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Carbohydrates as targeting compounds to produce infusions resembling espresso coffee brews using quality by design approach

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ABSTRACT

All coffee brews are prepared with roasted coffee and water, giving origin to espresso, instant, or filtered coffee, exhibiting distinct physicochemical properties, depending on the extraction conditions. The different relative content of compounds in the brews modulates coffee body, aroma, and colour. In this study it was hypothesized that a coffee infusion allows to obtain extracts that resemble espresso coffee (EC) physicochemical properties. Carbohydrates (content and composition) were the target compounds as they are organoleptically important for EC due to their association to foam stability and viscosity. The freeze-drying of the extracts allowed better dissolution properties than spray-drying. Instant coffee powders were obtained with chemical overall composition resembling espresso, although with lower lipids content. The extracts were able to produce the characteristic foam through CO_2 injection or salts addition. Their redissolution at espresso concentration allowed a viscosity, foamability and volatile profile representative of an espresso coffee, opening new exploitation possibilities.

1. Introduction

Espresso coffee (EC) is defined as a coffee brew of reduced volume and distinct sensorial properties such as body, aroma, taste, and colour, with a characteristic persistent foam that covers the liquid (Illy & Viani, 2005; Nunes, Coimbra, Duarte, & Delgadillo, 1997). 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 brew composition has been shown to depend on the preparation method, as EC, filter, instant, or moka (Angeloni et al., 2019; Caporaso, Genovese, Canela, Civitella, & Sacchi, 2014; Cordoba, Fernandez-Alduenda, Moreno, & Ruiz, 2020; Gloess et al., 2013). Nonetheless, for all methods of coffee preparation, coffee and water are the crucial starting materials, as all coffee brews are composed by hot water soluble carbohydrates, caffeine, chlorogenic acids, protein, lipids, and melanoidins. There is not a restricted composition range for each type of coffee brew. Even within the same extraction procedure, the range of values found for the number and concentration of compounds in a coffee brew has a wide variation. However, there are some distinctive features for certain coffee brews, as the lower amount of lipids in filtered brews (Gloess et al., 2013; Moeenfard, Silva, Borges, Santos, & Alves, 2015; Silva, Borges, Santos, & Alves, 2012; Speer & Kölling-Speer, 2006), or an overall higher carbohydrate content in instant coffee promoted by the severe extraction conditions used (Blanc, Davis, Parchet, & Viani, 1989; Capek, Paulovičová, Matulová, Mislovičová, Navarini, & Suggi-Liverani, 2014; Leloup, 2006; Lopes, Passos, Rodrigues, Teixeira, & Coimbra, 2020).

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 the properties of coffee brews. On the other hand, several variables as extraction time and temperature, weight/volume ratio or grinding degree affect coffee extraction processes, from espresso to infusion or filtered ones (Andueza, Paz de Peña, & Cid, 2003; Andueza, Vila, Paz de Peña, & Cid, 2007; Angeloni et al., 2019; Cordoba, Pataquiva, Osorio, Moreno, & Ruiz, 2019; Lopes, Passos, Rodrigues, Teixeira, & Coimbra, 2019; Ludwig et al., 2014). This opens the possibility of modulating the extraction conditions to obtain coffee brews with pre-desired

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characteristics, even when they are usually associated to other extraction processes. As a major coffee brew component, representing 12–24% of espresso coffee brew material (Lopes et al., 2016; Nunes et al., 1997) and with crucial impact on espresso properties as viscosity and foam stability, carbohydrates should be chosen as target compounds for developing extracts with EC characteristics.

Carbohydrates are the major group of compounds in green and roasted powder, as well as in coffee brews, having a considerable impact on brew properties. Galactomannans (GM) and arabinogalactans (AG) are the main carbohydrates in coffee brews (Moreira, Nunes, Domingues, & Coimbra, 2015). GM, a linear polysaccharide composed mainly by mannose residues branched with single residues of galactose, are related to the viscosity verified in coffee brews, and the amount of carbohydrates is associated to EC foam stability (Nunes et al., 1997), evidencing their importance in EC. In instant coffee, AG assume a preponderant abundance due to the extreme extraction conditions applied, which consequently lead to a relative decrease in the content of other compounds, such as caffeine and chlorogenic acids (Blanc et al., 1989; Leloup, 2006; Lopes et al., 2020; Villalón-López, Serrano-Contreras, Téllez-Medina, & Gerardo Zepeda, 2018).

In this study, it was hypothesized that the modulation of an infusion process having as target the carbohydrate content and composition of an EC allows to obtain extracts whose composition resemble EC. To verify the hypothesis, several steps were set: (a) establishment of the experimental guidelines to be replicated through a quality by design approach of the infusion process with the definition of a relative composition of coffee compounds in an EC cup; (b) preparation of coffee infusions resembling EC according to the optimized conditions of extraction; (c) comprehensive comparison of EC and infusion extracts composition testing the influence of freeze- and spray-drying processing; (d) evaluation of the capacity of the coffee extracts for producing foam, the most distinguishable EC property, through CO_2 injection and the addition of compounds able to release CO_2 when dissolved in water; (e) analysis of the volatile profile of the brews prepared with the coffee extracts; and (f) holistic comparison of extracts with other EC samples and commercial instant coffee samples, including one labelled as “espresso”, to check their resemblance with the infusion samples prepared.

2. Material and methods

2.1. Chemicals and materials

For sugars analysis were used 1-methylimidazole ($C_4H_6N_2$, ≥99%, Sigma-Aldrich), 2-deoxy-D-glucose ($C_6H_{12}O_5$, ≥99%, Sigma-Aldrich), ammonium hydroxide solution (NH_4OH, 25%, Sigma-Aldrich), acetic anhydride ($C_4H_6O_3$, ≥99%, Carlo Erba Reagents), acetic acid glacial ($C_2H_4O_2$, ≥99%, Carlo Erba Reagents), dichloromethane (CH_2Cl_2, 99.8%, Fischer Scientific), dimethyl sulfoxide ($(CH_3)_2SO$, 99.7%, Fischer Scientific), hydrochloric acid (HCl, 37%, Sigma-Aldrich), iodomethane (CH_3I, ≥99%, Sigma-Aldrich), sodium borodeuteride ($NaBD_4$, >90%, Sigma-Aldrich), sodium borohydride ($NaBH_4$, >95%, Fischer Scientific), sodium hydroxide (NaOH, 98%, José Manuel Gomes dos Santos), sulfuric acid (H_2SO_4, 98%, Biochem Chemopharma) and trifluoroacetic acid ($C_2HF_3O_2$, 99%, Alfa Aesar). For lipids analysis was used *n*-hexane (C_6H_{14}, 95%, Fischer Scientific). For caffeine/5-CQA determinations Milli-Q water, formic acid (Honeywell) and methanol (Fischer Scientific) were HPLC- grade reagents and as standards were used 5-CQA ($C_{16}H_{18}O_9$, ≥95%, Sigma-Aldrich) and caffeine ($C_8H_{10}N_4O_2$, ≥99%, Sigma-Aldrich). For foam properties experiments were used citric acid ($C_6H_8O_7$, 99.5%, Honeywell Fluka) and sodium bicarbonate ($NaHCO_3$, ≥99.7%, Sigma-Aldrich).

2.2. Coffee samples

A commercial blend of roasted coffee Delta® *Lote Chávena* was used to perform the coffee infusion extraction experiments, and a coffee grinder (Flama, 1231 FL) was used to grind the roasted coffee beans, as described in Lopes et al. (2019). The particle profile is shown as Supplementary Material (Fig. S1). The same roasted coffee was used to prepare the espresso coffee (6.0 g, 40 ± 2 mL) that after freeze-drying was used as reference (EC1). Distilled water and a home brewing device (Flama, Sigma 10 − 1226FL) were used. Further commercial single-dose coffee capsules (6.0 g) were prepared on a Delta Q® QOSMO machine. Different blends were used: EC2 (labelled intensity 5), EC3 (labelled intensity 10), and two equal coffee blends with different roasting degrees: EC4 (light) and EC5 (dark). After extraction, EC samples were frozen, freeze-dried, and stored until characterisation. A 100% instant coffee sample (IC1) was also analysed, as well as a commercial instant coffee powder, referred as “espresso” in the label (IC2). The significant differences were assessed by analysis of variance (ANOVA) through Tukey’s range test ($\alpha = 0.05$) using Minitab and GraphPad Prism 5.00.

