AUTHOR(S)
Ollennu-Chuasam, P., Ahmed, H., Koistinen, V., Hanhineva, K., Linderborg, K. M., & Suomela, J.-P.

TITLE
Lipophilic and Hydrophilic Metabolites as Descriptors of Different Coffee Beverages

YEAR
2024

DOI
10.1021/acs.jafc.4c03347

CITATION
Ollennu-Chuasam, P., Ahmed, H., Koistinen, V., Hanhineva, K., Linderborg, K. M., & Suomela, J.-P. (2024). Lipophilic and Hydrophilic Metabolites as Descriptors of Different Coffee Beverages. *Journal of Agricultural and Food Chemistry*, 72(29), 16461–16474. https://doi.org/10.1021/acs.jafc.4c03347

VERSION
Accepted Manuscript

# Lipophilic and hydrophilic metabolites as descriptors of different coffee beverages

Priscilla Ollennu-Chuasam[a], Hany Ahmed[a], Ville Koistinen[ab], Kati Hanhineva[ab], Kaisa M. Linderborg[a], Jukka-Pekka Suomela[a*]

[a]Food Sciences, Department of Life Technologies, University of Turku, Finland

[b]Department of Public Health and Clinical Nutrition, University of Eastern Finland, Finland

*Corresponding author

E-mail address: jusuom@utu.fi

ABSTRACT **(196 words)**

Coffee is a widely consumed beverage rich in bioactive phytochemicals. This study investigated the effect of brewing method on the profile of potential bioactive compounds in different coffee beverages using metabolomics and lipidomics based on UHPLC-MS/QTOF. The oil content of the espresso, pot, instant and filter coffee beverages studied were 0.13% ± 0.002, 0.12% ± 0.001, 0.04% ± 0.002, and 0.03% ± 0.003 respectively. Univariate analysis indicated significant differences ($p < 0.001$) in oil content when espresso and pot beverages were compared with instant and filter beverages. Principal Component Analysis revealed similarities in the lipid profiles of filter and espresso coffee beverages and the hydrophilic profiles of pot and filter coffee beverages. The espresso beverage had the highest intensity of hydrophilic compounds such as adenine, theobromine, chlorogenic acid, and caffeine. The pot beverage was the most abundant in triglycerides, phosphatidylcholine, and diterpenes. Cafestol and kahweol esters, but not their free forms, were the most abundant diterpene in the pot beverage. This work provides information on the differences in the profile of potentially bioactive compounds in four commonly consumed coffee beverage types and, thus, on the possible differences in the health effects of these coffee beverage types.

Keywords: coffee, brewing method, metabolomics, lipophilic, hydrophilic, cafestol, kahweol

## 1. Introduction

Coffee is popular worldwide with an annual consumption of about 10.7 million tons in 2022/2023 (1). The two most cultivated coffee species are *Coffea arabica* and the *Coffea canephora* variety robusta (also referred to as robusta). Robusta is primarily cultivated at low latitudes in Brazil, Western and Central Africa, and *Coffea arabica* in Southeast Asia and Latin America and Africa. Latin America had the highest regional percentage share (48.3%) of the world's coffee production in 2023 followed by Asia and Oceania (29.6%), Caribbean, Central America, and Mexico (11.4%), and the least was Africa (10.6%) (1, 2, 3).

Coffee is mostly consumed for its sensory appeal and stimulating properties (4). Nevertheless, it also contains bioactive compounds such as chlorogenic acid, caffeine, trigonelline, phospholipids, and diterpenes, which are potentially beneficial to human health (5, 6). Coffee consumption has been associated with a decrease in inflammation and reduced incidence of cardiovascular disease (7, 8). However, post-harvest processes, roasting conditions, the level of grinding, and the method of brewing affect the type and content of bioactive compounds present in coffee beverages (9, 10, 11). For example, the brewing method has been reported to affect the contents of chlorogenic acid, caffeine, kahweol, and cafestol in different coffee beverages (12, 13). Ratnayake et al. (14) in their research on filter, boiled, Turkish, espresso, and instant coffee beverages reported that boiled and espresso beverages contained the highest amount of lipid content and filter beverage the least. They also reported that triglycerides and diterpene alcohols were the most abundant lipid classes in the beverages, constituting 86-92% and 6-12% of total lipids respectively.

The choice of coffee brewing method is influenced by personal preference, habits, culture, tradition, and availability (15). For example, Italy and Spain are known for drinking espresso

and moka; drip filter coffee is prevalent in America and Finland; Scandinavian pot brewing is the traditional method of making coffee beverage in Scandinavia; and Turkish coffee is mostly consumed in Turkey, Eastern Europe, and the Middle East (16, 17). The process of making coffee beverage is categorized as solid-liquid extraction. Coffee brewing is carried out in three main steps; (i) incorporation of water into the ground coffee; (ii) transport of soluble compounds from the ground coffee into the water; and (iii) resultant coffee extract and residue separation (18). Although coffee extraction generally takes a few minutes, it has a huge impact on the chemical and sensory properties of coffee beverages (11, 12). The extraction method for coffee brewing is influenced by variables such as volume and temperature of water, pressure, particle size of coffee grounds, and brewing time (19). Coffee brewing results in the extraction of mainly water-soluble compounds, but even though the lipid fraction of coffee is insoluble in water, high temperature and pressure when used in brewing allow some lipids to be incorporated as well into the coffee beverage (20, 21).

Coffee extraction methods can largely be grouped as hot and cold brewing (21, 22, 23). The hot brew technique utilizes water near boiling point with a short extraction time for coffee brewing (24). A cold brewing technique (immersion or drip) done at room temperature over several hours is also emerging. Cold and hot brew techniques produce some differences in the chemical and sensory properties of their resultant coffee beverages with respect to bitterness, sweetness, and content of chlorogenic acid, amongst others (13, 25, 26). Coffee brewing is generally divided into pressure, instant, drip, and decoction methods (10, 27). The pressure method requires a driving force to push water through compact coffee grounds, as in the case of espresso and moka. In the instant method, hot water is poured over soluble coffee powder. The drip method uses a drip coffee maker with filter paper placed in its cone. Coffee grounds are placed in the filter paper, water added to the tank and coffee machine turned on to brew

(22). Finally, in the decoction method, coffee grounds are simply boiled in water and decanted (28). Irrespective of the many coffee extraction methods available, studies on espresso and filter coffee brewing methods are often reported compared to other brewing methods (29, 30). This is possibly so because espresso and filter coffee beverages are commonly consumed in most countries, while other types of coffee beverages remain geographically restricted (10).

Metabolomics is an omics approach used for the comprehensive characterization of the composition of small molecules within biological samples. This approach helps to provide a general idea of both known and unknown compounds present in a medium without prior characterization (31, 32). Few studies have employed metabolomics in coffee research. These studies reported on biomarkers of coffee intake and bioactive compounds in coffee beans and coffee beverages (33, 34, 35). The most extensive study on coffee beverage in metabolomics thus far has been by Chan et al. (36) who used an untargeted metabolomics approach to assay the bioactive compounds in probiotic fermented coffee brew using LC-QTOF-MS/MS.

Regardless of the success of untargeted metabolomics in the assaying of some bioactive compounds in different food matrices (34, 35, 36), published scientific findings on the profile of lipophilic and hydrophilic compounds in typically prepared and ready to be consumed coffee beverages are scarce. Thus, the aim of this study is to use untargeted metabolomics approach to identify, profile, and compare potentially bioactive compounds in coffee beverages from pot coffee, espresso coffee, filter coffee, and instant coffee by using UHPLC-QTOF/MS.

## 2. Materials and Methods

105 2.1. *Chemicals and reagents*

106 LC-MS grade acetonitrile, acetone, 2-propanol, methanol, and water were purchased from
107 Honeywell/ Riedel de Haën (Seelze, Germany). Formic acid was obtained from VWR
108 (Leuven, Belgium). Ammonium acetate was purchased from Sigma-Aldrich, Steinheim,
109 Germany. Cafestol palmitate standard (≥ 97% purity) was acquired from Santa Cruz
110 Biotechnology (Dallas, Texas, USA).

111

112 2.2. *Coffee samples*

113 All coffee samples were commercial. The coffee samples utilized in this study were pot
114 coffee (PC, dark and light roast), filter coffee (FC, dark and light roast), espresso coffee (EC)
115 and instant coffee (IC, medium and dark roast). Dark roast PC, dark roast FC and EC were
116 blends of arabica and robusta (90%: 10%). They originated from Brazil, Colombia, Central
117 America, and India. The dark roast level was 4/5 on manufacturer's scale 1-5. The coffees
118 were obtained ground with the following size averages: dark roast PC: 1040 µm, dark roast
119 FC: 800 µm, EC: 320 µm. Light roast PC and light roast FC were blends or arabica: robusta
120 (95%: 5%). They originated from South and Central America and East Africa. The light roast
121 level was 1/5 on manufacturer's scale 1-5. The types of instant coffee (IC) utilized were
122 prepared from lyophilized coffee powder. The medium roasted IC originated from South and
123 Central America. The roast level was 7/10 on the manufacturer's scale: 1-10. The dark
124 roasted IC was 4/5 on the manufacturer's scale 1-5.

125

126 2.3. *Coffee beverage preparation*

127 This study analysed coffee beverages from dark roast PC, EC, FC, light roast PC and FC, as
128 well as medium and dark roast IC. The PC beverage was prepared in a decanter volumetric
129 flask by the addition of 6 g of roasted ground coffee to 100 mL of boiled water, stirred,

brought to a boil, lifted off the stove, kept for 5 min and decanted. The coffee brew volume was 85 mL. The EC beverage was prepared from 7 g of roasted ground coffee with an espresso-making machine (Delonghi, dedica style, 15 bar, China). The brew time was 30 sec with a coffee brew volume of 30 mL. The FC beverage was prepared from 6 g of roasted ground coffee with 100 mL of cold fresh water using a coffee maker machine (Moccamaster, Technivorm, Netherlands). Brewing time was 1 min 30 sec with a coffee brew volume of 90 mL. The IC beverage was prepared by the addition of 2 g of lyophilized coffee powder to 150 mL of boiled water (80 °C). All prepared coffee beverages were allowed to cool to RT and immediately stored at -80 °C for further analysis. Each coffee beverage type was prepared in triplicate.

2.4. *Sample extraction for UHPLC- QTOF/MS analysis*

2.4.1. *General metabolomics*

Coffee extracts were prepared for untargeted metabolite profiling by slightly modifying the protocol by Klåvus et al. (31). 100 µL of coffee extracts were added to 400 µL of cold acetonitrile and mixed by pipetting. The resultant mixture was centrifuged at 7000 × g, 4 °C (VWR, Micro Star 17R, Germany) for 5 min. The supernatant was collected into 1 mL plastic syringe and filtered through 0.2 µm PTFE-filter into an autosampler sample vial for injection or stored at -20 °C until analysed.

2.4.2 *Lipid extraction*

The total lipids from the coffee beverages were determined by the modification of the Matyash et al. (37) method with respect to the sample to solvent ratio. Briefly, 5 mL of coffee beverage was extracted with 5 mL of MTBE/methanol (10:3, v/v), mixed (Stuart roller mixer STR1, power 50 W, Bibby Sterilin LTD, UK) for 20 min, and centrifuged (5 min; 700 × g;

RT). The supernatant was separated from the residue. The residue was further extracted with 2 mL of extraction solvent mixture (MTBE/methanol/water 10:3:2.5, v/v), mixed for 1 min, and centrifuged (5 min; 700 × g; RT). The supernatant was collected and added to the previous supernatant. To the combined supernatant, 1.5 mL of ultra-purified water was added, mixed for 1 min, and centrifuged (5 min; 700 × g; RT) to induce phase separation. The organic phase containing the extracted lipids was collected, evaporated to complete dryness under moderate nitrogen flow at 40 °C, weighed, dissolved in 10 mL of the extraction solvent mixture (MTBE/methanol (10:3, v/v)) and stored at -20 °C until analysed.

2.4.3. *External standard preparation*

A cafestol palmitate standard was prepared by weighing 30 µg of cafestol palmitate powder (scale: Mettler Toledo, XSE205 DualRange, max: 81g/220g, readability: 0.01mg/0.1mg) into an Eppendorf tube,1 mL of solvent mixture (MTBE/methanol 10:3, v/v) was added, mixed, and filtered through a 0.2 µm PTFE-filter into an autosampler vial for analysis.

2.5. *Metabolite analysis (UHPLC-QTOF/MS)*

UHPLC-QTOF/MS (Elute UHPLC, mass spectrometer: Impact II QTOF, Bruker Daltonic, Bremen, Germany) with Reversed-Phase (RP) and hydrophilic interaction chromatography (HILIC) separation was used for the analyses of hydrophilic and lipophilic compounds in coffee beverages. Calibrant (sodium formate) was introduced into the source through an external pump (New Era Pump Systems, Inc., model number: 300, USA). Triplicates of extraction blanks were injected prior to coffee sample analysis and an extraction blank injected after every group of coffee beverage type was analysed.

