in Annex A.III.2) was incompatible to the ones that allowed to equalize the other parameters as, contrary to the high temperature used to produce instant coffee, promoting degraded AG, in MAE this degradation was not so pronounced.

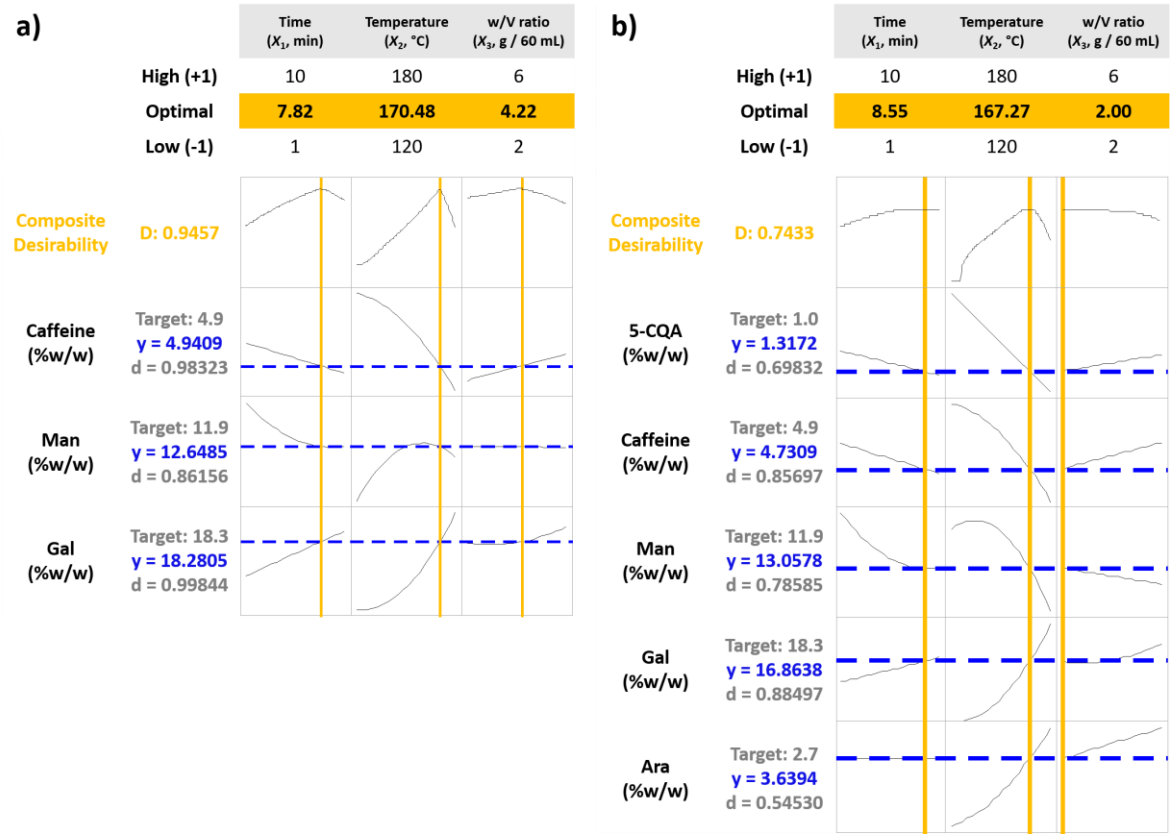


Figure III.2.16. Representation of desirability approach using instant coffee as a hypothetical goal for MAE conditions aiming to equal its composition through four parameters studied, using three (a) or five (b) parameters.

According to the desirability approach, the conditions of MAE run 1 (10 min, 180 ºC, 4g / 60 mL) were the ones that better approach the composition of commercial instant coffee sample among the runs performed. FTIR spectra allow to quickly compare the samples without their destruction or pre-treatment, providing an overall chemical fingerprint of samples composition in a fast and inexpensive way (Barbin, et al., 2014). It was already used to detect adulterations in roasted coffee and instant coffee or discriminate among coffee attributes as coffee species or sensorial quality. Figure III.2.17 evidences the overall great similarity between FTIR spectra of the samples, in accordance with the chemical analysis performed. The spectra have higher intensities in the carbohydrate region (800-1200 cm^{-1}) associated to their preponderance in the extracts (35-43 % of the samples) and resembling literature FTIR analysis of instant coffees (Capek et al., 2014; Mohammad, 2015).

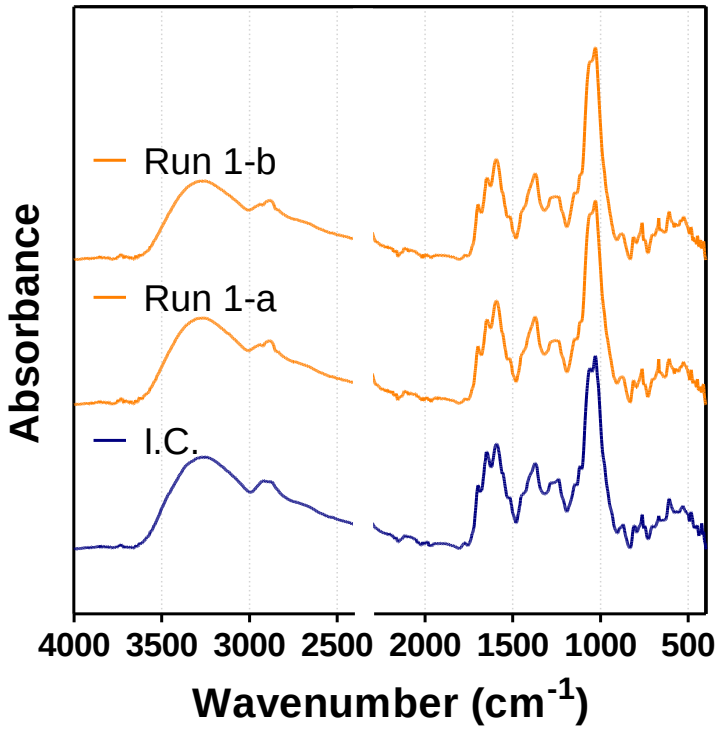


Figure III.2.17. FTIR spectra of run 1-a, run 1-b and IC samples in the 400-4000 cm^{-1} region.

III.2.3.6. Holistic analysis of the coffee MAE

To evaluate the extractability of the compounds, a holistic analysis of the extraction process was performed for the extracts obtained (Figure III.2.18a-c) and for the remaining residues (Figure III.2.18d,e and Table 1 in Annex III.3.2). The results were grouped by the extraction temperature, the variable that led to higher responses variability. Figure III.2.18a highlights the concomitant increase of extraction yield and carbohydrates, while caffeine and 5-CQA exhibit similar values across the temperatures tested. As the initial roasted coffee sample presented 1.9% w/w of caffeine, in accordance with literature values (0.8-2.6 %w/w) (Gant et al., 2015; Hečimović et al., 2011; Ludwig et al., 2014a), it was possible to infer that caffeine was effectively extracted throughout all MAE conditions tested. The same behaviour can be observed for 5-CQA.

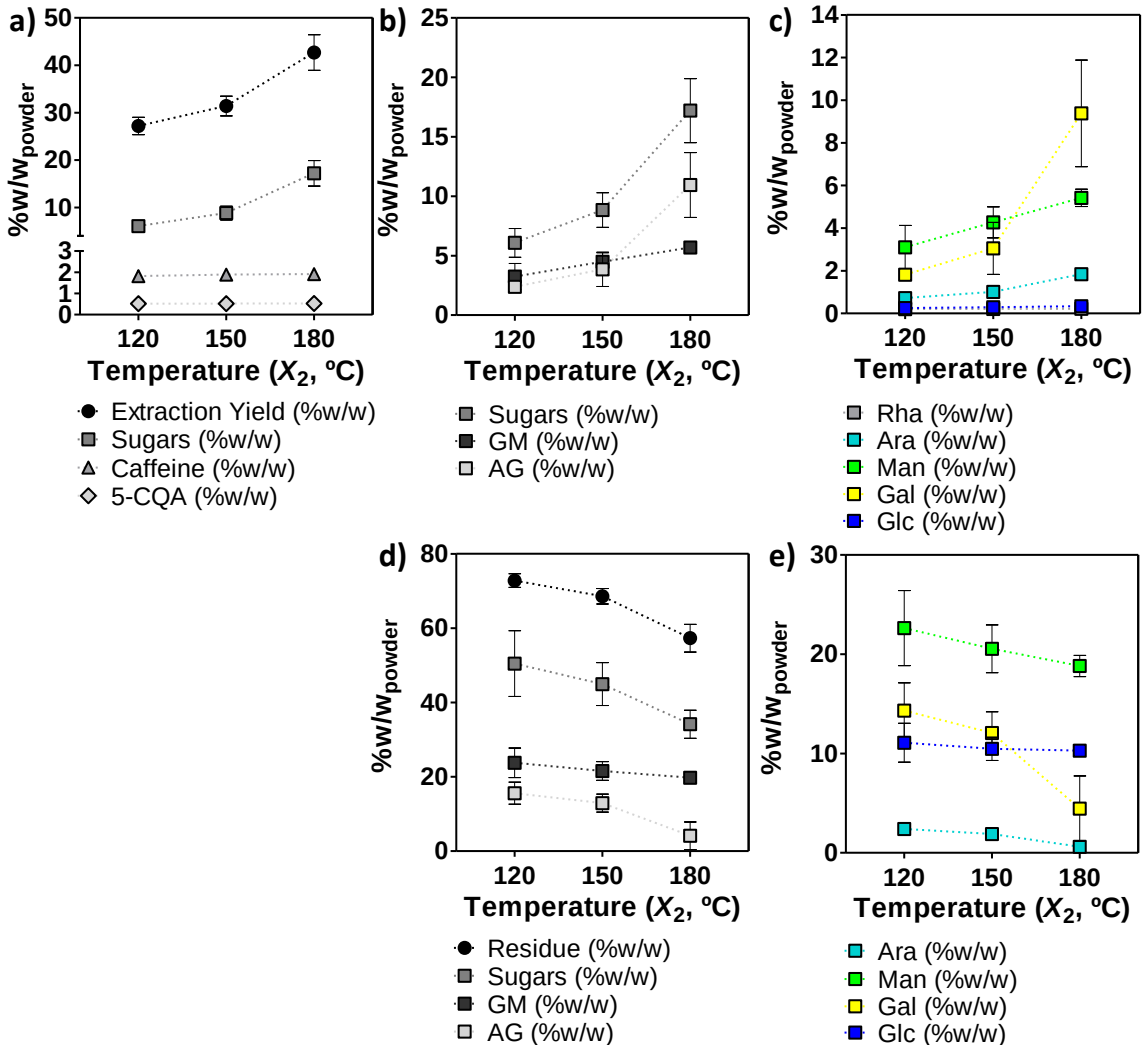


Figure III.2.18. Representation of the content of compounds extracted from coffee powder (a-c, %w/w_{powder}) and content of compounds that remained in the residue (d,e, %w/w_{powder}). The runs were grouped by extraction temperature. Results were expressed as mean ± standard deviation of 8 (120, 180 ºC) and 14 (150 ºC) runs.

Regarding carbohydrates, Figure III.2.18a, and Figure 19, reveals that although the considerable carbohydrates extraction, the residue left after MAE still contained a great fraction of carbohydrates to be extracted, namely GM, even at 180 ºC (Figure III.2.11d and Figure III.2.19). On the other hand, almost all AG content (more than 90 % in some runs) ended up in the extract in one step of MAE at 180 ºC, which does not occur with milder conventional extractions. This justifies the extraction of AG from spent coffee grounds after a first MAE procedure, while predominantly GM are recovered only after successive extraction steps (Passos & Coimbra, 2013; Passos et al., 2014a). Moreover, the GM cannot be extracted without the previous removal of AG. MAE may also be viewed as a rapid

methodology to remove almost all galactose (ending with ≈5% mol), providing a residue with a great predominance of GM and cellulose.

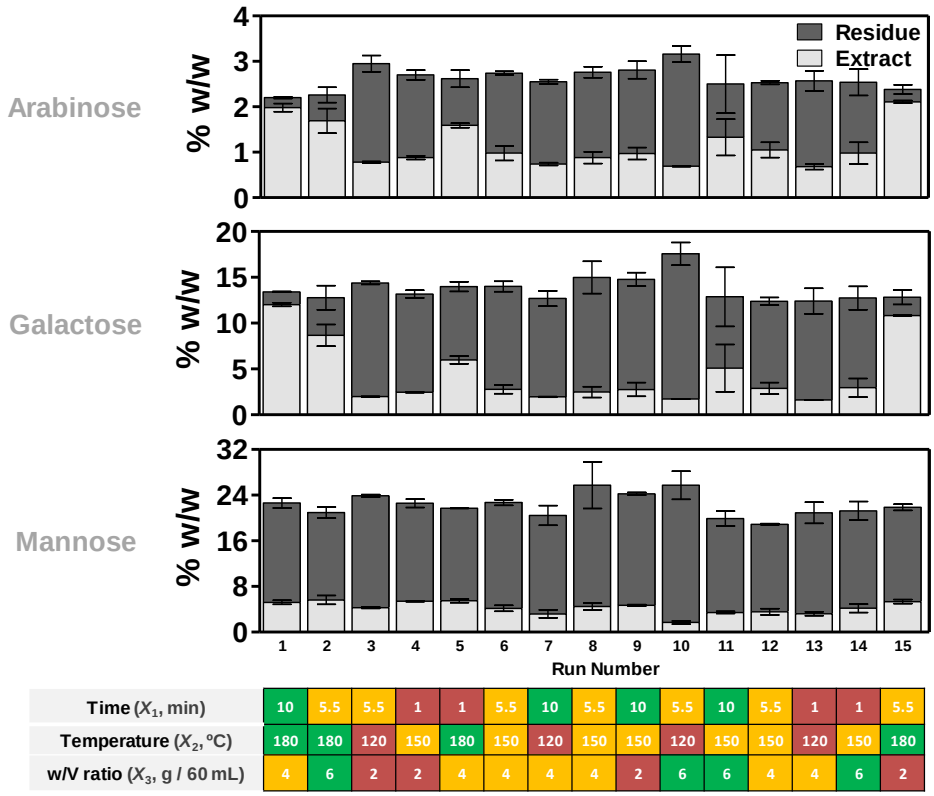


Figure III.2.19. Representation of the content (%w/w) of arabinose, galactose and mannose extracted from roasted powder that ends up in the extract (lighter) and residue (darker) after MAE experiments.

The different content of GM and AG all over the design space, ranging the ratio of GM/AG from 0.4 to 1.9, highlighted that it is possible to modulate the preponderance of each of the structures, according to possible applications for providing different viscosity of coffee brews or even for health promoting effects due to their immunomodulatory activities (Nosáľová et al., 2011; Simões et al., 2009). Figure III.2.18c,e and Figure 19 highlight that the pattern followed by galactose was accompanied by arabinose, showing a strong positive correlation between the extractability of the two sugar residues along the experiments, both in extracts (r=0.97, p<0.0001) and residues (r=0.98, p<0.0001), in accordance with the occurrence of AG. The analysis of the residues confirmed that the greatest amount of mannose and mainly glucose remains in the residue after MAE.

III.2.4. Concluding remarks

This study shows that MAE can be used to obtain extracts with different carbohydrates, caffeine, chlorogenic acids and coloured compounds, approaching instant coffee composition with high extraction temperatures (180 ºC), while in milder conditions at 120 ºC and 150 ºC the extracts are still comparable to home brewing methods. The higher extraction at 180 ºC is associated to a substantial increase in AG extraction. Indeed, temperature exerted a crucial role in coffee extracts differentiation, although time and w/V ratio also lead to different values in the responses. The composition of extracts obtained with higher yields comparing to conventional infusion method is substantially different from the composition of espresso coffee, hindering MAE application to obtain espresso-like extracts. Nevertheless, using less than 10 min of extraction, it was possible to obtain an extract with instant coffee-like composition. On the other hand, under a circular economy concept, MAE of roasted coffee also provides a galactomannan and cellulose rich residue able to be a source of valuable polysaccharides.

Figure III.2.20 shows that even though the coffee MAE resulted in higher polysaccharides yields than conventional method, in those conditions (mainly at 180 ºC, GM/AG ratio from 0.4-0.9), the extracts composition differed from EC (GM>AG), and approach instant coffee (AG>GM). Therefore, the next step was performed focusing the study on the in-depth analysis of the conventional infusion method.

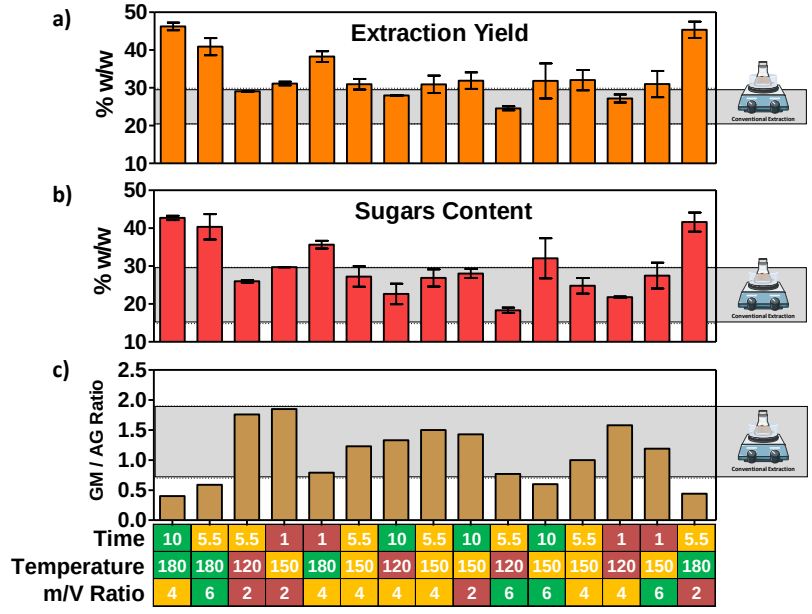


Figure III.2.20. Representation of extraction yield (a, %w/w), sugars content (b, %w/w), and GM/AG ratio (c) for the MAE runs with the illustration of the values obtained with the conventional method (grey region).

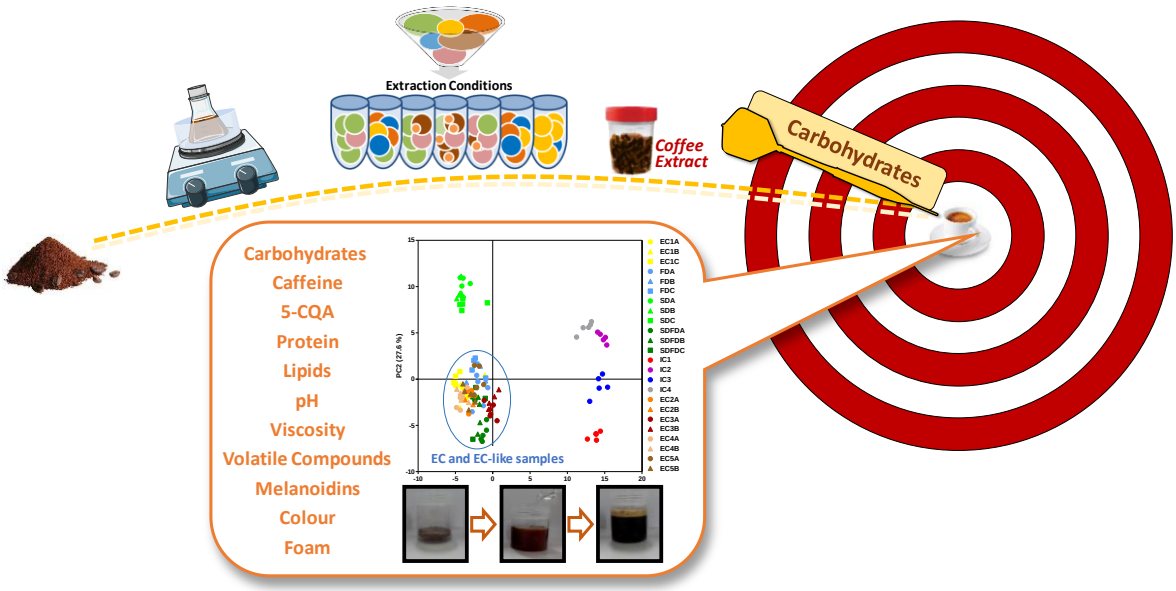
III.3. MODULATION OF INFUSION PROCESS TARGETING ESPRESSO PROPERTIES

The content of this chapter was submitted for publication:

Lopes, G. R., Passos, C. P., Rodrigues, C., Teixeira, J. A., & Coimbra, M. A. (2019). Coffee brews with espresso properties using quality by design approach targeting carbohydrates.

III.3.1. Abstract

Coffee brews as espresso, instant, or filtered coffee exhibit distinct physico-chemical properties, depending on the preparation method and extraction conditions. Thus, the different relative amount and composition of carbohydrates, proteins, chlorogenic acids, caffeine and lipids of coffee brews should affect body, colour, viscosity and/or organoleptic characteristics. This allows to hypothesize that a quality-by-design approach can be used to modulate coffee infusion process and obtain coffee extracts that closely resemble espresso-like physico-chemical properties. The results showed that espresso coffee composition can be clearly distinguished from instant coffee by their higher galactomannans/arabinogalactans ratio. The influence of freeze- and spray-drying processing methodologies on sample properties was also evaluated. The strategy used allowed to obtain instant coffee powders with espresso composition regarding carbohydrates (17-19%w/w, mannose/galactose>1), caffeine (8.7-8.8%w/w) and 5-caffeoylquinic acid (2.4-2.5%w/w). The brews prepared by their redissolution at espresso concentration had viscosity, pH, and foamability capacity resembling the desired espresso coffee characteristics, as well as a volatile compounds profile similar to espresso extracts, opening the possibility for further exploitation of these extracts.



III.3.2. Aim of the study

The espresso coffee (EC) is commonly defined as a coffee brew of reduced volume and distinct sensorial properties such as body, smell, taste and intense colour, with a characteristic persistent foam that covers the liquid (Illy & Viani, 2005; Nunes et al., 1997). The EC preparation supposes that hot water passes through compacted roasted coffee under pressure during a short extraction time, originating a concentrated brew (Illy & Viani, 2005). Coffee brews composition have been shown to depend on the preparation methods, including EC, filter, instant, Moka and coffee cold brew (Angeloni et al., 2019; Bell et al., 1996; Caporaso et al., 2014; Gloess et al., 2013; Ludwig et al., 2012). Nevertheless, all coffee brews are composed by carbohydrates, caffeine, chlorogenic acids, protein, lipids, and melanoidins, among other components.

For extraction studies, the use of the same coffee product avoids variations related to features as coffee species, geographical origin, or roasting degree that affect the composition of the roasted beans and consequently the properties of coffee brews (Illy & Viani, 2005; Maeztu et al., 2001a). Even within the same extraction procedure, the range of values found for coffee compounds may present a wide variation. Several variables as time, temperature, w/V ratio or grinding affect the coffee extraction processes, from espresso to infusion, or filtered ones, as discussed in Chapter I.2.2. and Chapter III.1 (Albanese et al., 2009; Andueza et al., 2002, 2003a, 2003b, 2007; Crozier et al., 2012; Ludwig et al., 2014b; McCusker et al., 2003). Indeed, there is no clear definition of a restricted composition range for each coffee brew type as discussed in Chapter I. This opens the possibility of modulating the extraction conditions to obtain coffee brews with pre-desired characteristics, even when they are usually associated to other extraction methodologies, as the coffee machine in EC. As a major coffee brew component and with impact on espresso properties as viscosity and foam stability, carbohydrates should be seen as target compounds with the purpose of developing coffee brews with specific properties.

Herein, it was hypothesized that the modulation of the carbohydrate content and composition of a coffee infusion process through a quality by design approach allows to obtain extracts with EC-like composition. The use of the same product for conventional EC preparation with an EC machine allows defining a composition profile, establishing the relative occurrence of coffee compounds in an EC cup, used as a guideline to be replicated

by the infusion process. For comparison purposes, other EC samples and also commercial instant coffee samples, some of them labelled as "espresso", were characterized and their similarity with the EC-like samples prepared was discussed. The influence of freeze- and spray-drying processing methodologies was also evaluated. Moreover, it was tested the capacity of the coffee extract for producing the most distinguishable EC property, the foam, through CO_2 injection and the addition of salts able to release CO_2 when dissolved in water. Furthermore, a volatile compounds profile (71 compounds among them 19 key odorants) of the coffee extracts was performed comparing the extracts with a fresh EC brew.

III.3.3. Results and discussion

III.3.3.1. Espresso coffee reference

The analysis of EC, obtained either by a conventional espresso machine or with single-dose espresso coffee capsules, are presented in Table III.3.1. The extraction process originated brews containing 1.19-1.35 g of total solids *per* cup of 40 mL, with a concentration of 29.8-33.8 $mg.mL^{-1}$. These contents were similar to those reported in literature (0.92-1.29 g, 23.0-32.3 $mg.mL^{-1}$) using equal amount of coffee powder (6 g) and water (40 mL) (Lopes et al., 2016; Nunes et al., 1997).

Table III.3.1. Sugars content and composition obtained after acid hydrolysis of espresso (6.0 g of coffee powder, 40 ± 2 mL of distilled water) and instant coffees brews.