2.3. Infusion preparations

The infusion preparations were performed in 100 mL Erlenmeyer flasks as described in Lopes et al. (2019) with freshly grounded coffee (grinding level 1–3) and distilled water (30 mL). The experiments were settled according to a central composite design (CCD) with four factors and three levels (time (X_1) − 10, 185, and 360 min, temperature (X_2) − 20, 50, and 80 °C, w/v ratio (X_3) − 0.03, 0.12, and 0.20 g mL^{-1}, and grinding level (X_4) - level 1, 2, and 3, Table S1). The data obtained were fitted to second-order polynomial models described by Eq. (1):

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

where Y represents the response observed for the dependent variable of interest, and β_0, β_i, β_{ii}, and β_{ij} represent the constant, linear, quadratic, and two-factor interaction regression coefficients, respectively, while x_i represents the factors studied in a dimensionless coded form. The extraction yields (%, w/w_{powder}) of the different carbohydrate residues and the composition of the coffee extracts (mol%) were studied as the responses. Experimental data were analysed with Statistica v12 and Minitab v17, with analysis of variance (ANOVA) at 95% significance level (*p*-value).

The condition that better resembled EC composition was performed at a larger scale (1.5 L) in the conditions established (10 min, 50 °C, 0.12 g mL^{-1}, grinding level 3), using the same coffee product (3 independent extractions). The infusion was filtrated and frozen. Then, half of the filtrate was freeze-dried (FD) and the remaining filtrate processed by spray-drying (SD), using, in both cases, a low solids content solution (0.03 g mL^{-1}). The spray-drying process conditions were settled as follows: inlet temperature (150 °C), outlet temperature (80 °C), spray-gas flow (6 mL min^{-1}), pump (20%), and aspirator (95%).

2.4. Lipids

A Soxhlet methodology with glass fibre cartridges (4 h, *n*-hexane, 80 °C) was used to extract the total lipids ($n = 3$) from 1 g of coffee extracts (EC1, IC1, FD, SD) and initial roasted coffee. The hexane extract was rotary evaporated (<40 °C) to dryness. A clean-up step was performed for elimination of co-extracted compounds (*e.g.* caffeine) with liquid–liquid extractions (5 mL) with hexane/water (1:1) with the amount of lipids quantified by weight after hexane fraction evaporation under a gentle nitrogen stream.

2.5. Fractionation of coffee extracts

Defatted coffee samples (EC1, IC1, FD, 3 replicates each) were dissolved in distilled water and dialysed (MW cut off 12–14 kDa, Visking size 8, Medicell International Ltd., London, UK) against distilled water

(4 °C) with constant stirring (Lopes et al., 2016). After dialysis, the volume inside the dialysis bag volume was adjusted to 30 mL with distilled water and a fraction (1 mL) was frozen and freeze-dried, for the estimation of 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 ($HMWM_{sol}$) and insoluble ($HMWM_{ins}$) in cold water, respectively.

2.6. Characterisation of coffee extracts

2.6.1. Carbohydrate analysis

The coffee extracts and the initial ground roasted coffee were evaluated for their carbohydrate content and composition after acid hydrolysis (2 M H_2SO_4, 1 h, 120 °C) and derivatization of sugar residues to alditol acetates (Lopes et al., 2016). The main sugars present (Rha - rhamnose; Ara - arabinose; Man - mannose; Gal - galactose; Glc - glucose) were quantified as equivalents of 2-deoxyglucose used as internal standard for quantification.

The glycosidic-linkages of carbohydrates were determined through a methylation procedure. The coffee extracts (FD and EC, 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 1 M HCl. 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 hydrolysed (2 M TFA, 1 h, 121 °C), and the resultant monosaccharides reduced ($NaBD_4$) and acetylated as described for neutral sugars (Lopes et al., 2016). The partially methylated alditol acetates (PMAA) were analysed and identified by gas chromatography-mass spectrometry (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, Folsom, CA, USA), following chromatography conditions described by Oliveira et al. (2017). The peak area was used to determine each PMAA relative amount. Three independent extracts were analysed for each coffee sample ($n = 3$).

2.6.2. Caffeine and 5-CQA analysis

For caffeine and 5-caffeoylquinic acid (5-CQA) determination, aliquots (10 mg mL^{-1} in Milli-Q water) were filtered (0.22 μm) prior to HPLC injection. The runs were performed on a HPLC-DAD apparatus equipped with a C18 column (Waters Sherisorb S10 ODS2, 4.6 mm × 250 mm, 10 μm) equilibrated with 5% formic acid (eluent A) and eluted also with methanol (eluent B), based on the method of Nunes, Cruz, and Coimbra (2012). The caffeine was detected at 280 nm and 5-CQA at 325 nm, and for quantification purposes, calibration curves of caffeine ($R^2 = 0.997$) and 5-CQA ($R^2 = 0.993$) were prepared.

2.6.3. Protein content

The polymeric fractions (HMWM, $HMWM_{sol}$ and $HMWM_{ins}$) were used to determine the nitrogen content by elemental analysis in a Truspec 630–200-200 elemental analyser with a TDC detector. The nitrogen content was converted to protein content (%, $w/w_{extract}$) using the 5.5 factor (Bekedam, Schols, van Boekel, & Smit, 2006).

2.6.4. Colour measurements

Samples colour (solid state and in aqueous solution − 30 mg mL^{-1}) was assessed with Konica Minolta CM 2300d spectrophotometer and computed through SpectraMagic™ NX software, obtaining the CIE*Lab* 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 $h_{ab} = \tan^{-1}(b^*/a^*)$. Extracts brown colour was also spectrophotometrically evaluated through the specific extinction coefficient at 405 nm ($K_{mix,405nm}$) determined in a microplate reader using several dilutions of the coffee extracts (0–1 mg mL^{-1} in distilled water) (Bekedam et al., 2006; Lopes et al., 2016). Simultaneously, the measure was performed at 280 nm and 325 nm allowing to determine the $K_{mix,280nm}$ and $K_{mix,325nm}$.

2.6.5. Density, viscosity, pH and electrical conductivity measurements

The density of coffee solutions (FD, SD, EC1, and IC1) at 30 mg mL^{-1} was determined by weighing the solution at 20 °C ($n = 6$). A Cannon-Fenske routine viscometer (Size 50) was used to perform viscosity measurements (30 mg mL^{-1} in distilled water), in a thermostatic water bath at 25 °C. It was recorded the efflux time ($n = 3$) for each independent extraction with an electronic digital stopwatch. For kinematic viscosity determination, the efflux time was multiplied by the constant provided by the manufacturer. The samples were then used to determine pH and electrical conductivity with a Crison pH-meter at 25 °C ($n = 3$).

2.6.6. Foam analysis

Foamability of coffee extracts was tested using an adaptation of the Bikerman method (Mosalux device), as described in Coelho, Rocha, and Coimbra (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^{-1}) and pressure (1 bar) were maintained constant for 50 s and then detached. Foamability was evaluated by measuring the foam height increase on the top of coffee solution (in cm) and then converted to mL using a calibration curve. Foamability was also evaluated with an effervescent formulation approach: sodium bicarbonate (72 mg), citric acid (60 mg) and extracts (1.2 g, EC1, FD, SD1, IC1, 3 replicates) were weighed and mixed before the addition of water at 70 °C, after preliminary tests with different quantities of the compounds. The foamability was evaluated measuring the foam volume in the cup (height increase converted in mL). The foam stability was measured as the time required for appearance of the halo beneath the foam of the coffee solution. The variation in pH after salts addition was evaluated with a Crison pH-meter when the solution cooled down to 25 °C.