*2.5.1. Hydrophilic analysis*

Both reversed-phase (RP) and hydrophilic interaction chromatography (HILIC) were used for the analysis of hydrophilic compounds in acetonitrile extracts. Analysis with RP column (Waters Acquity UHPLC column BEH C18 (2.1 x 100 mm column, 1.7 µm particle size) utilized solvent A which comprised of water with 0.1% formic acid and solvent B which comprised of methanol with 0.1% formic acid. HILIC column (Acquity UHPLC HILIC column BEH Amide (2.1 x 100 mm column, 1.7 µm particle size) utilized acetonitrile and water (1:1 by vol) with 20 mM ammonium formate as solvent A. Solvent B consisted of acetonitrile and water (9:1 by vol) with 20 mM ammonium formate. Sodium formate was used as calibrant for instrument calibration. The LC method used for HILIC and RP columns was the method developed by Klåvus A. et al. (31). Briefly for RP column: the initial start ratio of solvent A to B (98%: 2.0%, A: B) was increased to 100% B at 10 min, kept for 4.5 min, reduced to 2.0% B, and kept for 2 min. The total run time was 16.5 min, flow rate of 0.4 mL/min at an injection volume of 2 µL. The LC method for HILIC column used initial start ratio of solvent A to B (0%: 100%, A: B), kept at 100% B for 2.5 min, reduced to 0% B at 10 min, increased to 100% B, and kept for 2.5 min. The total run time was 12.5 min, flow rate 0.6 mL/min at injection volume of 2 µL. The autosampler and column temperatures were set at 4 °C and 50 °C, respectively. An electrospray ionization (ESI) source was used for positive and negative ionization modes. The ESI end plate offset was set at 500 V, the dry temperature at 325 °C, the capillary voltage 3500 V, the dry gas ($N_2$) flow at 10.0 L/min and nebulizer pressure at 3.1 bar. An MS/MS scanning mode over the mass range of 50-1600 *m/z* was applied using CID energies of 10, 20 and 40 eV in separate runs. In the MS/MS scans, 4 pre-cursors were selected for fragmentation with active precursor ion exclusion enabled for 0.25 min. MS/MS analysis was performed for one sample per the different coffee beverage types for structural confirmation.

205 2.5.2. *Lipophilic analysis*

206 An equal amount (0.25 mg) of oil extracted from the coffee beverages was analysed for EC,
207 PC, IC, and FC. RP column (Waters Acquity UHPLC column BEH C18 (2.1 x 100 mm
208 column, 1.7 µm particle size) was utilized for the lipidomics analysis. Solvent A was water
209 with 1% ammonium acetate (1 M) and 0.1% formic acid added. Solvent B constituted of
210 acetonitrile and isopropanol (1:1 by vol) with 1% ammonium acetate (1 M) and 0.1% formic
211 acid added. The LC method gradient used was as follows: 0 to 2 min 0-35% B, from 2 to 7
212 min 100% B, kept from 7-14 min and decreased to 35% B for 7 min. The total run time was
213 21 min, acquisition time was 16.5 min, column equilibration time was 7 min, and flow rate
214 0.4 mL/min at 2 µL injection volume. The set temperatures for the column and autosampler
215 were 50 °C and 4 °C respectively. Analysis was done in the positive mode using an ESI
216 source. The ESI end plate offset was set at 500 V, the capillary at 4500 V, the dry
217 temperature at 220 °C, the dry gas (N2) flow at 8.0 L/min and nebulizer pressure at 1.8 bar.
218 Automatic MS scanning mode over the mass range of 50-1300 *m/z* was employed. Cafestol
219 palmitate standard (30 µg/mL) was analysed under the same conditions as oil extracted from
220 EC, FC, PC, and IC beverages. EC beverage spiked with 500 µL of 30 µg/mL cafestol
221 palmitate standard was analysed to aid with the identification of cafestol palmitate in the
222 coffee beverages.

223

224 2.6. *Metabolomics data processing*

225 Data acquired on lipophilic and hydrophilic compounds was processed with Bruker Compass
226 DataAnalysis 5.1 (Bruker Daltonic GmbH, Bremen, Germany). MS-DIAL (version 4.80) was
227 used for peak picking. The peak picking parameters used in MS-DIAL were $MS^1$ mass
228 tolerance of 0.01 Da, $MS^2$ mass tolerance 0.025 Da, an unlimited retention time window, a
229 minimum peak height of 300 amp, gap filling by compulsion applied and sample to blank

ratio of 5-fold change. Feature reduction was achieved by manual selection of adducts for the different ionization modes (positive: $[M+H]^+$, $[M+NH_4]^+$, $[M+Na]^+$, $[M+CH_3OH+H)]^+$, $[M+K]^+$, $[M+ACN+H]^+$, $[M+H-H_2O]^+$, $[2M+H]^+$, negative: $[M-H]^-$, $[M-H_2O-H]^-$, $[M+Cl]^-$, $[M+FA-H]^-$, $[2M-H]^-$, $[3M-H]^-$). The criteria selected for peak spot viewing in MS-DIAL were blank filter, $MS^2$ acquired, reference match, suggested and unknown compounds. Compounds were selected based on their precursor ion mass-to-charge ratio (*m/z*), retention time, mass spectra, fragmentation pattern, and intense feature when comparing the same compound in different ionization modes (positive and negative ionization modes). The peak shape of compounds was manually inspected during the identification process. Data was exported from MS-DIAL to excel for data analysis. Compound identification was achieved by matching spectra against metabolomics database (Lipid maps: https://www.lipidmaps.org/), Metlin (https://metlin.scripps.edu/), and the Human Metabolome Database (https://hmdb.ca).

2.7 *Statistical and chemometric analysis*

Only compounds with statistical significance ($P < 0.05$) in at least one of the beverage types were considered for further analysis. Comparison of the oil content in the coffee beverage types was done with ANOVA (single factor, level of significance: $P < 0.05$) and post-hoc (Tukey test) using R-studio (R; ver. 4.3.1, Statistical package R). ANOVA and post-hoc (Tukey test) were applied to compare diterpene intensities in the coffee beverage types using MetaboAnlayst ver. 6.0 (https://www.metaboanalyst.ca/). Simca®18 (Multivariate Data Analysis Solution), SRplot online software (http://www.bioinformatics.com.cn/srplot), and VolcaNoseR online software (https://huygens.science.uva.nl/VolcaNoseR/) were used for chemometric data analysis. Simca®18 was used for Principal Component Analysis (PCA) of the whole peak data exported from MS-DIAL to excel (row: variables, column:

observations). For data preprocessing, missing values were excluded from data, data was checked for outliers, and normalization done by median. Data standardization was done with unit variance scaling (mean-centered and divided by the standard deviation of each variable). The linearity of the data was checked using scatter plot. Autofit in Simca®18 was used for cross validation to determine the number of significant components to retain in the PCA plots. SRplot online software was used for bar chat and heatmap analysis. All metabolites that showed significance for the coffee beverage types ($P < 0.05$) were used for plotting the bar chat. For heatmap analysis, all identified hydrophilic compounds were used. All lipophilic compounds that showed statistically significant difference ($P < 0.05$) were selected and their P-values corrected using False Discovery Rate online calculator (http://www.sdmproject.com/utilities/?show=FDR). The initial lipophilic compounds with the most statistical significance were chosen for the heatmap analysis. The cluster orientation for heatmap analysis was bidirectional and the complete cluster method was used. For VolcaNoseR online software, significance level (P-values) after initial calculation were corrected using False Discovery Rate approach (FDR) to obtain their resultant q-values. The values were log transformed for fold change (log 2) and q-values (-log 10) before loaded for volcano plotting. A metabolite abundance would qualify as a descriptor within a fold change threshold under -1.5 or higher than 1.5 and significance threshold of q-value not more than 0.05. Data used for volcano plot analysis was a combination of all identified lipophilic and hydrophilic metabolites in the coffee beverage types.

## 3. Results and Discussion

### 3.1. *Lipophilic compound analysis*

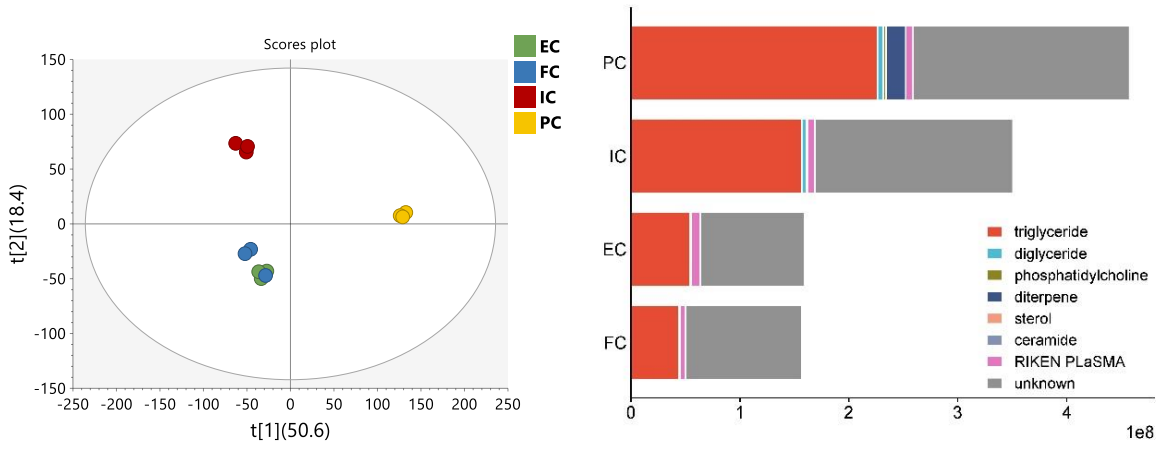
This study focused on the dark roast PC, EC, FC, and the medium roast IC beverages which are reported and discussed here on in this article. Data on light roast PC and FC (South and

Central America and East Africa), and dark roast IC beverages are reported as supplementary findings. The dark roast EC, FC, and PC beverages were brewed from coffee of the same origin, composition, and roast level, different from the medium roast IC beverage. The oil content (w/w) of the coffee beverages were 0.13% ± 0.002 (EC), 0.12% ± 0.001 (PC), 0.04% ± 0.002 (IC) and 0.03% ± 0.003 (FC). Univariate analysis showed statistically significant differences between the oil content of the coffee beverage types (ANOVA, $P < 0.001$). Significant differences in the oil content between EC and FC, EC and IC, PC and FC, and PC and IC beverages were observed (Supporting information Table S1). Brewing method affected the amount of coffee oil in all beverages. The oil content of EC and PC beverages were higher compared to that of IC and FC beverages.

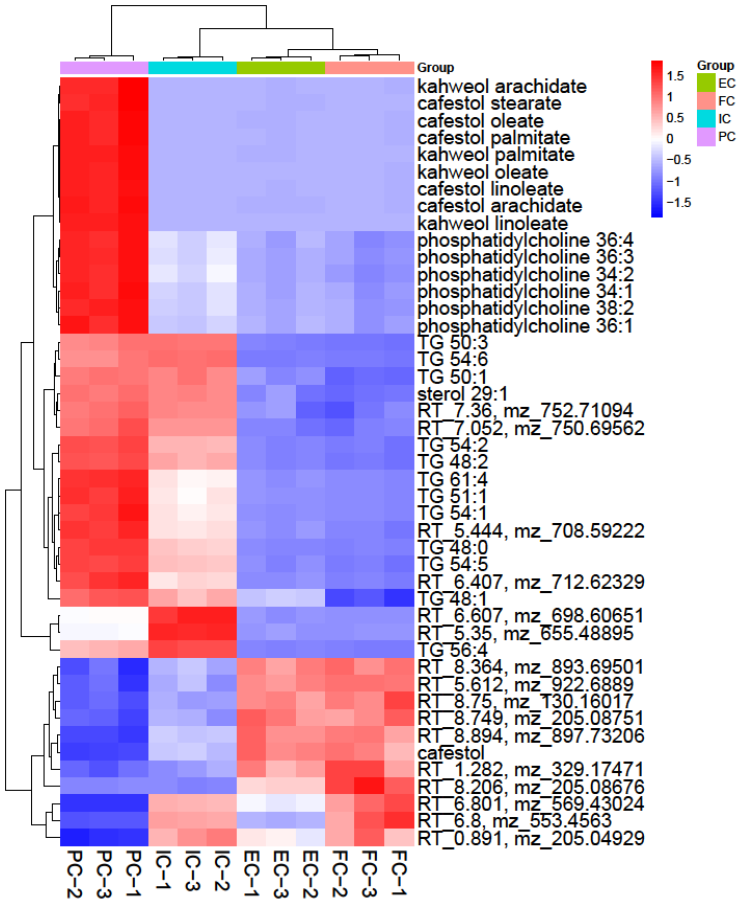
The low proportion of oil content (0.03-0.13%) in the coffee beverages resulted from the way in which coffee brewing method favours the extraction of water-soluble compounds. Nevertheless, extraction of some amount of coffee oil from coffee grounds was possible due to the pressure and high temperatures applied (21). EC and PC beverages recorded high oil contents compared to other beverages, which in the case of EC could have resulted from the pressure (15 bar) applied (38) in the espresso machine and the small particle size (320 µm) of espresso coffee grounds compared to filter (800 µm) and pot (1040 µm) coffee grounds. Although the same coffee/water ratio (6 g/100 mL) was used for PC and FC brewing, the absence of a filtering step in the case of PC probably explains the higher oil content in PC beverage compared to FC beverage (14). IC and FC beverages recorded low oil contents. The manufacturing process of IC soluble powder, which involves the extraction of the soluble contents of coffee beans before concentrating them into soluble coffee powder, could explain the low oil content of IC beverage.