Sample	Total Solids	Total Carbohydrates	Sugars Composition				
			Rha	*Ara*	*Man*	*Gal*	*Glc*
	(g *per* 40 mL)	(% w/w)[A]	(%mol)[B]				
EC1*	1.32±0.09a	16.2±1.6[a]	3.5±0.5[a]	14.5±1.0[a]	46.9±2.5[a]	30.3±1.3[a,b]	4.8±0.9[a]
EC2**	1.31±0.01a	18.1±2.2[a]	4.4±0.1[b]	16.8±0.4[b]	40.9±0.3[b]	32.1±0.5[a,c]	5.8±0.2[a,b]
EC3**	1.35±0.01a	15.3±0.9[a]	5.0±0.0[b,c]	17.2±0.2[b]	36.4±1.4[c,d]	29.1±1.1[b]	12.4±0.3[c]
EC4**	1.19±0.01b	15.5±1.0[a]	5.3±0.6[c]	19.4±0.6[c]	36.8±0.5[c,d]	32.0±0.3[a,c]	6.5±0.5[b]
EC5**	1.25±0.02b	18.0±1.3[a]	4.6±0.1[b,c]	16.3±0.3[b]	41.1±0.7[b]	33.0±1.1[c]	5.1±0.2[a]
IC1#	-	34.5±1.1[b]	1.5±0.1[d]	9.4±0.6[d]	33.9±1.0[d,e]	52.1±1.5[d]	3.1±0.4[d]
IC2##	-	37.3±3.2[b]	1.2±0.0[d]	10.0±0.1[d]	40.2±0.3[b,c]	45.6±0.3[e]	3.1±0.1[d]
IC3##	-	36.7±2.4[b]	1.4±0.0[d]	9.6±0.3[d]	30.9±0.1[d,e]	55.0±0.3[f]	3.1±0.1[d]
IC4##	-	41.8±1.0[c]	1.4±0.0[d]	12.8±0.1[e]	33.7±0.8[e]	49.6±0.5[d]	2.5±0.1[d]

[A]: relative content of the compounds in relation to the total solids extracted; [B]: relative molar content of sugar residues in the espresso coffee samples; *values used as reference for EC optimization through infusion process as the mean of the assays performed using two grinding levels; **commercial single-dose espresso coffees capsules; # instant coffee used as reference; ## commercial instant coffee samples labelled as "espresso". Columns with different characters in each row indicate samples with significant difference (p<0.05).

Table III.3.1 shows that carbohydrates constitute 15.3-18.1 %w/w of EC, within the literature range for these coffee brews (11.7-20.0%), (Nunes et al., 1997) and significantly lower than the amount of IC (34.5-41.8 %w/w). The ICs labelled as espresso coffee were also constituted by higher content of carbohydrates (36.7-41.8 %w/w), in accordance with literature reports for IC brews (35-39 %w/w),(Blanc et al., 1989; Capek et al., 2014; Leloup, 2006) but not for EC. Sugars composition (Table III.3.1) shows that in all EC, mannose was the major sugar residue (36.4-46.9 %mol) followed by galactose (28.8-33.0 %mol). This contrasted with the sugars composition of IC, where galactose was the main sugar residue (45.6-55.0 %w/w), followed by mannose (30.9-40.2 %mol), in accordance with literature for IC samples (Capek et al., 2014). Arabinose was also a sugar residue relatively abundant in both type of samples, although with relative higher abundance in EC (13.0-19.4 %mol) than IC (9.4-12.8 %mol), with the same trend occurring with minor sugar residues as glucose (EC: 4.7-12.4 %mol, IC:2.5-3.1 %mol) and rhamnose (EC: 3.1-5.3 %mol, IC: 1.2-1.5 %mol). In EC samples, mannose and galactose represented 5.8-7.8 %w/w and 4.6-6.2 %w/w of brew solids content, respectively, which are within the ranges defined in literature (3.8-14.3 %w/w for mannose and 1.4-8.0 %w/w for galactose) (Nunes et al., 1997). In IC, mannose represented 11.5-15.3 %w/w, while galactose represented 17.3-20.6 %w/w, in accordance with literature (10.2-19.7 %w/w for mannose and 13.0-24.7 %w/w for galactose) (Blanc et al., 1989; Leloup, 2006). Thus, the mass ratio of Man/Gal in the EC analysed had a range of 1.1-1.8, while for the IC it was 0.6-0.9. In Chapter III.1, it was shown that the modulation of operational parameters of the infusion process allows to obtain extracts with Man/Gal ratio within the range 0.9-2.4, depending on the extraction conditions, with impact in coffee viscosity. The Man/Gal ratio depends mainly on temperature and amount of coffee to be extracted. A finer grinding (EC1G1) was associated to an EC extract with higher Man/Gal ratio and higher viscosity (1.09±0.02 cSt for EC1G1 and 1.06±0.01 cSt for EC1G3 at 30 $mg.mL^{-1}$) (Table 1 in Annex A.III.3.). Thus, it should be possible to modulate the infusion process to obtain an extract with Man/Gal ratio, carbohydrate content, and viscosity similar to EC. To fulfil this hypothesis, a comprehensive study of the coffee infusion process was settled according to a central composite design, as discussed in Chapter III. The espresso carbohydrate composition, as the major class of compounds of EC brew and exhibiting important organoleptic properties, was chosen as target to define the operational extraction conditions. To

eliminate the variability that could occur by the use of different blends, with distinct coffee species and/or roasting degree, the starting material used for the reference (EC1) and infusion experiments, was the same. The following conditions were studied: time (10, 185, and 360 min), temperature (20, 50, and 80 ºC), w/V ratio (1, 3.5, and 6 g/30 mL), and grinding level (1-3). Significant models (p<0.0001) with non-significant lack-of-fit and high determination coefficient (R^2 = 0.82-0.95, Table III.3.2) were developed for overall sugars content (%w/w) and for the molar composition (%mol) of the three main sugar residues (Table 2 in Annex A.III.1 and Tables 2-4 in Annex A.III.3).

Table III.3.2. Models developed for the sugar related variables: sugars content (%w/w), arabinose (%mol), mannose (%mol) and galactose (%mol), as function of the parameters studied (X_1 - time, X_2 - temperature, X_3 - w/V ratio) with the corresponding coefficients of determination (R^2). The models are expressed in terms of coded values ((-1), (0), (+1)).

Response	Model Equation	R^2
Sugars (%w/w)	$Y_1 = 22.02 + 0.10\ X_1 + 2.94\ X_2 - 0.85\ X_3 - 1.67\ X_4 - 1.31\ X_1^2 - 1.64\ X_1.X_2 - 1.22\ X_2.X_3$	0.82
Ara (%mol)	$Y_2 = 14.23 + 0.18\ X_1 - 2.14\ X_2 + 1.13\ X_3 + 0.58\ X_4 + 2.70\ X_2^2 + 1.26\ X_1.X_2 + 0.58\ X_1.X_3$	0.88
Man (%mol)	$Y_3 = 49.30 - 0.92\ X_1 + 5.19\ X_2 - 3.30\ X_3 - 1.29\ X_4 + 5.19\ X_2^2 - 4.34\ X_1.X_2 - 1.78\ X_1.X_3 - 2.66\ X_2.X_3$	0.95
Gal (%mol)	$Y_4 = 28.85 + 1.03\ X_1 - 1.65\ X_2 + 1.96\ X_3 + 0.26\ X_4 + 2.20\ X_2^2 + 2.44\ X_1.X_2 + 1.01\ X_1.X_3 + 1.90\ X_2.X_3$	0.86

Figure III.3.1 illustrates the two approaches applied used in the optimization strategy: a) a desirability approach, where the desired values (those from EC1) were established as goals and b) the overlaid contour plot strategy, where a range for EC1 was defined as the objective (all minimum and maximum values were considered).

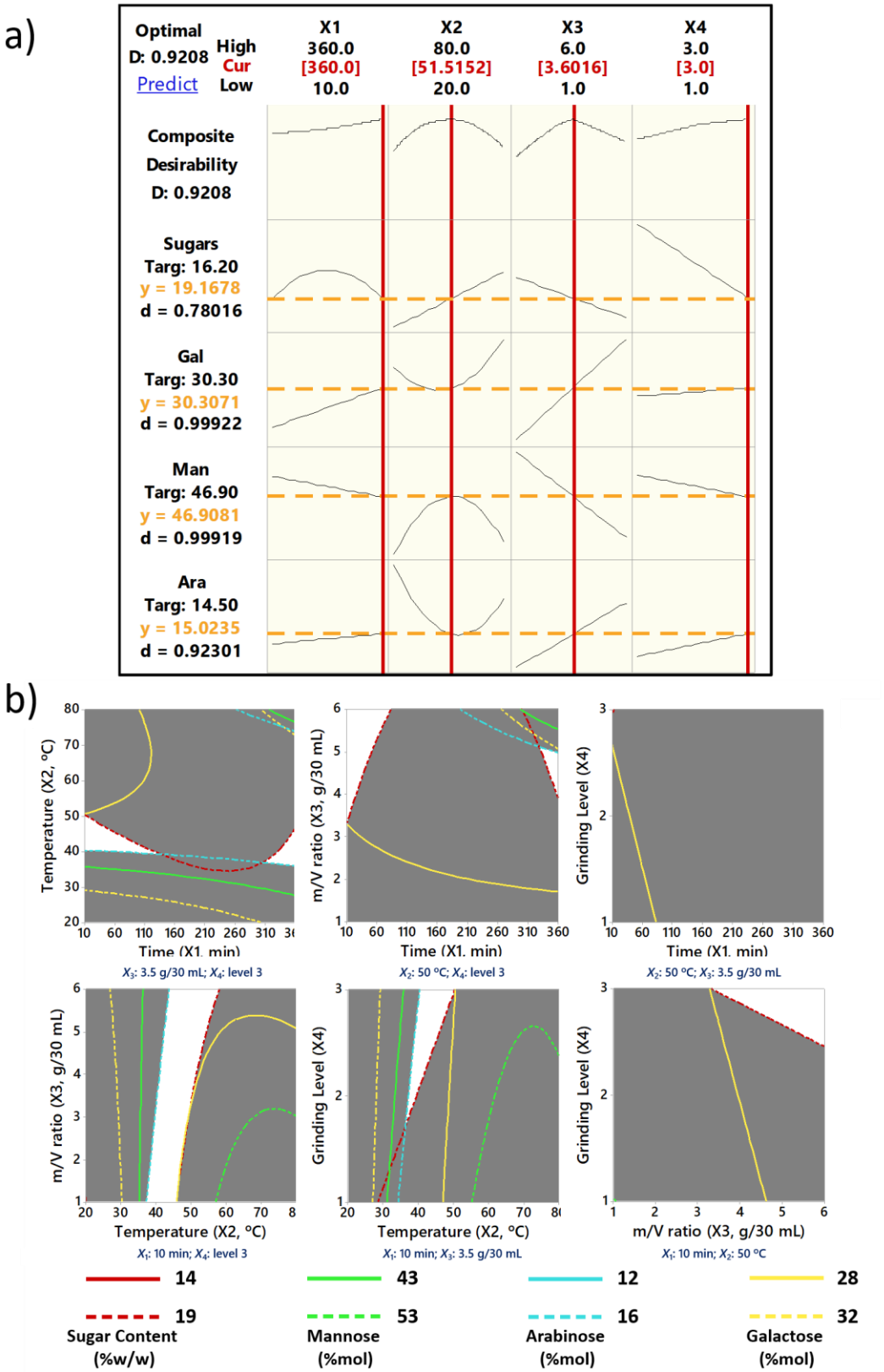


Figure III.3.1. Optimization plots using the sugars content and composition (the three main sugars) as parameters representing the a) response optimizer strategy according to desirability function and having the mean values for espresso coffee as reference and b) overlaid contour plot strategy taking into account the minimum and maximum values for espresso coffee used as reference, fixing two independent variables while varying the remaining two. The area of interest is highlighted in white.

The desirability approach showed that a coffee infusion extraction during 360 min, at 51.5 ºC, 3.6 g *per* 30 mL and coarser particles (level 3) would fit EC1 composition with an overall desirability of 0.92. This would originate an extract with slightly higher amount of sugars content (19.2 %) than EC1, although within the average reported content for EC. The overlaid contour plot strategy shows the acceptable range when the requirements established are fulfilled. Crossing the data from the two strategies and considering also economic reasons (less energy consumption) lead us to define 10 min as the optimum time for extraction, while maintaining 50 ºC as extraction temperature, 3.5 g *per* 30 mL and level 3 of grinding. The overall desirability approach in these conditions lowered to 0.80, with a predictable composition of 18.9 %w/w of sugars content, 48.9, 28.1 and 14.6 %mol for mannose, galactose, and arabinose, respectively. Such composition is still quite similar to EC1, as well as to EC2-EC5, the different commercial EC samples used for comparison, and much different from all IC samples, even those labelled as espresso.

III.3.3.2. Infusion extraction process

The operational conditions previously defined were tested in a 50 times larger extraction experiment using 1.5 L as extraction volume and, performing three independent extractions. Table III.3.3 shows the overall coffee extracts characterization processed via freeze-drying and spray-drying, accompanied by performing control EC extraction with the machine using the same package of coffee product. The infusion process extracted 19.8±0.2 %w/w of coffee compounds, a value slightly higher than EC1, but still comparable to EC brews described in literature (11.7-20.0%) (Nunes et al., 1997). Concerning the dehydration step, while with the freeze-drying method all coffee material was recovered, nearly half of the content was lost via spray-drying processing (stuck in the drying chamber of the apparatus). This problem would result in an overall extraction yield decrease to 10.8 ± 0.3 %w/w. However, it was related to the drying process and not to the extraction step. This could be a problem only noticeable in small scale experiments as during the processing of larger quantities this waste would end up negligible.

Table III.3.3. Composition of EC1, IC1, and roasted coffee infusion obtained through optimization procedure and processed by freeze- (FD) and spray-dried (SD) methodologies.

Parameter		EC1	IC1	Infusion FD	Infusion SD
					
Total Carbohydrates (%w/w)[A]		17.6±0.9[a]	34.5±1.1[b]	19.3±1.5[c]	16.8±0.9[a]
Rha (% mol)		4.3±0.5[a]	1.5±0.1[b]	4.3±0.5[a]	4.4±0.2[a]
Ara (% mol)		15.7±0.5[a]	9.4±0.6[b]	15.7±0.6[a]	15.0±1.1[a]
Man (% mol)		44.4±1.8[a]	33.9±1.0[b]	43.0±1.0[a]	44.8±4.1[a]
Gal (% mol)		29.8±1.3[a]	52.1±1.5[b]	31.6±1.1[a]	33.0±3.4[a]
Glc (% mol)		5.8±0.6[a]	3.1±0.4[b]	5.4±0.6[a]	5.7±0.5[a]
Total Lipids (%w/w)[A]		0.92±0.05[a]	0.05±0.02[b]	0.10±0.04[b]	0.10±0.01[b]
Caffeine (%w/w)[A]		8.83±0.42[a]	4.92±0.38[b]	8.83±0.64[a]	8.67±0.64[a]
5-CQA (%w/w)[A]		2.39±0.15[a]	1.01±0.16[b]	2.47±0.17[a]	2.37±0.16[a]
True density (g cm^{-3})		n.d.	n.d.	1.55±0.17[a]	1.59±0.04[a]
Density (g cm^{-3})[B]		1.007±0.003[a]	1.008±0.004[a]	1.008±0.004[a]	1.008±0.006[a]
Colour (Powder)	*L**	15.9±3.1[a]	8.9±0.7[b]	21.7±3.1[c]	38.7±2.9[d]
	*a**	7.2±0.4[a]	10.8±0.5[b]	7.6±1.2[a]	6.9±0.6[a]
	*b**	14.9±2.1[a]	12.8±0.4[a]	17.9±2.3[b]	23.1±0.7[c]
	*C**	16.6±2.0[a]	16.8±0.5[a]	19.4±2.6[b]	24.2±0.8[c]
	h_{ab}	64.0±2.3[a]	50.4±0.4[b]	66.9±1.1[c]	73.4±1.0[d]
Colour (Brew)[B]	*L**	36.9±0.8[a]	36.7±0.9[a]	37.0±1.1[a]	38.1±2.4[a]
	*a**	1.4±0.1[a]	1.5±0.1[a]	3.2±0.1[b]	3.7±0.8[c]
	*b**	1.4±0.1[a]	1.3±0.1[a]	1.5±0.2[a]	1.6±0.9[a]
	*C**	2.0±0.1[a]	1.9±0.1[a]	3.5±0.2[b]	4.1±1.1[b]
	h_{ab}	43.8±3.3[a]	40.9±3.3[a]	24.6±2.5[b]	21.5±6.8[b]
Colour ($K_{mix,\ 405\ nm}$)		0.69±0.03[a]	0.66±0.08[a]	0.44±0.02[b]	0.46±0.01[b]
Kinematic Viscosity (cSt)[B]		1.06±0.01[a]	1.03±0.00[b]	1.05±0.01[a]	1.06±0.01[a]
Electrical conductivity (mS cm^{-1})[B]		3.56±0.31[a]	2.33±0.21[b]	3.83±0.36[a]	3.85±0.14[a]
pH[B]		5.75±0.12[a]	4.88± 0.04[b]	6.09±0.09[c]	5.87±0.04[a]

[A]: relative content of the compounds in relation to the total solids extracted; [B]: analysis performed after redissolution of freeze-dried samples in water (30 $mg.mL^{-1}$). Columns with different characters in each row indicate samples with significant difference (p<0.05). n.d. - not determined.

Furthermore, the appearance of the dried samples was distinct: the freeze-dried ones were comparable to fluffy brown cotton, while spray-dried samples were yellowish powders (as presented in Table III.3.3 and Figure III.3.2). This was supported by the variation in colour parameters (CIELab coordinates) with higher *L** (lightness) and *b** (shifting in the yellower coordinate) associated to SD samples (Table III.3.3). This distinction was not so evident in solution at EC concentration, as both FD and SD showed a similar brown colour not perceived by naked eye, with similar *L** and *b** values, although showing different chroma (*C**) and hue angle (h_{ab}). The dissolution of the samples in cold water resulted in a more translucid/transparent solution with FD and SD extracts when compared to EC and IC that formed more foggy/cloudy coffee solutions.

Moreover, although the freeze-dried extracts (both EC and infusion) dissolved almost instantaneously after the addition of water to obtain a coffee solution with EC solids concentration (30 mg.mL^{-1}), the spray-dried extracts did not immediately dissolve, fading away in the solution upon agitation (Figure III.3.2). The SD samples seem to act as a more hydrophobic material, suggesting a different organization of molecules during the drying process. SD processing usually confers smaller particles compared to FD, with smaller spaces between the particles (Padma Ishwarya & Anandharamakrishnan, 2015). Thus, as SD was a more compact structure, it could hinder the penetration of water inside the powder, while the more disorganized FD structure allowed an easier contact with water. According to literature, the SD process leads to air trapping inside the particles, which could result in lowering of density that may cause particles floating, preventing their dissolution in water (Burmester et al., 2011; Goula & Adamopoulos, 2008). Nevertheless, the true density analysis performed showed similar values for both samples (1.6 g cm^{-3}), in accordance with values reported for instant coffee products (Imison, 2011).

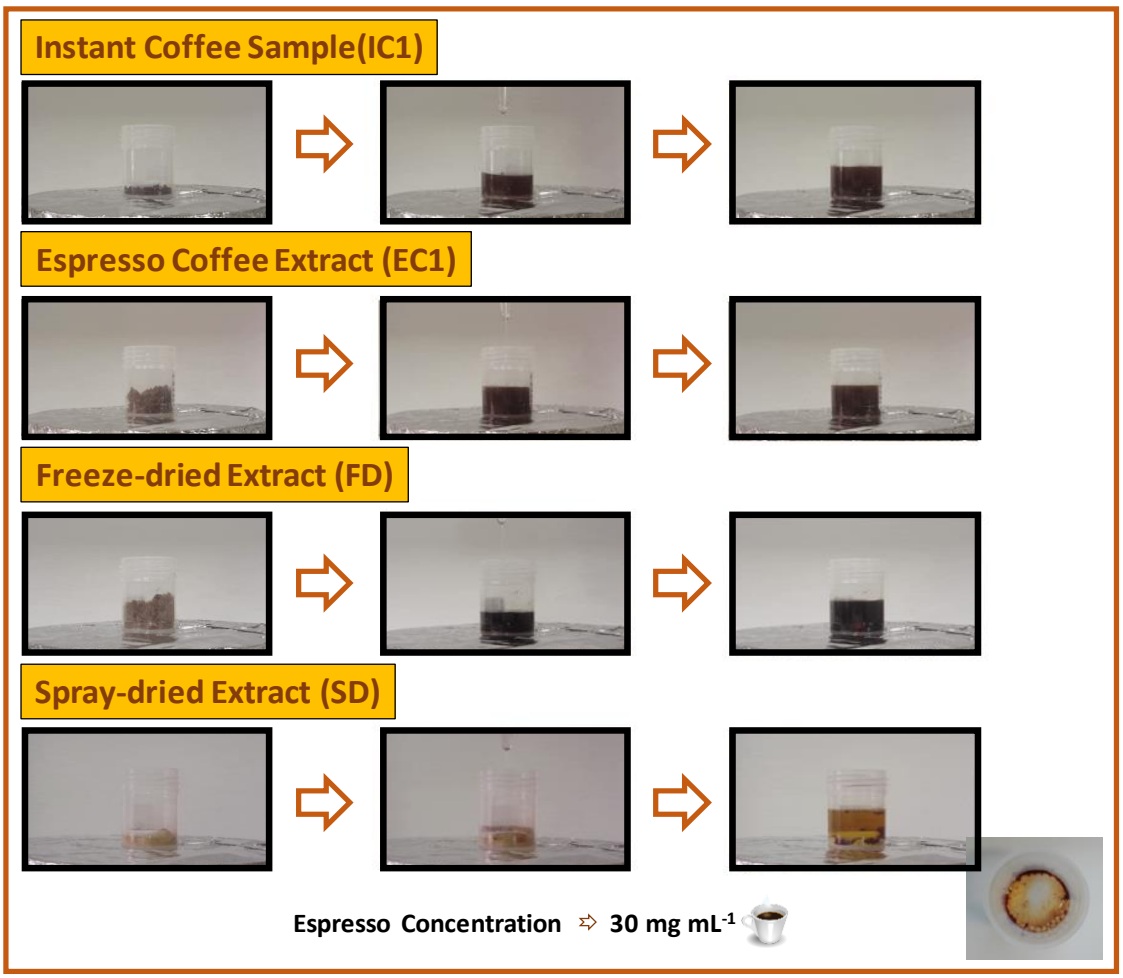


Figure III.3.2. Representation of experiments of samples dissolution in cold water (30 mg.mL^{-1}).

SD samples had slightly lower content of total sugars, despite the same extraction step possible due to a preferential interaction of carbohydrates with the drying chamber. In general, the sugars composition of FD and SD were statistically similar between them and with EC1 (Table III.3.3), suggesting equal overall sugars composition of infusion process and EC. As FD samples were easily dissolved compared to SD, which is a considerable advantage for product development, further in-depth sugars analysis was performed using the freeze-dried samples. Table III.3.4 shows the glycosidic linkage analysis performed to EC1 and FD using three samples from independent extractions. Generally, no significant differences were observed between the two groups of samples analysed, suggesting that the carbohydrate structures present in the EC1 and FD were similar, as shown in the chromatogram of methylation analysis (Figure III.3.3).

Table III.3.4. Glycosidic linkage composition of the sugars present in the coffee extracts.

linkage	EC1	FD
T-Rha*p*	3.8±0.2[a]	3.8±0.3[a]
Total	**3.8 (4.3)**	**3.8 (4.3)**
T-Ara*f*	6.4±0.2[a]	6.6±0.9[a]
5-Ara*f*	7.7±0.1[a]	8.0±0.2[a]
Total	**14.1 (15.7)**	**14.6 (15.7)**
T-Man*p*	4.3±0.3[a]	4.6±0.2[a]
4-Man*p*	40.4±1.2[a]	39.7±2.6[a]
4,6-Man*p*	2.3±0.2[a]	2.6±0.2[a]
Total	**47.1 (44.4)**	**46.8 (43.0)**
T-Gal*p*	6.1±0.6[a]	6.1±0.6[a]
3-Gal*p*	9.3±0.5[a]	9.2±1.1[a]
6-Gal*p*	4.0±0.1[a]	4.3±0.5[a]
3,6-Gal*p*	6.8±0.6[a]	6.8±1.5[a]
Total	**26.2 (29.8)**	**26.5 (31.6)**
T-Glc*p*	6.3±0.2[a]	5.7±0.2[b]
4-Glc*p*	1.6±0.2[a]	1.7±0.4[a]
6-Glc*p*	0.9±0.1[a]	1.0±0.3[a]
Total	**8.8 (5.8)**	**8.4 (5.4)**
DB $_{GM}$	4.9±0.5[a]	5.5±0.5[a]
DB $_{AG}$	28.3±1.1[a]	28.4±1.9[a]

DB - degree of branching. The sugars composition for each sugar residue obtained by derivatization to alditol acetates is shown in brackets. Two samples t-test was used to compare coffee means, with statistical differences indicated with different letters (p<0.05).