2.6.7. 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 °C) and humidity (35%). Samples were placed on the crystal of the attenuated total reflectance accessory (ATR) and cleaned with aqueous ethanol (70%) between measurements. Five replicates spectra were obtained for each sample in a random order. The FTIR spectra were baseline and SNV (standard normal deviate) corrected before principal component analysis (PCA) performed using MetaboAnalyst 4.0 (web interface - https://www.metaboanalyst.ca/). Graphs were performed using GraphPad Prism 5.00 and MS Excel software.

2.6.8. Volatile profile analysis

A headspace solid phase microextraction (HS-SPME) followed by gas chromatography coupled to quadrupole mass spectrometry detection (GC–qMS) methodology was used to study the volatile composition of coffee samples. A short extraction time was used (3 min) to simulate the consumer's perception during fresh coffee brew consumption (Akiyama et al., 2008). All details related with the GC analysis and the identification strategy are presented in Supplementary Material (volatile analysis section). For each HS-SPME assay, 1.2 g of coffee extract was dissolved in 40 mL of distilled water, kept at 70 °C, and placed into a 120 mL glass vial ($1/\beta = 0.5$, $n = 3$). Each glass vial was previously placed during 5 min at 60.0 ± 0.1 °C in a thermostatic bath. The sample was introduced in the vial, which was capped. The SPME fibre was manually inserted into the sample headspace vial for 3 min, at constant stirring (400 rpm). The SPME fibre (50/30 μm DVB/CAR/PDMS) was

manually inserted into the GC injection port at 250 °C and kept 3 min for desorption. The HS-SPME analysis allowed to putatively identify 71 compounds in the vapour phase of the liquid coffee samples through comparison of mass spectra with software-included library and comparison of retention indexes with those reported in literature. The data (GC peak areas, expressed as arbitrary units, a.u.) were handled using MetaboAnalyst 4.0 (web interface). Heatmap representations were created using the GC peak areas of the samples analysed, with a data scaling to attribute equal importance to each compound. Such representations highlight samples differences through a chromatic scale, from a dark blue (lower) to a dark red (higher) scale.

3. Results and discussion

3.1. Characteristics of the espresso coffee used as reference

To define a composition profile able to be used as reference to prepare coffee infusions resembling espresso coffee (EC), a freeze-dried EC sample (EC1) was obtained using a conventional espresso machine and two distinct grinding levels. The EC brews contained 1.3 ± 0.1 g of total solids *per* cup of 40 mL (Table S2), a content similar to those reported in literature (0.9–1.3 g) using equal amount of coffee powder (6 g) and water (40 mL) (Lopes et al., 2016; Nunes et al., 1997). Thus, the reference used contained 21 ± 2% of coffee compounds extracted. Carbohydrates represented up to 3.4 ± 0.4% (w/w_{powder}), constituting 16 ± 1% ($w/w_{extract}$) of EC1, which was within the literature range for this type of coffee brews (12–24%) (Lopes et al., 2016; Nunes et al., 1997), but significantly lower than the relative amount present in instant coffee (IC) brews (35–39%, $w/w_{extract}$) (Blanc et al., 1989; Capek et al., 2014; Leloup, 2006).

EC1 exhibited mannose as major sugar residue (48 mol%), followed by galactose (30 mol%) and arabinose (14 mol%) (Fig. 1a). EC1 Man/Gal ratio was 1.6, representing mannose and galactose 8% ($w/w_{extract}$) and 5% ($w/w_{extract}$) of brew solids content, respectively, within the ranges defined in literature (4–14%, $w/w_{extract}$ for mannose and 1–8%, $w/w_{extract}$ for galactose) (Nunes et al., 1997). Recently, it was shown that the modulation of operational parameters of the infusion process allows to obtain coffee extracts with Man/Gal ratio within the range of 0.9–2.4, depending on the extraction conditions, with impact in coffee properties as viscosity, for instance (Lopes et al., 2019). In the present study, a finer grinding was associated to an EC with higher Man/Gal ratio and higher viscosity (Table S2). Thus, it should be possible to modulate the infusion process to obtain an extract with a Man/Gal ratio, carbohydrate content, and viscosity similar to EC. To fulfil this hypothesis, a comprehensive study of the coffee infusion process was established according to a central composite design (CCD, Table S1). To eliminate the variability that could occur using different blends due to 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 °C), w/v ratio (0.03, 0.12, and 0.20 g mL^{-1}), and grinding level (1–3). 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. It was considered the extraction of the main sugar residues (%, w/w_{powder}) and the proportion of these residues in the coffee extract obtained (mol%). From the models developed, after backward elimination ($\alpha = 0.1$), they were considered the significant ones ($p < 0.0001$) with high determination coefficients ($R^2 > 80\% - 86–95\%$) (Fig. S2 and Table S3). Fig. 1b illustrates the optimization strategy applied through a desirability approach, where the desired values (those from EC1) were established as goals. The operational conditions that resemble EC1 composition with an overall desirability of 0.86 were an extraction time of 360 min, at 50 °C, with 0.12 g of coffee powder *per* mL of water, using coarser particles (level 3). The major variations were observed, in decreasing order, for temperature (X_2),

a)

EC1	mol%	%w/w$_{powder}$
Rha	3.4 ± 0.4	0.1 ± 0.0
Ara	14.0 ± 1.4	0.4 ± 0.1
Man	48.0 ± 3.1	1.7 ± 0.1
Gal	29.8 ± 1.4	1.1 ± 0.2
Glc	4.8 ± 0.1	0.2 ± 0.0

b)

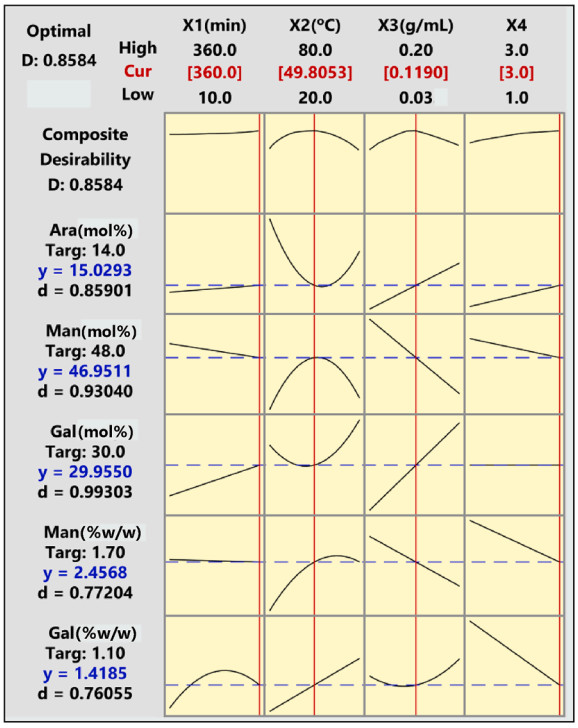


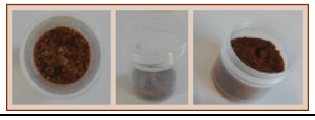
Fig. 1. Carbohydrate composition of coffee samples (Rha, rhamnose; Ara, arabinose; Man, mannose; Gal, galactose; Glc, glucose). a) freeze-dried espresso coffee (EC1); b) plots of response optimization strategy applied according to desirability function (X_1, extraction time; X_2, temperature; X_3, coffee powder/water ratio; X_4, grinding level). The responses were the extraction of the main sugar residues (%, w/w_{powder}) and the proportion of these residues in the coffee extract obtained (mol%), for models with high determination coefficients ($R^2 > 80\% - 86–95\%$).

ratio of coffee powder/water (X_3), and coffee particles size (X_4). As the effect of time (X_1) was very low, to minimize energy consumption, 10 min was defined as the optimum time for extraction, maintaining all other parameters. This decision slightly decreased the desirability value (D = 0.82), allowing to predict an overall composition of the extract still quite similar to EC1. Fig. 1b allows to verify that the trend for molar composition of arabinose and galactose is similar, evidencing the presence of arabinogalactans (AG), structures easily extracted compared to galactomannans (GM), composed mostly by mannose, whose extraction is more dependent on extraction conditions, mainly temperature (Lopes et al. 2019). The extraction of GM is favored with increasing temperatures (at atmospheric pressure, <100 °C), but the increase in the weight/water ratio applying prolonged extraction times would result in a predominance of arabinogalactans in the brew, which is not usually verified in EC brews (Lopes et al., 2016; Nunes et al., 1997).