Lipophilic analysis of the different coffee beverages was carried out with equal amounts of oil (0.25 mg). Normalization of the amount of oil used for analysis was done to aid the direct comparison of the lipid profiles of the different coffee types. Over a thousand lipophilic molecular features were extracted from coffee beverages using UHPLC-QTOF-MS in positive mode. In total, 134 lipophilic compounds were annotated, of which 115 were identified as metabolites (Table 1) and the remaining 19 unknown metabolites with a RIKEN ID of known unidentified metabolites given (Supporting information Table S2).

**A** **B**



315 **C**




316

317 Figure 1. (A) The Principal Component Analysis (PCA) scores plot of lipophilic compound
318 intensities obtained in positive ionization mode (matrix:12180) for coffee brewed with
319 different methods. **(**B) Profile of lipid classes in EC, FC, PC, and IC coffee beverages.
320 Intensity refers to the total intensities of all metabolites in a lipid class. RIKEN PlaSMA
321 refers to those uncharacterized molecular features, which received a spectral match with a
322 known unknown molecular feature in the RIKEN PlaSMA database of Plant Specialized
323 Metabolome Annotation. (C) The heatmap of hierarchical clustering analysis of intensities of
324 lipophilic compounds obtained in a positive mode (matrix: 45, column: observations, row:
325 variables) for coffee brewed with four different methods. EC, espresso coffee; FC, filter
326 coffee; PC, pot coffee; IC, instant coffee. Rt is retention time and *m/z* is mass-to-charge ratio.

327

For PCA analysis, the first and second principal components explained 51% and 18% respectively of the variation between the different coffee beverage types. The cumulative $R^2$ value for the first three components was 0.803, which explained the level of fit for the PCA model. The estimated predictive ability of the model ($Q^2$) was 0.651. The loadings plot for the PCA is provided as supporting information (Figure S1 A). PCA analysis showed three distinct groups of coffee beverages. FC and EC beverages formed one group. PC and IC beverages formed the other two groups (Figure 1 A). The correlation between groups of lipid extracts of different coffee beverage types was independently determined based on metabolite abundance using heatmap analysis (Figure 1 C). The PC and IC groups were clustered next to each other, which implies some similarities in their lipid profiles. On the other hand, the PC group clustered away from the EC and FC groups, which indicates differences in their lipid profiles. Although EC, PC, and FC beverages were prepared from the same coffee material, the group outcome shows that the brewing method influenced their lipid profile, with triglycerides (TG), phosphatidylcholines, and diterpene esters as key contributors to the clustering. The oil extract from the PC beverage recorded relatively high intensities of most identified lipophilic metabolites compared to the other beverage types (Figure 1 C). The direct infusion and long contact time of coffee grounds with water (25) might explain this occurrence. It is noteworthy that most of the lipophilic compounds of high intensity in the FC and EC beverages remain unidentified. However, their retention time (Rt) and mass-to-charge ratio (*m/z*) have been provided in Figure 1 C. In general, the brewing method affected the intensities of lipid metabolites in the coffee beverages.

Triglycerides (TG), diglycerides (DG), phosphatidylcholines, sterols, diterpenes, and ceramides were the lipid classes identified in the coffee beverages (Figure 1 B). TG was the predominant lipid class identified with 82 metabolites, followed by DG (12), diterpenes (11)

and phosphatidylcholines (8), sterols (1), and ceramides (1) respectively. Other authors have similarly reported TG as the most abundant lipid class in coffee beverages (14). EC and PC beverages were different in their lipid profile. PC beverage contained all the identified lipid classes, with TG as the most dominant, followed by diterpenes. The main lipid class in EC and FC beverages was TG. Diterpenes, phosphatidylcholines, ceramides, and sterols were in trace amounts in IC, FC, and EC beverages. In the case of the FC beverage, filter paper created a barrier effect retaining most of the lipids in the paper. Research has shown that filter paper retains most of the coffee oil during brewing (39). Furthermore, the sample origin of coffee material used for the IC beverage (South and Central America) could have accounted for variations in the lipid profile of IC when compared to PC, FC, and EC beverages. It is worth mentioning that not all lipids were identified, and these unidentified lipids could have contributed to the lipid proportions and distribution in the beverage types. A volcano plot analysis revealed that diterpene esters were statistically significant metabolites in PC beverage when compared to the other beverage types (Supporting information Figure S2 A, S2 B). Furthermore, TG 54:3 and TG 48:2 showed statistical significance in PC beverage when compared to FC beverage, and TG 54:3 and TG 56:4 when compared to EC beverage (Supporting information Figure S2 A, S2 B). Overall, PC contained the highest intensities of most of the identified lipid classes compared to other brewing methods. The proportion of TGs and diterpenes in the IC beverage was higher than in FC and EC beverages. The lipid metabolites reported were identified in all the coffee beverage types. No lipid was unique to or missing from any beverage type, however, the lipid proportions varied resulting in differences in the lipid profiles of EC, FC, IC, and PC beverages. For instance, oil from the PC beverage had a higher proportion of TGs, phosphatidylcholines, and diterpenes compared to other beverages (Figure 1B). The differences in the lipid profiles of the coffee beverage types suggest possible differences in their physiological effects such as reduced inflammation

and increase in serum cholesterol levels (5). This is e.g. because phosphatidylcholines which have been reported to potentially reduce inflammation and enhance liver function (6) were higher in PC beverage while diterpenes with their cholesterol-elevating tendencies (5) were lower in EC, IC, and FC beverages (Figure 1C).

382 Table 1. Identified lipophilic compounds in espresso, pot, filter, and instant coffee beverages using the reversed-phase chromatography

383 method in the positive ionization mode.