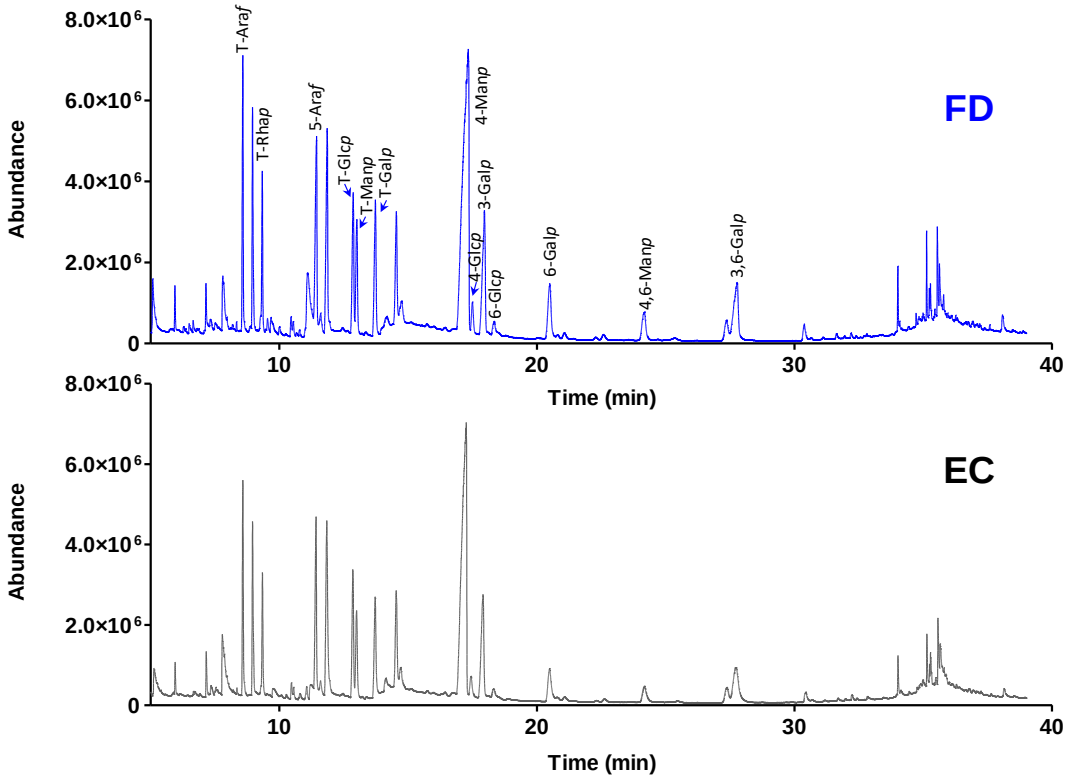


Figure III.3.3. Chromatograms of methylation analysis of FD and EC1 samples.

The estimation of galactomannans (GM) through the sum of mannosyl residues and the contribution of T-Gal*p*, assessed as the amount of the 4,6-Man*p* (Gniechwitz et al., 2007a; Passos et al., 2019a), indicates that EC1 and the infusion had 49.4±1.1% and 49.3±2.7% of GM, respectively. For arabinogalactans (AG) estimation, it was accounted the arabinosyl and galactosyl residues, subtracting the amount of T-Gal*p* in GM. Table III.3.4 indicates that EC1 and the infusion present 38.0±1.1% and 38.5±2.8% of AG, respectively. Thus, the ratio of GM/AG for the two methods was similar (1.3). Such ratio is reported to vary from 1.3-3.3 in coffee infusions processes, from 0.9-1.5 in drip brew, being 1.1 in French Press brew, 2.6 in pad coffee and ranging from 1.6-2.0 with a stovetop espresso, depending the variation on the properties of the roasted coffee used (Gniechwitz et al., 2007a; Nunes & Coimbra, 2002a, 2002b). This variability can be reduced to the ratio 1.0-1.6 if the same coffee is used (Gniechwitz et al., 2007a) highlighting that the extraction conditions may be modulated to obtain similar proportions even with different methods. For IC, literature shows that this ratio lowered to 0.4, while it is reported an estimation of 1.1 for an instant espresso sample (Gniechwitz et al., 2007a). In the present study, the sugars analysis of instant espresso coffee samples (IC2-IC4) showed a composition

resembling more the IC samples than the EC ones. Moreover, the estimation of the branching degree of GM showed similar values for both extraction methodologies, approximately 5%, in accordance with other infusion processes (3.6-5.4%) (Nunes & Coimbra, 2001, 2002a, 2002b), other extraction methods (drip brew, instant espresso, coffee pads; 3.1-4.0%) or even IC (4.4%), or extracts obtained from spent coffee grounds (2-7%) (Gniechwitz et al., 2007a; Passos et al., 2019a, 2019b). The degree of branching for AG were also similar between the samples analysed and in accordance with other infusion results (22-38%), depending on coffee processing parameters as the roasting degree.

A dialysis step was employed to analyse the polymeric material of the samples and evaluate the similarities between EC1 and FD. The IC commercial sample (IC1) was also tested for comparison purposes using the same amount of starting material. Table III.3.5 shows that low molecular weight compounds represent 74.8-80.3% of the three samples tested, higher for EC1 and FD samples when compared to IC. In the first 6 h of dialysis, in all samples, only 31.4-39.5% of material was recovered, explained by adsorption phenomena of low molecular weight compounds to polysaccharides (Lopes et al., 2016). Despite the higher carbohydrates content of IC compared to EC1 and FD (Table III.3.3), the polymeric material did not reflect a significant difference, ranging all samples from 19.7 to 25.2%. This suggests that in IC a considerable fraction of low molecular weight carbohydrates (<12-14 kDa) diffused through the dialysis membrane. The predominance of low molecular weight compounds in IC (<1 kDa compounds accounting for nearly 40%) was in accordance with literature (Ferreira et al., 2018; Passos et al., 2014b).

Table III.3.5. Distribution of low and high molecular weight material for the espresso coffee and the infusion samples.

Fraction	EC1	IC1	FD
LMWMRecovered (%w/w)	37.0±1.5	31.4±2.0	39.5±0.6
LMWMTotal Est.* (%w/w)	77.6±0.3	74.8±4.2	80.3±0.5
HMWMTotal (%w/w)	22.4±0.3	25.2±4.2[a]	19.7±0.5[a]
Total Carbohydrates (%w/w HMWM)	56.7±3.5	68.6±0.7	59.0±8.2
Rha (% mol)	4.4±0.0	1.7±0.1	4.1±0.2
Ara (% mol)	12.8±0.2	6.9±0.0	12.6±0.7
Man (% mol)	50.5±0.9	28.3±0.4	50.6±2.2
Gal (% mol)	30.4±0.4	60.9±0.2	31.2±1.3
Glc (% mol)	1.9±0.2	2.2±0.5	1.4±0.0
Protein (%w/w)	16.8±0.5	15.5±1.1	13.4±0.3
Melanoidins (% w/w HMWM)	26.4±0.5	19.2±0.1	27.6±0.3
HMWMSol. (%w/w)	17.6±0.2	20.8±2.1	18.8±0.3
Total Carbohydrates (%w/w HMWM)	58.7±0.9	78.8±10.9	62.0±0.0
Rha (% mol)	5.0±0.2	1.8±0.2	4.2±0.1
Ara (% mol)	14.5±0.4	7.5±0.3	12.6±0.1
Man (% mol)	44.9±1.4	16.0±0.1	50.4±0.0
Gal (% mol)	34.1±0.9	72.5±0.1	31.4±0.1
Glc (% mol)	1.5±0.0	2.3±0.1	1.4±0.1
Protein (%w/w HMWM)	12.6±0.5	11.4±0.1	12.5±0.0
Kmix, 280 nm	4.87±0.20	4.35±0.29	4.62±0.33
Kmix, 325 nm	3.95±0.17	3.36±0.22	3.68±0.28
Kmix, 405 nm	1.14±0.07	0.91±0.05	1.24±0.11
HMWMInsol. (%w/w)	4.8±0.3	4.4±2.1	0.8±0.2
Total Carbohydrates (%w/w HMWM)	11.8±2.1	68.7±5.1	31.2±8.8
Rha (% mol)	5.5±0.6	0.6±0.0	3.3±0.5
Ara (% mol)	17.1±2.2	2.1±0.1	9.7±1.7
Man (% mol)	37.6±1.6	86.5±0.3	63.4±6.0
Gal (% mol)	29.7±2.1	9.2±0.3	19.8±2.4
Glc (% mol)	10.2±3.3	1.6±0.1	3.8±1.4
Protein (%w/w HMWM)	36.1±0.4	12.2±0.1	25.8±1.5

The carbohydrate composition showed that the polymeric material EC1 and FD exhibited great similarity, while IC1 sample was richer in galactose and poorer in mannose, arabinose and rhamnose. Such differences were also observed in the soluble HMWM fractions that represent at least 78% of the HMWM material (Table III.3.5). On the other hand, the cold-water insoluble fractions (precipitated after centrifugation of HMWM material) represented higher amounts in EC1 and IC1 (4.8 and 4.4%, respectively), when compared to FD which content was almost absent (0.8% of coffee solids). In these fractions, mannose was the major sugar even in IC sample. The higher proportion of insoluble compounds in EC1 sample compared to FD may be due to the extraction of small roasted coffee particles directly to the brew, absent in FD due to the filtration step. This hypothesis is reinforced by the higher glucose content in EC1, as well as by the similarity of the carbohydrate composition with the roasted coffee powder (Table

III.1.2 in Chapter III.1.3.1). Although such fraction may be important for EC organoleptic properties, the almost total cold-water solubility of FD sample was important in view of their posterior dissolution application.

Protein has been associated to foamability in EC (Nunes et al., 1997). Table III.3.5 shows that EC sample exhibited higher relative protein content in HMWM (16.8%) when compared to FD (13.4%), with IC1 presenting an intermediate content (15.5%). Literature values for infusions were comparable to those obtained for FD (8-12%) (Bekedam et al., 2008b, 2008c; Nunes & Coimbra, 2001). EC1 (12.6%) and FD (12.5%) revealed similar content for the major polymeric fraction, in accordance with literature for EC samples when applying the same methodology (Lopes et al., 2016). Considering the mass of compounds, the results showed that EC1 contained 37.7 mg of protein *per* g of brew solids, while FD and IC exhibited 26.4 mg and 30.7 mg, respectively. The distinction comes from the insoluble fraction (EC1: 17.4 mg; FD: 2.2 mg; IC: 6.8 mg), as the soluble one showed similar values among the samples (EC1: 22.2 mg; FD: 23.5 mg; IC: 23.7 mg), values comparable with literature reports for EC (Lopes et al., 2016).

Melanoidins are brown nitrogen-containing polymeric material, whose estimation is usually performed by difference between the total polymeric material and the one determined as protein and carbohydrates. Table III.3.5 shows that EC1 and FD had similar content of melanoidins. The estimation of the amount *per* brew (1.2 g of total solids as a typical EC total solids value) shows that the EC1 analysed had nearly 71 mg *per* brew, and FD extract exhibited 65 mg, in accordance with literature reports for EC brews (Vitaglione et al., 2012). The brown characteristic of melanoidins was measured through the specific extinction coefficient at 405 nm ($K_{mix,\ 405\ nm}$). Table III.3.5 shows a resemblance between the $K_{mix,\ 405\ nm}$ values EC (1.1) and FD (1.2), suggesting a similar colour of extracts.

The lipids content in EC1 (0.92%w/w) was significantly higher than those of IC1, FD and SD (0.05, 0.10 and 0.10%, respectively, Table III.3.3). Moreover, roasted powder showed 11.1% of lipids, allowing to infer that EC procedure may extract nearly 2% of the coffee lipids. It was reported that pressure favours lipids extraction, while filtration steps, as occurred at the end of the infusion process, hinder the passage of these compounds to the brew. On the other hand, the amount of caffeine and the major chlorogenic acid (5-CQA) in EC1 and FD/SD extracts were similar, while the amount in IC1 was significantly lower, due to the higher relative abundance of other compounds, such as carbohydrates.

The dissolution of the extracts at a concentration of EC brews (30 $mg.mL^{-1}$) showed that EC1, FD and SD extracts exhibited similar kinematic viscosity, while the IC sample had lower viscosity, probably due to the different Man/Gal ratio verified in these samples. On the same conditions, the EC1, FD and SD exhibited similar electrical conductivity, which could be an indication of comparable amount of ions present, exhibiting IC1 a lower value. For pH, and in the same conditions of analysis for all brews, the dissolution of the EC1/FD and SD extracts originate solutions with approximate pH 6, while for the IC it was nearly 5, showing to be a more acidic beverage.

III.3.3.3. Foam analysis

To evaluate the possibility to develop a consistent foam using the coffee extracts obtained (EC1, FD and SD), it was injected CO_2 through coffee solutions prepared at EC concentration (30 $mg.mL^{-1}$, Figure III.3.4).

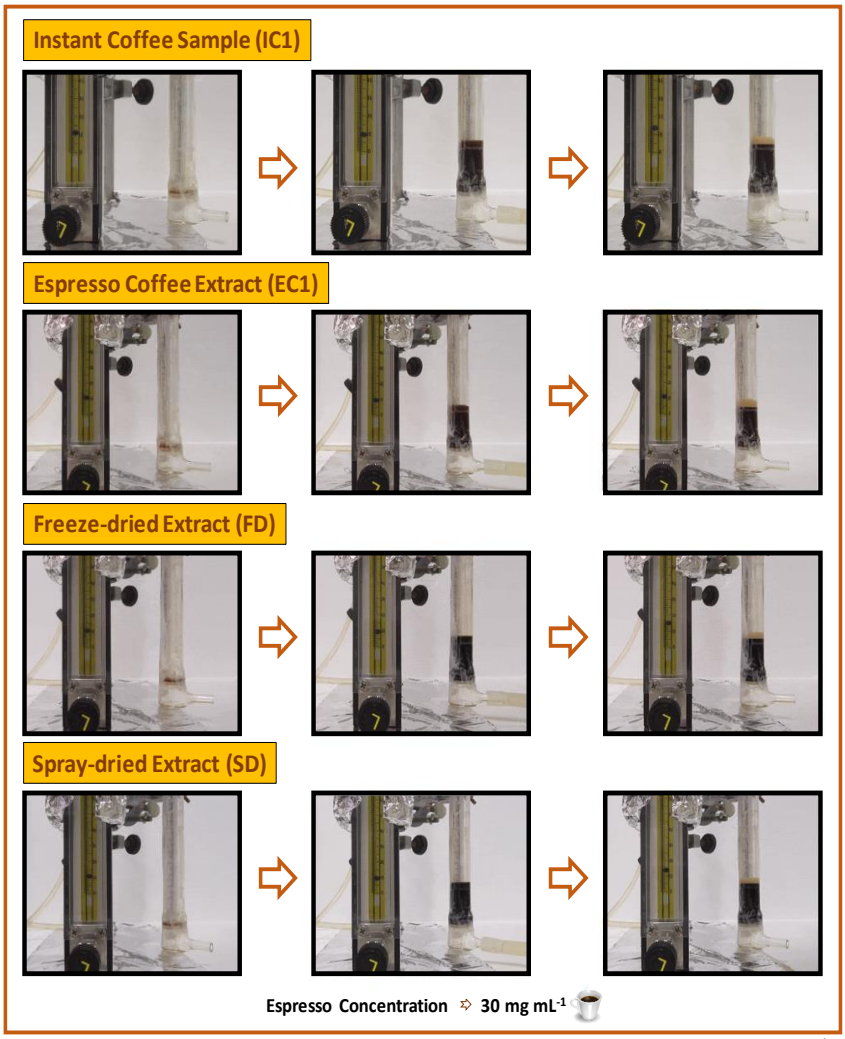


Figure III.3.4. Representation of experiments of CO_2 injection through samples solutions (30 $mg.mL^{-1}$).

This methodology was already applied in the study of wine compounds foamability and foam stability (Coelho et al., 2011). It was verified that the freeze-dried EC1 sample, when dissolved in water (25 ºC), was able to produce a foam index of 10.2% in the column, with 10% as the minimum acceptable for a good EC (Illy & Viani, 2005). Moreover, the foam was stable for approximately 9.9 min. The application of the same procedure to FD extract showed a foam index of 12.3%, with a foam stable during 13.3 min. These results allowed to conclude that the coffee extracts were able to produce foam. As the intent was to generate CO_2 *in situ* and evaluate the foamability of the coffee products, it was conducted a series of experiments with effervescent formulations using the effervescent properties of the sodium bicarbonate/citric acid mixtures. The IC1 sample was used to determine the amount of reagents needed to attain the desired level of foam-index (at least 10%) with the addition of water at 70 ºC to the coffee formulation. The best formulation tested consisted of 1:9 of effervescent mixture of 1.2:1.0 %w/w sodium bicarbonate:citric acid and coffee extract (1.2 g) (Figure III.3.5).

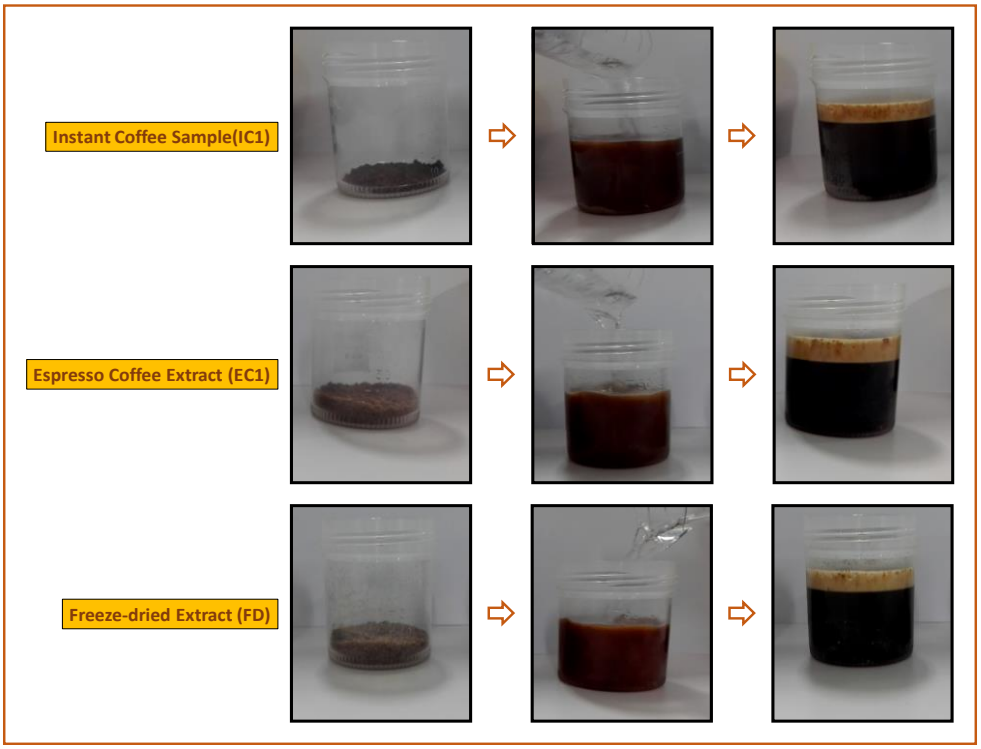


Figure III.3.5. Representation of experiments of addition of effervescent mixture to coffee samples prior to addition of water (70 ºC) achieving espresso coffee concentration (30 $mg.mL^{-1}$).

The dissolution of the EC1, IC1 and FD formulations with hot water readily formed a foam layer in the top of the brew that was stable for at least one minute for all samples (Table III.3.6). On the other hand, the lower solubility of SD sample hindered the formation of the

foam layer. The addition of the salts led to a variation in the pH of coffee solutions, with a decrease of 0.14 pH units in FD sample, maintaining the pH values for EC samples, and increasing the pH for IC1 (approximately 0.35 pH units) due to the buffering effect of the bicarbonate/citrate effervescent mixture. Indeed, the addition of these pH-regulator compounds to coffee have been reported to extend the shelf life of coffee brews, keeping longer their cup quality and even increasing antioxidant activity (Pérez-Martínez et al., 2008; 2010).

Table III.3.6. Foam and pH properties obtained from effervescent formulations. Columns with different characters in each row indicate samples with significant difference (p<0.05).

Sample	Foamability (mL)	Foam index (%)	Foam Stability (s)	pH
EC1	8.1±2.1[a]	20.3±5.2[a]	68.8±8.4[a]	5.76±0.09[a]
IC1	8.1±0.4[a]	20.3±1.0[a]	79.4±28.2[a]	5.23±0.05[b]
FD	7.2±1.4[a]	18.0±3.6[a]	80.2±22.6[a]	5.95±0.09[c]
SD	-	-	-	5.69±0.06[a]

III.3.3.4. Volatile compounds analysis

The volatile compounds were studied after the dissolution of the coffee samples (EC1 and FD) in hot water (70 ºC, 40 mL) through the analysis of the vapour phase above the coffee samples. The methodology adopted (HS-SPME) did not require a solvent extraction step and/or sample clean-up and detected only the compounds released from the liquid sample. On the other hand, the use of a mixed coating fibre (divinilbenzene/carboxen/polydimethylsiloxane - DVB/CAR/PDMS) allowed to extract compounds with different polarities. The intent was to study the aroma perceived while taking the coffee brews, so a short extraction time (3 min) was chosen to simulate the consumer perception. For comparison, espresso coffees were extracted just before the HS-SPME analysis with a conventional coffee machine (EC Machine), using the same coffee blend used to produce EC1 and FD1. Moreover, two instant coffee samples (IC1 - instant coffee and IC2 - instant "espresso" coffee) were studied for comparison purposes. In Figure III.3.6 are represented the chromatograms obtained by HS-SPME analysis. The SD samples presented dissolution problems and were discarded from the analyses, besides their volatile profile exhibited lower intensities (data not shown) as expected for solutions with concentrations lower than 50 %w/w (Clarke, 2001). The HS-SPME analysis allowed to putatively identify 71 compounds in the vapour phase of the liquid coffee samples

(Table III.3.7). Due to their chemical nature the compounds were grouped in families: aldehydes, furans, indole compounds, phenols, pyrazines, pyridines, pyrazines, and pyrroles. The compounds not included in any of the previous families were classified as others. Figure III.3.8 shows the total GC peak area for the samples analysed grouping the compounds by their chemical family.

The espresso coffee brews from EC machine have higher peak intensities than the dissolved extracts (EC1, FD, IC1, and IC2), explained by the loss of coffee volatile compounds during the previous extraction methodology followed by concentration steps (freeze or spray drying). On the other hand, the EC1 and FD samples exhibited higher total peak intensities than the instant coffee samples (IC1 and IC2). The lower volatiles amount in instant coffees compared to other brews were reported in literature (Rhoades, 1960; Sanz et al., 2002; Semmelroch & Grosch, 1995; Wang et al., 1983).

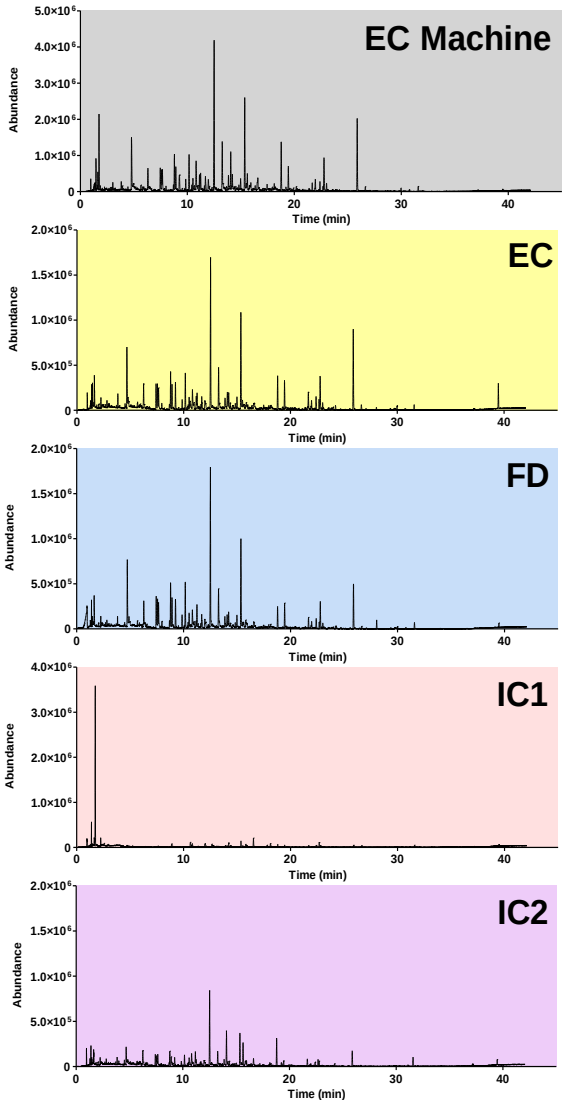
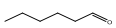
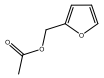
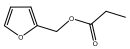
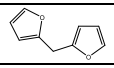
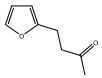
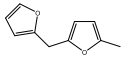
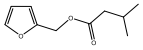
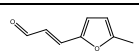
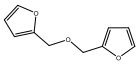
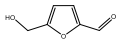
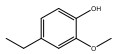
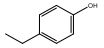
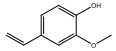
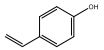


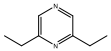
Figure III.3.6. Chromatograms of the HS-SPME volatile compounds analysis**.**

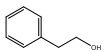
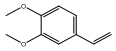
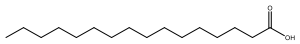
Table III.3.7. Chromatographic data of the compounds identified in coffee brews and extracts using HS-SPME/GC-MS.