3.2. Physicochemical characterisation of infusions with EC-like sugars composition

The defined operational conditions to prepare infusions with EC-like sugars composition were scaled-up in a 50 times larger extraction experiment using 1.5 L of water in three independent extractions. Table 1 shows the overall characterisation of infusion coffee extracts

Table 1
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	SD
				
Total Carbohydrates (%, w/$w_{extract}$)[1]	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/$w_{extract}$)[1]	0.92 ± 0.05[a]	0.05 ± 0.02[b]	0.10 ± 0.04[b]	0.10 ± 0.01[b]
Caffeine (%, w/$w_{extract}$)[1]	8.83 ± 0.42[a]	4.92 ± 0.38[b]	8.83 ± 0.64[a]	8.67 ± 0.64[a]
5-CQA (%, w/$w_{extract}$)[1]	2.39 ± 0.15[a]	1.01 ± 0.16[b]	2.47 ± 0.17[a]	2.37 ± 0.16[a]
Density (g cm^{-3})	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)[2] 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)[2]	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})[2]	3.56 ± 0.31[a]	2.33 ± 0.21[b]	3.83 ± 0.36[a]	3.85 ± 0.14[a]
pH[2]	5.75 ± 0.12[a]	4.88 ± 0.04[b]	6.09 ± 0.09[c]	5.87 ± 0.04[a]
Foamability (mL)[2]	8.1 ± 2.1[a]	8.1 ± 0.4[a]	7.2 ± 1.4[a]	-[3]
Foam index (%)[2]	20.3 ± 5.2[a]	20.3 ± 1.0[a]	18.0 ± 3.6[a]	-[3]
Foam Stability (s)[2]	68.8 ± 8.4[a]	79.4 ± 28.2[a]	80.2 ± 22.6[a]	-[3]
pH (after effervescence)	5.76 ± 0.09[a]	5.23 ± 0.05[b]	5.95 ± 0.09[c]	5.69 ± 0.06[a]

[1]: relative content of the compounds in relation to the total solids extracted; [2]: analysis performed after redissolution of freeze-dried samples in water (30 mg mL^{-1}). [3]: the extract did not form the foam. n.d.: not determined. Columns with different characters ([a–d]) in each row indicate samples with significant difference ($p < 0.05$). (Rha, rhamnose; Ara, arabinose; Man, mannose; Gal, galactose; Glc, glucose)

processed via freeze-drying (FD) and spray-drying (SD).

The scale-up experiment was performed using the same coffee sample, although from a different lot than the one used for CCD experiments. To compare the extracts obtained with the EC reference, additional EC1 samples were prepared with the new lot of coffee (Table 1). The optimized infusion process extracted 20% of coffee compounds, a value similar to EC1 21% (w/w_{powder}), and in line with EC brews described in literature for related extraction conditions (6 g, 40 mL, 19–21%) (Lopes et al., 2016). This suggests that the quantity of compounds extracted, in absolute values, was equivalent by the two methods.

Concerning the dehydration step, while the freeze-drying method enables the recovery of all coffee material, under the conditions used, nearly half of the content was lost during the processing of the sample via spray-drying, stuck in the drying chamber of the apparatus. This problem would decrease the overall extraction yield to 11% (w/w_{powder}), although not directly related to the extraction process. Furthermore, the appearance of the samples was distinct: the freeze-dried ones were fluffy brown, while spray-dried samples were yellowish powders (Table 1 and Fig. S3). This was supported by the variation in powder colour parameters (*Cielab* coordinates) with higher L^* (lightness) and b^* (shifting in the yellower coordinate) associated to SD samples, in accordance with literature (Padma Ishwarya & Anandharamakrishnan, 2015). This distinction was not so evident when the powder was dissolved in water (brew) at EC concentration (30 mg mL^{-1}), as both FD and SD showed a similar brown colour not perceived by naked eye, with similar L^* and b^* values. The dissolution of FD and SD extracts produced more translucid solutions when compared to EC and IC (foggy/cloudy coffee). In addition, although the freeze-dried extracts (both EC and infusion) dissolved almost instantaneously, the spray-dried extract did not (Fig. S3). The SD extract seem to act as a more hydrophobic material, suggesting a different organization of the molecules during the drying process. SD processing usually confers smaller particles compared to FD, with smaller spaces between the particles. Thus, as SD was a more compacted 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, Pietsch, & Eggers, 2011).

Table 1 shows that SD had slightly lower content of total sugars in the extract, possibly caused by a preferential interaction/retention of carbohydrates in the drying chamber. Overall, the sugars composition of FD and SD were statistically similar between them and with EC1 (Table 1), suggesting similar sugars composition of infusion and EC solids. On the other hand, sample IC1 exhibited a substantially higher content of carbohydrates (34.5%, w/$w_{extract}$) and a distinct composition, with galactose as the main sugar residue (52.1 mol%, 18.3%, w/$w_{extract}$), followed by mannose (33.9 mol%, 11.9%, w/$w_{extract}$), in accordance with literature for IC samples (10.2–19.7%, w/$w_{extract}$ for mannose and 13.0–24.7%, w/$w_{extract}$ for galactose) (Blanc et al., 1989; Capek et al., 2014; Leloup, 2006). While in EC1, FD and SD the Man/Gal ratio was 1.4–1.5, it lowered to 0.7 in IC1. Arabinose was also relatively abundant in all type of samples analysed (2.3%, w/$w_{extract}$ in EC1; 2.5%, w/$w_{extract}$ in FD; 2.1%, w/$w_{extract}$ in SD; and 2.7%, w/$w_{extract}$ in IC1), although with a lower relative molar ratio in IC1 (9.4 mol%) than in the other ones (15.0–15.7 mol%).

A further in-depth sugar analysis was performed using FD sample, as it was easily dissolved than SD, a decisive advantage for product

development. Generally, glycosidic linkage analysis performed to EC1 and FD (Table S4) did not show significant differences between the two groups of samples, suggesting similar carbohydrate structures in EC1 and FD extracts. 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, Brueckel, Reichardt, Blaut, Steinhart, & Bunzel, 2007; Passos, Rudnitskaya, Neves, Lopes, Evtuguin, & Coimbra, 2019), indicated 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. EC1 and the infusion present 38.0 ± 1.1% and 38.5 ± 2.8% of AG, respectively (Table S4). Thus, the ratio of GM/AG for the two methods was similar (1.3). This ratio is reported to vary from 0.9 to 2.8 in different coffee brews, including infusions, drip brew, or espresso, for instance (Gniechwitz et al., 2007; Nunes & Coimbra, 2001; 2002). Indeed, the extraction conditions may be modulated to obtain similar proportions even with different methods. In the case of instant coffee, literature shows a lower GM/AG ratio (0.4), in line with the molar composition obtained for sample IC1 (Table 1). Moreover, the estimation of the branching degree of GM showed similar values for both extraction methodologies, approximately 5% for EC1 and FD, in accordance with other infusion processes (4–5%) (Nunes & Coimbra, 2001, 2002), other extraction methods (drip brew, instant espresso, coffee pods; 3.1–4.0%), IC samples (4.4%), or extracts obtained from spent coffee grounds (2–7%) (Gniechwitz et al., 2007; Passos, Rudnitskaya, Neves, Lopes, & Coimbra, 2019).