384

| Compound name | Lipid class | Adduct | [a]Rt | Formula | Observed [b]*m/z* | Mass Difference (mDa) | [c]MS/MS Fragments |
|---|---|---|---|---|---|---|---|
| Cer 43:1;4O | Ceramide | $[M+H-H_2O]^+$ | 7.714 | $C_{43}H_{85}NO_5$ | 678.63898 | 0.55 | 298.3766,280.2643,262.2537 |
| DG 32:0 | Diglycerol | $[M+NH_4]^+$ | 7.061 | $C_{35}H_{68}O_5$ | 586.53802 | 2.51 | 551.5024,313.2733 |
| DG 34:1 | Diglycerol | $[M+NH_4]^+$ | 7.021 | $C_{37}H_{70}O_5$ | 612.55347 | 2.74 | 577.5182,33.274, 339.2902 |
| DG 34:2 | Diglycerol | $[M+NH_4]^+$ | 6.675 | $C_{37}H_{68}O_5$ | 610.53894 | 1.59 | 575.5024,313.2737,337.2737 |
| DG 36:0 | Diglycerol | $[M+NH_4]^+$ | 7.702 | $C_{39}H_{76}O_5$ | 642.59979 | 3.3 | 607.5634,313.2725,369.3316 |
| DG 36:2 | Diglycerol | $[M+NH_4]^+$ | 7.053 | $C_{39}H_{72}O_5$ | 638.57214 | 0.36 | 603.5363,341.3052,337.2744 |
| DG 36:3 | Diglycerol | $[M+NH_4]^+$ | 6.671 | $C_{39}H_{70}O_5$ | 636.55414 | 2.07 | 601.5177,339.2888,337.2739,619.5306 |
| DG 36:4 | Diglycerol | $[M+NH_4]^+$ | 6.301 | $C_{39}H_{68}O_5$ | 634.54028 | 0.25 | 599.5021,337.2734,617.5121 |
| DG 36:5 | Diglycerol | $[M+NH_4]^+$ | 5.968 | $C_{39}H_{66}O_5$ | 632.52496 | 0.06 | 597.4868,337.2741,335.258,615.4984 |
| DG 38:2 | Diglycerol | $[M+NH_4]^+$ | 7.383 | $C_{41}H_{76}O_5$ | 666.60236 | 0.73 | 631.5652,369.3357,337.2739 |
| DG 42:0 | Diglycerol | $[M+NH_4]^+$ | 8.462 | $C_{45}H_{88}O_5$ | 726.69434 | 2.68 | 691.6602,369.3365,397.3691, 709.6705 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| DG 44:0 | Diglycerol | $[M+NH_4]^+$ | 8.741 | $C_{47}H_{92}O_5$ | 754.72632 | 1.95 | 719.6918,397.3675,737.6982 |
| DG 46:0 | Diglycerol | $[M+NH_4]^+$ | 9.06 | $C_{49}H_{96}O_5$ | 782.76007 | 0.49 | 747.7261,397.367.425.4002 |
| PC 32:0 | Phosphatidylcholine | $[M+H]^+$ | 6.474 | $C_{40}H_{80}NO_8P$ | 734.56915 | 0.25 | 184.0742,478.3295 |
| PC 34:1 | Phosphatidylcholine | $[M+H]^+$ | 6.464 | $C_{42}H_{82}NO_8P$ | 760.58502 | 0.06 | 184.0743,496.3421 |
| PC 34:2 | Phosphatidylcholine | $[M+H]^+$ | 6.062 | $C_{42}H_{80}NO_8P$ | 758.56818 | 1.22 | 184.0731,502.3341 |
| PC 36:1 | Phosphatidylcholine | $[M+H]^+$ | 6.896 | $C_{44}H_{86}NO_8P$ | 788.61578 | 0.61 | 184.0728,518.3625 |
| PC 36:2 | Phosphatidylcholine | $[M+H]^+$ | 6.497 | $C_{44}H_{84}NO_8P$ | 786.59937 | 1.34 | 184.0737,520.3348 |
| PC 36:3 | Phosphatidylcholine | $[M+H]^+$ | 6.055 | $C_{44}H_{82}NO_8P$ | 784.58551 | 0.43 | 184.0726,522.3473 |
| PC 36:4 | Phosphatidylcholine | $[M+H]^+$ | 5.644 | $C_{44}H_{80}NO_8P$ | 782.56873 | 0.67 | 184.0734,502.3293,520.3434 |
| PC 38:2 | Phosphatidylcholine | $[M+H]^+$ | 6.949 | $C_{46}H_{88}NO_8P$ | 814.63074 | 1.28 | 184.0724,552.4085,534.3955 |
| ST 29:1;O | Sterol | $[M+H-H_2O]^+$ | 8.979 | $C_{29}H_{50}O$ | 397.38138 | 1.52 | 147.1155,81.0693,95.0855,161.131, |
| TG 16:0 | Triglycerol | $[M+H]^+$ | 14.143 | $C_{53}H_{98}O_6$ | 848.76953 | 0.67 | 553.5082,575.5046 |
| TG 48:0 | Triglycerol | $[M+NH_4]^+$ | 9.004 | $C_{51}H_{98}O_6$ | 824.76843 | 1.77 | 551.5035,807.7445 |
| TG 48:1 | Triglycerol | $[M+NH_4]^+$ | 8.659 | $C_{51}H_{96}O_6$ | 822.74945 | 5.07 | 549.4873,523.4717,577.5219 |
| TG 48:2 | Triglycerol | $[M+NH_4]^+$ | 8.376 | $C_{51}H_{94}O_6$ | 820.73724 | 1.65 | 547.4709,523.4706,575.5032 |
| TG 49:2 | Triglycerol | $[M+NH_4]^+$ | 8.492 | $C_{52}H_{96}O_6$ | 834.75281 | 1.71 | 561.4874,575.5031,537.4883,817.7279 |
| TG 49:4;1O | Triglycerol | $[M+NH_4]^+$ | 8.238 | $C_{52}H_{92}O_7$ | 846.71631 | 1.77 | 573.4882,551.5039,261.2219,313.2733 |
| TG 50:0 | Triglycerol | $[M+NH_4]^+$ | 9.349 | $C_{53}H_{102}O_6$ | 852.79895 | 2.56 | 579.5341,551.5031,835.7665 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| TG 50:1 | Triglycerol | $[M+NH_4]^+$ | 8.936 | $C_{53}H_{100}O_6$ | 850.78448 | 1.35 | 577.5188,551.5031,833.7623 |
| TG 50:2 | Triglycerol | $[M+NH_4]^+$ | 8.626 | $C_{53}H_{98}O_6$ | 848.77252 | 2.32 | 575.5021,551.502,831.746 |
| TG 50:2;1O | Triglycerol | $[M+NH_4]^+$ | 8.032 | $C_{53}H_{98}O_7$ | 864.76147 | 3.61 | 591.4984,551.5031,279.2314,573.4875 |
| TG 50:3 | Triglycerol | $[M+NH_4]^+$ | 8.389 | $C_{53}H_{96}O_6$ | 846.75385 | 0.67 | 573.4895,551.5049,829.7311 |
| TG 50:3;1O | Triglycerol | $[M+NH_4]^+$ | 7.815 | $C_{53}H_{96}O_7$ | 862.74768 | 1.71 | 829.7282,573.4892,261.2212,551.5043 |
| TG 50:4 | Triglycerol | $[M+NH_4]^+$ | 8.111 | $C_{53}H_{94}O_6$ | 844.73682 | 2.07 | 547.4717,599.502,827.7135 |
| TG 51:1 | Triglycerol | $[M+NH_4]^+$ | 9.088 | $C_{54}H_{102}O_6$ | 864.79858 | 2.93 | 591.5326,577.5165,565.517,847.7837 |
| TG 51:2 | Triglycerol | $[M+NH_4]^+$ | 8.754 | $C_{54}H_{100}O_6$ | 862.78406 | 1.77 | 575.505,589.5217,565.5217,845.7587 |
| TG 51:3 | Triglycerol | $[M+NH_4]^+$ | 8.492 | $C_{54}H_{98}O_6$ | 860.76581 | 4.39 | 563.4983,587.5017,575.499,843.7436 |
| TG 51:4 | Triglycerol | $[M+NH_4]^+$ | 8.223 | $C_{54}H_{96}O_6$ | 858.7525 | 2.02 | 561.4879,599.5054,841.72227 |
| TG 52:0 | Triglycerol | $[M+NH_4]^+$ | 9.742 | $C_{55}H_{106}O_6$ | 880.82892 | 3.9 | 607.5639, 579.53 |
| TG 52:1 | Triglycerol | $[M+NH_4]^+$ | 9.265 | $C_{55}H_{104}O_6$ | 878.81293 | 4.15 | 577.5204,605.552, 579.5355,861.7945 |
| TG 52:2 | Triglycerol | $[M+NH_4]^+$ | 8.896 | $C_{55}H_{102}O_6$ | 876.80243 | 0.92 | 603.5333,575.5021,579.5329,859.7763 |
| TG 52:2;1O | Triglycerol | $[M+NH_4]^+$ | 8.207 | $C_{55}H_{102}O_7$ | 892.79651 | 0.12 | 599.5049,577.5208,619.5296,593.5734 |
| TG 52:3 | Triglycerol | $[M+NH_4]^+$ | 8.575 | $C_{55}H_{100}O_6$ | 874.78766 | 1.83 | 575.5042,601.5199,577.5193,857.7635 |
| TG 52:3;1O | Triglycerol | $[M+NH_4]^+$ | 7.997 | $C_{55}H_{100}O_7$ | 890.78101 | 0.31 | 575.5045,617.5133,599.5039,591.4976 |
| TG 52:4 | Triglycerol | $[M+NH_4]^+$ | 8.331 | $C_{55}H_{98}O_6$ | 872.76776 | 2.44 | 576.5043,601.5203, 573.4887 |
| TG 52:4;1O | Triglycerol | $[M+NH_4]^+$ | 7.798 | $C_{55}H_{98}O_7$ | 888.76038 | 4.7 | 575.037,615.4984,591.4987,279.2323 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| TG 52:5 | Triglycerol | $[M+NH_4]^+$ | 8.128 | $C_{55}H_{96}O_6$ | 870.75415 | 0.37 | 575.5023,573.4869,597.4872,853.7299 |
| TG 52:5;1O | Triglycerol | $[M+NH_4]^+$ | 7.602 | $C_{55}H_{96}O_7$ | 886.74841 | 0.98 | 575.5028,613.4816,571.4756,277.2164 |
| TG 52:6 | Triglycerol | $[M+NH_4]^+$ | 7.921 | $C_{55}H_{94}O_6$ | 868.73578 | 3.11 | 573.4907,595.4706,851.7171 |
| TG 53:1 | Triglycerol | $[M+NH_4]^+$ | 9.44 | $C_{56}H_{106}O_6$ | 892.83118 | 1.64 | 593.5505,577.5193,619.5668 |
| TG 53:2 | Triglycerol | $[M+NH_4]^+$ | 9.054 | $C_{56}H_{104}O_6$ | 890.81451 | 2.57 | 617.5547,575.5058,593.5491,873.793 |
| TG 53:3 | Triglycerol | $[M+NH_4]^+$ | 8.707 | $C_{56}H_{102}O_6$ | 888.79932 | 2.19 | 591.5322,601.5176,589.5177 |
| TG 53:4 | Triglycerol | $[M+NH_4]^+$ | 8.444 | $C_{56}H_{100}O_6$ | 886.78351 | 2.32 | 589.5178,599.503,869.7559 |
| TG 53:5 | Triglycerol | $[M+NH_4]^+$ | 8.225 | $C_{56}H_{98}O_6$ | 884.76855 | 1.65 | 587.5024,597.4869,589.5192,867.74 |
| TG 54:0 | Triglycerol | $[M+NH_4]^+$ | 10.193 | $C_{57}H_{110}O_6$ | 908.86121 | 2.86 | 635.5952,607.5638,579.5314 |
| TG 54:1 | Triglycerol | $[M+NH_4]^+$ | 9.636 | $C_{57}H_{108}O_6$ | 906.84552 | 2.87 | 577.5198,607.567,633.582 |
| TG 54:2 | Triglycerol | $[M+NH_4]^+$ | 9.218 | $C_{57}H_{106}O_6$ | 904.8324 | 0.42 | 605.5436,603.5347 |
| TG 54:3 | Triglycerol | $[M+NH_4]^+$ | 8.844 | $C_{57}H_{104}O_6$ | 902.81653 | 0.55 | 603.5333,605.5486,601.5181,885.7914 |
| TG 54:3;1O | Triglycerol | $[M+NH_4]^+$ | 8.187 | $C_{57}H_{104}O_7$ | 918.80811 | 3.9 | 603.5325,619.53,601.5169,883.7731 |
| TG 54:4 | Triglycerol | $[M+NH_4]^+$ | 8.554 | $C_{57}H_{102}O_6$ | 900.80139 | 0.12 | 603.5338,601.5182,883.7757 |
| TG 54:4;1O | Triglycerol | $[M+NH_4]^+$ | 7.993 | $C_{57}H_{102}O7$ | 916.79199 | 4.4 | 601.5749,617.508,619.5268,599.5003 |
| TG 54:5 | Triglycerol | $[M+NH_4]^+$ | 8.291 | $C_{57}H_{100}O_6$ | 898.78564 | 0.19 | 601.5185,599.5049 |
| TG 54:5;1O | Triglycerol | $[M+NH_4]^+$ | 7.756 | $C_{57}H_{100}O_7$ | 914.77594 | 4.76 | 601.5162,617.512,615.4962,597.48.53 |
| TG 54:6 | Triglycerol | $[M+NH_4]^+$ | 8.071 | $C_{57}H_{98}O_6$ | 896.77112 | 0.92 | 599.5021,879.7443 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| TG 54:6;1O | Triglycerol | $[M+NH_4]^+$ | 7.492 | $C_{57}H_{98}O_7$ | 912.76294 | 2.14 | 597.4871,599.5014,615.496,887.7282 |
| TG 54:7 | Triglycerol | $[M+NH_4]^+$ | 7.873 | $C_{57}H_{96}O_6$ | 894.75244 | 2.08 | 597.4882,599.5041 |
| TG 54:7;1O | Triglycerol | $[M+NH_4]^+$ | 7.333 | $C_{57}H_{96}O_7$ | 910.74658 | 2.81 | 613.4824,599.4995,595.4676,277.2145 |
| TG 54:8 | Triglycerol | $[M+NH_4]^+$ | 7.668 | $C_{57}H_{94}O_6$ | 892.73566 | 3.23 | 597.4896,595.4716,875.7142 |
| TG 55:1 | Triglycerol | $[M+NH_4]^+$ | 9.846 | $C_{58}H_{110}O_6$ | 920.86041 | 3.66 | 647.5972,577.5208,621.5815 |
| TG 55:2 | Triglycerol | $[M+NH_4]^+$ | 9.399 | $C_{58}H_{108}O_6$ | 918.84509 | 3.3 | 575.5033,621.5823,645.6829 |
| TG 55:4 | Triglycerol | $[M+NH_4]^+$ | 8.696 | $C_{58}H_{104}O_6$ | 914.81366 | 3.42 | 617.5483,599.5022 |
| TG 56:0 | Triglycerol | $[M+NH_4]^+$ | 10.731 | $C_{59}H_{114}O_6$ | 936.89258 | 2.81 | 663.6295,551.5032,919.857 |
| TG 56:1 | Triglycerol | $[M+Na]^+$ | 10.079 | $C_{59}H_{112}O_6$ | 939.83252 | 2.56 | 599.4992,657.5735,638.5883 |
| TG 56:2 | Triglycerol | $[M+NH_4]^+$ | 9.589 | $C_{59}H_{110}O_6$ | 932.86151 | 2.56 | 635.5978,631.5671,603.5352 |
| TG 56:3 | Triglycerol | $[M+NH_4]^+$ | 9.153 | $C_{59}H_{108}O_6$ | 930.84509 | 3.3 | 633.5791,631.5646,601.5172,913.8211 |
| TG 56:4 | Triglycerol | $[M+NH_4]^+$ | 8.825 | $C_{59}H_{106}O_6$ | 928.83136 | 1.46 | 631.5646,599.5023,911.8076 |
| TG 56:4;1O | Triglycerol | $[M+NH_4]^+$ | 8.204 | $C_{59}H_{106}O_7$ | 944.82318 | 4.52 | 631.5651,615.4998,647.5597,629.5463 |
| TG 56:5 | Triglycerol | $[M+NH_4]^+$ | 8.504 | $C_{59}H_{104}O_6$ | 926.8147 | 2.38 | 629.5512,599.5046,909.791 |
| TG 57:1 | Triglycerol | $[M+NH_4]^+$ | 10.317 | $C_{60}H_{114}O_6$ | 948.89178 | 3.61 | 649.6099,675.6273,577.5176,931.8693 |
| TG 57:2 | Triglycerol | $[M+Na]^+$ | 9.796 | $C_{60}H_{112}O_6$ | 951.83148 | 3.6 | 597.4826,695.5945,671.6955 |
| TG 57:3 | Triglycerol | $[M+NH_4]^+$ | 9.328 | $C_{60}H_{110}O_6$ | 944.86047 | 3.6 | 601.5184,645.5816,647.5952, 927.8311 |
| TG 57:4 | Triglycerol | $[M+NH_4]^+$ | 8.996 | $C_{60}H_{108}O_6$ | 942.84418 | 4.21 | 645.582,599.504,925.8222 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| TG 58:1 | Triglycerol | $[M+NH_4]^+$ | 10.589 | $C_{61}H_{116}O_6$ | 962.90747 | 3.54 | 577.5154,663.6275,689.6374,945.8778 |
| TG 58:2 | Triglycerol | $[M+NH_4]^+$ | 10.014 | $C_{61}H_{114}O_6$ | 960.89178 | 3.61 | 663.6297, 575.5047,687.6293, 943.8707 |
| TG 58:3 | Triglycerol | $[M+NH_4]^+$ | 9.506 | $C_{61}H_{112}O_6$ | 958.87683 | 2.87 | 601.5203,661.615,656.6003,941.8517 |
| TG 58:4 | Triglycerol | $[M+NH_4]^+$ | 9.128 | $C_{61}H_{110}O_6$ | 956.85986 | 4.21 | 659.5991,599.5047,939.8338 |
| TG 59:1 | Triglycerol | $[M+NH_4]^+$ | 10.861 | $C_{62}H_{118}O_6$ | 976.92444 | 2.26 | 677.6434,577.5218,703.6577,959.9008 |
| TG 59:2 | Triglycerol | $[M+NH_4]^+$ | 10.245 | $C_{62}H_{116}O_6$ | 974.91058 | 0.43 | 575.5051,677.6442,701.6457 |
| TG 59:3 | Triglycerol | $[M+NH_4]^+$ | 9.696 | $C_{62}H_{114}O_6$ | 972.88983 | 5.56 | 601.5198,675.6274,673.6129 |
| TG 59:4 | Triglycerol | $[M+NH_4]^+$ | 9.3 | $C_{62}H_{112}O_6$ | 970.87567 | 4.03 | 673.6148,599.5065,953.8558 |
| TG 60:1 | Triglycerol | $[M+NH_4]^+$ | 11.181 | $C_{63}H_{120}O_6$ | 990.93915 | 3.17 | 691.657,577.5204,717.6783 |
| TG 60:13 | Triglycerol | $[M+NH_4]^+$ | 8.77 | $C_{63}H_{96}O_6$ | 966.7561 | 1.58 | 645.4901,647.5048,949.7336 |
| TG 60:14 | Triglycerol | $[M+NH_4]^+$ | 8.478 | $C_{63}H_{94}O_6$ | 964.73853 | 0.36 | 645.4879,643.4734,947.7137 |
| TG 60:15 | Triglycerol | $[M+NH_4]^+$ | 8.119 | $C_{63}H_{92}O_6$ | 962.72302 | 0.19 | 643.472,946.697 |
| TG 60:2 | Triglycerol | $[M+NH_4]^+$ | 10.495 | $C_{63}H_{118}O_6$ | 988.927 | 0.3 | 691.6622,603.5349,687.6308, 971.905 |
| TG 60:3 | Triglycerol | $[M+NH_4]^+$ | 9.918 | $C_{63}H_{116}O_6$ | 986.90826 | 2.75 | 601.5181,687.6263,689.64.21,969.8647 |
| TG 60:4 | Triglycerol | $[M+NH_4]^+$ | 9.459 | $C_{63}H_{114}O_6$ | 984.89111 | 4.28 | 687.7303,599.5026 |
| TG 61:2 | Triglycerol | $[M+NH_4]^+$ | 10.785 | $C_{64}H_{120}O_6$ | 1002.9392 | 3.17 | 705.6727,603.5298,701.6387 |
| TG 61:3 | Triglycerol | $[M+NH_4]^+$ | 10.123 | $C_{64}H_{118}O_6$ | 1000.9252 | 1.53 | 701.642,601.5186,703.66,983.9055 |
| TG 61:4 | Triglycerol | $[M+NH_4]^+$ | 9.675 | $C_{64}H_{116}O_6$ | 998.90729 | 3.72 | 701.6431,599.5028,981.8845 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| TG 62:2 | Triglycerol | $[M+NH_4]^+$ | 11.08 | $C_{65}H_{122}O_6$ | 1016.9543 | 3.73 | 719.6912,603.5319,715.6591 |
| TG 62:3 | Triglycerol | $[M+NH_4]^+$ | 10.418 | $C_{65}H_{120}O_6$ | 1014.938 | 4.33 | 717.6724,601.5167,715.6583 |
| TG 62:4 | Triglycerol | $[M+NH_4]^+$ | 9.887 | $C_{65}H_{118}O_6$ | 1012.923 | 3.67 | 715.6601,599.5038,995.8904 |
| | | | | | | | |
| Cafestol | Diterpene | $[M+H]^+$ | 1.765 | $C_{20}H_{28}O_3$ | 317.2113 | N/A | 292.202,281.1887,253.196 |
| Cafestol linoleate | Diterpene | $[M+H-H_2O]^+$ | 5.531 | $C_{38}H_{58}O_4$ | 561.4301 | N/A | 281.1906,147.1173,173.0965 |
| Cafestol oleate | Diterpene | $[M+H-H_2O]^+$ | 5.973 | $C_{38}H_{60}O_4$ | 563.4458 | N/A | 281.1904,147.117,173.0965,239.1439 |
| Cafestol palmitate | Diterpene | $[M+H-H_2O]^+$ | 5.965 | $C_{36}H_{58}O_4$ | 537.43018 | N/A | 281.1905,147.1171,239.2375,173.096 |
| Cafestol stearate | Diterpene | $[M+H-H_2O]^+$ | 6.462 | $C_{38}H_{62}O_4$ | 565.46179 | N/A | 281.1906,147.1173,173.0964,239.1438 |
| Cafestol arachidate | Diterpene | $[M+H-H_2O]^+$ | 6.9 | $C_{40}H_{66}O_4$ | 593.49207 | N/A | 281.19,147.1168,173.0957,239.1423 |
| Kahweol | Diterpene | $[M+H]^+$ | 1.298 | $C_{20}H_{26}O_3$ | 315.19 | N/A | 297.1248,279.1039 |
| Kahweol linoleate | Diterpene | $[M+H-H_2O]^+$ | 5.467 | $C_{38}H_{56}O_4$ | 559.41486 | N/A | 279.1753,131.0496,157.0653,183.0811 |
| Kahweol oleate | Diterpene | $[M+H-H_2O]^+$ | 5.909 | $C_{38}H_{58}O_4$ | 561.43018 | N/A | 279.1754,131.0496,157.0651,223.1125 |
| Kahweol palmitate | Diterpene | $[M+H-H_2O]^+$ | 5.898 | $C_{36}H_{56}O_4$ | 535.41516 | N/A | 279.1752,131.0495,233.1125,183.0809 |
| Kahweol arachidate | Diterpene | $[M+H-H_2O]^+$ | 6.848 | $C_{40}H_{64}O_4$ | 591.47668 | N/A | 279.1748,131.0493,223.1117,183.0812 |