Peak Number	t_R[a] (min)	Compound	CAS number	Structure	RI_{Calc}	RI_{Lit}	Ref.	E/B/RG	Ref.	Aroma Descriptors	Ref.
		Aldehydes									
1	1.632	2-Methylbutanal*	96-17-3		914	914	[1]	E/B/RG	[1-23]	chocolate-like, fruity, fermented, pungent, malty, cocoa, buttery, oily, sweet	[1, 5, 13, 14, 19, 22-25]
2	1.649	3-Methylbutanal*	590-86-3		917	919	[1]	E/B/RG	[1-4, 7-23]	acrid-pungent, fruity, malty, sweet, buttery, oily, peach, apple, chocolate, burned, almond	[1, 7, 13, 14, 19, 22-27]
3	3.027	Hexanal*	66-25-1		1086	1083	[1]	E/B/RG	[5, 7-14, 16, 23, 28]	grassy, raw bean, fruity, leaf-like, fatty-green, rancid butter	[1, 7, 13, 14, 23, 25, 27, 28]
4	11.970	Benzaldehyde	100-52-7		1516	1520	[3]	E/B/RG	[2, 3, 23, 28]	bitter almond, sweet	[23-25, 27]
5	20.756	2-Phenyl-2-butenal	4411-89-6		1929	1922	[29]	B/RG	[7, 17, 23]	-	-
		Furans									
6	1.463	2-Methylfuran	534-22-5		885	876	[30]	E/B/RG	[3-6, 19, 23, 31]	ethereal, sickly, unpleasant	[23, 24]
7	1.865	2,5-Dimethylfuran*	625-86-5		952	952	[32]	E/B/RG	[3, 5, 14, 16, 19, 23]	coffee, ethereal	[23, 24]
8	2.961	2-Vinylfuran	1487-18-9		1079	1085	[3]	E/B/RG	[3-5, 23, 28, 31]	-	-
9	3.935	2-Vinyl-5-methylfuran	10504-13-9		1145	1152	[3]	B/RG	[3, 23]	coffee	[24]
10	5.643	2-(Methoxymethyl)furan*	13679-46-4		1236	1241	[3]	E/B/RG	[2-5, 14, 16, 19, 23, 28, 31]	burnt, pungent, chemical	[28]
11	10.812	Furfural*	98-01-1		1466	1468	[3]	E/B/RG	[2-5, 13, 17-19, 23, 28, 31, 33-35]	pungent, sweet, caramellic, bready, cinnamon-almond-like, almond/bitter, cooked pea, chemical, smoky	[13, 23-25, 28]
12	11.159	Furfuryl methyl sulfide*	1438-91-1		1481	1492	[3]	E/B/RG	[2-6, 14, 16-19, 23, 28]	coffee, smoke-roast, burnt, sulfury, cooked, cabbage, onion, leather-like, toasty, garlic	[5, 14, 19, 23, 27, 28]
13	11.684	2-Acetylfuran	1192-62-7		1504	1512	[3]	E/B/RG	[3, 5, 6, 18, 19, 34, 35]	balsamic-sweet, floral undertones of balsamic-cinnamic character, roasty, tobacco-like, spicy	[5, 23-25, 27]

Peak Number	t_R[a] (min)	Compound	CAS number	Structure	RI_{Calc}	RI_{Lit}	Ref.	E/B/RG	Ref.	Aroma Descriptors	Ref.
14	12.512	Furfuryl acetate*	623-17-6		1540	1539	[3]	E/B/RG	[1-3, 5, 6, 14, 16-19, 31, 33-35]	ethereal-floral, herbal-spicy, nutty, fruity, banana	[23-25, 27]
15	13.264	5-Methylfurfural	620-02-0		1574	1582	[3]	E/B/RG	[2, 3, 5, 6, 17-19, 31, 33-35]	sweet, spicy, warm, caramel	[23-25]
16	13.819	Furfuryl propionate	623-19-8		1598	1603	[3]	E/B/RG	[2, 3, 5, 17, 31, 35]	spicy, floral, fruity	[5, 27]
17	14.075	2-Furfurylfuran	1197-40-6		1610	1615	[3]	E/B/RG	[3, 5, 17, 19, 28, 35]	caramel	[23]
18	14.828	1-(2-Furanyl)-3-butanone	699-17-2		1644	1640	[36]	RG	[23]	-	-
19	15.355	Furfuryl alcohol*	98-00-0		1668	1666	[3]	E/B/RG	[1-3, 5, 6, 14, 16-19, 28, 31, 33, 34]	slightly caramellic, warm-oily, burnt, smoked, unpleasant	[14, 23-25, 27, 28]
20	15.630	5-Methyl-2-furfurylfuran	13678-51-8		1680	1682	[37]	E/RG	[6, 19]	-	-
21	15.959	Furfuryl isovalerate	13678-60-9		1695	-	-	RG	[23]	fruity, berry, ripe	[23]
22	16.607	*m/z* 140, 111, 139, 97, 124	-	-	1726	-	-	-	-	-	-
23	19.226	3-Phenylfuran	13679-41-9		1850	1859	[38]	B/RG	[23, 39]	jasmine	[23]
24	19.709	1-(5-methyl-2-furanyl)-1-propen-3-al	5555-90-8		1874	1856	[40]	RG	[23]	-	-
25	21.965	Difurfuryl ether	4437-22-3		1998	1986	[5]	E/B/RG	[4, 5, 17]	unpleasant, salicylate, coffee-like note	[23, 24]
26	31.111	5-Hydroxymethylfurfural	67-47-0		2521	2528	[41]	RG	[23]	warm-herbaceous, winey-ethereal	[23]
		Indole compounds									
27	29.971	Indole	120-72-9		2456	2450	[42]	E/B/RG	[5, 6, 17, 18, 23]	floral notes	[23, 25, 27]

Peak Number	t_R[a] (min)	Compound	CAS number	Structure	RI_{Calc}	RI_{Lit}	Ref.	E/B/RG	Ref.	Aroma Descriptors	Ref.
28	30.723	3-Methylindole	83-34-1		2499	2490	[43]	RG	[23]	unpleasant, mothball-like, sweet, warm, over-ripe fruit	[23, 25, 27, 43]
		Phenols									
29	19.437	2-Methoxyphenol*	90-05-1		1860	1864	[5]	E/B/RG	[1, 2, 4, 5, 7-23]	phenolic, burnt, smoky, spicy, harsh, earthy, sweet	[1, 5, 13, 14, 19, 22, 24, 26, 27]
30	22.392	Phenol	108-95-2		2022	2007	[24]	E/B/RG	[4, 17, 18, 23]	smoky, sickeningly sweet, irritating	[7, 27]
31	22.774	4-Ethyl-2-methoxyphenol*	2785-89-9		2044	2048	[44]	E/B/RG	[2, 4-6, 13, 14, 16-23]	spicy, medicinal, phenolic, smoky, roasted, green, ethereal, clove-like, vanilla-like, sweet	[13, 14, 17-19, 23, 24, 26, 27]
32	23.976	3-Methylphenol	108-39-4		2113	2116	[44]	B/RG	[23]	medicinal–leathery, asphalt-like, wood preservative-like	[23, 25, 27]
33	25.528	4-Ethylphenol	123-07-9		2202	2195	[44]	E/B/RG	[5, 23]	woody-phenolic, sweet	[23, 25, 27]
34	25.876	4-Vinyl-2-methoxyphenol*	7786-61-0		2222	2225	[41]	E/B/RG	[2, 4-6, 13, 14, 16-23]	spicy, apple, rum, roasted peanut, smoky, medicinal, phenolic, eugenol-like, sweet, clove-like	[13, 14, 17-19, 22-27]
35	29.319	4-Vinylphenol	2628-17-3		2419	2415	[44]	RG	[23]	vanilla	[27]
		Pyrazines									
36	6.259	2-Methylpyrazine	109-08-0		1265	1274	[3]	E/B/RG	[2-6, 17-19, 23, 34, 35]	toasted, nutty, cocoa, green, roasted, chocolate, meaty	[24, 27]
37	7.426	2,5-Dimethylpyrazine	123-32-0		1318	1320	[45]	E/B/RG	[3-6, 17-19, 23, 35]	chocolate, roasted nuts, earthy, grassy,	[23, 27]
38	7.554	2,6-Dimethylpyrazine	108-50-9		1324	1326	[45]	E/B/RG	[2-5, 17, 19, 23, 33-35]	chocolate, roasted nuts, fried potatoes, nutty	[23-25, 27]
39	7.644	2-Ethylpyrazine*	13925-00-3		1328	1330	[45]	E/B/RG	[3-11, 15, 17, 19, 23, 35]	nutty, green, sweet, buttery, rum, toasted, peanut butter, woody	[23, 24, 27]
40	7.959	2,3-Dimethylpyrazine	5910-89-4		1342	1341	[45]	E/B/RG	[2, 5, 6, 17, 23, 28]	toasted, earthy, smoky, unpleasant, nutty, cocoa-like, green, roasted	[23, 24, 27]
41	8.774	2-Ethyl-6-methylpyrazine*	13925-03-6		1377	1381	[45]	E/B/RG	[1-11, 14-19, 23, 35]	toasted, earth, mould, flowery, fruity, hazelnut-like	[1, 5, 14, 24]
42	8.905	2-Ethyl-5-methylpyrazine*	13360-64-0		1383	1386	[45]	E/B/RG	[2-6, 14, 16, 17, 19, 23, 28]	rubber, smoky, chemical, greasy, onion, toasted, coffee-like	[14, 24, 28]

Peak Number	t_R[a] (min)	Compound	CAS number	Structure	RI_{Calc}	RI_{Lit}	Ref.	E/B/RG	Ref.	Aroma Descriptors	Ref.
43	9.236	2-Ethyl-3-methylpyrazine	15707-23-0		1398	1400	[45]	E/B/RG	[2, 5, 23, 35]	nutty, roasted, peanut-like, raw-potato, earthy	[5, 23, 24, 27]
44	9.506	2-Propylpyrazine	18138-03-9		1409	1404	[46]	E/B/RG	[5, 14, 16, 23, 28]	brothy, sulfury, smoky, beany, green, vegetable, herbal	[5, 14, 27, 28]
45	9.850	2,6-Diethylpyrazine	13067-27-1		1424	1440	[29]	E/B/RG	[2, 4, 5, 14, 16, 23]	potato-like, hazelnut-like, toasted	[5, 14]
46	10.153	3-Ethyl-2,5-dimethylpyrazine	13360-65-1		1437	1449	[29]	E/B/RG	[2, 4, 6, 17, 19, 23, 34]	-	-
47	10.349	2,3-Diethylpyrazine	15707-24-1		1446	1449	[45]	RG	[23]	nutty, hazelnut, earthy	[23, 27]
48	10.418	2,5-Diethylpyrazine	13238-84-1		1449	1449	[45]	RG	[23]	hazelnut-like	[23]
49	10.510	2-Ethyl-3,5-dimethylpyrazine*	13925-07-0		1453	1455	[45]	E/B/RG	[1, 2, 5, 7-23, 33]	paper, burnt, earthy, toasty, nutty-roast, meaty, green, potato-like, almond, nuts	[1, 13, 14, 18, 19, 22, 26]
50	11.247	2,3-diethyl-5-methylpyrazine*	18138-04-0		1485	1488	[45]	E/B/RG	[20-23, 26]	earthy, roasty, nutty, meaty, roasted hazelnut	[22, 23, 26, 27]
51	17.777	*m/z* 95, 43, 138, 39, 207	-	-	1780	-	-	-	-	-	-
		Pyridines									
52	4.720	Pyridine	110-86-1		1191	1193	[3]	E/B/RG	[2-6, 13, 17-19, 23]	burnt, pungent, smokey, fishy	[13, 23-25]
53	8.663	3-Ethylpyridine	536-78-7		1372	1375	[47]	B/RG	[17, 23]	toasted	[24]
		Pyrroles									
54	3.811	1-Methylpyrrole*	96-54-8		1138	1140	[3]	E/B/RG	[3, 5, 13, 17, 19, 23]	coffee, roasty, smokey, sweet, woody-herbaceous	[13, 23-25]
55	4.478	1-Ethylpyrrole	617-92-5		1177	1178	[29]	B/RG	[19, 23]	-	-
56	8.305	*m/z* 81, 80, 137, 93, 108, 207	-	-	1357	-	-	-	-	-	-

Peak Number	t_R[a] (min)	Compound	CAS number	Structure	RI_{Calc}	RI_{Lit}	Ref.	E/B/RG	Ref.	Aroma Descriptors	Ref.
57	14.209	2-Formyl-1-methylpyrrole	1192-58-1		1616	1626	[5]	E/B/RG	[3-5, 17-19, 28]	Cracker-popcorn-like, buttery	[23, 24]
58	18.137	*m/z* 81, 80, 53, 137	-	-	1797	-	-	-	-	-	-
59	18.788	*N*-Furfurylpyrrole	1438-94-4		1829	1822	[29]	E/B/RG	[2, 5, 6, 17-19, 23]	vegetable, pleasant green, earthy, hay-like, mushroom-like	[23, 27]
60	21.668	2-Acetylpyrrole	1072-83-9		1981	1975	[5]	E/B/RG	[2, 5, 13, 18, 23, 34]	bread, walnut, licorice	[27]
61	22.672	1H-pyrrole-2-carboxaldehyde	1003-29-8		2038	2030	[29]	E/B/RG	[5, 17, 18, 23]	corny, pungent	[23]
62	24.218	*m/z* 109, 108, 80, 53, 188	-	-	2127	-	-	-	-	-	-
63	26.632	1-Furfuryl-2-formylpyrrole	13788-32-4		2265	2254	[5]	E/B/RG	[5, 23]	green, misty	[23]
		Others									
64	2.814	2,3-Pentanedione*	600-14-6		1065	1062	[3]	E/B/RG	[3-23, 28, 34, 35]	sweet, buttery, oily, milky, caramel-like	[2, 5, 13, 14, 17-19, 22-28]
65	2.937	Dimethyl disulfide	624-92-0		1077	1078	[3]	E/B/RG	[2, 3, 23]	onion-like	[23, 25]
66	10.595	Acetic acid	64-19-7		1456	1465	[48]	E/B/RG	[4, 12, 17, 18, 23, 28]	pungent, sour, vinegar	[23-25, 27, 49]
67	17.474	Methyl salicylate	119-36-8		1766	1755	[50]	B/RG	[23, 39]	sweet, rooty-fruity, minty, spicy, wintergreen-like	[23, 25, 27]
68	20.426	Benzeneethanol	60-12-8		1910	1912	[50]	RG	[23]	rose-like, honey-like, pleasant floral-woody	[23, 25, 27]
69	23.008	3,4-Dimethoxystyrene	6380-23-0		2058	-	-	RG	[23]	-	-
70	37.145	Palmitic acid	57-10-3		2866	2871	[51]	RG	[23]	-	-

71	39.463	*m/z* 194, 109, 45, 67	-	-	2999	-	-	-	-	-	-	

[a]Retention time (t_R); RI_{Calc}: Calculated Retention Index; RI_{Lit}: Literature Retention Index; E/R/B: compound already identified in the headspace of espresso coffee brew (E), compound already identified in the headspace of other coffee brews (B) and compound previously identified as roasted and ground coffee volatiles (RG) well summarized by Flament [23]. *: described as key odorant in espresso coffee brew.

1- Caprioli et al. (2012); 2- Gloess et al. (2013); 3- Shimoda and Shibamoto (1990); 4- Piccone et al. (2012); 5- López-Galilea et al. (2006); 6- Rocha et al. (2004); 7- Maeztu et al. (2001b); 8- Andueza et al. (2007); 9- Andueza et al. (2003b); 10- Andueza et al. (2003a); 11- Andueza et al. (2002); 12- Sopelana et al. (2013); 13- Mestdagh et al. (2014); 14- Caporaso et al. (2014); 15- Parenti et al. (2014); 16- Genovese et al. (2014); 17- Akiyama et al. (2008); 18- Akiyama et al. (2007); 19- Akiyama et al. (2009); 20- Mayer et al. (2000); 21- Semmelroch & Grosch (1996); 22- Semmelroch & Grosch (1995); 23- Flament (2001); 24- Gonzalez-Rios et al. (2007); 25- Arctander (1967); 26- Blank et al. (1992); 27- Burdock (2004); 28- Chin et al. (2011); 29- Shimoda et al. (1996); 30- Bianchi et al. (2007); 31- Petisca et al. (2013); 32- Galindo-Cuspinera et al. (2002); 33- Moreno et al. (2015); 34- Bicchi et al. (2002); 35- Roberts et al. (2000); 36- Madruga & Mottram (1998); 37- Ishikawa et al. (2004); 38- Liu et al. (2001); 39- Budryn et al. (2011); 40- Baltes & Bochmann (1987); 41- Moon & Shibamoto (2009); 42-Rychlik et al. (1998); 43- Sanz et al. (2002); 44- Ferreira et al. (2001); 45- Wong & Bernhard (1988); 46- Du et al. (2008); 47- Horiuchi et al. (1998); 48- Riu-Aumatell et al. (2004); 49- Ranau & Steinhart (2005); 50- Nagarajan et al. (2001); 51- Suarez et al. (1991).

Figure III.3.7 allows to perceive the number of compounds for each chemical family and their contribution to the overall peak intensities of coffee samples. Furans are the family with higher number of compounds in all samples and with predominance contribution in EC machine (45%), EC1 (37%), FD1 (36%) and IC2 (48%), which does not occur with IC1. In this sample, pyrroles, compounds that are derived from the thermal degradation of Amadori intermediates, from furans and from amino acid derivatives degradation products, had a preponderant role (30%) (Caporaso et al., 2014; Flament, 2001). The predominance of furans over other compounds was also described in literature for different coffee brews as espresso coffee, French-press coffee, and Turkish coffee, being described as the main responsible for characteristic coffee brew aroma (Amanpour & Selli, 2016; Caporaso et al., 2014; Flament, 2001; Petisca et al., 2013; Rocha et al., 2004). The levels of furans in brews as espresso and American coffee is higher compared to Neapolitan or Moka coffee (Caporaso et al., 2014). These compounds are associated to caramel, nutty, ethereal, and smoked notes, when sulphur is absent from the structure, while sulphur confers a garlic odour (Caporaso et al., 2014) (Table III.3.7). Pyrazines represent the following predominant class in coffee samples (except in IC1) concerning the contribution of peak intensities to total area: 22% for EC Machine and IC2, 23% for EC1, and 29% for FD1 (Figure III.3.8). Such compounds are key aroma, namely the alkylpyrazines, as they confer hazelnut, nutty, roasted notes to coffee (Caporaso et al., 2014; Flament, 2001) (Table III.3.7). Phenolic compounds also greatly contribute to total GC peak area, mainly in EC Machine, EC1, and FD (13-16%) comparing to instant samples (6-11%) (Figure III.3.7). These compounds are associated to smoky, roasted and spicy notes (Table III.3.7) contributing to the typical coffee flavour (Caporaso et al., 2014; Flament, 2001). Indeed, generally instant coffee is associated to sweetish-caramel notes in relation to roast and smoky attributed to brews as filtered coffee (Sanz et al., 2002).

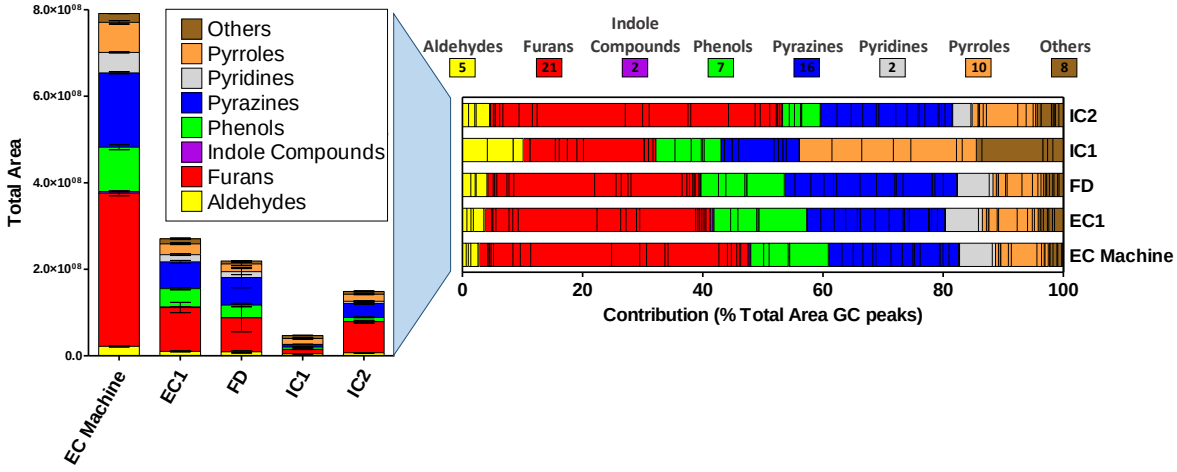


Figure III.3.7. Total GC peak area for the different samples analysed grouped by chemical family (left) and contribution of each family of compounds for the overall total area (right). Number inside the box represent the number of compounds found in each family group. IC - instant coffee samples, FD - freeze-dried sample, EC - espresso coffee samples.

Figure III.3.8 illustrates the overall profile of volatile compounds putatively identified in the coffee samples allowing to perceive in more detail the contribution of each compound. Furfuryl acetate was the major compound in the coffee samples analysed, representing 13.1-14.7% of the overall peak intensities, in line with literature for espresso brews (10.5-13.6%) (Petisca et al., 2013), except in IC1 that exhibited a lower level (0.6%). In such sample, acetic acid was predominant, constituting 10.1% of total peak area. This was in accordance with results for agglomerated instant coffee (powder), whose volatile composition was composed by 6-7% of acetic acid and furfuryl acetate does not exceed 0.1% (Leobet et al., 2019). Furthermore, the compounds with major contribution for total area (more than 5%) were the same and in the same order for EC machine, EC1 and FD: furfuryl acetate, furfuryl alcohol (8.5-9.3%), 4-vinyl-2-methoxyphenol (6.2-7.9%) and pyridine (5.3-5.5%), which were samples obtained from the same coffee blend. Literature reports furfuryl acetate as the most abundant coffee volatile compound followed by furfuryl alcohol in freshly brewed espresso, American coffee, Neopalitan coffee and Moka coffee as occurred in EC machine, EC1, and FD (Akiyama et al., 2009; Caporaso et al., 2014). Indeed, furfuryl acetate has been highlighted as a major difference between the samples, with higher preponderance in espresso, with furfuryl alcohol with equal contributions (Caporaso et al., 2014). However, it was also observed an inverted trend (furfuryl alcohol > furfuryl acetate) when different SPME fibre exposure time was used and in other coffee brews as French press and Turkish coffee (Akiyama et al., 2009; Amanpour & Selli, 2016). Regarding furfuryl alcohol, its contribution for overall area was

also considerable in instant coffee samples (IC1 - 10.1%, IC2 - 6.3%). This compound is formed by the thermal degradation of sugars or coffee acids, with their amount depending on the roasting process (time and temperature), although a considerable fraction is lost in this step (Albouchi & Murkovic, 2018; Amanpour & Selli, 2016; Moon & Shibamoto, 2010). Moreover, the amount of furfuryl alcohol is clearly major in ground coffee compared to furfuryl acetate (Petisca, et al., 2013). Nevertheless, the area values of EC machine ($7x10^7$), EC and FD ($3x10^7$ and $2x10^7$, respectively) were much higher than those obtained for instant coffee samples (IC1 - $5x10^6$, IC2 - $9x10^6$). In instant coffee samples, other compounds as 2-furfurylfuran (6.5% in IC2), N-furfurylpyrrole (5.3% for IC1 and 5.2% for IC2), and 1H-pyrrole-2-carboxaldehyde, 2-ethyl-5-methylpyrazine, 2-formyl-1-methylpyrrole (7.6%, 5.9%, 5.5%, respectively in IC1) constitute more than 5% of GC total area.

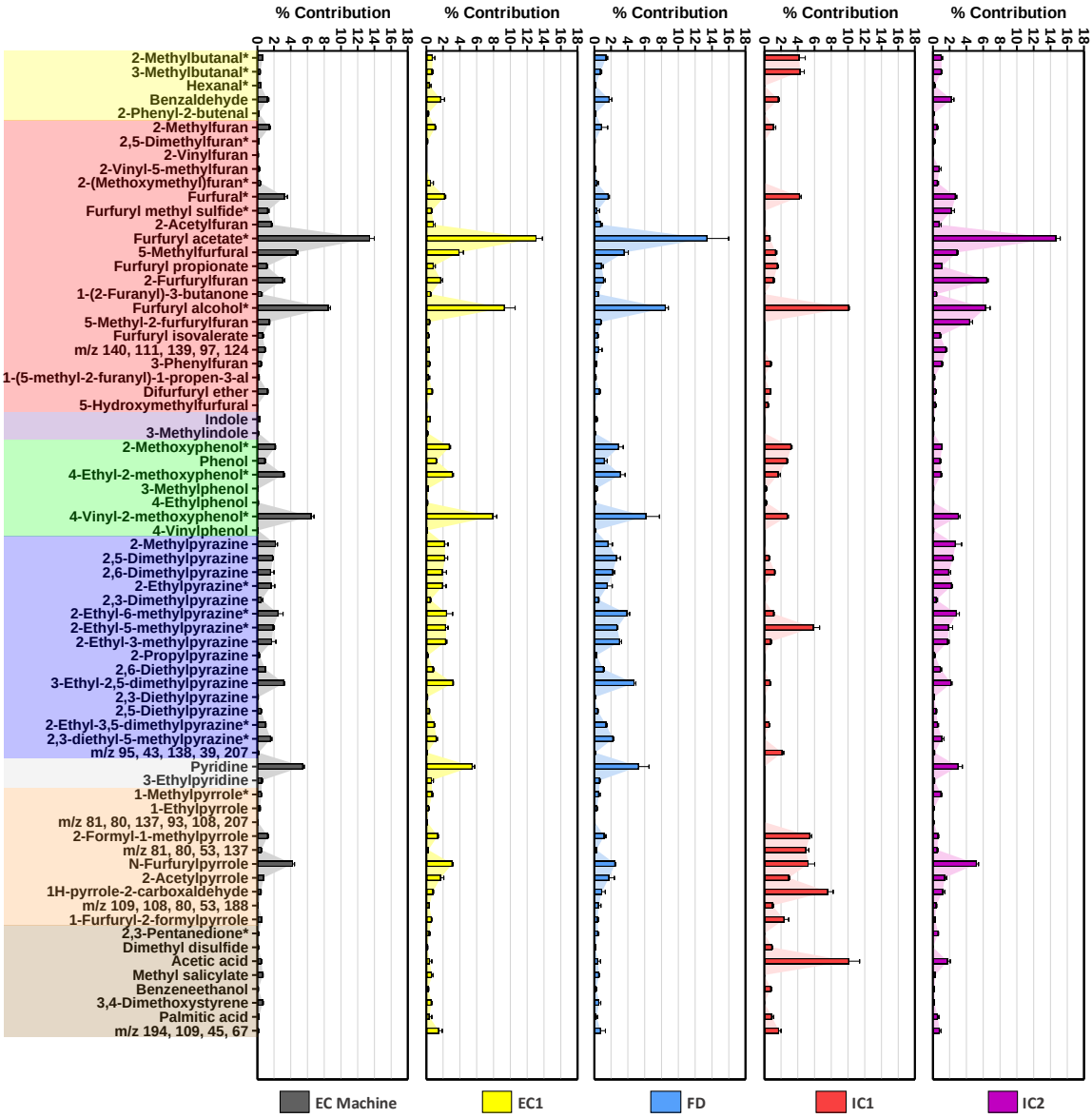


Figure III.3.8. Contribution profile of volatile compounds detected for the overall GC peak area determined for the different samples analysed. Compounds described as key odorants in literature are marked with an asterisk (*) (Table III.3.7.)