A dialysis step was employed to obtain the polymeric material of the samples and evaluate the similarities between EC1 and FD. The IC1 sample was also tested for comparison purposes using the same amount of starting material. Despite the higher carbohydrates of IC1 when compared to EC1 and FD (Table 1), the polymeric material did not reflect a significant difference, with all samples ranging from 19.7 to 25.2%. This suggests that in IC1 a considerable fraction of low molecular weight carbohydrates diffused through the dialysis membrane (<12–14 kDa). The predominance of low molecular weight compounds in instant coffees agrees with literature (<1 kDa compounds accounting for nearly 40%) (Ferreira et al., 2018; Passos et al., 2014).

The carbohydrate composition of the polymeric material showed that EC1 and FD exhibited great similarity, richer in mannose, while IC1 sample was richer in galactose and poorer in mannose and arabinose. Such differences were also observed in the soluble high molecular weight material (HMWM) fraction that represented at least 78% of the HMWM material of the samples (Table 2). On the other hand, higher amount of cold-water insoluble fraction ($HMWM_{Insol}$) was found in EC1 and IC1 (4.8 and 4.4%, w/$w_{extract}$, respectively), when compared to FD (0.8%, w/$w_{extract}$). The higher proportion of insoluble compounds in EC1 sample may be due to the presence of small roasted coffee particles directly extracted to the brew, not found 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 S1).

Protein has been associated to foamability in EC (Nunes et al., 1997). Table 2 shows that EC1 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 (9–12%) (Bekedam, Roos, Schols, Van Boekel, & Smit, 2008; Nunes and Coimbra, 2001). The major polymeric fraction revealed similar percentages in EC1 (12.6%, w/$w_{HWMWSol}$) and FD (12.5%, w/$w_{HWMWSol}$) and agrees with literature for EC samples when applying the same procedure of analysis (Lopes et al., 2016). Considering the mass of compounds, the results showed that EC1 contained 38 mg of protein *per* g of brew solids, while FD and IC1 exhibited 26 mg and 31 mg, respectively. The distinction came from the insoluble fraction (EC1: 17 mg; FD: 2 mg; IC1: 7 mg), as the soluble one showed similar values among the samples (EC1: 22 mg; FD: 24 mg; IC1: 24 mg), values comparable with literature reports for EC (Lopes et al., 2016).

Table 2
High molecular weight material for the espresso coffee and the infusion samples. The estimated amount (in mg) is shown in brackets *per* g of sample.

Fraction	EC1	IC1	FD
$HMWM_{Total}$ (%, w/$w_{extract}$)	22.4 ± 0.3 (224)	25.2 ± 4.2 (252)	19.7 ± 0.5 (197)
Total Carbohydrates (%, w/w_{HMWM})	56.7 ± 3.5 (127)	68.6 ± 0.7 (173)	59.0 ± 8.2 (116)
Rha (mol%)	4.4 ± 0.0 (5)	1.7 ± 0.1 (3)	4.1 ± 0.2 (4)
Ara (mol%)	12.8 ± 0.2 (14)	6.9 ± 0.0 (10)	12.6 ± 0.7 (12)
Man (mol%)	50.5 ± 0.9 (66)	28.3 ± 0.4 (50)	50.6 ± 2.2 (61)
Gal (mol%)	30.4 ± 0.4 (40)	60.9 ± 0.2 (107)	31.2 ± 1.3 (37)
Glc (mol%)	1.9 ± 0.2 (2)	2.2 ± 0.5 (4)	1.4 ± 0.0 (2)
Protein (%, w/w_{HMWM})	16.8 ± 0.5 (38)	12.2 ± 0.1 (31)	13.4 ± 0.3 (26)
Melanoidins (%, w/w_{HMWM})[1]	26.4 (59)	19.2 (48)	27.6 (54)
$HMWM_{Sol}$ (%, w/$w_{extract}$)	17.6 ± 0.2 (176)	20.8 ± 2.1 (208)	18.8 ± 0.3 (188)
Total Carbohydrates (%, w/$w_{HMWMSol}$)	58.7 ± 0.9 (103)	78.8 ± 10.9 (164)	62.0 ± 0.0 (117)
Rha (mol%)	5.0 ± 0.2 (5)	1.8 ± 0.2 (3)	4.2 ± 0.1 (5)
Ara (mol%)	14.5 ± 0.4 (13)	7.5 ± 0.3 (10)	12.6 ± 0.1 (12)
Man (mol%)	44.9 ± 1.4 (48)	16.0 ± 0.1 (27)	50.4 ± 0.0 (61)
Gal (mol%)	34.1 ± 0.9 (36)	72.5 ± 0.1 (121)	31.4 ± 0.1 (38)
Glc (mol%)	1.5 ± 0.0 (2)	2.3 ± 0.1 (4)	1.4 ± 0.1 (2)
Protein (%, w/$w_{HMWMSol}$)	12.6 ± 0.5 (22)	11.4 ± 0.1 (24)	12.5 ± 0.0 (24)
Melanoidins (%, w/$w_{HMWMSol}$)[1]	28.7 (50)	9.7 (20)	25.5 (48)
$K_{mix,280nm}$	4.87 ± 0.20	4.35 ± 0.29	4.62 ± 0.33
$K_{mix,325nm}$	3.95 ± 0.17	3.36 ± 0.22	3.68 ± 0.28
$K_{mix,405nm}$	1.14 ± 0.07	0.91 ± 0.05	1.24 ± 0.11
$HMWM_{Insol}$ (%, w/$w_{extract}$)	4.8 ± 0.3 (48)	4.4 ± 2.1 (44)	0.8 ± 0.2 (8)
Total Carbohydrates (%, w/$w_{HMWMInsol}$)	11.8 ± 2.1 (6)	68.7 ± 5.1 (31)	31.2 ± 8.8 (3)
Rha (mol%)	5.5 ± 0.6 (0)	0.6 ± 0.0 (0)	3.3 ± 0.5 (0)
Ara (mol%)	17.1 ± 2.2 (1)	2.1 ± 0.1 (1)	9.7 ± 1.7 (0)
Man (mol%)	37.6 ± 1.6 (2)	86.5 ± 0.3 (27)	63.4 ± 6.0 (2)
Gal (mol%)	29.7 ± 2.1 (2)	9.2 ± 0.3 (3)	19.8 ± 2.4 (1)
Glc (mol%)	10.2 ± 3.3 (1)	1.6 ± 0.1 (0)	3.8 ± 1.4 (0)
Protein (%, w/$w_{HMWMInsol}$)	36.1 ± 0.4 (17)	15.5 ± 1.1 (7)	25.8 ± 1.5 (2)
Melanoidins (%, w/$w_{HMWMInsol}$)[1]	52.0 (25)	15.8 (7)	43.0 (4)

[1]: values for melanoidins obtained from the difference between the total polymeric material and the material determined as carbohydrates (Rha, rhamnose; Ara, arabinose; Man, mannose; Gal, galactose; Glc, glucose) and proteins.

Melanoidins are brown nitrogen-containing polymeric material, whose estimation is usually performed by the difference between the total polymeric material and the one determined as protein and carbohydrates (Lopes et al., 2016). Table 2 shows that EC1 and FD had similar content of melanoidins, and higher than IC1. The estimation of the amount *per* brew (1.2 g of solids) 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, Fogliano, & Pellegrini, 2012). The brown characteristic colour of melanoidins was measured through the specific extinction coefficient at 405 nm ($K_{mix,405nm}$). Table 2 shows a resemblance between $K_{mix,405\ nm}$ values for EC1 (1.1) and FD (1.2), suggesting a similar brown colour of these extracts.