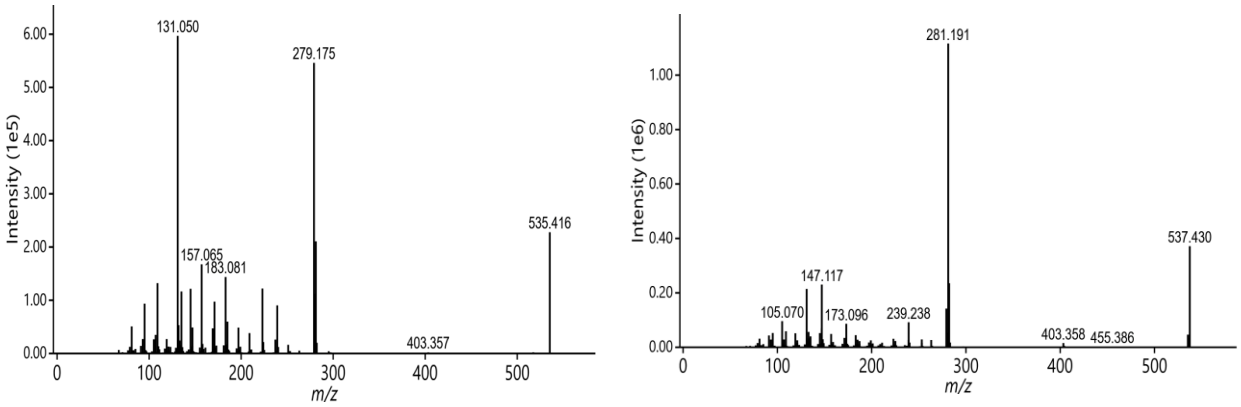
385

386 [a]Rt=retention time (min), [b]*m/z*=mass-to-charge ratio, [c]MS/MS=fragmentation mass spectra

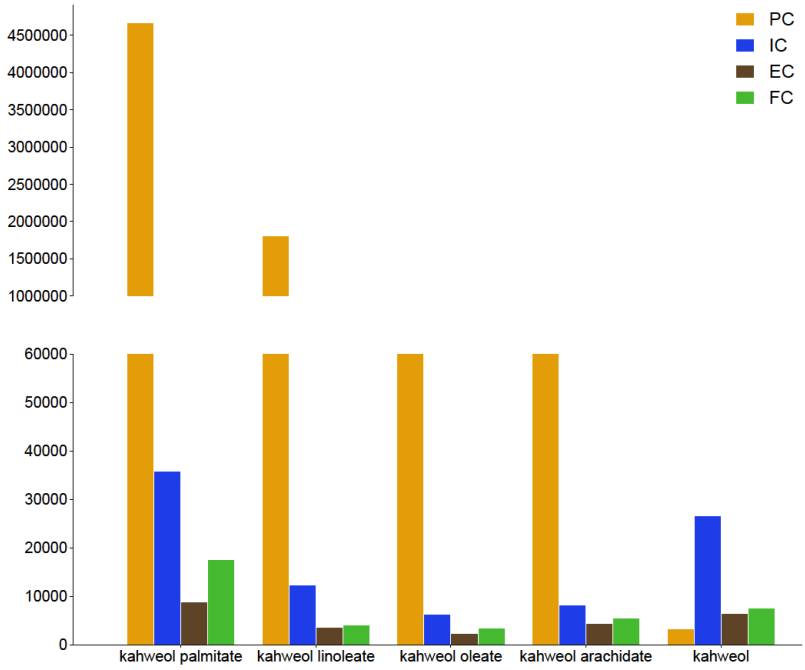
3.1.1. *Relative intensities of diterpenes in the lipid extract of the four coffee beverage types*

As the diterpenes cafestol and kahweol are unique to coffee plants (40), their analysis was of particular interest to the authors of this study. Cafestol palmitate was used as an external standard for the identification of cafestol, kahweol, and their esters present in the different coffee beverages (Figure 2 A, 2 B, 2 C, 2 D) (Supporting information Figure S3). The presence of a double bond structurally differentiates kahweol from cafestol. With this knowledge, kahweol with a lower molecular weight eluted before cafestol during UHPLC-QTOF/MS analysis. Diterpene esters: linoleate, palmitate, oleate, stearate, and arachidate except for kahweol stearate were identified in all the oils from the different beverage types (Figure 2 A, 2 B), with the palmitate form of kahweol and cafestol recording the highest intensities (Supporting information Figure S4 A, S4 B), which agrees with the findings of Moeenfard et al. (41).

**A** **B**

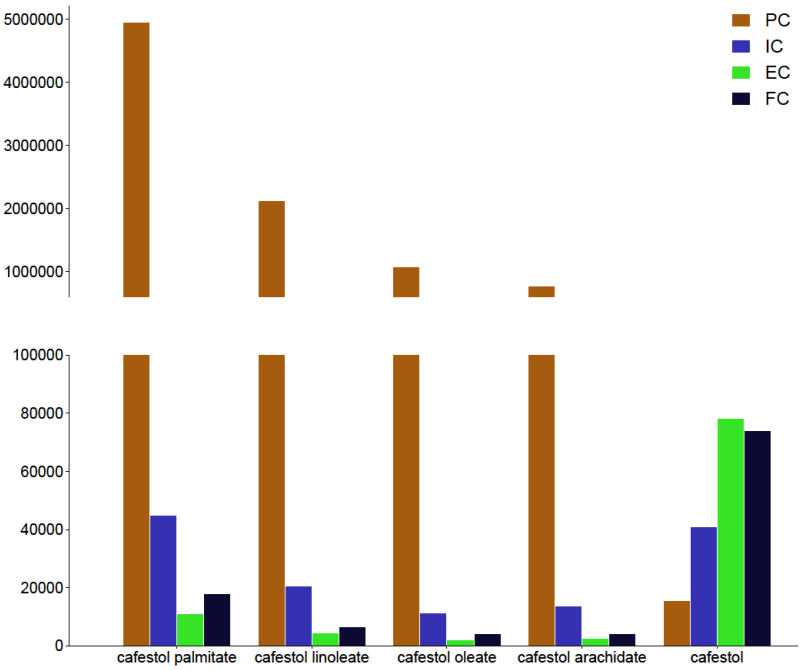



**C**




403

404 **D**




405

406 Figure 2. (A) Mass spectrum of kahweol palmitate from PC beverage $[M+H-H_2O]^+$, *m/z*

407 535.41516, (B) Mass spectrum of cafestol palmitate PC beverage $[M+H-H_2O]^+$, *m/z*

408 537.43018, (C) Distribution of kahweol and its esters, and (D) cafestol and its esters in oil

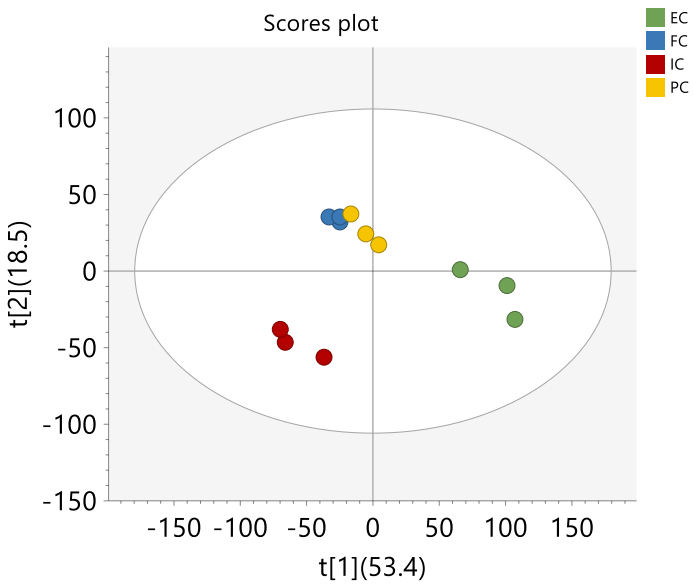
409 extracts of four coffee beverages. EC, espresso coffee; FC, filter coffee; PC, pot coffee; IC,
410 instant coffee.
411
412 Kahweol, cafestol and their esters were putatively annotated from the coffee beverage types
413 based on characteristic MS/MS fragments except for cafestol palmitate, which was identified
414 based on the cafestol palmitate reference standard. Cafestol palmitate standard had a
415 precursor ion *m/z* of 537.43182 with an adduct $[M+H-H_2O]^+$. The main fragment ions were
416 *m/z* 281.1918 and *m/z* 147.1179. The adduct $[M+H-H_2O]^+$ was detected for the precursor ions
417 of both kahweol palmitate and cafestol palmitate in all coffee beverage types. For kahweol
418 palmitate, the main fragment ions detected from the precursor ion *m/z* 537.43018 were *m/z*
419 279.157, *m/z* 131.050, *m/z* 157.065 and *m/z* 183.081 respectively (Figure 2 A) (Table 1). The
420 fragment ions *m/z* 281.191 followed by *m/z* 147.117 were the main ions detected from the
421 precursor ion 537.43018 for cafestol palmitate (Figure 2 B) (Table 1). Similar results on the
422 fragmentation pattern of kahweol palmitate and cafestol palmitate has been reported by
423 Kurzrock T. and Speer K. (42). Details of the fragment ions and fragmentation patterns for
424 kahweol, cafestol, and their esters identified in this study is provided as supplementary data
425 (Supporting information Figure S3).
426
427 Overall, PC beverage recorded the highest intensities of all esters of kahweol and cafestol as
428 reported in other studies (41). Statistical differences were observed in the intensity of
429 individual diterpene esters between PC beverage and, EC, FC, and IC beverages ($P < 0.001$).
430 The most significant difference observed in the intensity of the free forms of kahweol and
431 cafestol was between the IC beverage and the other coffee beverage types and between PC
432 and EC beverages ($P < 0.001$) (Supporting information Table S3). Although the same coffee
433 material and composition were used for EC, FC, and PC brewing, the intensity of individual

diterpene esters in EC and FC oil compared to PC oil was negligible. Some authors (43) have associated the high diterpene ester contents in PC beverage with the decanting of the beverage. Additionally, a longer contact time between the coffee and water and direct boiling of the coffee grounds in water, which differed from other brewing methods where hot water was added to coffee grounds, could explain the high intensities of diterpene esters in PC beverage. As regards filter brewing, the retention of lipids in the filter paper could explain the recorded low levels of diterpenes in the resultant beverage. According to Ahola M. et al. (44), filter paper retains about 80% of lipids during coffee brewing. In the case of EC beverage, the low level of diterpene esters could be the result of the short contact time between the coffee grounds and water, and the possibility of lipid retention in the portafilter used in espresso machines. Moeenfard M. et al. (41) have reported trace amounts of diterpene esters in FC and IC brews. Over the years, research has named diterpenes, kahweol and cafestol, as the culprits for the serum cholesterol-elevating effect in coffee beverages, with the most observed in PC beverage (45, 46). In this study, the unesterified forms of kahweol and cafestol were present in significantly lower proportions in the oil of PC beverage compared to their esterified forms (Figure 2 C, 2 D), which agrees with other studies (41). Furthermore, the unesterified forms of kahweol and cafestol were high in EC and FC beverages compared with PC beverage. Considering that the cholesterol-elevating effects of coffee beverage are associated with PC (41, 44) but not EC nor FC, we suggest that it is not the free forms of kahweol and cafestol but their esterified forms that contribute to the tendency of PC beverage consumption to increase cholesterol levels. This hypothesis warrants verification from in vivo studies.