The heatmap representation in Figure III.3.9a was created using the peak area of the different samples analysed, performing a data scaling of the results to attribute equal importance to each variable (compound) analysed. Such representation allows a quick assessment of the relative abundance of the compounds, highlighting samples differences through colour distinction. Thus, according to peak intensities, the samples were represented in a blue to red scale, with blue associated with lower GC peak area abundance and red to higher intensities. Figure III.3.9a evidenced that EC machine has the highest peak intensities in almost all compounds, except for 5-hydroxymethylfurfural (last line in

furans - present exclusively in instant sample). The dendrogram (Figure III.3.9a) highlights the different EC machine composition compared to the other samples. Nevertheless, it also shows the similarity between FD and EC sample and discernible difference from the two instant coffee analysed. Figure III.3.9b graphically distribute the samples according to the principal component analysis (PCA) of the data allowing to understand the difference and similarity of the samples. PC1 and PC2 were able to explain 90% of total variance of the samples, with PC1 explaining 83.2%. PC1 clearly separated EC Machine (PC1 negative) from the remaining coffee samples. The loadings plot shows 5-hydroxymethylfurfural as the only compound in positive PC1, which constitute a decisive feature for samples differentiation, as it was absent in EC Machine sample. This compound is described as one of the major volatile compounds (18-22%) in agglomerated instant coffee samples (powder), probably due to the thermal processing associated to their production (Leobet et al., 2019). The PC2, explaining 6.8% of samples variability, confirms the similarity between EC1 and FD samples and the difference of these samples in relation to instant coffee ones (IC1 and IC2). Instant coffees were considerable different between them namely due to acetic acid and 1H-pyrrole-2-carboxaldehyde, compounds reported both in instant coffee and different brew samples (Akiyama et al., 2007; 2008; Amanpour & Selli, 2016; Leobet et al., 2019; López-Galilea et al., 2006; Padma Ishwarya & Anandharamakrishnan, 2015).

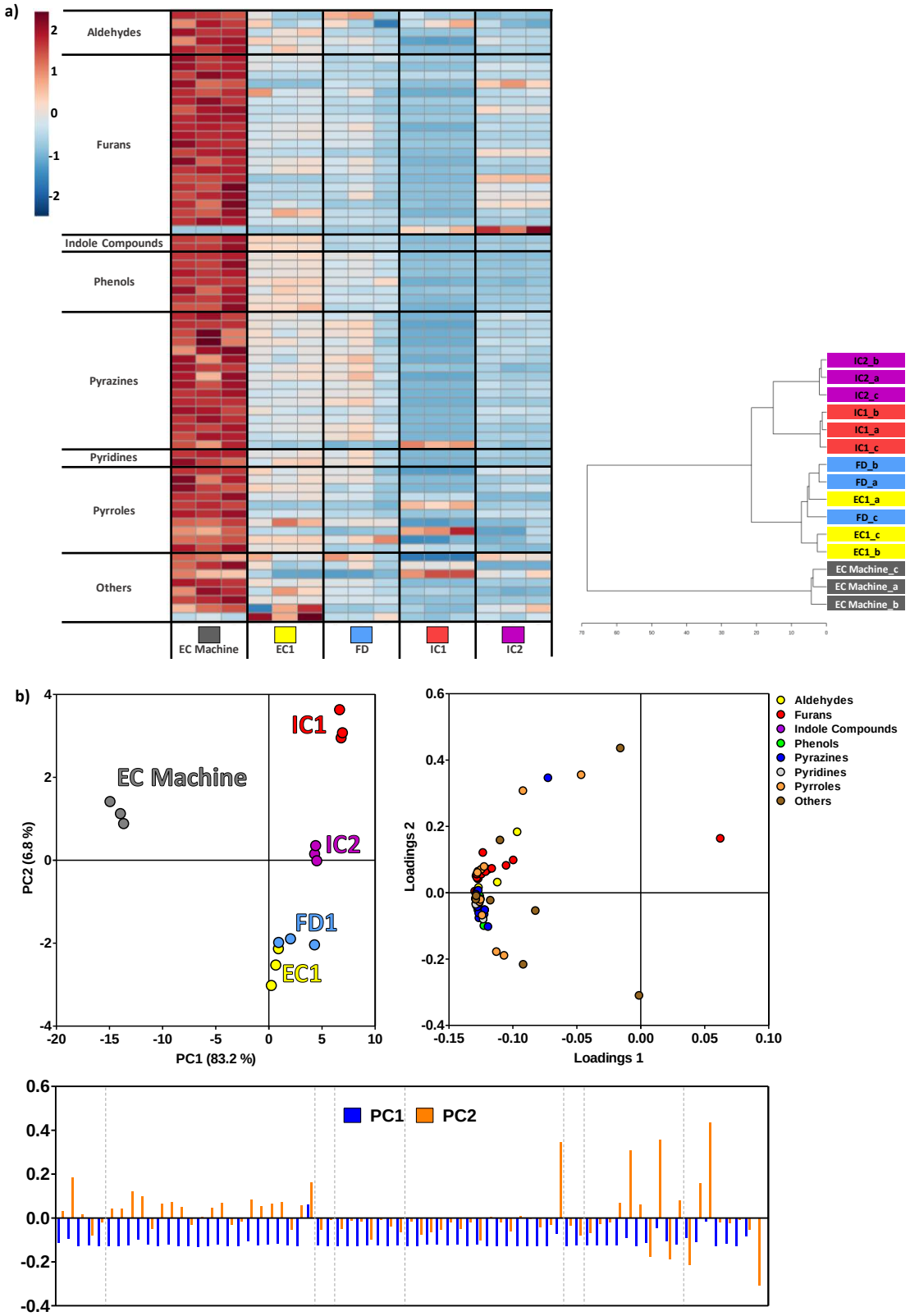


Figure III.3.9. a) Heatmap representation of the compounds identified in 5 coffee samples analysed grouped by chemical families considering GC peak areas after mean-centred the data for each variable and dividing by the standard deviation (autoscaling). The dendrogram using Ward's cluster algorithm and Euclidean distance was also presented. b) Principal component analysis (PCA) of the volatile compounds identified in the coffee samples analysed presenting the distribution of the samples (scores, left) and compounds (loadings, right and below).

The multivariate analysis (Figure III.3.9) evidenced that EC machine was clearly the most dissimilar sample concerning volatile profile, associated to the fresher extraction process of coffee, without the loss of compounds from a first extraction and drying process. Therefore, the data was re-analysed excluding the EC machine sample to better understand the similarities or differences between the re-dissolved extracts (FD1, EC1, IC1, and IC2). The heatmap presented in Figure III.3.10a highlights the higher overall intensity associated to EC1 and FD, that some compounds were more intense in IC2 and the poorer global intensity of IC1. The differentiation of FD and EC1 compared to instant samples (IC1 and IC2) was evidenced by the dendrogram in Figure III.3.10. The PCA, explaining 83% of data variability, reinforces the differences of the samples. PC1, representing 65.0% of the variability of the samples separated EC1 from IC1, explained by the lower intensity of IC1 in most of the compounds, while IC2 was positioned at an intermediate level. The PC2, that explained 17.7% of data variability, separated IC2 (negative PC2) from the remaining samples (positive PC2) mainly due to the higher intensities associated to some furan compounds, as viewed in heatmap.

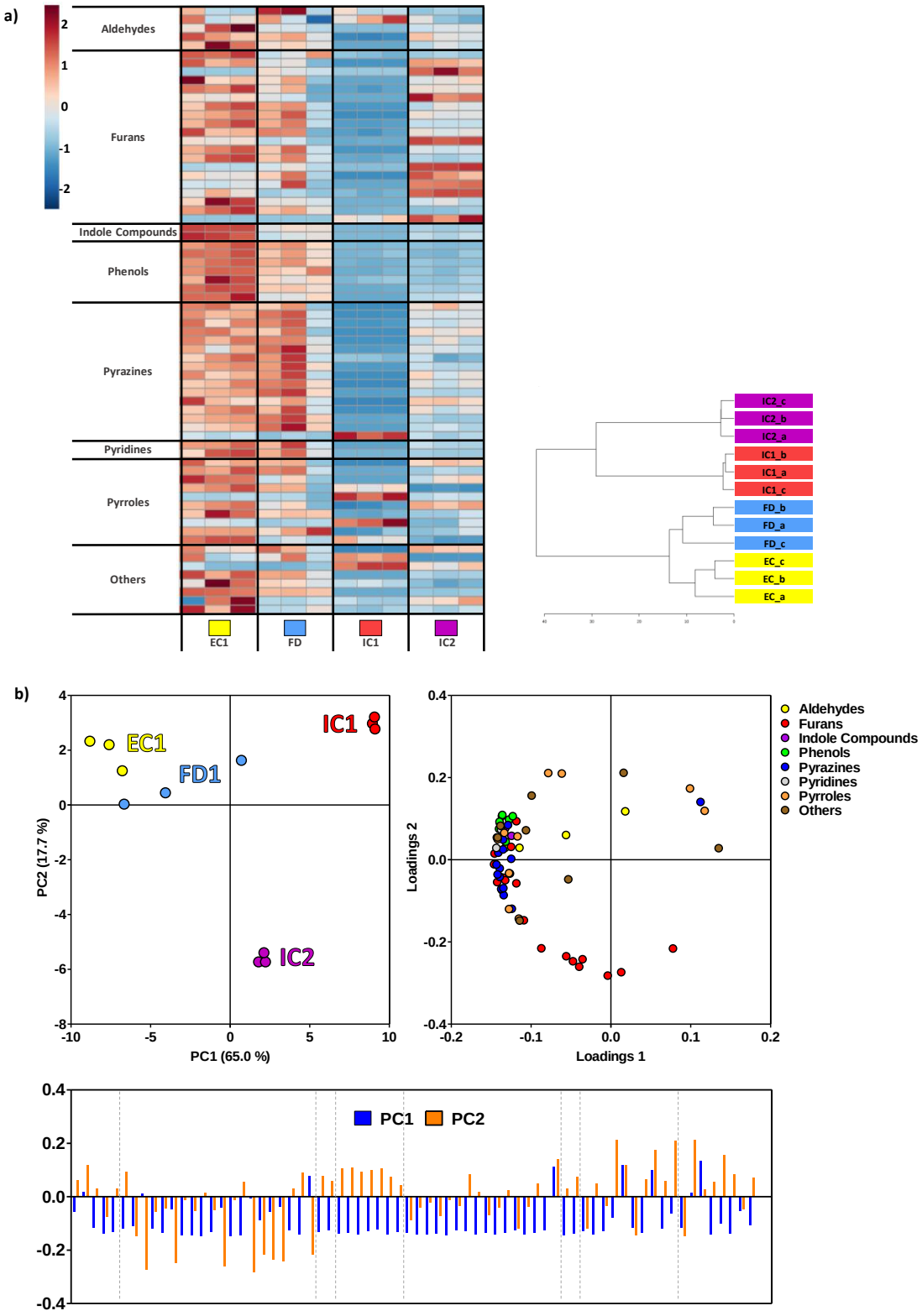


Figure III.3.10. a) Heatmap representation of the compounds identified in 4 coffee samples analysed grouped by chemical families considering GC peak areas after mean-centred the data for each variable and dividing by the standard deviation (autoscaling). The dendrogram using Ward's cluster algorithm and Euclidean distance was also presented. b) Principal component analysis (PCA) of the volatile compounds identified in the coffee samples analysed presenting the distribution of the samples (scores, left) and compounds (loadings, right and below).

An in-depth analysis was performed through the selection of compounds identified in the coffee samples that has already been described in literature as odorants, having impact in coffee brews aroma, namely in espresso coffee. Although 56 of the compounds identified in the coffee samples have aroma descriptors associated (Table III.3.7), only 19 were already described in literature as important odorants for coffee brews (Table III.3.8.). Some of them have a positive effect on coffee aroma, associated to freshness, fruity, or spicy odours, others are negative key odorants associated to fermented, burnt, or mouldy notes, while some of them do not properly affected coffee aroma, but are present in high amount in coffee brews, and are usually monitored (Caprioli et al., 2012). In the later fraction are found the more abundant compounds (furfuryl acetate, furfuryl alcohol), although nutty, caramel, and burnt are some of the aroma descriptors associated to these compounds (Table III.3.7) (Amanpour & Selli, 2016; Chin et al., 2011).

Table III.3.8. Odorants found in the analysis of coffee samples and respective aroma descriptors reported in literature (based on Table III.3.7.)

Compound	Structure	Aroma Descriptors	Compound	Structure	Aroma Descriptors
2-Methylbutanal		chocolate-like, fruity, fermented, pungent, malty, cocoa, buttery, oily, sweet	4-Ethyl-2-methoxyphenol		spicy, medicinal, phenolic, smoky, roasted, green, ethereal, clove-like, vanilla-like, sweet
3-Methylbutanal		acrid-pungent, fruity, malty, sweet, buttery, oily, peach, apple, chocolate, burned, almond	4-Vinyl-2-methoxyphenol		spicy, apple, rum, roasted peanut, smoky, medicinal, phenolic, eugenol-like, sweet, clove-like
Hexanal		grassy, raw bean, fruity, leaf-like, fatty-green, rancid butter	2-Ethylpyrazine		nutty, green, sweet, buttery, rum, toasted, peanut butter, woody
2,5-Dimethylfuran		coffee, ethereal	2-Ethyl-6-methylpyrazine		toasted, earth, mould, flowery, fruity, hazelnut-like
2-(Methoxymethyl)furan		burnt, pungent, chemical	2-Ethyl-5-methylpyrazine		rubber, smoky, chemical, greasy, onion, toasted, coffee-like
Furfural		pungent, sweet, caramellic, bready, cinnamon-almond-like, almond/bitter, cooked pea, chemical, smoky	2-Ethyl-3,5-dimethylpyrazine		paper, burnt, earthy, toasty, nutty-roast, meaty, green, potato-like, almond, nuts
Furfuryl methyl sulfide		coffee, smoke-roast, burnt, sulfury, cooked, cabbage, onion, leather-like, toasty, garlic	2,3-diethyl-5-methylpyrazine		earthy, roasty, nutty, meaty, roasted hazelnut
Furfuryl acetate		ethereal-floral, herbal-spicy, nutty, fruity, banana	1-Methylpyrrole		coffee, roasty, smokey, sweet, woody-herbaceous
Furfuryl alcohol		slightly caramellic, warm-oily, burnt, smoked, unpleasant	2,3-Pentanedione		sweet, buttery, oily, milky, caramel-like
2-Methoxyphenol		phenolic, burnt, smoky, spicy, harsh, earthy, sweet			

The heatmap built using the compounds known as odorants (Figure III.3.11) evidences EC machine sample with the highest peak intensities in all the compounds identified. Compared to the later sample, EC1 extract exhibited a reduction of 33-83% along the compounds, FD 30-91%, IC2 31-94% and IC1 27%-100%. In IC1 sample, 8 out of 19 compounds were not detected. All processing steps of instant coffee production (freezing and concentration by freeze- or spray-drying) have impact on the retention (or

loss) of aroma compounds (Petersen et al., 1973). The 2,3-pentanedione had the least percentage of reduction comparing EC Machine with EC1 (33%), FD (30%), and IC2 (31%) extracts although it ceases to appear in IC1 sample. This compound is pointed out as key aroma coffee odorant, associated to buttery notes in coffee brews, as espresso (Andueza et al., 2003b; Maeztu et al., 2001b). Indeed, IC1 differentiation from all other extract samples was confirmed in PCA (Figure III.3.11b). On the other hand, in this sample, 3-methylbutanal was also responsible for samples differentiation (compound highlighted in negative PC2), with the most approximate level to EC Machine values, and a reduction of 27% in IC1, and higher levels in EC1 (33%), FD1 (42%) and IC2 (45%). This compound is associated to fruity (peach, apple), sweet and malty notes, being described as having a positive impact for coffee aroma (Table III.3.7) and pointed out as a major compound in coffee brew (infusion) volatiles (9.7 %) (Caporaso et al., 2014; Caprioli et al., 2012; Gonzalez-Rios et al., 2007; Shimoda & Shibamoto, 1990). Considering EC Machine as reference, 2-methylbutanal appeared in considerable amount in FD sample (37% reduction) comparing to the remaining (EC1-60%, IC1-61%, IC2-69%). This compound was already found as the major compound of headspace volatiles of infusion coffee, representing 17.7% of GC peak area (Shimoda & Shibamoto, 1990). This aldehyde has been negatively associated to coffee aroma, described as a fermented odour, although other reports referred it as sweet and fruity (Table III.3.7) (Caporaso et al., 2014; Caprioli et al., 2012; Gonzalez-Rios et al., 2007). Indeed, both 2- and 3-methylbutanal are described as responsible for the malty notes of coffee (Caporaso et al., 2014; Semmelroch & Grosch, 1995). Then, all other compounds showed more than 53% decrease level comparing to EC Machine, a richer coffee aroma sample. Contrary to the previous PCA's, Figure III.3.11b evidenced a reasonable similarity between EC1, FD, and IC2 extracts. However, such results may be affected by the prevalent higher intensities of EC Machine peak areas compared to other samples.

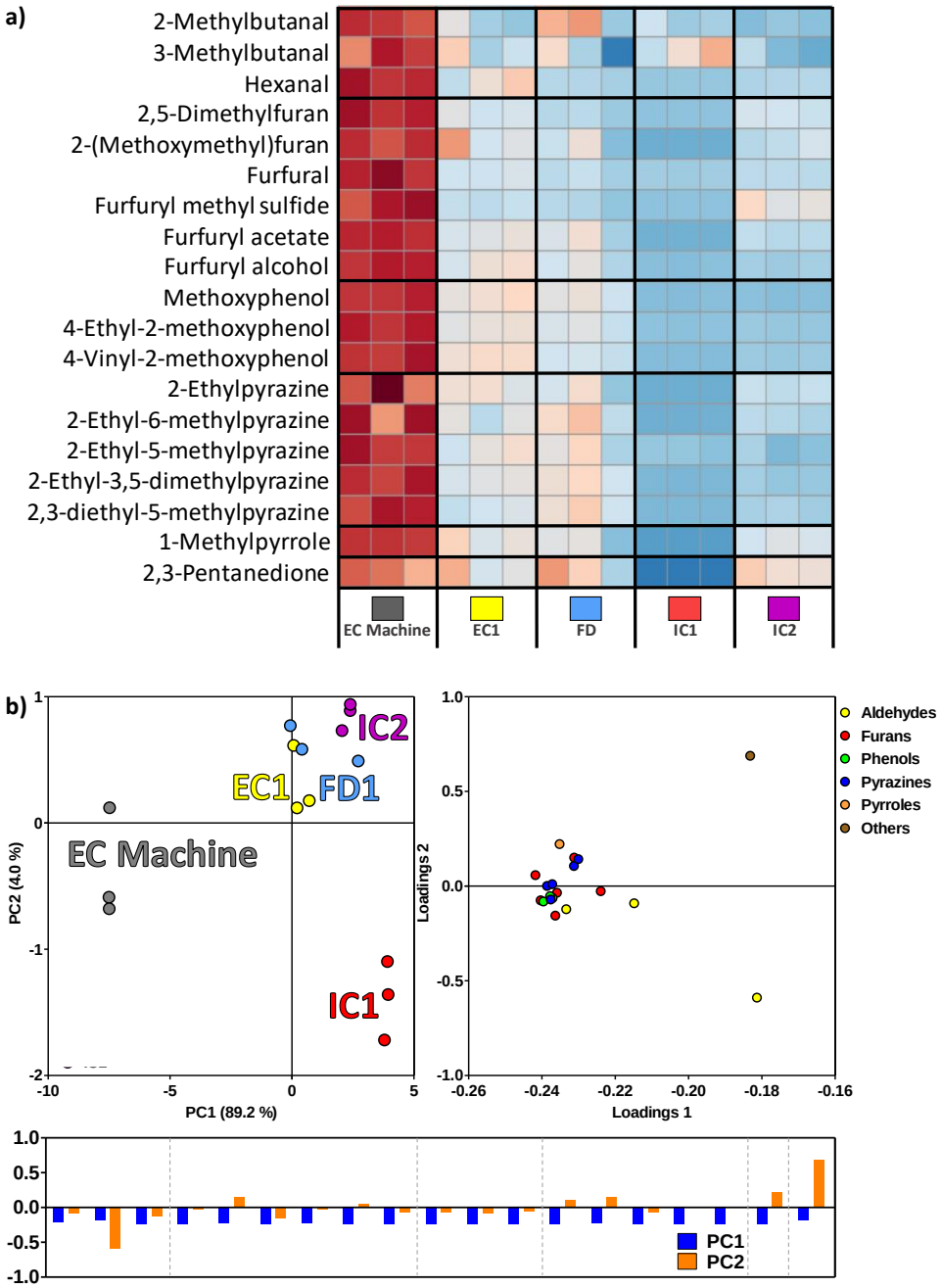


Figure III.3.11. a) Heatmap representation of the odorants identified in 5 coffee samples analysed grouped by chemical families considering GC peak areas mean-centred the data for each variable and dividing by the standard deviation (autoscaling). The dendogram using Ward's cluster algorithm and Euclidean distance was also presented. b) Principal component analysis (PCA) of the volatile compounds identified in the coffee samples analysed presenting the distribution of the samples (scores, left) and compounds (loadings, right and below).

The comparison of only extract samples (without EC Machine) concerning the odorants identified evidenced the similarity of EC1 and FD1 extracts (heatmap and PCA in Figure III.3.12) and discrepancy to IC1 sample. PC1 that explained 67.2% of samples variability, separated FD and EC1 (negative PC1) from the samples IC1 and IC2 (positive

PC1) due to overall higher level in odorant compounds peak areas. PC2, that explain 14.5% of samples variability separating IC2 (PC2 negative) from the remaining samples associated, for instance, to higher level of furfuryl methyl sulphide compared to all others. On the other hand, the higher level of 2-methylbutanal distinguish FD1 sample.

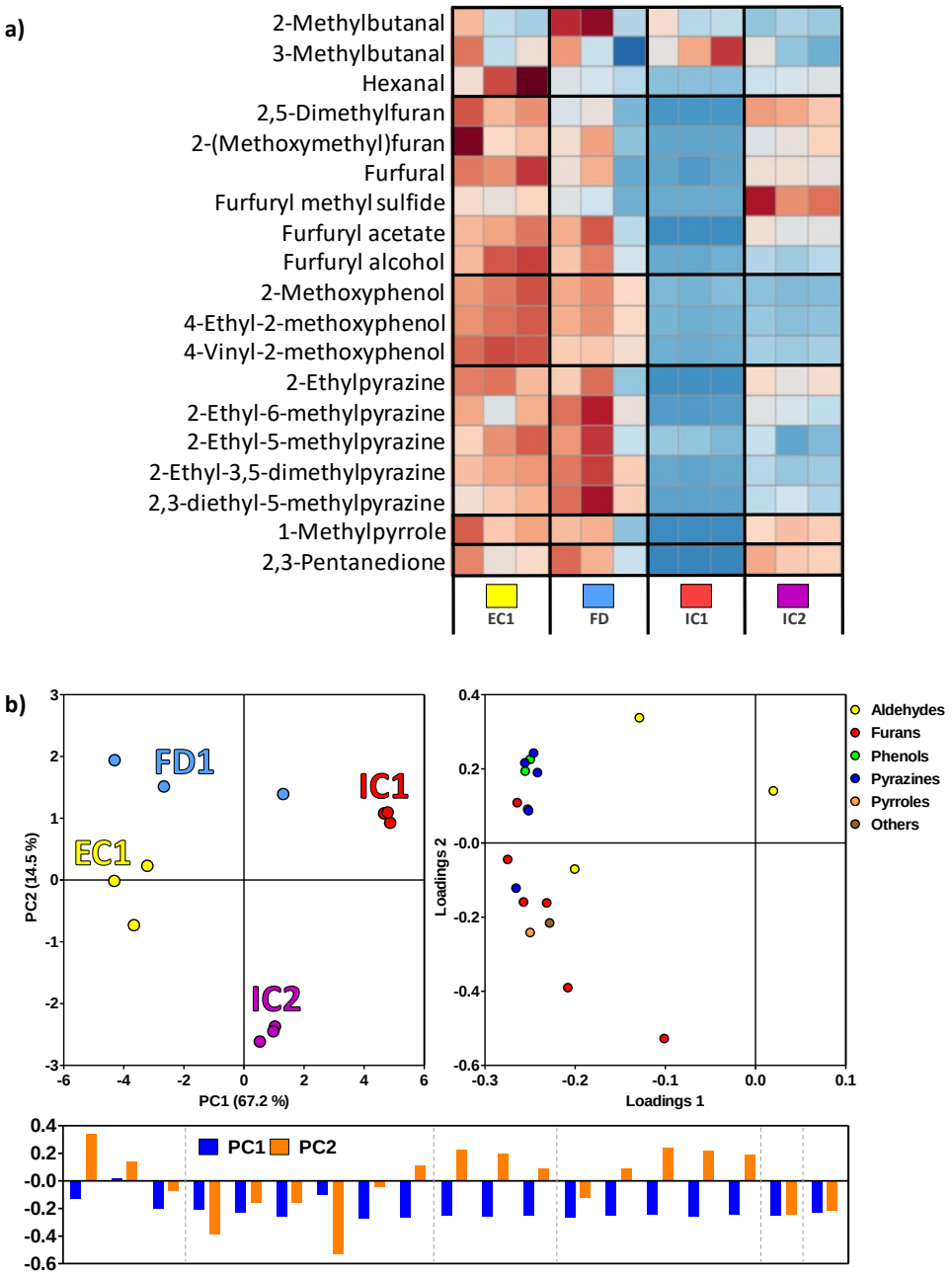


Figure III.3.12. a) Heatmap representation of the odorants identified in 4 coffee samples analysed grouped by chemical families considering GC peak areas after mean-centred the data for each variable and dividing by the standard deviation (autoscaling). The dendogram using Ward's cluster algorithm and Euclidean distance was also presented. b) Principal component analysis (PCA) of the volatile compounds identified in the coffee samples analysed presenting the distribution of the samples (scores, left) and compounds (loadings, right and below).