The lipids content in EC1 (0.92%, w/$w_{extract}$, Table 1) was significantly higher than IC1, FD, and SD (0.05, 0.10, and 0.10%, w/$w_{extract}$, respectively). Moreover, roasted powder contained 11.1% (w/w_{powder})

of lipids, showing that EC procedure may extract nearly 2% of the coffee lipids present in the coffee powder. It was reported that pressure favours lipids extraction, while filtration steps, as performed after 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 was similar, while the amount in IC1 was significantly lower, due to the higher relative abundance of other compounds 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 values, probably due to the different Man/Gal ratio verified in these samples. Under the same conditions, EC1, FD, and SD exhibited similar electrical conductivity, which could be an indication of comparable amount of ions present, with a lower value observed in IC1. Concerning pH, the dissolution of EC1, FD, and SD extracts, at the same conditions, originated solutions with pH 5.7–6.0 (Table 1), in line with values for EC brews (5.4–5.9) (Andueza et al., 2007; Caporaso et al., 2014), while the IC1 sample had pH 5.2, thus more acidic, in accordance with values for these brews (4.9–5.2) (da Silveira, Tavares, & Glória, 2007; Welna, Szymczycha-Madeja, & Zyrnicki, 2013).

3.3. Foam experiments

The dried coffee samples (EC1, FD, and SD) foamability and foam stability was evaluated through the injection of CO_2 using brews prepared at EC concentration (30 mg mL^{-1}, Fig. S4). This methodology was already applied in the study of wine compounds foamability and foam stability (Coelho et al., 2011). The EC1 sample, when dissolved in water (25 °C), was able to produce a foam index of 10.2% in the column, with 10% as the indicated acceptable value 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 stability of 13.3 min. These results show that the coffee extracts can produce consistent foam. As the goal was to generate CO_2 *in situ* and evaluate the foamability of the coffee products, series of experiments were conducted with effervescent formulations using the effervescent properties of sodium bicarbonate/citric acid mixtures. IC1 sample was used to determine the quantity of reagents needed to attain the desired level of foam-index (at least 10%) with the addition of water at 70 °C 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) (Fig. S5). 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 1). On the other hand, the lower instant solubility of SD sample hindered the formation of the foam layer. Thus, SD sample was not considered in further experiments. The addition of the salts led to a variation in the pH of coffee solutions, with a decreasing of 0.14 pH units with FD sample, maintaining the pH values for EC1, and increasing the

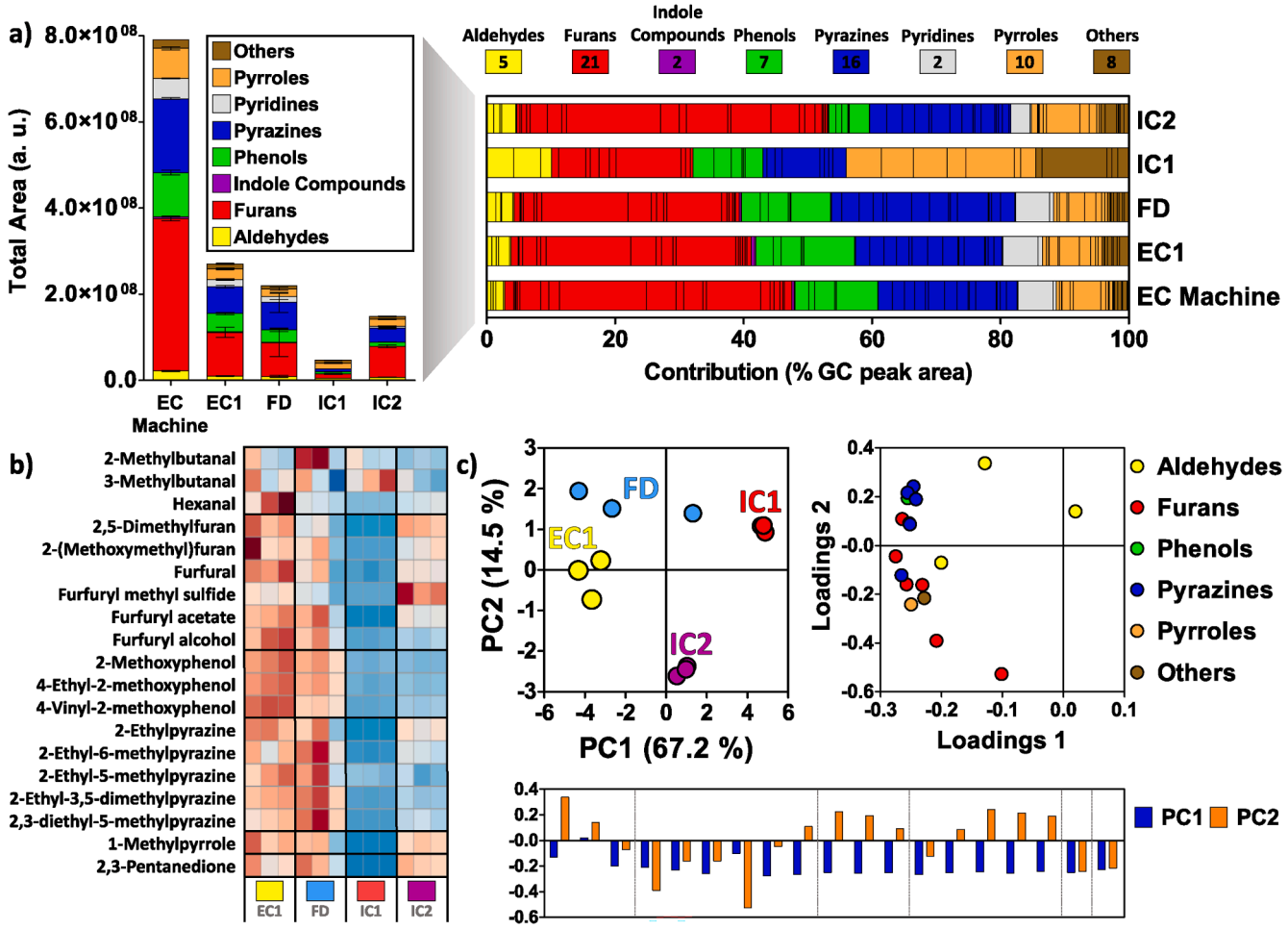


Fig. 2. Coffee volatile profile analysis. a) Total GC peak area grouped by chemical family (left) and contribution of each family for the total area (right - the number inside the box represents the number of compounds in each chemical family). b) Heatmap representation of the aroma contributing volatile compounds identified, grouped by chemical families, considering the GC peak areas after mean-centred the data for each variable and dividing by the standard deviation (autoscaling). c) Principal component analysis (PCA) of the volatile compounds identified, presenting the distribution of the samples (scores, left) and compounds (loadings, right and below).

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 has been reported to extend the shelf life of coffee brews, keeping longer their cup quality and even increasing antioxidant activity (Pérez-Martínez, Caemmerer, De Peña, Cid, & Kroh, 2010).