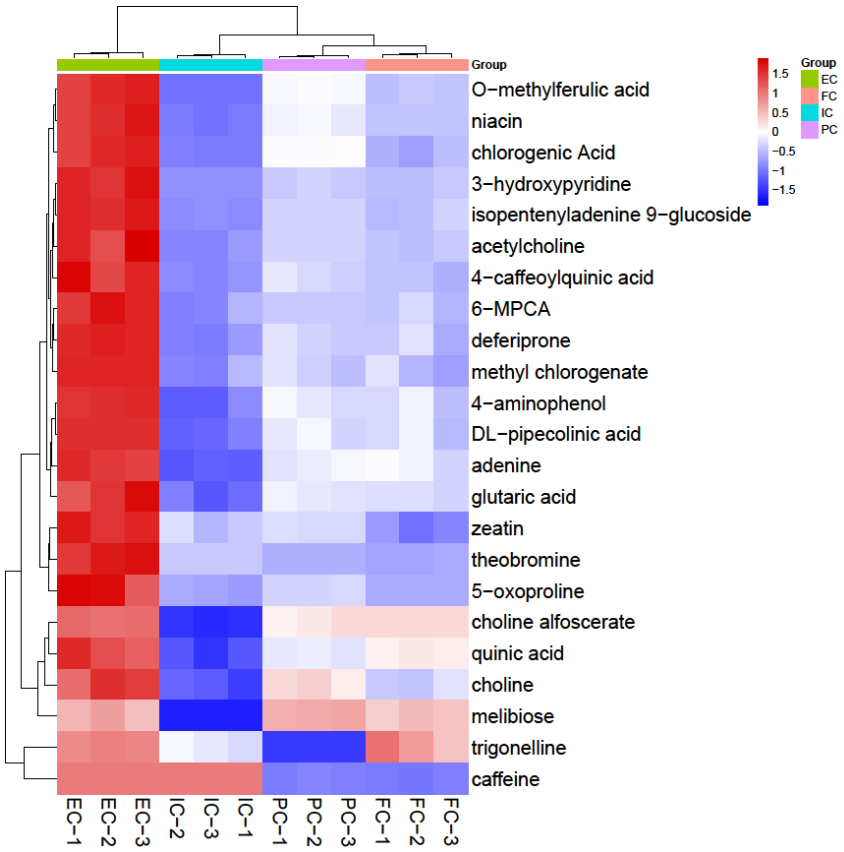
3.2. *Hydrophilic compounds*

A total of 22 hydrophilic metabolites were identified in positive and negative ionization modes (Table 2) for EC, PC, IC, and FC beverages. The compound classes of metabolites present in the coffee beverage types were amino acids, purines, cholines, carbohydrates, amines, vitamins, polyphenols, pyridines, carboxylic acids, alkaloids and glycosylamines (Table 2). The primary metabolites present in the coffee beverage types were niacin, adenine, melibiose, zeatin, choline, 6-MPCA, 4-aminophenol, acetylcholine and choline alfoscerate. The secondary metabolites DL-pipecolinic acid, 4-caffeoylquinic acid, methyl chlorogenate, O-methylferulic acid, trigonelline, chlorogenic acid, 3-hydroxypyridine, isopentenyladenine 9-glucoside, 5-oxoproline, theobromine, caffeine, glutaric acid, and quinic acid. It is noteworthy that majority of hydrophilic compounds in the coffee beverage types remained unidentified.

**A**




**B**




473

474 Figure 3. (A) The Principal Component Analysis (PCA) scores plot of combined intensities
475 of hydrophilic compounds obtained in positive and negative ionization modes (matrix: 6696),
476 for the EC, FC, PC and IC coffee beverages. (B) The heatmap of hierarchical clustering
477 analysis of hydrophilic compound intensities obtained in positive and negative ionization
478 modes (matrix: 22, column: observations, row: variables) for the different coffee beverages.
479 EC, espresso coffee; FC, filter coffee; PC, pot coffee; IC, instant coffee.

480

481 The differences between the hydrophilic profile of the beverage types were determined using
482 PCA. The first two principal components explained 53.4% and 18.5% respectively of the
483 variation in the data. PC1 and PC2 were adequate to show differences in the coffee
484 beverages. The cumulative $R^2$ value for the first three components of the PCA model was
485 0.719 and the estimated predictive ability of the model ($Q^2$) was 0.577. Information on the

PCA loadings plot is provided as supporting information (Figure S1 B). Overall, PC and FC beverages grouped together, which indicated similarities in the profile of their hydrophilic compounds. EC and IC beverages formed two separate groups and were different from PC and FC beverages (Figure 3 A). Heatmap evaluation (Figure 3 B) of metabolite intensities in the different coffee beverages showed three groups: 1) EC, 2) PC and FC, and 3) IC beverages. PC and FC beverages recorded a low relative intensity of the most identified hydrophilic compounds in coffee beverages. The EC beverage recorded the highest intensity of most identified hydrophilic compounds. Pressure employed, particle size, and the higher concentration of solid coffee in EC beverage compared to other beverages could explain this outcome (38, 47). It is worth mentioning that the shape of the caffeine peak observed for EC beverage during analysis suggests moderate saturation of the peak in the detector; thus, the actual difference of caffeine in EC compared to FC, PC, and IC may be higher than reported in this study.

A volcano plot analysis of identified hydrophilic metabolites showed that theobromine, caffeine and 4-caffeoylquinic acid were statistically significantly higher in EC beverage when compared to PC beverage (Supporting information Figure S2 A). When comparing PC and IC beverages, melibiose was statistically significantly higher in PC, and caffeine higher in IC (Supporting information Figure S2 C). Overall, EC beverage contained the highest intensity of polyphenols and alkaloids compared to the other coffee beverage types. Furthermore, the EC beverage recorded higher intensities of metabolites that exhibit antioxidant and anti-inflammatory properties such as chlorogenic acid, methyl chlorogenate, caffeine, quinic acid, and 4-caffeoylquinic acid (7) (Figure 3 B). In agreement with other studies, these types of components were highest in coffee brewed with an espresso machine when compared to filter, French press, and moka brews (13).

Preliminary data in our study exist to support similarities in the hydrophilic profile of light roast coffee grounds of PC and FC from the same coffee material (arabica: robusta: 95:5) and dark roast IC lyophilized powder purchased from the Finnish market (Supporting information Figure S5 A, S5 B) and also the differences in the lipophilic profile of bioactive compounds in PC and FC beverages (Supporting information Figure S6 A, S6 B).

This study provides in-depth scientific knowledge on the chemical composition, similarities, and differences in the profile of coffee beverage types commonly consumed. It was possible to identify different lipophilic and hydrophilic metabolites in ready-to-drink coffee beverages through omics approach employed in this research. The results can be used e.g. in product development of coffee containing foods, and as background in assessments of coffee beverages in nutrition. According to authors' knowledge, this is the first study of its kind to report on the lipophilic and hydrophilic profile of potential bioactive compounds in coffee beverage types processed for the Northern European market.

It is worth mentioning that the participation of sensory studies is needed in coffee research. Future studies could focus on optimizing the coffee brewing process to achieve coffee beverages of desired chemical and sensory characteristics (48). For sustainability's sake, also the possibilities of cell-culture coffee in preparation of coffee beverages should be explored in the future.

532 Table 2. Identified hydrophilic bioactive compounds in espresso, pot, filter, and instant coffee beverages.

533

| | Compound name | Compound class | Metabolite [a]ID | Adduct | [b]Rt | Formula | Observed [c]*m/z* | Mass Difference (mDa) | MS/MS Fragments |
|---|---|---|---|---|---|---|---|---|---|
| Positive | | | | | | | | | |
| HILIC | 4-aminophenol | amines | HMDB0001169 | $[M+H]^+$ | 1.949 | $C_6H_7NO$ | 110.06026 | 0.22 | 82.0662,80.0502,92.0502 |
| | DL-pipecolinic acid | amino acids | HMDB0000070 | $[M+H]^+$ | 4.156 | $C_6H_{11}NO_2$ | 130.08664 | 0.38 | 84.0817 |
| | 6-MPCA | amino acids | [e]16396232 | $[M+H]^+$ | 3.057 | $C_7H_{13}NO_2$ | 144.102 | 2.09 | 98.0974 |
| | melibiose | carbohydrates | HMDB0000048 | $[M+Na]^+$ | 7.314 | $C_{12}H_{22}O_{11}$ | 365.10696 | 1.52 | 203.0526 |
| | acetylcholine | cholines | HMDB0000895 | $[M]^+$ | 1.027 | $C_7H_{16}NO_2$ | 146.11719 | 0.36 | 87.045,88.0484 |
| | choline alfoscerate | cholines | HMDB0000086 | $[M]^+$ | 6.229 | $C_8H_{20}NO_6P$ | 258.11111 | 1.55 | 104.107,184.0735,125,86.0966 |
| | 4-caffeoylquinic acid | polyphenols | HMDB0030653 | $[M+H]^+$ | 4.163 | $C_{16}H_{18}O_9$ | 355.10272 | 2.71 | 163.0387,164.0421 |
| | methyl chlorogenate | polyphenols | [e]6476139 | $[M+H]^+$ | 2.092 | $C_{17}H_{20}O_9$ | 369.11783 | 0.18 | 177.056,178.0594,145.0295,149.0585 |
| | zeatin | purines | HMDB0012204 | $[M+H]^+$ | 1.968 | $C_{10}H_{13}N_5O$ | 220.11905 | 0.24 | 202.1094,148.0627,136.0629 |
| [d]RP | trigonelline | alkaloids | HMDB0000875 | $[M+H]^+$ | 0.793 | $C_7H_7NO_2$ | 138.05502 | 0.06 | 94.0652,92.0496,78.0339 |
| | caffeine | alkaloids | HMDB0001847 | $[M+H]^+$ | 4.56 | $C_8H_{10}N4O_2$ | 195.0884 | 0.76 | 138.0669,139.0699,110.072 |
| | theobromine | alkaloids | HMDB0002825 | $[M+H]^+$ | 3.924 | $C_7H_8N_4O_2$ | 181.07118 | 0.83 | 163.0395,138.0661,110.0705,135.0659 |
| | 5-oxoproline | amino acids | HMDB0000267 | $[M+H]^+$ | 1.091 | $C_5H_7NO_3$ | 130.04984 | 0.03 | 84.0446 |
| | choline | cholines | HMDB0000097 | $[M]^+$ | 0.702 | $C_5H_{14}NO$ | 104.10715 | 0.7 | 60.0815 |
| | Isopentenyladenine 9-glucoside | glycosylamines | HMDB0012240 | $[M+H]^+$ | 5.38 | $C_{16}H_{23}N5O_5$ | 366.1748 | 2.39 | 204.1255,177.0559,148.0617,136.0649 |
| | chlorogenic acid | polyphenols | HMDB0003164 | $[M+H]^+$ | 4.378 | $C_{16}H_{18}O_9$ | 355.10059 | 1.77 | 163.0388,164.0424, 135.0434,145.0293 |
| | O-methylferulic acid | polyphenols | [e]5357283 | $[M+H]^+$ | 6.317 | $C_{11}H_{12}O_4$ | 209.0795 | 1.34 | 191.0712,163.0762,192.0744,176.0478 |
| | 3-hydroxypyridine | pyridines | HMDB0013188 | $[M+H]^+$ | 0.853 | $C_5H_5NO$ | 96.0444 | 0.01 | 78.0339,68.05,79.0411 |
| | niacin | vitamins | HMDB0001406 | $[M+H]^+$ | 1.088 | $C_6H_5NO_2$ | 124.03927 | 0.04 | 80.0496,78.0341,96.0453,106.029 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Negative HILIC | glutaric acid | carboxylic acids | HMDB0000661 | $[M-H]^-$ | 1.23 | $C_5H_8O_4$ | 131.03197 | 3.02 | 87.0427,131.0309 |
| | adenine | purines | HMDB0000034 | $[M-H]^-$ | 1.367 | $C_5H_5N_5$ | 134.04378 | 3.45 | 107.0337,92.0239 |
| [d]RP | quinic acid | polyphenols | HMDB0003072 | $[M-H]^-$ | 0.999 | $C_7H_{12}O_6$ | 191.0493 | 6.81 | 85.0266,93.031 |

534

535 [a]ID=identification, [b]Rt=retention time (min), [c]*m/z*=mass-to-charge ratio, [d]RP=reverse phase, [e]=PubChem, HILIC= Hydrophilic interaction liquid

536 chromatography

## Supporting information

The supporting information is attached (PDF) with the following information.