The comparison of EC1 and FD samples only significantly differed in two compounds: 2,5-dimethylfuran (p<0.05) and 4-vinyl-2-methoxyphenol (p<0.01) (Figure III.3.13). These samples have the same coffee blend origin and were both concentrated via freeze-drying after extraction using different methodologies (espresso and infusion). The identical pattern suggested that the aroma created when dissolved the samples in hot water was similar. Similar overall volatile profile was also found in literature for distinct coffee brews as Turkish coffee or French press, for instance (Amanpour & Selli, 2016).

The compound 4-vinyl-2-methoxyphenol is described as conferring a spicy, phenolic odour to coffee, and it was already found by some authors in coffee brew and absent or clearly diminished in instant coffee (Sanz et al., 2002; Semmelroch & Grosch, 1995). In this study, besides the substantial difference comparing to EC Machine ($5.1x10^7$), the level in EC1 ($2.1x10^7$) and FD ($1.3x10^7$) was quite higher than the level found in IC1 ($1.3x10^6$) and IC2 ($4.6x10^6$). Furthermore, this compound is highlighted in a study for being the most affected by volume *per* sip of espresso coffee brew, with the greatest increase in headspace concentration from the odorants analysed (Genovese, et al., 2014).

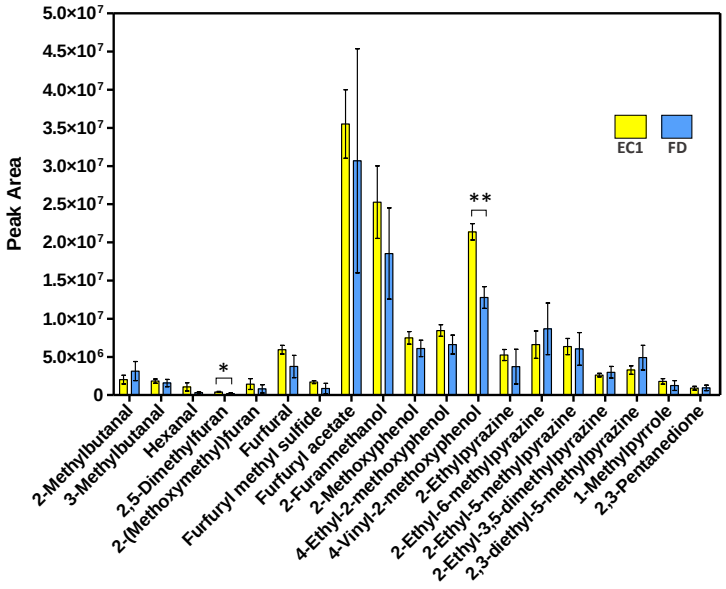


Figure III.3.13. GC peak areas of the odorants identified in coffee samples (EC1 (yellow) and FD (blue)) highlighting the compounds with significant different levels (*p<0.05, **p<0.01).

The presence of other phenolic compounds, as 4-ethyl-2-methoxyphenol, that also confers spicy notes, and 2-methoxyphenol, with burnt and smoky odor, are reported to be present in coffee brews and absent/minor in instant coffee. This is consistent with the

results obtained in this study, with a clear decrease in IC samples ($7.8x10^5$-$1.5x10^6$) compared to EC1/FD ($6.6x10^6$-$8.5x10^6$) (Sanz et al., 2002; Semmelroch & Grosch, 1995). The same trend occurred with 3-methylindole which is in accordance with literature (Sanz et al., 2002). A series of reports monitored 13 structures as key aroma compounds to EC aroma, constituting 7.0-11.9% of total peak area, comprising 6 aldehydes, 2 ketones, 3 pyrazines, 1 sulphur compound (methanethiol) and a phenolic compound (2-methoxyphenol) (Andueza et al., 2002, 2003a, 2003b, 2007; Maeztu et al., 2001b). Among these compounds the aldehydes and ketones were associated to buttery, fruity, and malty odour, while pyrazines were related to roasty, toasted and earthy, and 2-methoxyphenol is associated to spicy, smoky and burnt odours, for example, contributing all of them for distinct coffee aroma (Table III.3.7) (Andueza et al., 2003a, 2003b). In the EC1 and FD, 8 of these compounds were identified, namely all pyrazines (2-ethylpyrazine, 2-ethyl-6-methylpyrazine, and 2-ethyl-3,5-dimethylpyrazine), 3 aldehydes (2-methylbutanal, 3-methylbutanal, and hexanal), 1 ketone (2,3-pentanedione), and 2-methoxyphenol corresponding to 10.3% (EC1/FD) and 12.5% (EC1/FD) of the GC compounds total area. These results were of the same magnitude of the reported studies, and were similar between the samples tested. In this study, no statistical differences were observed between EC1 and FD considering these compounds. Thus, although EC machine representing the aroma of an espresso coffee revealed higher intensities, the profile of this sample, a freeze-dried EC1 or one obtained from an infusion process (FD) was similar. The change of operation parameters as the slower freezing process may help to increase the retention of volatiles (Petersen et al., 1973). The addition of flavour extracts to enrich the aroma profile may approximate the FD aroma to the aroma of a fresh coffee machine extraction. It was possible to simulate a coffee flavour by mixing the most potent coffee odorants in an oil/water mixture, for instance, with an aroma profile close to roasted coffee and brews (Czerny et al., 1999; Semmelroch & Grosch, 1996). The authors pointed out 4-vinyl-2-methoxyphenol, 2-ethyl-3,5-dimethylpyrazine, 2,3-diethyl-5-methylpyrazine, the “malty” smelling 2- and 3-methylbutanal and 2,3-pentanedione found in EC1 and FD samples, as part of the main compounds responsible for coffee flavour. In the present study, the addition of roasted coffee lipid extracts may overcome both the lack of lipids and aroma in the FD developed instant espresso coffee formulation.

III.3.3.5. Global Analysis

The principal component analysis (PCA) of the FTIR spectra from the extracts allowed to infer that the espresso coffee samples (E1-E5) and the freeze-dried extracts (FD) have similar overall composition (Figure III.3.14). On the other hand, the SD samples were separated from the freeze-dried ones, explained mainly by a shift in the 1029 cm^{-1} peak to 1032 cm^{-1}. This is an effect of the drying process, as when SD samples were dissolved in water and freeze-dried, samples were placed together with all other FD samples in new PCA (SDFD in Figure III.3.14). Moreover, the IC samples, both IC1 and also the labelled espresso ones, exhibited a different FTIR spectra profile with higher intensity in the carbohydrate region (800-1200 cm^{-1}). This is associated to their higher content in that samples in accordance with Table III.3.1 data, being the wavenumber at 1029 cm^{-1} the one that explained the major variation in PC1. On the other hand, EC/FD/SD samples showed greater peak intensities at 1580, 1645 and 1699 cm^{-1}, related to higher caffeine and chlorogenic acids content, explaining the shift towards negative PC1 (Barbin, et al., 2014; Singh, et al., 1998). EC samples (EC1-EC5) showed a higher peak intensity at 2923 cm^{-1}, associated to lipids, in accordance with their higher content in EC samples. The FTIR analysis demonstrated that, overall, the extracts produced (mainly FD) were chemically close to EC samples and greatly distinct from IC samples, even the ones labelled as espresso, possibly because they were obtained using drastic conditions of extraction that could hinder their similarity to EC.

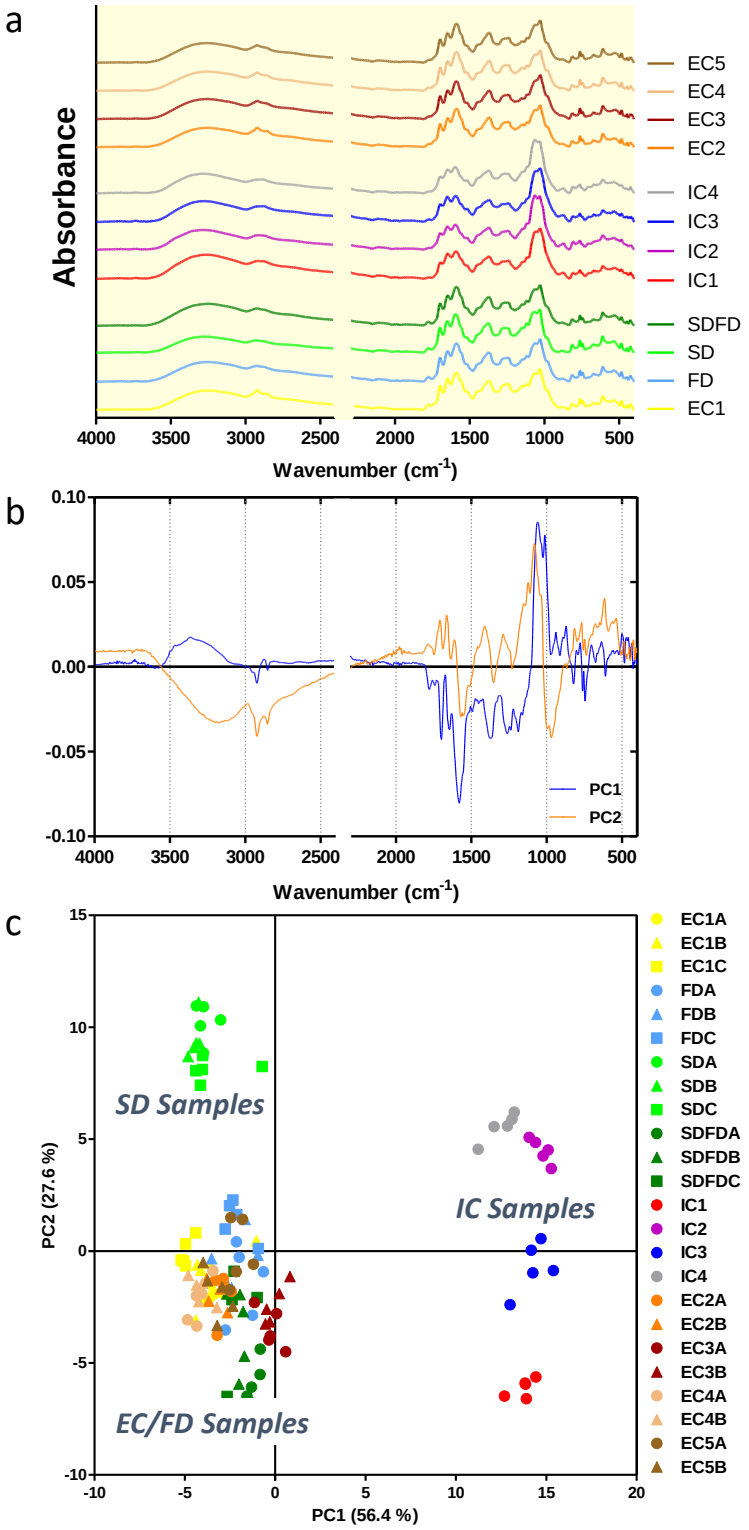


Figure III.3.14. FTIR analysis of coffee powders. a) FTIR spectra (SNV-corrected), b) PCA loadings and c) scores.

Moreover, it was performed a PCA of all compounds determined and a heatmap representation of the data for EC1, FD and IC1 that allowed to perceive the similarity of them (Figure III.3.15). The PC1, explaining 60% of data variability, separated the EC1 and

FD sample from IC1, evidencing extracts similarity in most of the compounds. Carbohydrates (mainly galactose) differentiated IC1 sample explained by their higher amount. Moreover, EC1 sample was separated from FD due to lipids as well as melanoidins content, although the differences in the levels for the two extracts was low (5.4% of total solids in FD, comparing to 5.9 % in EC1). The EC sample had also a higher protein content (3.8% comparing to 2.6% in FD sample), however the foam analysis does not evidence considerable differences in foamability. The heatmap representation highlights the similarity of EC1 and FD extracts in most of the parameters analysed and the considerable difference to IC1.

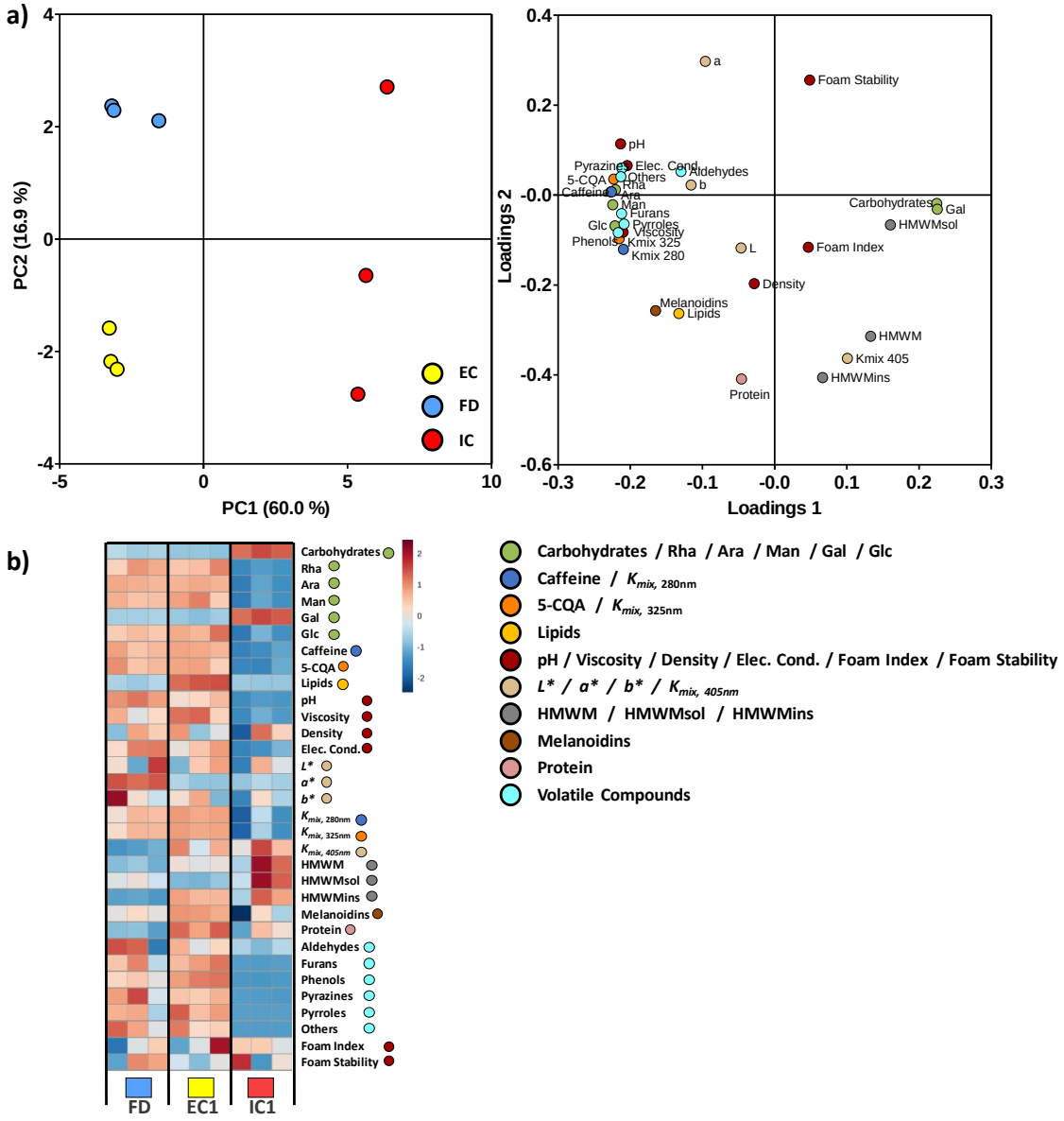


Figure III.3.15. Principal component analysis a) and b) heatmap representation of all the compounds and properties determined for EC, FD, and IC samples.

III.3.3.6. Concluding remarks

EC consumer habits depend greatly on personal taste preference related to roasting degree or coffee concentration. For example, when higher amounts of coffee powder (6.5-8.5 g) are used for the same amount of water (40 mL), as in the present study, the concentration of total solids content tend to increase (27.1-45.3 $mg.mL^{-1}$), (Andueza et al., 2002, 2003a, 2003b; 2007; López-Galilea et al., 2007; Maeztu et al., 2001a) which is even more marked (40.3-73.7 $mg.mL^{-1}$) when lower amount of water (25-30 mL, 5.5-8.0 g) is used to obtain the brew (Albanese et al., 2009; Caporaso et al., 2014; Gloess et al., 2013; Parenti et al., 2014; Severini et al., 2015) and discussed in Chapter III.1. Thus, EC exhibits a broad range of concentration values (23.0-73.7 $mg.mL^{-1}$). Such values may be achieved by changing the amount of coffee extract to be dissolved in water.

In the EC samples studied, 20.8-22.5 %w/w of the coffee compounds end up in the brew extract, which is an amount similar to the one obtained with a regular infusion extraction. The composition of freeze-dried EC and infusion extracts was similar in many of the parameters analysed. The processing by spray-drying was not favourable to extracts with low concentration of solids due to undesirable properties (dissolution). The FD extracts lacked lipids content due to higher extractability of this fraction with EC devices. The FD sample contains an aroma representative of espresso coffee, considering that the compounds are still present in the extract, although in considerably lower amount. Nevertheless, the addition of flavour extracts to enrich the aroma profile may approximate the FD aroma to the aroma of a fresh coffee machine extraction. Indeed, it is possible to simulate a coffee flavour by mixing the most potent coffee odorants in an oil/water mixture, with an aroma profile close to real coffee samples (Czerny et al., 1999, Semmelroch & Grosch, 1996). On the other hand, the addition of roasted coffee lipid extracts may overcome both the lack of lipids and aroma in the FD developed instant espresso coffee formulation.

A high fraction of compounds remains unextracted. The posterior extraction of such residue in more drastic conditions could be used to the production of instant coffee, taking into consideration the quantity and composition of compounds, namely galactomannans and arabinogalactans, that remain in the coffee residue. This would lead to

the total exploitation of the coffee powder in two distinct products, EC and IC, by a two-steps extraction process.

Chapter IV

Conclusions and Future work

In this thesis, the comprehensive study of coffee infusion process was carried out aiming to develop an extract that chemically resemble espresso coffee. The process was conducted studying carbohydrates, caffeine and 5-CQA content, as well as the colour of the extracts.

Temperature was the main factor affecting both conventional and microwave-assisted extractions. The wide range of conditions tested allowed to extract a maximum of 30% of coffee compounds with the conventional method, while with MAE these values may increase to 47%. In both systems, the increased extraction yield was associated to higher extraction of polysaccharides. The carbohydrates were evaluated as their content and composition regarding the extracts total solids. For conventional system:

1) Arabinogalactans were extracted regardless the conditions used (7.5-12%), while galactomannans depended mostly on the increase of extraction temperature (5.4-18.3%), suggesting different extractability of coffee polysaccharides;
2) Sugars content was positively correlated with galactomannans, evidencing that the extraction conditions are responsible for their higher/lower extraction:

    2.1) At lower temperatures (cold brews), the extraction of arabinogalactans was predominant, while prolonged times increased galactomannans extraction;

    2.2) At higher temperatures, the galactomannans were the predominant polysaccharides, although higher w/V ratios and prolonged times seemed to favour arabinogalactans extraction;
3) The overall mass extraction yield seemed to be related to the brown color of the coffee extracts.
4) Caffeine and 5-CQA were extensively extracted in the conditions tested, while their content in each extract varied due to the concentration/dilution in relation to other extracted compounds.

When applying the MAE at high temperatures (120-180 ºC):

1) An increase in overall yield of extraction (and sugars content) was obtained and resulted in higher arabinogalactans extraction, suggesting their preferential extraction over galactomannans;

2) Almost all arabinose and galactose presented in roasted coffee powder was extracted during experiments at 180 °C, showing that MAE was an effective method for a quick arabinogalactans extraction from coffee;
3) Mannose extraction did not exceed the 27% of the amount available in the coffee powder, evidencing that galactomannans were more difficult to extract than the arabinogalactans;
4) MAE resulted in yellowish extracts with increased extraction yield, suggesting that the additional compounds extracted in relation to conventional methods are non-coloured or degraded to less brown compounds;
5) Increase of extraction yield (and sugars content in the extract) decreased the relative content of caffeine and 5-CQA, suggesting that the content in the extract was more dependent on the overall extraction of carbohydrates than on the compounds themselves;
6) The MAE allowed to quickly obtain an extract that chemically resembled instant coffee;
5) The increment in extraction yield was associated to arabinogalactans and it was not feasible to achieve a high extraction yield with a positive ratio of galactomannans/arabinogalactans even when using low extraction times (< 10 min).

As from the results obtained a galactomannan/arabinogalactan ratio higher than 1 was easily achieved using conventional extraction, it was possible to scale up the infusion process for comparison of the extracts obtained with commercial espresso and instant coffee. The extracted coffee solutions were processed via spray- and freeze-drying. The spray-dried sample exhibited lower potential to be applied as it had lower tendency to dissolve. The carbohydrates (content, composition, and glycosidic linkages) of infusion extracts and espresso reference were similar, as well as their caffeine and 5-CQA content and density or kinematic viscosity. However, the extracts lack the lipids content. Furthermore, the extracts showed to be able to produce foam when subjected to CO_2 injection or by adding effervescent agents to the formulation.

Concerning the volatile profile, the freeze-dried extracts obtained from the scale up condition showed the same volatile profile and the presence of key odorants as the espresso

coffee extract. However, both abovementioned samples showed a loss of volatile intensity in comparison with fresh espresso coffee sample.

Diverse future studies may be conducted following on the results obtained: sensorial analysis of the extracts is essential to confirm the potentiality of the extracts regarding their resemblance to espresso coffee. Due to the lack of lipids and volatile compounds in the extracts, the feasibility should be tested to obtain espresso-like extracts by infusion and also obtain the oil fraction that remain in the residue, that should contain a considerable amount of liposoluble volatile compounds. It should be tested the addition of the two extracts prior to the concentration step. Some preliminary tests were conducted with roasted coffee showing that the lipid fraction may be easily extracted by solid-liquid extraction with *n*-hexane at room temperature.

The residue left is still a source of lipids. Moreover, the high fraction of polysaccharides still remaining in the residue can be used as source of dietary fibre. The use of these resources would result in a total use of coffee powder in more than one product. The extracts produced besides the possible utilization in the preparation of coffee brews, may be used also as flavorings in food industry (ice cream, cakes, desserts or sweet cookies). In this sense, it could be interesting to study the aroma developed in MAE extracts obtained at higher temperatures, as even if it is perceivable that the aroma was different from the obtained in conventional methods. The aroma analysis for such extracts was not performed yet once their composition differs greatly from espresso coffee. The lower extraction times (<10 min) comparing to the used in industrial soluble factories, could be interesting for industrial application. Further improvement in the espresso technology would be the production of an extract that ideally could produce heat and foam *in situ* in order to avoid the need of an espresso coffee machine. Edible coatings may be useful to the success of this step, allowing the protection of coffee compounds from those required for the development of heat (exothermic reaction). Indeed, coffee polysaccharides are already used as edible coatings. The encapsulated compounds would only be released in contact with water. On the other hand, coatings may also increase the shelf-life of the product and/or promote the retention of coffee volatile compounds. This would be the ultimate goal of an espresso-instant coffee product.