3.4. Volatile profile analysis

The volatile profile of each coffee was studied after the dissolution of the samples (EC1 and FD, 1.2 g) in hot water (70 °C, 40 mL), analysing the vapour phase above the coffee brews. As the intent was to study the aroma perceived while drinking a coffee brew, a short extraction time (3 min) was selected to simulate the consumers' perception. For comparison, EC were extracted right before the analysis with a conventional coffee machine (EC Machine), using the same coffee blend used to produce EC1 and FD samples. Moreover, two instant coffee samples (IC1 - instant coffee and IC2 - instant coffee labelled "espresso" by the manufacturer) were studied for comparison purposes. As SD sample presented dissolution problems, it was discarded from this analysis. The HS-SPME/GC-qMS analysis (chromatograms in Fig. S6) allowed to putatively identify 71 compounds in the headspace of the coffee samples studied (Table S5). Globally, similar volatile profiles were observed for the coffee brews analysed under the HS-SPME conditions used. The fresh espresso coffee (EC Machine) brew exhibited higher GC peak intensities than the extracts, whose previous concentration step (freeze drying process) explain the general intensity loss of the volatile compounds. EC1 and FD samples showed higher total GC peak intensities than the instant coffee samples (IC1 and IC2). In fact, the lower volatiles in instant coffees compared to other brews is in accordance with literature (Sanz, Czerny, Cid, & Schieberle, 2002; Semmelroch & Grosch, 1995). According to their chemical nature, the compounds were grouped in the most relevant coffee chemical families, as aldehydes, furans, indole compounds, volatile phenols, pyrazines, pyridines, pyrazines, and pyrroles. The compounds not included in any of the previous chemical families were classified as "others". Fig. 2a shows the total GC peak area for the samples analysed grouped by their chemical family and the contribution of each peak to the overall intensity.

Furans were the chemical family with higher number of compounds determined in all samples and with a predominant contribution of their GC peak areas in the EC machine sample (45%), EC1 (37%), FD (36%), and IC2 (48%). For IC1, pyrroles were the preponderant chemical family (30% of total GC peak area). The predominance of furans over other compounds was already described in literature for different coffee brews, as the principal contributors for characteristic coffee brew aroma (Caporaso et al., 2014). Pyrazines represent the following predominant chemical family in the coffee samples studied (except in IC1 which is furans): 22% (EC Machine and IC2), 23% (EC1), and 29% (FD) (Fig. 2a). These compounds are key aroma compounds, namely the alkylpyrazines, as they confer hazelnut, nutty, and roasted notes to coffee (Caporaso et al., 2014; Flament, 2001) (Table S5). Volatile phenolic compounds also greatly contribute to the total GC peak area, mainly in the EC Machine, EC1, and FD (13–16%) comparing to instant samples (6–11%) (Fig. 2a). These compounds are associated to smoky, roasted, and spicy notes (Table S5), contributing to the typical coffee aroma associated to coffee brews.

Furfuryl acetate was the major compound detected in coffee samples, representing 13.1–14.7% of the overall GC peak intensities, in line with literature for espresso coffee (10.5–13.6%) (Petisca, Pérez-Palacios, Farah, Pinho, & Ferreira, 2013) and other freshly brews (American, Neopalitan, and Moka) (Akiyama et al., 2009; Caporaso et al., 2014). This was not observed for IC1 that exhibited a lower level of furfuryl acetate (0.6%). In IC1, acetic acid was predominant (10.1% of total peak

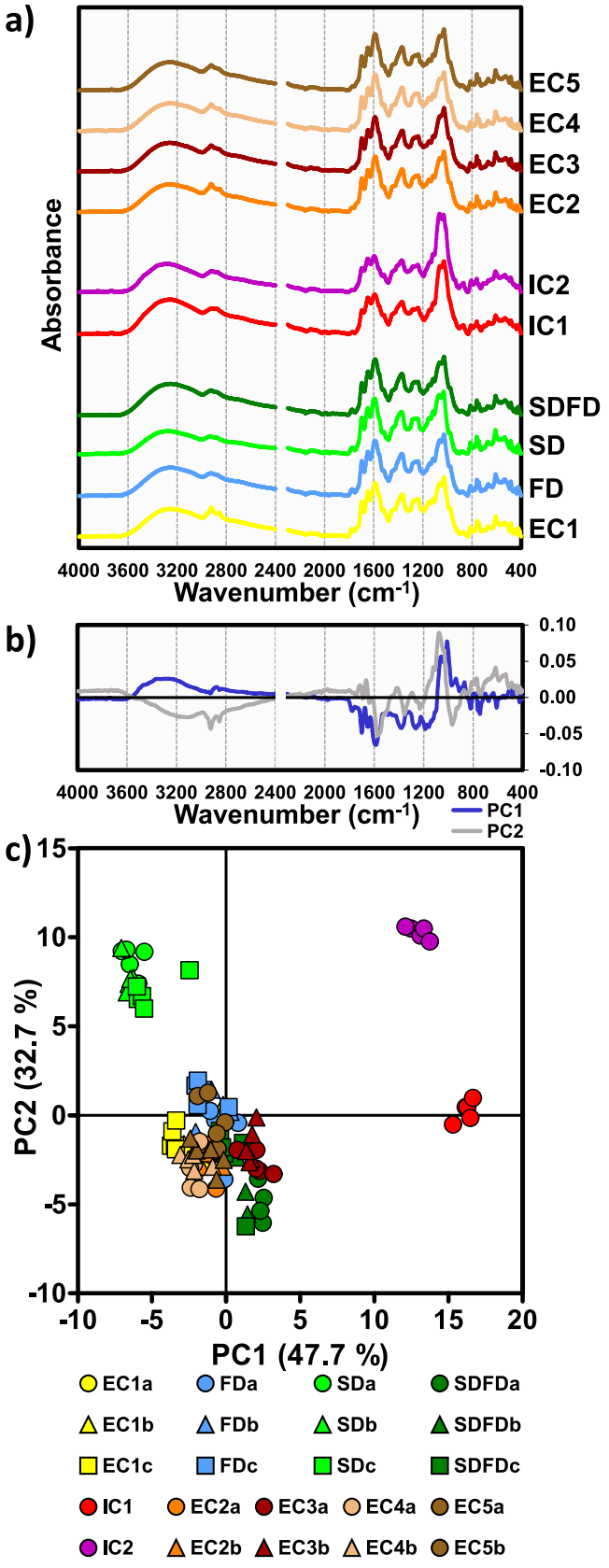


Fig. 3. FTIR analysis of the different coffee extracts. a) FTIR spectra (SNV-corrected), b) PCA loadings and c) scores.

area), in accordance with results for agglomerated instant coffee (powder), composed by 6–7% of acetic acid and where furfuryl acetate does not exceed 0.1% (Leobet et al., 2019). Furthermore, the compounds with major contribution for the total GC peak area (>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%). Indeed, the higher preponderance of furfuryl acetate in espresso coffee has been highlighted as diagnostic between different coffee brews (Caporaso et al., 2014). Although EC Machine exhibited the highest GC peak areas for almost all compounds (67 out of 71), there were some exceptions as 5-hydroxymethylfurfural, whose presence was only observed in instant coffee samples. This compound is one of the major volatile compounds (18–22%) in agglomerated instant coffee powder (Leobet et al., 2019), probably due to the thermal extraction processing.

To explore the similarities/differences between the extracts (FD, EC1, IC1, and IC2), masked by the substantial higher peak abundance of fresh sample, the data was re-analysed excluding EC machine sample. The heatmap (Fig. S7a) highlights the higher overall intensity associated to EC1 and FD samples, where some compounds were more intense in IC2 while the poorer global intensity was observed for IC1. The differentiation of FD and EC1 when compared to instant samples (IC1 and IC2) was evidenced by the dendrogram and PCA (Fig. S7). PC1, representing 65.0% of samples variability, separated instant coffees, mainly IC1, from EC1 and FD due to higher GC peak areas determined in most compounds of the latter ones.

Although 56 of the compounds identified in the coffee samples have associated aroma descriptors (Table S5), only 19 (Fig. 2b) were already described as important aroma contributors for coffee brews (Caprioli et al., 2012). The PCA of GC peak areas of the 19 coffee aroma contributors without EC Machine showed a similarity between EC1 and FD extracts (Fig. 2b and 2c), and their difference from IC1. PC1, that explained 67.2% of samples variability, separated FD and EC1 (negative PC1) from IC1 and IC2 (positive PC1). PC2, that explained 14.5% of samples variability, separated IC2 from the remaining samples, which was associated, for instance, to the higher level of furfuryl methyl sulphide.