ANOVA and post-hoc (Tukey test) analysis of oil (Table 1); identified RIKEN ID metabolites in espresso, pot, filter, and instant coffee beverages using reverse phase analysis in positive mode (Table 2); Loadings plot of principal component analysis of lipophilic and hydrophilic compounds in dark roast espresso, filter, pot, and medium roast instant coffee beverages (Figure 1); volcano plots of the level of significance for identified lipophilic and hydrophilic compounds intensities to discriminate espresso, filter, and instant coffee beverages with respect to pot coffee beverage (Figure 2); mass spectra of diterpenes in espresso, filter, instant, and pot coffee beverages (Figure 3); extracted ion chromatogram (EIC) of diterpenes in pot coffee beverage (Figure 4); ANOVA and post-hoc (Tukey test) analysis of diterpene intensities of the coffee beverage types (Table 3); scores and loadings plot from principal component analysis of hydrophilic compounds in filter, instant, and pot coffee beverages (Figure 5); scores and loadings plot from principal components analysis of lipophilic compounds in filter, instant, and pot coffee beverages (Figure 6).

## Acknowledgement

The authors thank Paulig Finland Ltd., Meira Oy, Finland and Suomen Nestlé Oy for providing coffee materials for this work.

## Conflict of interest

Authors declare no conflict of interest.

## Funding sources

This research received financial support from Finnish Food Research Foundation, Coffee Roasters' Association member companies, Finland, and Turun Seudun Osuuspankki-Foundation (TOP-Säätiö), Finland.

## Abbreviations

PC, pot coffee; EC, espresso coffee; IC, instant coffee; FC, filter coffee; QTOF, quadrupole time-of-flight; RP, reversed-phase; HILIC, hydrophilic interaction liquid chromatography; MS, mass spectrometry; UHPLC, ultra-high performance liquid chromatography; PCA, principal component analysis; CID, Collision-induced dissociation; BEH, ethylene bridged hybrid; EIC, extracted ion chromatogram; DG, diglyceride; TG, triglyceride; ANOVA, analysis of variance; T-Test, statistical method for comparing the differences between two groups; RIKEN, the institute of physical and chemical research; PTFE, polytetrafluoroethylene

## References

1. International Coffee Organization (2023). Coffee report and outlook. URL (https://icocoffee.org/documents/cy2023-24/Coffee_Report_and_Outlook_December_2023_ICO.pdf) (Accessed: 7.03.2024)

2. Volk G.M. and Krishnan S. (2020). Case study: Coffee wild species and cultivars. In: Volk, G.M., Byrne, P., editors. Crop Wild Relatives in Genebanks. Fort Collins, Colorado: Colorado State University.URL (https://colostate.pressbooks.pub/cropwildrelatives/chapter/case-study-coffee-wild-species-and-cultivars/). (Date viewed: 25.02.2023)

584 3. Davis A. P., Chadburn H., Moat J., O'Sullivan R., Hargreaves S. and Nic Lughadha E.
585 (2019). High extinction risk for wild coffee species and implications for coffee sector
586 sustainability. Science Advances, 5(1), eaav3473–eaav3473. Doi.org/10.1126/sciadv.aav3473
587 4. Seninde D. R. and Chambers E. (2020). Coffee flavor: A review. *Beverages*, 6(44), 1-
588 25. Doi.org/10.3390/beverages6030044

589 5. Wu H., Gu J., Amrit B.K., Nawaz M. A., Barrow C. J., Dunshea F. R. and Suleria H. A. R.
590 (2022). Effect of processing on bioaccessibility and bioavailability of bioactive compounds in
591 coffee beans. Food Bioscience, 46, 101373. Doi.org/10.1016/j.fbio.2021.101373
592 6. Schneider H., Braun A., Füllekrug J., Stremmel, W. and Ehehalt R. (2010). Lipid based
593 therapy for ulcerative colitis—modulation of intestinal mucus membrane phospholipids as a
594 tool to influence inflammation. International Journal of Molecular Sciences. 11, 4149-
595 4164.Doi.org/10.3390/ijms11104149.

596 7. Chieng D., Canovas R., Segan L, Sugumar H., Voskoboinik A., Prabhu S., Ling L-H., Lee
597 G., Morton, David J.B., Kaye M., Kalman J.M and Kistler P.M. (2022). The impact of coffee
598 subtypes on incident cardiovascular disease, arrhythmias, and mortality: long-term outcomes
599 from the UK Biobank. European Journal of Preventive Cardiology, 29 (17), 2240–
600 2249.Doi.org/10.1093/eurjpc/zwac189
601 8. Tajik N., Tajik M., Mack I. and Enck P. (2017). The potential effects of chlorogenic acid,
602 the main phenolic components in coffee, on health: a comprehensive review of the literature.
603 European Journal of Nutrition, 56(7):2215-2244. Doi: 10.1007/s00394-017-1379-1
604 9. Głowacka R., Górska A., Wirkowska-Wojdyła, M., Wołosiak, R., Majewska, E. and
605 Derewiaka D. (2019). The influence of brewing method on bioactive compounds residues in
606 spent coffee grounds of different roasting degree and geographical origin. International
607 Journal of Food Science and Technology, 54(11), 3008–3014. Doi.org/10.1111/ijfs.14213

608 10. Rodriguez Y. F. B., Guzman N. G. and Hernandez J. G. (2020). Effect of the postharvest
609 processing method on the biochemical composition and sensory analysis of arabica coffee.
610 Engenharia Agrícola, 40(2), 177–183. Doi.org/10.1590/1809-4430-eng.agric.v40n2p177-
611 183/2020
612 11. Córdoba N., Moreno F. L., Osorio C., Velásquez S., Fernandez-Alduenda M. and Ruiz-
613 Pardo Y. (2021). Specialty and regular coffee bean quality for cold and hot brewing:
614 Evaluation of sensory profile and physicochemical characteristics. Food Science and
615 Technology, 145, 111363. Doi.org/10.1016/j.lwt.2021.111363
616 12. Stanek N., Zarebska M., Bilos L., Barabosz K., Nowakowska-Bogdan E., Semeniuk I.,
617 Blaszkiewicz J., Kulesza R., Matejuk R. and Szkutnik K. (2021). Influence of coffee brewing
618 methods on the chromatographic and spectroscopic profiles, antioxidant, and sensory
619 properties. Scientific Reports, 11(1), 21377–21377. Doi.org/10.1038/s41598-021-01001-2
620 13. Angeloni G., Guerrini L., Masella P., Bellumori M., Daluiso S., Parenti A. and Innocenti
621 M. (2019). What kind of coffee do you drink? An investigation on effects of eight different
622 extraction methods. Food Research International, 116, 13271335.
623 Doi:10.1016/j.foodres.2018.10.022
624 14. Ratnayake W. M. N., Hollywood R., O'Grady E. and Stavric B. (1993). Lipid content and
625 composition of coffee brews prepared by different methods. Food and Chemical Toxicology,
626 31(4), 263–269. Doi.org/10.1016/0278-6915(93)90076-B
627 15. Samoggia A. and Riedel B. (2018). Coffee consumption and purchasing behavior review:
628 Insights for further research. Appetite, 129, 70-81. Doi: 10.1016/j.appet.2018.07.002
629 16. Kristine B. (2020). The top coffee consuming countries. URL
630 (https://www.worldatlas.com/articles/top-10-coffee-consuming-nations.html/) (Accessed:
631 9.03.2023)

632 17. Derossi A., Ricci I., Caporizzi R., Fiore A. and Severini C. (2018). How grinding level
633 and brewing method (Espresso, American, Turkish) could affect the antioxidant activity and
634 bioactive compounds in a coffee cup. Journal of the Science of Food and Agriculture, 98(8),
635 3198–3207. Doi.org/10.1002/jsfa.8826
636 18. Petracco M. (2001). Chapter 7-technology IV: Beverage preparation: Brewing trends for
637 the new millennium. In R. J. Clarke, & O. G. Vitzthum (Eds.). Coffee: Recent developments
638 (pp. 140–164). John Wiley & Sons.
639 19. Wang X., William J., Fu Y. and Lim L.-T. (2016). Effects of capsule parameters on
640 coffee extraction in single-serve brewer. Food Research International, 89 (1), 797–805.
641 Doi.org/10.1016/j.foodres.2016.09.031
642 20. Farah A., Monteiro M. C., Calado V., Franca A. S. and Trugo L. C. (2006). Correlation
643 between cup quality and chemical attributes of Brazilian coffee. Food Chemistry, 98(2), 373–
644 380. Doi.org/10.1016/j.foodchem.2005.07.032
645 21. Cordoba N., Fernandez-Alduenda M., Moreno F. L. and Ruiz Y. (2020). Coffee
646 extraction: A review of parameters and their influence on the physicochemical characteristics
647 and flavour of coffee brews. Trends in Food Science and Technology, 96, 45–60.
648 Doi.org/10.1016/j.tifs.2019.12.004
649 22. Janda K., Jakubczyk K., Baranowska-Bosiacka I., Kapczuk P., Kochman J., Rebacz-
650 Maron, E. and Gutowska I. (2020). Mineral composition and antioxidant potential of coffee
651 beverages depending on the brewing method. Foods, 9(2), 121.
652 Doi.org/10.3390/foods9020121
653 23. Muzykiewicz-Szymanska A., Nowak A., Wira D. and Klimowicz A. (2021). The effect of
654 brewing process parameters on antioxidant activity and caffeine content in infusions of
655 roasted and unroasted arabica coffee beans originated from different countries. Molecules
656 (Basel, Switzerland), 26(12), 3681. Doi.org/10.3390/molecules26123681

657 24. Blumberg S., Frank O. and Hofmann T. (2010). Quantitative studies on the influence of
658 the bean roasting parameters and hot water percolation on the concentrations of bitter
659 compounds in coffee brew. Journal of Agricultural and Food Chemistry, 58(6), 3720–3728.
660 Doi.org/10.1021/jf9044606
661 25. Cordoba N., Pataquiva L., Osorio C., Moreno F. L. M. and Ruiz R. Y. (2019). Effect of
662 grinding, extraction time and type of coffee on the physicochemical and flavour
663 characteristics of cold brew coffee. Scientific Reports, 9(1), 8440–8440.
664 Doi.org/10.1038/s41598-019-44886-w
665 26. Rao N. Z. and Fuller M. (2018). Acidity and antioxidant activity of cold brew coffee.
666 Scientific Reports, 8(1), 16030–16039. Doi.org/10.1038/s41598-018-34392-w
667 27. Moroney K. M., Lee W. T., O'Brien S. B. G., Suijver F. and Marra J. (2015). Modelling
668 of coffee extraction during brewing using multiscale methods: An experimentally validated
669 model. Chemical Engineering Science, 137, 216–234. Doi.org/10.1016/j.ces.2015.06.003
670 28. Uslu N. (2022). The influence of decoction and infusion methods and times on
671 antioxidant activity, caffeine content and phenolic compounds of coffee brews. European
672 Food Research and Technology, 248, 2021–2030. Doi.org/10.1016/J.FOODRES.2012.06.032
673 29. Caprioli G., Cortese M., Maggi F., Minnetti C., Odello L., Sagratini G. and Vittori S.
674 (2014). Quantification of caffeine, trigonelline and nicotinic acid in espresso coffee: The
675 influence of espresso machines and coffee cultivars. International Journal of Food Sciences
676 and Nutrition, 65(4). Doi.org/10.3109/09637486.2013.873890
677 30. Stiefel C., Lindemann B. and Morlock G. E. (2022). Non-target bioactive compound
678 profiles of coffee roasts and preparations. Food Chemistry, 391, 133263.
679 Doi.org/10.1016/J.FOODCHEM.2022.133263
680 31. Klåvus A., Kokla M., Noerman S., Koistinen V. M., Tuomainen M., Zarei I., Meuronen
681 T., Häkkinen M. R., Rummukainen S., Babu A. F., Sallinen T., Kärkkäinen O., Paananen J.,