Chapter V

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Annexes

A.III.1. ANNEX FROM III.1

A.III.2. ANNEX FROM III.2

A.III.3. ANNEX FROM III.3

A.III.1. ANNEX FROM III.1

Table 1. ANOVA table for the CCD model related to *Extraction Yield* (Y_1, %w/w).

a) Full Model

Variables	Sum of Squares	DF	Mean Square	*F* value	*p*-value
Model	112.0375	14	8.0027	50.075	< 0.0001
X_1	10.2756	1	10.2756	64.297	< 0.0001
X_2	67.6672	1	67.6672	423.413	< 0.0001
X_3	9.5339	1	9.5339	59.656	< 0.0001
X_4	7.3472	1	7.3472	45.974	< 0.0001
X_1^2	0.4845	1	0.4845	3.032	0.1021
X_2^2	0.3790	1	0.3790	2.371	0.1444
X_3^2	0.0008	1	0.0008	0.005	0.9446
X_4^2	0.0863	1	0.0863	0.540	0.4739
$X_1 \times X_2$	0.7225	1	0.7225	4.521	0.0505
$X_1 \times X_3$	5.5225	1	5.5225	34.556	< 0.0001
$X_1 \times X_4$	0.2025	1	0.2025	1.267	0.2780
$X_2 \times X_3$	4.8400	1	4.8400	30.285	< 0.0001
$X_2 \times X_4$	0.0400	1	0.0400	0.250	0.6241
$X_3 \times X_4$	0.1600	1	0.1600	1.001	0.3329
Error	2.3972	15	0.1598		
Lack-of-Fit	1.3222	10	0.1322	0.615	0.7603
Pure Error	1.0750	5	0.2150		
Total	114.4347	29			
R^2	0.9791				
R^2_{adj}	0.9595				
R^2_{pred}	0.9173				

b) Reduced Model

Variables	Sum of Squares	DF	Mean Square	*F* value	*p*-value
Model	105.1864	6	17.5311	43.599	< 0.0001
X_1	10.2756	1	10.2756	25.555	< 0.0001
X_2	67.6672	1	67.6672	168.285	< 0.0001
X_3	9.5339	1	9.5339	23.710	0.0001
X_4	7.3472	1	7.3472	18.272	0.0003
$X_1 \times X_3$	5.5225	1	5.5225	13.734	0.0012
$X_2 \times X_3$	4.8400	1	4.8400	12.037	0.0021
Error	9.2483	23	0.4021		
Lack-of-Fit	8.1733	18	0.4541	2.112	0.2088
Pure Error	1.0750	5	0.2150		
Total	114.4347	29			
R^2	0.9192				
R^2_{adj}	0.8981				
R^2_{pred}	0.8517				

Table 2. ANOVA table for the CCD model related to *Sugars Content* (Y_2, %w/w).

a) Full Model

Variables	Sum of squares	DF	Mean Square	*F* value	*p*-value
Model	329.5654	14	23.5404	9.653	< 0.0001
X_1	0.1800	1	0.1800	0.074	0.7896
X_2	156.0556	1	156.0556	63.990	< 0.0001
X_3	13.0050	1	13.0050	5.333	0.0356
X_4	50.3339	1	50.3339	20.639	0.0004
X_1^2	12.7005	1	12.7005	5.208	0.0375
X_2^2	4.4737	1	4.4737	1.834	0.1957
X_3^2	5.3424	1	5.3424	2.191	0.1595
X_4^2	3.3434	1	3.3434	1.371	0.2599
$X_1 \times X_2$	42.9025	1	42.9025	17.592	0.0008
$X_1 \times X_3$	0.4900	1	0.4900	0.201	0.6604
$X_1 \times X_4$	4.8400	1	4.8400	1.985	0.1793
$X_2 \times X_3$	24.0100	1	24.0100	9.845	0.0068
$X_2 \times X_4$	8.4100	1	8.4100	3.448	0.0831
$X_3 \times X_4$	4.6225	1	4.6225	1.895	0.1888
Error	36.5813	15	2.4388		
Lack-of-Fit	31.8613	10	3.1861	3.375	0.0958
Pure Error	4.7200	5	0.9440		
Total	366.1467	29			
R^2	0.9001				
R^2_{adj}	0.8068				
R^2_{pred}	0.5034				

b) Reduced Model

Variables	Sum of Squares	DF	Mean Square	*F* value	*p*-value
Model	298.7592	7	42.6799	13.934	< 0.0001
X_1	0.1800	1	0.1800	0.059	0.8107
X_2	156.0556	1	156.0556	50.947	< 0.0001
X_3	13.0050	1	13.0050	4.24574	0.0514
X_4	50.3339	1	50.3339	16.433	0.0005
X_1^2	12,2722	1	12.2722	4.007	0.0578
$X_1 \times X_2$	42.9025	1	42.9025	14.006	0.0011
$X_2 \times X_3$	24.0100	1	24.0100	7.839	0.0104
Error	67.3875	22	3.0631		
Lack-of-Fit	62.6675	17	3.6863	3.905	0.0692
Pure Error	4.7200	5	0.9440		
Total	366.1467	29			
R^2	0.8160				
R^2_{adj}	0.7574				
R^2_{pred}	0.6246				

Table 3. ANOVA table for the CCD model related to *GM Content* (Y_3, %w/w).

a) Full Model

Variables	Sum of squares	DF	Mean Square	*F* value	*p*-value
Model	287.4664	14	20.5333	14.578	< 0.0001
X_1	1.9339	1	1.9339	1.373	0.2596
X_2	122.7222	1	122.7222	87.127	< 0.0001
X_3	29.6450	1	29.6450	21.046	0.0004
X_4	22.6689	1	22.6689	16.094	0.0011
X_1^2	5.2904	1	5.2904	3.756	0.0717
X_2^2	9.1471	1	9.1471	6.494	0.0223
X_3^2	2.9722	1	2.9722	2.110	0.1669
X_4^2	1.3471	1	1.3471	0.956	0.3436
$X_1 \times X_2$	46.9225	1	46.9225	33.313	< 0.0001
$X_1 \times X_3$	1.0000	1	1.0000	0.710	0.4127
$X_1 \times X_4$	1.5625	1	1.5625	1.109	0.3089
$X_2 \times X_3$	28.6225	1	28.6225	20.321	0.0004
$X_2 \times X_4$	3.2400	1	3.2400	2.300	0.1501
$X_3 \times X_4$	2.7225	1	2.7225	1.933	0.1847
Error	21.1282	15	1.4085		
Lack-of-Fit	17.4949	10	1.7495	2.408	0.1721
Pure Error	3.6333	5	0.7267		
Total	308.5947	29			
R^2	0.9315				
R^2_{adj}	0.8676				
R^2_{pred}	0.6363				

b) Reduced Model

Variables	Sum of squares	DF	Mean Square	*F* value	*p*-value
Model	271.3330	7	38.7619	22.886	< 0.0001
X_1	1.9339	1	1.9339	1.142	0.2969
X_2	122.7222	1	122.7222	72.458	< 0.0001
X_3	29.6450	1	29.6450	17.503	0.0004
X_4	22.6689	1	22.6689	13.384	0.0014
X_2^2	18.8180	1	18.8180	11.111	0.0030
$X_1 \times X_2$	46.9225	1	46.9225	27.704	< 0.0001
$X_2 \times X_3$	28.6225	1	28.6225	16.899	0.0005
Error	37.2617	22	1.6937		
Lack-of-Fit	33.6283	17	1.9781	2.7222	0.1361
Pure Error	3.6333	5	0.7267		
Total	308.5947	29			
R^2	0.8793				
R^2_{adj}	0.8408				
R^2_{pred}	0.7542				

Table 4. ANOVA table for the CCD model related to to $K_{mix,\ 405\ nm}$ (Y_4, $mL^{-1}\ mg^{-1}\ cm^{-1}$)**.**

a) Full Model

Variables	Sum of squares	DF	Mean Square	*F* value	*p*-value
Model	0.0816	14	0.0058	17.439	< 0.0001
X_1	0.0078	1	0.0078	23.437	0.0002
X_2	0.0260	1	0.0260	77.766	< 0.0001
X_3	0.0283	1	0.0283	84.524	< 0.0001
X_4	0.0010	1	0.0010	3.083	0.0995
X_1^2	0.0004	1	0.0004	1.225	0.2859
X_2^2	< 0.0001	1	< 0.0001	0.019	0.8911
X_3^2	0.0009	1	0.0009	2.691	0.1217
X_4^2	0.0002	1	0.0002	0.668	0.4266
$X_1 \times X_2$	0.0014	1	0.0014	4.230	0.0575
$X_1 \times X_3$	0.0049	1	0.0049	14.639	0.0017
$X_1 \times X_4$	< 0.0001	1	< 0.0001	0.099	0.7575
$X_2 \times X_3$	0.0054	1	0.0054	16.141	0.0011
$X_2 \times X_4$	0.0018	1	0.0018	5.519	0.0329
$X_3 \times X_4$	0.0022	1	0.0022	6.694	0.0206
Error	0.0050	15	0.0003		
Lack-of-Fit	0.0041	10	0.0004	2.210	0.1973
Pure Error	0.0009	5			
Total	0.0866	29			
R^2	0.9421				
R^2_{adj}	0.8881				
R^2_{pred}	0.6684				

b) Reduced Model

Variables	Sum of squares	DF	Mean Square	*F* value	*p*-value
Model	0.0775	8	0.0097	22.257	< 0.0001
X_1	0.0078	1	0.0078	18.003	0.0004
X_2	0.0260	1	0.0260	59.735	< 0.0001
X_3	0.0283	1	0.0283	64.925	< 0.0001
X_4	0.0010	1	0.0010	2.368	0.1387
$X_1 \times X_3$	0.0049	1	0.0049	11.245	0.0030
$X_2 \times X_3$	0.0054	1	0.0054	12.399	0.0020
$X_2 \times X_4$	0.0018	1	0.0018	4.239	0.0521
$X_3 \times X_4$	0.0022	1	0.0022	5.142	0.0340
Error	0.0091	21	0.0004		
Lack-of-Fit	0.0082	16	0.0005	2.775	0.1319
Pure Error	0.0009	5	0.0002		
Total	0.0866	29			
R^2	0.8945				
R^2_{adj}	0.8543				
R^2_{pred}	0.7182				

A.III.2. ANNEX FROM III.2

Table 1. Resume of sugars analysis of coffee residues after MAE according to Box-Behnken design with three levels and three independent factors, and extracts $K_{mix,\ 280nm}$ and $K_{mix,\ 325nm}$.

Run Order	Process Variables[a]			Residues					Extracts	
	Time	Temperature	w/V ratio	Sugars[b]	Ara	Man	Gal	Glc	$K_{mix,\ 280nm}$	$K_{mix,\ 325nm}$
	(min)	(°C)	(g/ 60 mL)	(%w/w)		%mol			mL mg^{-1} cm^{-1}	mL mg^{-1} cm^{-1}
1-a	10.0 (+1)	180 (+1)	4 (0)	59.9	0.9	61.2	5.2	32.7	6.4275	4.4215
1-b	10.0 (+1)	180 (+1)	4 (0)	61.3	1.0	61.8	4.7	32.5	6.3620	4.1058
2-a	5.5 (0)	180 (+1)	6 (+1)	54.3	2.1	54.6	11.9	31.5	5.5180	3.8227
2-b	5.5 (0)	180 (+1)	6 (+1)	56.6	2.8	52.8	16.6	27.8	6.2852	4.3750
3-a	5.5 (0)	120 (-1)	2 (-1)	78.5	5.7	44.1	28.3	21.9	8.1951	6.0809
3-b	5.5 (0)	120 (-1)	2 (-1)	70.9	6.4	44.8	27.8	21.0	8.7967	6.5179
4-a	1.0 (-1)	150 (0)	2 (-1)	64.9	5.7	44.1	27.5	22.7	7.7634	5.8398
4-b	1.0 (-1)	150 (0)	2 (-1)	68.1	5.8	44.3	27.6	22.3	8.1264	6.1132
5-a	1.0 (-1)	180 (+1)	4 (0)	64.9	4.1	46.1	23.9	26.0	6.8754	5.0720
5-b	1.0 (-1)	180 (+1)	4 (0)	64.1	3.2	47.1	22.2	27.5	6.5574	4.8730
6-a	5.5 (0)	150 (0)	4 (0)	68.0	5.2	44.5	27.4	22.9	7.7047	5.6733
6-b	5.5 (0)	150 (0)	4 (0)	66.9	5.3	45.2	26.8	22.7	8.3984	6.0884
7-a	10.0 (+1)	120 (-1)	4 (0)	55.0	6.0	43.9	27.8	22.4	8.4215	6.2493
7-b	10.0 (+1)	120 (-1)	4 (0)	62.6	5.5	45.2	27.7	21.6	8.1598	6.0461
8-a	5.5 (0)	150 (0)	4 (0)	67.5	5.4	44.8	27.4	22.4	8.0105	5.8285
8-b	5.5 (0)	150 (0)	4 (0)	85.3	4.6	46.0	26.2	23.2	8.1676	5.9621
9-a	10.0 (+1)	150 (0)	2 (-1)	73.7	5.5	43.9	28.5	22.1	8.0265	5.9223
9-b	10.0 (+1)	150 (0)	2 (-1)	72.3	4.8	45.2	26.4	23.6	7.1357	5.3260
10-a	5.5 (0)	120 (-1)	6 (+1)	77.6	5.6	43.1	28.9	22.4	9.1585	6.9335
10-b	5.5 (0)	120 (-1)	6 (+1)	87.1	5.5	44.3	28.7	21.5	8.9588	6.6617
11-a	10.0 (+1)	150 (0)	6 (+1)	52.8	3.0	52.7	18.7	25.6	6.6977	4.6664
11-b	10.0 (+1)	150 (0)	6 (+1)	59.7	5.2	45.7	26.5	22.6	7.8498	5.7556
12-a	5.5 (0)	150 (0)	4 (0)	61.7	5.1	44.6	26.7	23.6	7.7154	5.6517
12-b	5.5 (0)	150 (0)	4 (0)	57.6	5.3	43.5	28.0	23.1	7.9814	5.8678
13-a	1.0 (-1)	120 (-1)	4 (0)	59.3	5.9	45.5	27.3	21.4	8.3359	6.1547
13-b	1.0 (-1)	120 (-1)	4 (0)	62.4	5.9	44.6	27.7	21.8	8.2901	6.1385
14-a	1.0 (-1)	150 (0)	6 (+1)	58.2	4.7	45.2	25.2	24.8	7.1799	5.2171
14-b	1.0 (-1)	150 (0)	6 (+1)	60.4	5.4	45.7	26.8	22.1	8.0548	5.8683
15-a	5.5 (0)	180 (+1)	2 (-1)	56.5	0.9	60.8	5.4	32.9	6.2397	4.4350
15-b	5.5 (0)	180 (+1)	2 (-1)	58.5	1.5	58.9	8.8	30.9	6.0982	4.3230

[a] : The process variables are shown in real and (coded) values; [b] : mass of compound present in the residue left after extraction.

Table 2. ANOVA table for the BBD model related to *Extraction Yield* (Y_1, %w/w).

a) Full Model

Variables	Sum of Squares	DF	Mean Square	*F* value	*p*-value
Model	1129.9409	9	125.5490	24.118	< 0.0001
X_1	26.7806	1	26.7806	5.144	0.0345
X_2	962.5506	1	962.5506	184.904	< 0.0001
X_3	21.1600	1	21.1600	4.065	0.0574
X_1^2	0.0288	1	0.0288	0.006	0.9414
X_2^2	93.7212	1	93.7212	18.004	0.0004
X_3^2	0.0738	1	0.0738	0.014	0.9064
$X_1 \times X_2$	25.2050	1	25.2050	4.842	0.0397
$X_1 \times X_3$	0.0013	1	0.0013	0.000	0.9878
$X_2 \times X_3$	0.0013	1	0.0013	0.000	0.9878
Error	104.1138	20	5.2057		
Lack-of-Fit	34.7038	3	11.5679	2.833	0.0693
Pure Error	69.4100	17	4.0829		
Total	1234.0547	29			
R^2	0.9156				
R^2_{adj}	0.8777				
R^2_{pred}	0.7985				

b) Reduced Model

Variables	Sum of Squares	DF	Mean Square	*F* value	*p*-value
Model	1129.8423	5	225.9685	52.040	< 0.0001
X_1	26.7806	1	26.7806	6.168	0.0204
X_2	962.5506	1	962.5506	221.674	< 0.0001
X_3	21.1600	1	21.1600	4.873	0.0371
X_2^2	94.1460	1	94.1460	21.682	0.0001
$X_1 \times X_2$	25.2050	1	25.2050	5.805	0.0240
Error	104.2124	24	4.3422		
Lack-of-Fit	34.8024	7	4.9718	1.218	0.3459
Pure Error	69.4100	17	4.0829		
Total	1234.0547	29			
R^2	0.9156				
R^2_{adj}	0.8980				
R^2_{pred}	0.8692				

Table 3. ANOVA table for the BBD model related to *Sugars Content* (Y_2, %w/w).

a) Full Model

Variables	Sum of Squares	DF	Mean Square	*F* value	*p*-value
Model	1487.2604	9	165.2512	25.306	< 0.0001
X_1	29.1600	1	29.1600	4.466	0.0474
X_2	1279.8506	1	1279.8506	195.994	< 0.0001
X_3	12.7806	1	12.7806	1.957	0.1771
X_1^2	8.6334	1	8.6334	1.322	0.2638
X_2^2	81.3349	1	81.3349	12.455	0.0021
X_3^2	27.9003	1	27.9003	4.273	0.0519
$X_1 \times X_2$	19.2200	1	19.2200	2.943	0.1017
$X_1 \times X_3$	19.2200	1	19.2200	2.943	0.1017
$X_2 \times X_3$	20.8013	1	20.8013	3.185	0.0895
Error	130.6013	20	6.5301		
Lack-of-Fit	38.6563	3	12.8854	2.3824	0.1053
Pure Error	91.9450	17	5.4085		
Total	1617.8617	29			
R^2	0.9193				
R^2_{adj}	0.8830				
R^2_{pred}	0.8097				

b) Reduced Model

Variables	Sum of Squares	DF	Mean Square	*F* value	*p*-value
Model	1440.1871	6	240.0312	31.072	< 0.0001
X_1	29.1600	1	29.1600	3.775	0.0644
X_2	1279.8506	1	1279.8506	165.677	< 0.0001
X_3	12.7806	1	12.7806	1.654	0.2112
X_2^2	77.7694	1	77.7694	10.067	0.0042
X_3^2	25.7158	1	25.7158	3.329	0.0811
$X_2 \times X_3$	20.8013	1	20.8013	2.693	0.1144
Error	177.675	23	7.7250		
Lack-of-Fit	85.7296	6	14.2883	2.642	0.0537
Pure Error	91.9450	17	5.4085		
Total	1617.862				
R^2	0.8902				
R^2_{adj}	0.8615				
R^2_{pred}	0.8095				

Table 4. ANOVA table for the Box-Behnken model related to *GM* (Y_3, %w/w).

Variables	Sum of Squares	DF	Mean Square	*F* value	*p*-value
Model	167.1141	9	18.5682	10.873	< 0.0001
X_1	20.0928	1	20.0928	11.766	0.0027
X_2	11.0889	1	11.0889	6.493	0.0192
X_3	51.8040	1	51.8040	30.335	< 0.0001
X_1^2	4.8500	1	4.8500	2.840	0.1075
X_2^2	19.4001	1	19.4001	11.360	0.0030
X_3^2	1.0270	1	1.0270	0.601	0.4471
$X_1 \times X_2$	4.1616	1	4.1616	2.437	0.1342
$X_1 \times X_3$	0.0002	1	0.0002	0.000	0.9915
$X_2 \times X_3$	52.6851	1	52.6851	30.851	< 0.0001
Error	34.1548	20	1.7077		
Lack-of-Fit	2.1665	3	0.7222	0.384	0.7660
Pure Error	31.9882	17	1.8817		
Total	201.2689	29			
R^2	0.8303				
R^2_{adj}	0.7539				
R^2_{pred}	0.6515				

Table 5. ANOVA table for the BBD model related to *AG* (Y_4, %w/w).

a) Full Model

Variables	Sum of Squares	DF	Mean Square	*F* value	*p*-value
Model	1486.4976	9	165.1664	28.419	< 0.0001
X_1	101.9595	1	101.9595	17.543	0.0005
X_2	1097.7626	1	1097.7626	188.883	< 0.0001
X_3	14.2506	1	14.2506	2.452	0.1331
X_1^2	0.6157	1	0.6157	0.106	0.7482
X_2^2	188.9793	1	188.9793	32.516	< 0.0001
X_3^2	18.7278	1	18.7278	3.222	0.0878
$X_1 \times X_2$	44.5568	1	44.5568	7.667	0.0118
$X_1 \times X_3$	19.7506	1	19.7506	3.398	0.0801
$X_2 \times X_3$	8.2215	1	8.2215	1.415	0.2482
Error	116.2375	20	5.8119		
Lack-of-Fit	47.3725	3	15.7908	3.898	0.0274
Pure Error	68.8649	17	4.0509		
Total	1602.7349	29			
R^2	0.9275				
R^2_{adj}	0.8948				
R^2_{pred}	0.8207				

b) Reduced Model

Variables	Sum of Squares	DF	Mean Square	*F* value	*p*-value
Model	1477.6602	7	211.0943	37.130	< 0.0001
X_1	101.9595	1	101.9595	17.934	0.0003
X_2	1097.7626	1	1097.7626	193.091	< 0.0001
X_3	14.2506	1	14.2506	2.507	0.1276
X_2^2	188.4385	1	188.4385	33.145	< 0.0001
X_3^2	18.3174	1	18.3174	3.222	0.0864
$X_1 \times X_2$	44.5568	1	44.5568	7.837	0.0104
$X_1 \times X_3$	19.7506	1	19.7506	3.474	0.0757
Error	125.0747	22	5.6852		
Lack-of-Fit	56.2097	5	11.2419	2.775	0.0520
Pure Error	68.8649	17	4.0509		
Total	1602.7349	29			
R^2	0.9220				
R^2_{adj}	0.8971				
R^2_{pred}	0.8391				

Table 6. ANOVA table for the BBD model related to Ara (% w/w).

a) Full Model

Variables	Sum of Squares	DF	Mean Square	*F* value	*p*-value
Model	12.9574	9	1.4397	11.416	<0.0001
X_1	0.5587	1	0.5587	4.430	0.0481
X_2	10.8298	1	10.8298	85.875	<0.0001
X_3	0.2317	1	0.2317	1.837	0.1904
X_1^2	0.0014	1	0.0014	0.011	0.9161
X_2^2	0.6142	1	0.6142	4.870	0.0392
X_3^2	0.2462	1	0.2462	1.952	0.1776
$X_1 \times X_2$	0.0006	1	0.0006	0.005	0.9437
$X_1 \times X_3$	0.3170	1	0.3170	2.513	0.1286
$X_2 \times X_3$	0.2113	1	0.2113	1.676	0.2103
Error	2.5222	20	0.1261		
Lack-of-Fit	1.0804	3	0.3601	4.246	0.0207
Pure Error	1.4418	17	0.0848		
Total	15.4796	29			
R^2	0.8371				
R^2_{adj}	0.7637				
R^2_{pred}	0.6150				

b) Reduced Model

Variables	Sum of Squares	DF	Mean Square	*F* value	*p*-value
Model	12.4992	5	2.4998	20.130	<0.0001
X_1	0.5587	1	0.5587	4.499	0.0444
X_2	10.8298	1	10.8298	87.208	<0.0001
X_3	0.2317	1	0.2317	1.866	0.1846
X_2^2	0.5620	1	0.5620	4.526	0.0439
$X_1 \times X_3$	0.3170	1	0.3170	2.552	0.1232
Error	2.9804	24	0.1242		
Lack-of-Fit	1.5386	7	0.2198	2.592	0.0515
Pure Error	1.4418	17	0.0848		
Total	15.4796	29			
R^2	0.8075				
R^2_{adj}	0.7674				
R^2_{pred}	0.6671				

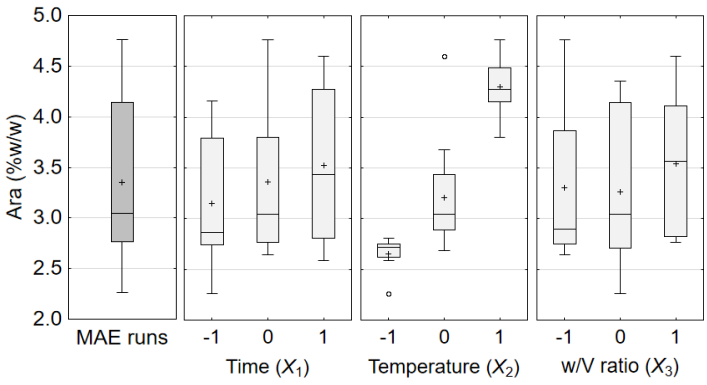

Figure 1. Box plot showing the effects of time (X_1), temperature (X_2), and w/V ratio (X_3) during the MAE experiments on the arabinose content in the extract (%w/w). In the box plot is represented the distribution of the raw data, containing the box the interquartile range and limiting the whisker the non-outlier range, with the median represented as (—) and the mean as ($^+$), while outliers (coeff. 1.5) are represented as (^{o}) and extremes (coeff. 3) as (*).