The results for EC1 (10.3% of GC peak areas) and FD (12.5%) were of the same magnitude (7.0–11.9%) as studies regarding the key odorants for EC aroma (Andueza et al., 2003, 2007; Maeztu, Sanz, Andueza, Paz De Peña, Bello, & Cid, 2001). On the other hand, the GC peak areas for EC1 and FD samples were not statistically different, except for 2,5-dimethylfuran ($p < 0.05$) and 4-vinyl-2-methoxyphenol ($p < 0.01$). The identical volatile pattern observed suggested that the aroma created when dissolving the samples in hot water was similar. These samples have the same coffee blend origin and were freeze-dried after extraction (espresso and infusion). The compound 4-vinyl-2-methoxyphenol is absent or clearly diminished in instant coffee (Sanz et al., 2002; Semmelroch & Grosch, 1995). In the present study, the GC peak areas in EC1 (2.1×10^7) and FD (1.3×10^7) was much higher than the peak areas found in IC1 (1.3×10^6) and IC2 (4.6×10^6). The same trend was observed for other volatile phenolic compounds, as 4-ethyl-2-methoxyphenol (EC1/FD: 6.6×10^6-8.5×10^6; IC1/IC2: 7.8×10^5-1.5×10^6), that also confers spicy notes and 2-methoxyphenol (EC1/FD: 6.1×10^6-7.5×10^6; IC1/IC2: 1.5×10^5-1.6×10^6), with burnt and smoky aroma notes, which were compounds reported to be present in coffee brews and absent/minor in instant coffee (Sanz et al., 2002; Semmelroch & Grosch, 1995). On overall, although EC machine revealed higher intensities, the volatile profile of this sample processed by freeze-drying (EC1) or one obtained from an infusion process (FD) was similar.

3.5. Global analysis

The analysis of FTIR spectra allow to comprehensively study the samples overall composition. Besides the espresso reference (EC1), the freeze- (FD) and spray-dried (SD) extracts and the instant samples (IC1 and IC2), other espresso coffee samples (E2-E5) were added to increase the robustness of the results. Fig. 3a evidenced that IC samples differed from all others. PCA (Fig. 3b,c) suggested similarity on overall composition between espresso coffee samples (E1-E5) and the freeze-dried extracts (FD). On the other hand, the SD sample was 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, once the dissolution of SD sample in water and its posterior freeze-drying (SDFD in Fig. 3c), placed this sample next to all other FD samples. Loading analysis showed that the carbohydrate region (800–1200 cm^{-1}) differentiated IC samples from the remaining samples, with the major variation in PC1 explained by the wavenumber 1029 cm^{-1} (56.4%). This is associated with higher carbohydrates content in IC samples (Table 1 and S6), even the one labelled as instant espresso coffee (IC2). The sugar composition of additional espresso samples (E2-E5, Table S6) was similar to EC1 and infusion extracts. The 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. Furthermore, EC1-EC5 samples 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 the extracts produced (mainly FD) were chemically close to EC samples and greatly distinct

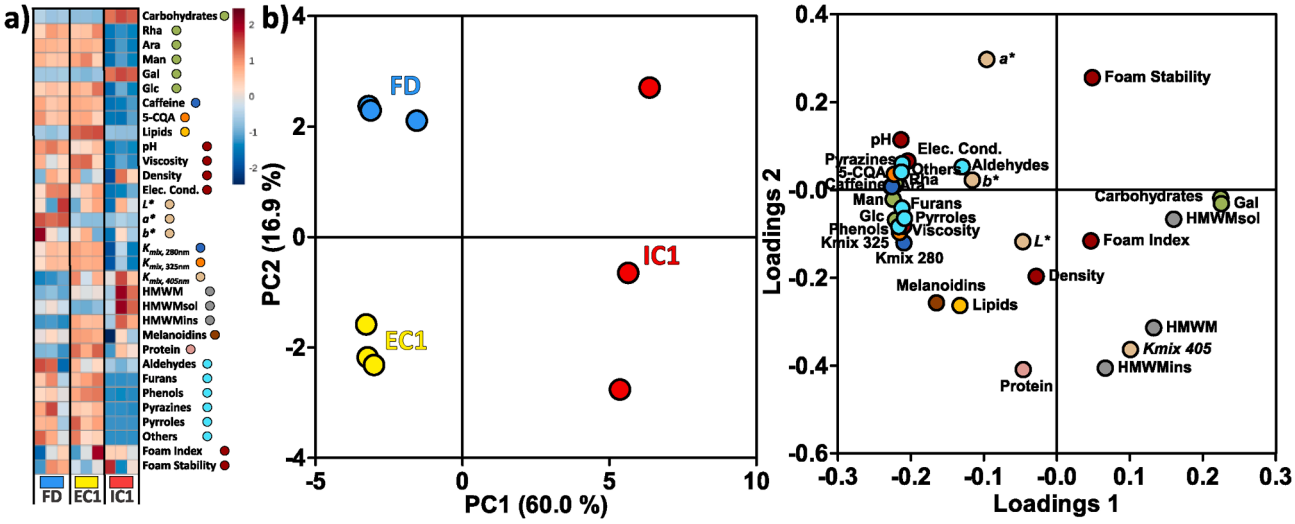


Fig. 4. a) Heatmap representation (a) and principal component analysis (b) of all the compounds and properties determined for EC1, FD and IC1 samples.

from IC samples, even the one labelled as espresso, possibly related to the drastic conditions of extraction used to obtain them which hinder their resemblance to EC.

Fig. 4a shows a heatmap representation covering all analyses performed for EC1, FD, and IC1 samples highlighting the similarity of EC1 and FD in most of the parameters analysed and the considerable difference to IC1. The PCA (Fig. 4b) shows that 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, lipids had a considerable influence on the separation between EC1 and FD samples. The addition of flavour extracts (as the unextracted roasted coffee lipid extract) could enrich both the lipids content and the aroma profile, approximating the FD aroma to the one of a fresh coffee. Furthermore, melanoidins and protein seem also to have influence, although the differences in their amounts between the two extracts was low (5.9% $w/w_{extract}$ in EC1 compared to 5.4% w/w in FD for melanoidins, and 3.8% $w/w_{extract}$ in EC1 compared to 2.6% in FD sample).

4. Concluding remarks

In the EC studied, 21 ± 2% of the coffee compounds end up in the brew extract, which represents an amount similar to the one obtained after modulation of a regular infusion extraction. These extracts had similar composition to EC in many of the parameters analysed (carbohydrates, caffeine, chlorogenic acid, pH, foamability or colour). However, the processing by spray-drying was not favourable to process extracts with low concentration of solids due to posterior poor instantly dissolution in low water volume used in EC. Moreover, the freeze-dried extract lacked lipids content due to higher extractability of this fraction with EC devices. However, the freeze-dried sample contained a volatile profile representative of an EC, considering that the compounds are still present in the extract, although in considerably lower amount. The results herein obtained could be used as a tool to create new coffee brew formulations approximating instant extract powders to espresso coffees.

The modulation of studied infusion process resulted also in a high fraction of unextracted compounds, namely carbohydrates. Thus, under a circular economy, the residue can be posteriorly extracted in more drastic conditions to produce instant coffee, leading to the total exploitation of the coffee powder in two distinct products, EC and IC, by a two steps extraction process.

CRediT authorship contribution statement

Guido R. Lopes: Conceptualization, Methodology, Investigation, Formal analysis, Writing - original draft. **Cláudia P. Passos:** Methodology, Writing - review & editing. **Sílvia Petronilho:** Methodology, Investigation, Writing - review & editing. **Carla Rodrigues:** Supervision, Writing - review & editing. **José A. Teixeira:** Supervision, Writing - review & editing. **Manuel A. Coimbra:** Conceptualization, Validation, Resources, Supervision, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2020.128613.

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