Table 7. ANOVA table for the BBD model related to Gal (% w/w).

a) Full Model

Variables	Sum of Squares	DF	Mean Square	*F* value	*p*-value
Model	1226.1829	9	136.2425	30.346	<0.0001
X_1	83.5014	1	83.5014	18.599	0.0003
X_2	899.8745	1	899.8745	200.436	< 0.0001
X_3	8.6822	1	8.6822	1.934	0.1796
X_1^2	0.7294	1	0.7294	0.162	0.6912
X_2^2	162.7051	1	162.7051	36.241	<0.0001
X_3^2	15.0149	1	15.0149	3.344	0.0824
$X_1 \times X_2$	43.5671	1	43.5671	9.704	0.0055
$X_1 \times X_3$	15.0728	1	15.0728	3.357	0.0818
$X_2 \times X_3$	4.2436	1	4.2436	0.945	0.3426
Error	89.7915	20	4.4896		
Lack-of-Fit	37.6870	3	12.5623	4.0987	0.0233
Pure Error	52.1046	17	3.0650		
Total	1315.9744	29			
R^2	0.9318				
R^2_{adj}	0.9011				
R^2_{pred}	0.8301				

b) Reduced Model

Variables	Sum of Squares	DF	Mean Square	*F* value	*p*-value
Model	1221.2099	7	174.4586	40.501	<0.0001
X_1	83.5014	1	83.5014	19.385	0.0002
X_2	899.8745	1	899.8745	208.910	<0.0001
X_3	8.6822	1	8.6822	2.016	0.1697
X_2^2	161.9921	1	161.9921	37.607	<0.0001
X_3^2	14.5965	1	14.5965	3.389	0.0792
$X_1 \times X_2$	43.5671	1	43.5671	10.114	0.0043
$X_1 \times X_3$	15.0728	1	15.0728	3.499	0.0748
Error	94.7645	22	4.3075		
Lack-of-Fit	42.6600	5	8.5320	2.784	0.0515
Pure Error	52.1046	17	3.0650		
Total	1315.9744	29			
R^2	0.9280				
R^2_{adj}	0.9051				
R^2_{pred}	0.8492				

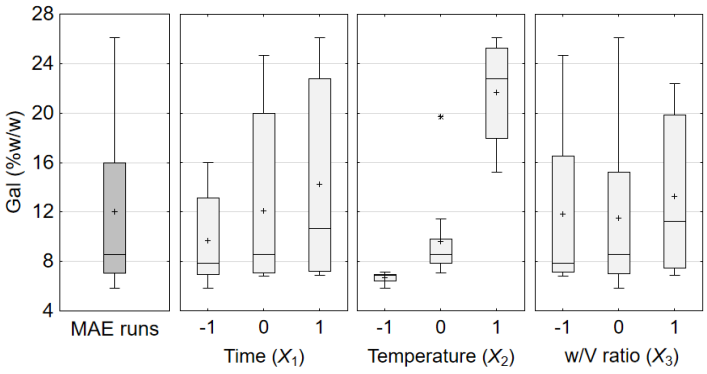

Figure 2. Box plot showing the effects of time (X_1), temperature (X_2), and w/V ratio (X_3) during the MAE experiments on the galactose content in the extract (%w/w). In the box plot is represented the distribution of the raw data, containing the box the interquartile range and limiting the whisker the non-outlier range, with the median represented as (—) and the mean as ($^+$), while outliers (coeff. 1.5) are represented as (^{o}) and extremes (coeff. 3) as (*).

Table 8 ANOVA table for the BBD model related to Man (% w/w).

a) Full Model

Variables	Sum of Squares	DF	Mean Square	*F* value	*p*-value
Model	151.4421	9	16.8269	10.863	<0.0001
X_1	18.1904	1	18.1904	11.744	0.0027
X_2	10.0442	1	10.0442	6.485	0.0192
X_3	46.9542	1	46.9542	30.314	< 0.0001
X_1^2	4.4092	1	4.4092	2.847	0.1071
X_2^2	17.5742	1	17.5742	11.346	0.0031
X_3^2	0.9297	1	0.9297	0.600	0.4476
$X_1 \times X_2$	3.7645	1	3.7645	2.430	0.1347
$X_1 \times X_3$	0.0002	1	0.0002	< 0.001	0.9919
$X_2 \times X_3$	47.7580	1	47.7580	30.832	< 0.0001
Error	30.9790	20	1.5490		
Lack-of-Fit	1.9703	3	0.6568	0.385	0.7653
Pure Error	29.0088	17	1.7064		
Total	182.4211	29			
R^2	0.8302				
R^2_{adj}	0.7538				
R^2_{pred}	0.6512				

b) Reduced Model

Variables	Sum of Squares	DF	Mean Square	*F* value	*p*-value
Model	146.7477	6	24.4579	15.769	<0.0001
X_1	18.1904	1	18.1904	11.728	0.0023
X_2	10.0442	1	10.0442	6.476	0.0181
X_3	46.9542	1	46.9542	30.273	<0.0001
X_1^2	4.1276	1	4.1276	2.661	0.1164
X_2^2	18.3099	1	18.3099	11.805	0.0023
$X_2 \times X_3$	47.7580	1	47.7580	30.791	<0.0001
Error	35.6735	23	1.5510		
Lack-of-Fit	6.6647	6	1.1108	0.651	0.6892
Pure Error	29.0088	17	1.7064		
Total	182.4211	29			
R^2	0.8044				
R^2_{adj}	0.7534				
R^2_{pred}	0.6874				

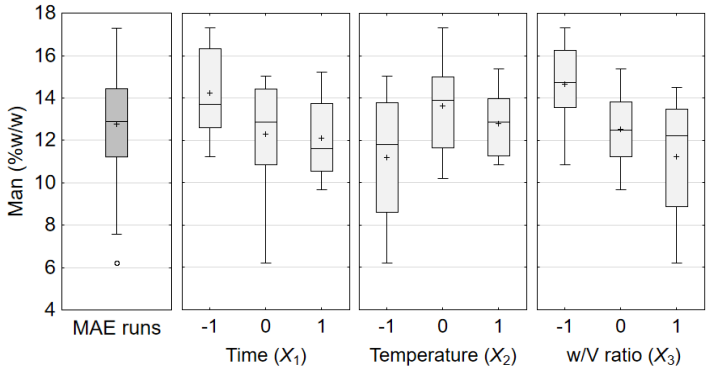

Figure 3. Box plot showing the effects of time (X_1), temperature (X_2), and w/V ratio (X_3) during the MAE experiments on the mannose content in the extract (%w/w). In the box plot is represented the distribution of the raw data, containing the box the interquartile range and limiting the whisker the non-outlier range, with the median represented as (—) and the mean as ($^+$), while outliers (coeff. 1.5) are represented as (^{o}) and extremes (coeff. 3) as (*).

Table 9. ANOVA table for the BBD model related to Caffeine (% w/w).

a) Full Model

Variables	Sum of Squares	DF	Mean Square	*F* value	*p*-value
Model	24.6943	9	2.7438	13.241	<0.0001
X_1	1.8544	1	1.8544	8.949	0.0072
X_2	19.5002	1	19.5002	94.103	<0.0001
X_3	1.4039	1	1.4039	6.775	0.0170
X_1^2	0.0026	1	0.0026	0.013	0.9121
X_2^2	1.3407	1	1.3407	6.470	0.0193
X_3^2	0.2298	1	0.2298	1.109	0.3048
$X_1 \times X_2$	0.2542	1	0.2542	1.227	0.2812
$X_1 \times X_3$	0.1492	1	0.1492	0.720	0.4062
$X_2 \times X_3$	0.0101	1	0.0101	0.049	0.8276
Error	4.1444	20	0.2072		
Lack-of-Fit	1.6059	3	0.5353	3.585	0.0357
Pure Error	2.5385	17	0.1493		
Total	28.8388	29			
R^2	0.8563				
R^2_{adj}	0.7916				
R^2_{pred}	0.6516				

b) Reduced Model

Variables	Sum of Squares	DF	Mean Square	*F* value	*p*-value
Model	24.0433	4	6.0108	31.336	<0.0001
X_1	1.8544	1	1.8544	9.667	0.0046
X_2	19.5002	1	19.5002	101.660	<0.001
X_3	1.4039	1	1.4039	7.319	0.0121
X_2^2	1.2849	1	1.2849	6.699	0.0158
Error	4.7954	25	0.1918		
Lack-of-Fit	2.2570	8	0.2821	1.889	0.1286
Pure Error	2.5385	17	0.1493		
Total	28.8388	29			
R^2	0.8337				
R^2_{adj}	0.8071				
R^2_{pred}	0.7538				

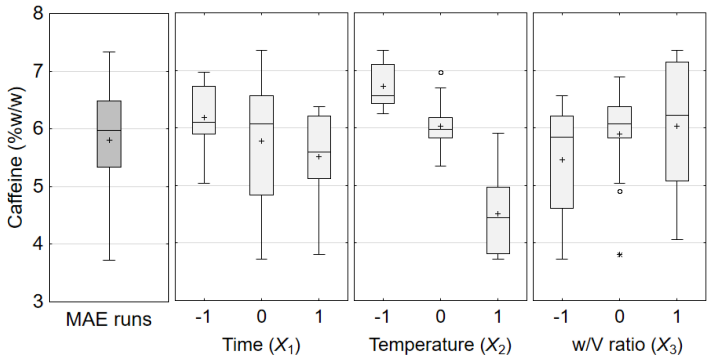

Figure 4. Box plot showing the effects of time (X_1), temperature (X_2), and w/V ratio (X_3) during the MAE experiments on the caffeine content in the extract (%w/w). In the box plot is represented the distribution of the raw data, containing the box the interquartile range and limiting the whisker the non-outlier range, with the median represented as (—) and the mean as ($^+$), while outliers (coeff. 1.5) are represented as (^{o}) and extremes (coeff. 3) as (*).

Table 10. ANOVA table for the BBD model related to 5-CQA (% w/w).

a) Full Model

Variables	Sum of Squares	DF	Mean Square	*F* value	*p*-value
Model	1.9207	9	0.2134	18.232	<0.0001
X_1	0.1017	1	0.1017	8.691	0.0080
X_2	1.6869	1	1.6869	144.111	<0.0001
X_3	0.0491	1	0.0491	4.194	0.0539
X_1^2	0.0207	1	0.0207	1.766	0.1989
X_2^2	0.0308	1	0.0308	2.629	0.1206
X_3^2	0.0240	1	0.0240	2.053	0.1674
$X_1 \times X_2$	0.0035	1	0.0035	0.302	0.5885
$X_1 \times X_3$	0.0001	1	0.0001	0.006	0.9414
$X_2 \times X_3$	0.0001	1	0.0001	0.012	0.9138
Error	0.2341	20	0.0117		
Lack-of-Fit	0.0522	3	0.0174	1.625	0.2208
Pure Error	0.1819	17	0.0107		
Total	2.1548	29			
R^2	0.8914				
R^2_{adj}	0.8425				
R^2_{pred}	0.7323				

b) Reduced Model

Variables	Sum of Squares	DF	Mean Square	*F* value	*p*-value
Model	1.8377	3	0.6126	50.227	<0.0001
X_1	0.1017	1	0.1017	8.342	0.0077
X_2	1.6869	1	1.6869	138.314	<0.0001
X_3	0.0491	1	0.0491	4.026	0.0553
Error	0.3171	26	0.0122		
Lack-of-Fit	0.1352	9	0.0150	1.403	0.2615
Pure Error	0.1819	17	0.0107		
Total	2.1548	29			
R^2	0.8528				
R^2_{adj}	0.8359				
R^2_{pred}	0.7967				

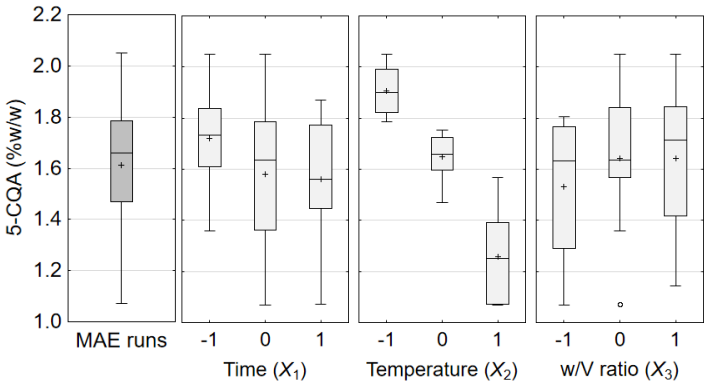

Figure 5. Box plot showing the effects of time (X_1), temperature (X_2), and w/V ratio (X_3) during the MAE experiments on the 5-CQA content in the extract (%w/w). In the box plot is represented the distribution of the raw data, containing the box the interquartile range and limiting the whisker the non-outlier range, with the median represented as (—) and the mean as ($^+$), while outliers (coeff. 1.5) are represented as (^{o}) and extremes (coeff. 3) as (*).

Table 11. ANOVA table for the BBD model related to $K_{mix,\ 405\ nm}$ (mL mg^{-1} cm^{-1}).

Variables	Sum of Squares	DF	Mean Square	*F* value	*p*-value
Model	0.1393	9	0.0155	8.454	< 0.0001
X_1	0.0156	1	0.0156	8.516	0.0085
X_2	0.0736	1	0.0736	40.226	< 0.0001
X_3	0.0308	1	0.0308	16.838	0.0006
X_1^2	0.0009	1	0.0009	0.475	0.4986
X_2^2	0.0094	1	0.0094	5.127	0.0348
X_3^2	0.0033	1	0.0033	1.802	0.1946
$X_1 \times X_2$	0.0018	1	0.0018	0.987	0.3324
$X_1 \times X_3$	0.0019	1	0.0019	1.064	0.3147
$X_2 \times X_3$	0.0008	1	0.0008	0.435	0.5169
Error	0.0366	20	0.0018		
Lack-of-Fit	0.0107	3	0.0036	2.350	0.1086
Pure Error	0.0259	17	0.0015		
Total	0.1759	29			
R^2	0.7918				
R^2_{adj}	0.6982				
R^2_{pred}	0.4947				

A.III.3. ANNEX FROM III.3

Table 1. Sugars content and composition obtained after acid hydrolysis of espresso samples (6.0 g of coffee powder, 40 ± 2 mL of distilled water).

Sample	Total Solids (g *per* 40 mL)	Total Carbohydrates (% w/w)	Sugar Composition					*Viscosity* (cSt)
			Rha	*Ara*	*Man* (%mol)	*Gal*	*Glc*	
EC1G1	1.22±0.15	15.4±1.7	3.1±0.2	13.0±0.5	50.2±2.2	28.8±1.0	4.8±0.8	1.09±0.02
EC1G3	1.35±0.04	16.7±1.4	3.7±0.5	15.0±0.6	45.8±1.7	30.7±1.2	4.7±1.0	1.06±0.01

Table 2. ANOVA table for the CCD model related to *Ara* (Y_2, %mol).

a) Full Model

Variables	Sum of Squares	DF	Mean Square	*F* value	*p*-value
Model	207.9228	14	14.8516	14.819	<0.0001
X_1	0.5583	1	0.5583	0.557	0.4670
X_2	82.5613	1	82.5613	82.379	<0.0001
X_3	23.0294	1	23.0294	22.979	0.0002
X_4	6.1367	1	6.1367	6.123	0.0258
X_1^2	2.0301	1	2.0301	2.026	0.1751
X_2^2	12.4851	1	12.4851	12.458	0.0030
X_3^2	0.9014	1	0.9014	0.899	0.3580
X_4^2	0.4253	1	0.4253	0.424	0.5246
$X_1 \times X_2$	25.2758	1	25.2758	25.220	0.0002
$X_1 \times X_3$	5.4173	1	5.4173	5.405	0.0345
$X_1 \times X_4$	0.6360	1	0.6360	0.635	0.4381
$X_2 \times X_3$	3.5250	1	3.5250	3.517	0.0803
$X_2 \times X_4$	1.3514	1	1.3514	1.348	0.2637
$X_3 \times X_4$	3.6005	1	3.6005	3.593	0.0775
Error	15.0331	15	1.0022		
Lack-of-Fit	7.5470	10	0.7547	0.504	0.8332
Pure Error	7.4861	5	1.4972		
Total	222.9559	29			
R^2	0.9326				
R^2_{adj}	0.8696				
R^2_{pred}	0.7682				

b) Reduced Model

Variables	Sum of Squares	DF	Mean Square	*F* value	*p*-value
Model	195.5098	7	27.9300	22.388	<0.0001
X_1	0.5583	1	0.5583	0.447	0.5105
X_2	82.5613	1	82.5613	66.179	<0.0001
X_3	23.0294	1	23.0294	18.460	0.0003
X_4	6.1367	1	6.1367	4.919	0.0372
X_2^2	52.5312	1	52.5312	42.107	<0.0001
$X_1 \times X_2$	25.2758	1	25.2758	20.260	0.0002
$X_1 \times X_3$	5.4173	1	5.4173	4.342	0.0490
Error	27.4461	22	1.2475		
Lack-of-Fit	19.9599	17	1.1741	0.784	0.6804
Pure Error	7.4861	5	1.4972		
Total	222.9559	29			
R^2	0.8769				
R^2_{adj}	0.8377				
R^2_{pred}	0.7560				

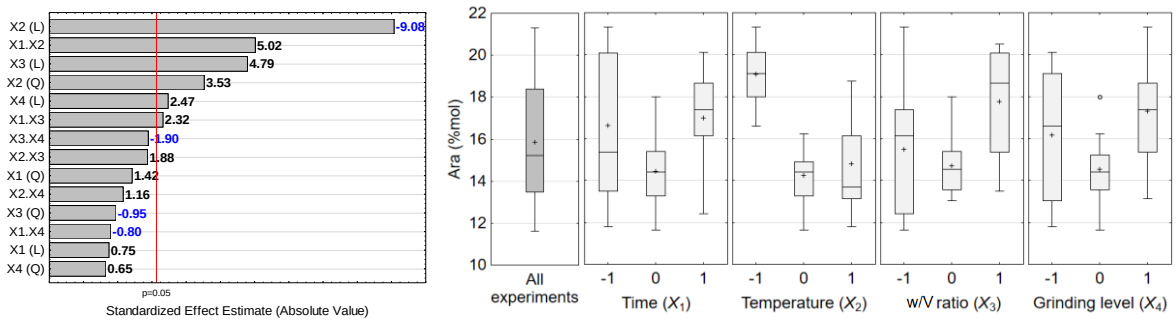

Figure 1. Box plot showing the effects of time (X_1), temperature (X_2), w/V ratio (X_3), and grinding level (X_4) during the infusion experiments on the arabinose content in the extract (%mol). In the box plot is represented the distribution of the raw data, containing the box the interquartile range and limiting the whisker the non-outlier range, with the median represented as (—) and the mean as (+), while outliers (coeff. 1.5) are represented as (º) and extremes (coeff. 3) as (*).

Table 3. ANOVA table for the CCD model related to *Man* (Y_3, %mol).

a) Full Model

Variables	Sum of Squares	DF	Mean Square	*F* value	*p*-value
Model	1484.279	14	106.0199	22.843	<0.0001
X_1	15.2536	1	15.2536	3.286	0.0899
X_2	485.4728	1	485.4728	104.598	<0.0001
X_3	195.7561	1	195.7561	42.177	<0.0001
X_4	29.9280	1	29.9280	6.448	0.0227
X_1^2	6.1516	1	6.1516	1.325	0.2676
X_2^2	102.6986	1	102.6986	22.127	0.0003
X_3^2	5.4784	1	5.4784	1.180	0.2944
X_4^2	0.0501	1	0.0501	0.011	0.9186
$X_1 \times X_2$	300.6756	1	300.6756	64.783	<0.0001
$X_1 \times X_3$	50.4810	1	50.4810	10.876	0.0049
$X_1 \times X_4$	0.1024	1	0.1024	0.022	0.8839
$X_2 \times X_3$	112.8906	1	112.8906	24.323	0.0002
$X_2 \times X_4$	0.5476	1	0.5476	0.118	0.7360
$X_3 \times X_4$	2.1756	1	2.1756	0.469	0.5040
Error	69.6195	15	4.6413		
Lack-of-Fit	48.0412	10	4.8041	1.113	0.4819
Pure Error	21.5783	5	4.3157		
Total	1553.8985	29			
R^2	0.9552				
R^2_{adj}	0.9134				
R^2_{pred}	0.7414				

b) Reduced Model

Variables	Sum of Squares	DF	Mean Square	*F* value	*p*-value
Model	1472.4332	8	184.0542	47.445	<0.0001
X_1	15.2536	1	15.2536	3.932	0.0606
X_2	485.4728	1	485.4728	125.144	<0.0001
X_3	195.7561	1	195.7561	50.462	<0.0001
X_4	29.9280	1	29.9280	7.715	0.0113
X_2^2	281.9755	1	281.9755	72.687	<0.0001
$X_1 \times X_2$	300.6756	1	300.6756	77.508	<0.0001
$X_1 \times X_3$	50.4810	1	50.4810	13.013	0.0017
$X_2 \times X_3$	112.8906	1	112.8906	29.101	<0.0001
Error	81.4653	21	3.8793		
Lack-of-Fit	59.8870	16	3.7429	0.867	0.6262
Pure Error	21.5783	5	4.3157		
Total	1553.8985	29			
R^2	0.9476				
R^2_{adj}	0.9276				
R^2_{pred}	0.8715				

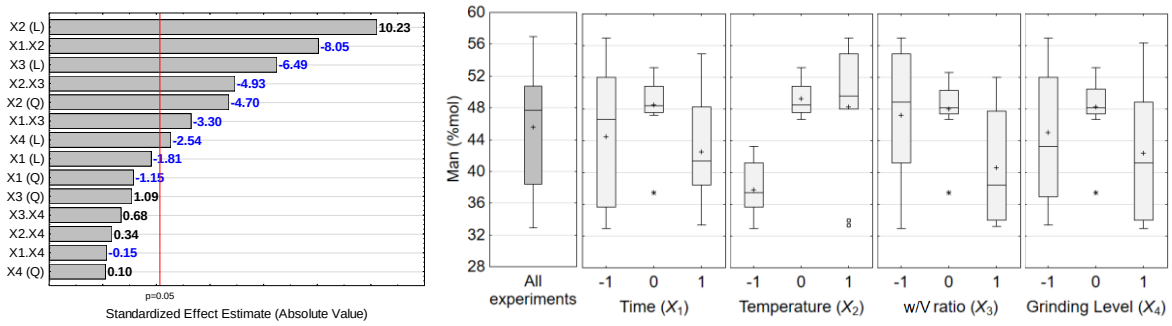

Figure 2. Box plot showing the effects of time (X_1), temperature (X_2), w/V ratio (X_3), and grinding level (X_4) during the infusion experiments on the mannose content in the extract (%mol). In the box plot is represented the distribution of the raw data, containing the box the interquartile range and limiting the whisker the non-outlier range, with the median represented as (—) and the mean as ($^+$), while outliers (coeff. 1.5) are represented as (^{o}) and extremes (coeff. 3) as (*).

Table 4. ANOVA table for the CCD model related to *Gal* (Y_4, %mol).

a) Full Model

Variables	Sum of Squares	DF	Mean Square	*F* value	*p*-value
Model	348.2268	14	24.8733	7.5710	0.0002
X_1	18.9728	1	18.9728	5.775	0.0296
X_2	48.7743	1	48.7743	14.846	0.0016
X_3	69.2664	1	69.2664	21.083	0.0004
X_4	1.1756	1	1.1756	0.358	0.5586
X_1^2	0.2021	1	0.2021	0.062	0.8075
X_2^2	19.3706	1	19.3706	5.896	0.0282
X_3^2	0.0080	1	0.0080	0.002	0.9612
X_4^2	2.3913	1	2.3913	0.728	0.4070
$X_1 \times X_2$	95.5506	1	95.5506	29.084	0.0001
$X_1 \times X_3$	16.4025	1	16.4025	4.993	0.0411
$X_1 \times X_4$	0.4290	1	0.4290	0.131	0.7229
$X_2 \times X_3$	57.7600	1	57.7600	17.581	0.0008
$X_2 \times X_4$	1.7030	1	1.7030	0.518	0.4826
$X_3 \times X_4$	0.4900	1	0.4900	0.149	0.7048
Error	49.2803	15	3.2854		
Lack-of-Fit	40.6878	10	4.0688	2.3676	0.1769
Pure Error	8.5925	5	1.7185		
Total	397.5071	29			
R^2	0.8760				
R^2_{adj}	0.7603				
R^2_{pred}	0.2572				

b) Reduced Model

Variables	Sum of Squares	DF	Mean Square	*F* value	*p*-value
Model	342.8118	8	42.8515	16.453	<0.0001
X_1	18.9728	1	18.9728	7.2845	0.0134
X_2	48.7743	1	48.7743	18.7266	0.0003
X_3	69.2664	1	69.2664	26.5945	<0.0001
X_4	1.1756	1	1.1756	0.4513	0.5090
X_2^2	34.9096	1	34.9096	13.4034	0.0015
$X_1 \times X_2$	95.5506	1	95.5506	36.6862	<0.0001
$X_1 \times X_3$	16.4025	1	16.4025	6.2977	0.0204
$X_2 \times X_3$	57.7600	1	57.7600	22.1767	0.0001
Error	54.6953	21	2.6045		
Lack-of-Fit	46.1028	16	2.8814	1.677	0.2967
Pure Error	8.5925	5	1.7185		
Total	397.5071	29			
R^2	0.8624				
R^2_{adj}	0.8100				
R^2_{pred}	0.6470				

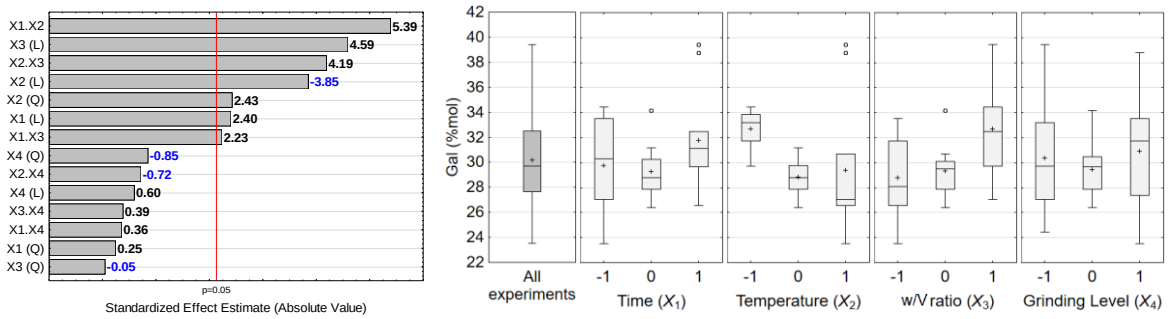

Figure 3. Box plot showing the effects of time (X_1), temperature (X_2), w/V ratio (X_3), and grinding level (X_4) during the infusion experiments on the galactose content in the extract (%mol). In the box plot is represented the distribution of the raw data, containing the box the interquartile range and limiting the whisker the non-outlier range, with the median represented as (–) and the mean as ($^+$), while outliers (coeff. 1.5) are represented as (^{o}) and extremes (coeff. 3) as (*).