682 Broadhurst D., Brunius C. and Hanhineva K. (2020). “Notame”: Workflow for non-targeted
683 LC-MS metabolic profiling. Metabolites, 10(4), 135. Doi.org/10.3390/metabo10040135
684 32. Özge C. Z., Cemil C. E., Tuba R., Sedef K. and Emirhan N. (2020). Integration of GC–
685 MS and LC–MS for untargeted metabolomics profiling. Journal of Pharmaceutical and
686 Biomedical Analysis, 190,113509. Doi.org/10.1016/j.jpba.2020.113509
687 33. Xu L., Lao F., Xu Z., Wang X., Chen F., Liao X., Chen A. and Yang S. (2019). Use of
688 liquid chromatography quadrupole time-of-flight mass spectrometry and metabolomic
689 approach to discriminate coffee brewed by different methods. Food Chemistry, 286, 106–
690 112. Doi.org/10.1016/j.foodchem.2019.01.154
691 34. Xi M., Berendsen A. A. M., Ernst M., Hu T., Vázquez-Manjarrez N., Feskens E. J. M.,
692 Dragsted L. O. and La Barbera G. (2021). Combined urinary biomarkers to assess coffee
693 Intake using untargeted metabolomics: Discovery in three pilot human intervention studies
694 and validation in cross-sectional studies. Journal of Agricultural and Food Chemistry, 69(25),
695 7230–7242. Doi.org/10.1021/acs.jafc.1c01155
696 35. Vezzulli F., Rocchetti G., Lambri M. and Lucini L. (2022). Metabolomics combined with
697 sensory analysis reveals the impact of different extraction methods on coffee beverages from
698 Coffea arabica and Coffea canephora var. robusta. Foods, 11(6), 807.
699 Doi. 10.3390/foods11060807
700 36. Chan M. Z. A., Lau H., Lim S. Y., Li S. F. Y. and Liu S.-Q. (2021). Untargeted LC-
701 QTOF-MS/MS based metabolomics approach for revealing bioactive components in
702 probiotic fermented coffee brews. Food Research International, 149, 110656–110656.
703 Doi.org/10.1016/j.foodres.2021.110656
704 37. Matyash V., Liebisch G., Kurzchalia T. V., Shevchenko A. and Schwudke D. (2008).
705 Lipid extraction by methyl-terf-butyl ether for high-throughput lipidomics. Journal of Lipid
706 Research, 49(5). Doi.org/10.1194/jlr.D700041-JLR200

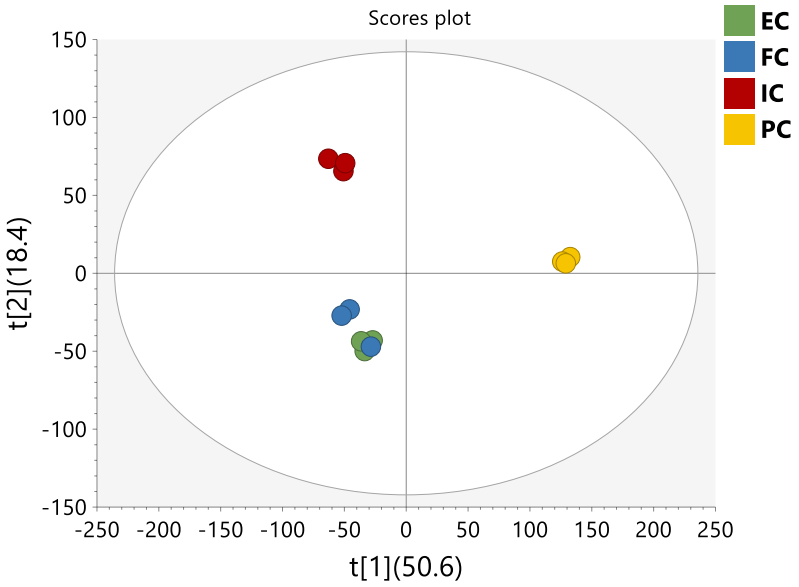
707 38. Cameron M. I., Morisco D., Hofstetter D., Uman E., Wilkinson J., Kennedy Z. C.,
708 Fontenot S. A., Lee W. T., Hendon C. H. and Foster J. M. (2020). Systematically improving
709 espresso: insights from mathematical modeling and experiment. Matter, 2(3).
710 Doi.org/10.1016/j.matt.2019.12.019
711 39. Rendón M. Y., dos Santos Scholz M. B. and Bragagnolo N. (2017). Is cafestol retained on
712 the paper filter in the preparation of filter coffee? Food Research International, 100.
713 Doi.org/10.1016/j.foodres.2017.08.013
714 40. Kitzberger C. S. G., Scholz M. B. dos S., Pereira L. F. P., Vieira L. G. E., Sera T., Silva J.
715 B. G. D. and Benassi M. de T. (2013). Diterpenes in green and roasted coffee of Coffea
716 arabica cultivars growing in the same edapho-climatic conditions. Journal of Food
717 Composition and Analysis, 30(1). Doi.org/10.1016/j.jfca.2013.01.007
718 41. Moeenfard M., Erny G. L. and Alves A. (2016). Variability of some diterpene esters in
719 coffee beverages as influenced by brewing procedure. Journal of Food Science and
720 Technology, 53(11), 3916–3927. Doi.org/10.1007/s13197-016-2378-6
721 42. Kurzrock T. and Speer K. (2001). Identification of kahweol fatty acid esters in Arabica
722 coffee by means of LC/MS. Journal of Separation Science, 24,843-848.
723 43. Gross G., Jaccaud E. and Huggett A. C. (1997). Analysis of the content of the diterpenes
724 cafestol and kahweol in coffee brews. Food and Chemical Toxicology, 35(6), 547–554.
725 Doi.org/10.1016/S0278-6915(96)00123-8
726 44. Ahola I., Jauhiainen M. and Aro A. (1991). The hypercholesterolaemic factor in boiled
727 coffee is retained by a paper filter. Journal of Internal Medicine, 230(4).
728 Doi.org/10.1111/j.1365-2796.1991.tb00447.x
729 45. Bak A. A. and Grobbee D. E. (1989). The effect on serum cholesterol levels of coffee
730 brewed by filtering or boiling. The New England journal of medicine, *321*(21), 1432–1437.
731 Doi.org/10.1056/NEJM198911233212103

732 46. Urgert R. and Katan M.B. (1996). The cholesterol-raising factor from coffee beans.
733 Journal of the Royal Society of Medicine, 89(11):618-23.
734 Doi: 10.1177/014107689608901107
735 47. Khamitova G., Angeloni S., Borsetta G., Xiao J., Maggi F., Sagratini G., Vittori S. and
736 Caprioli G. (2020). Optimization of espresso coffee extraction through variation of particle
737 sizes, perforated disk height and filter basket aimed at lowering the amount of ground coffee
738 used. Food Chemistry, 314. Doi.org/10.1016/j.foodchem.2020.126220
739 48. CarolinaVieira-Porto A., Cunha S.C., Rosa E.C., DePaula J., Cruz A.G., Freitas-Silva O.,
740 Fernandes J.O. and Farah A. (2024). Chemical composition and sensory profiling of coffees
741 treated with asparaginase to decrease acrylamide formation during roasting. Food Research
742 International, 186, 114333.Doi.org/10.1016/j.foodres.2024.114333

743

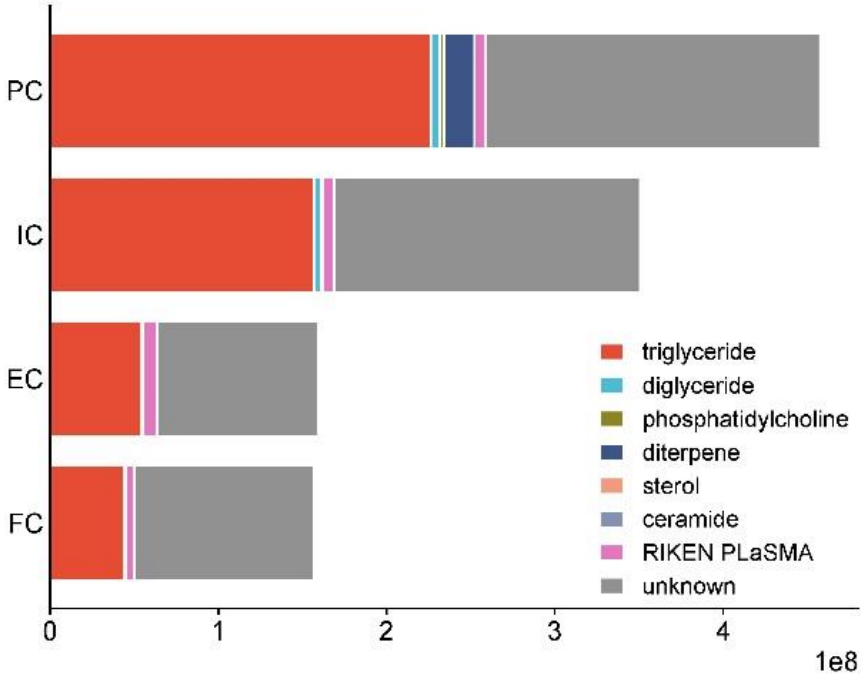
744 **Figures used in the manuscript**

745 Lipophilic compounds



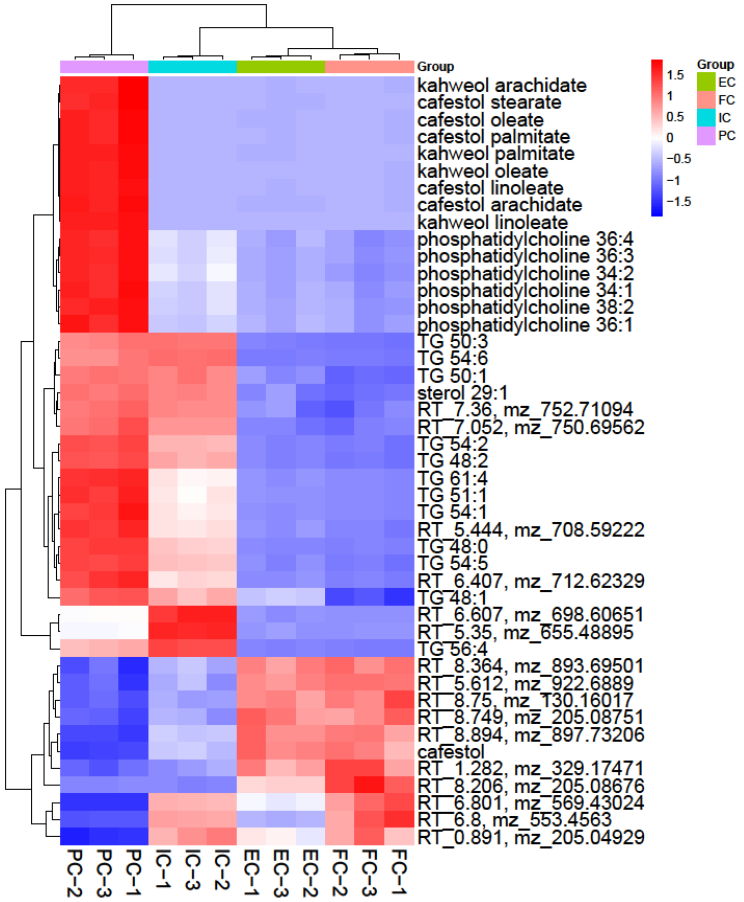

746

747



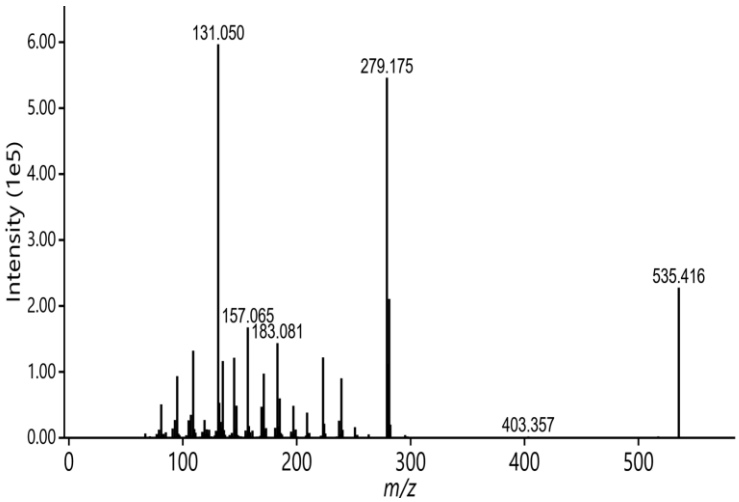

748

749



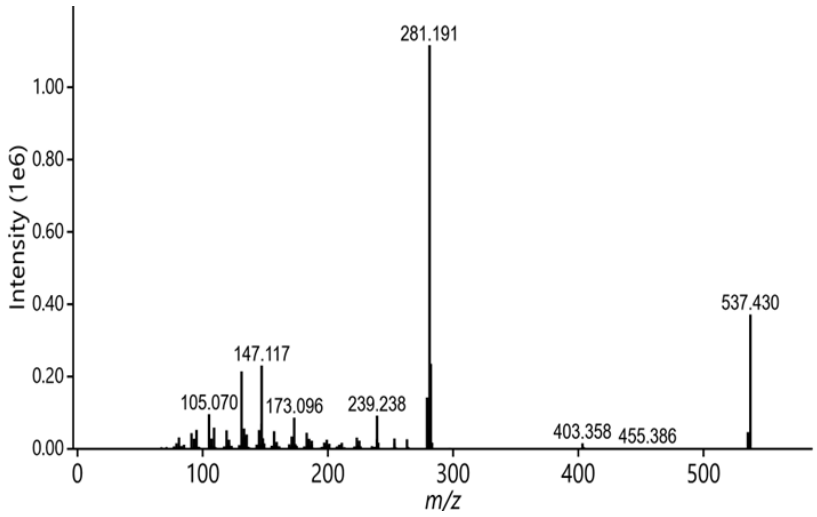

750

751



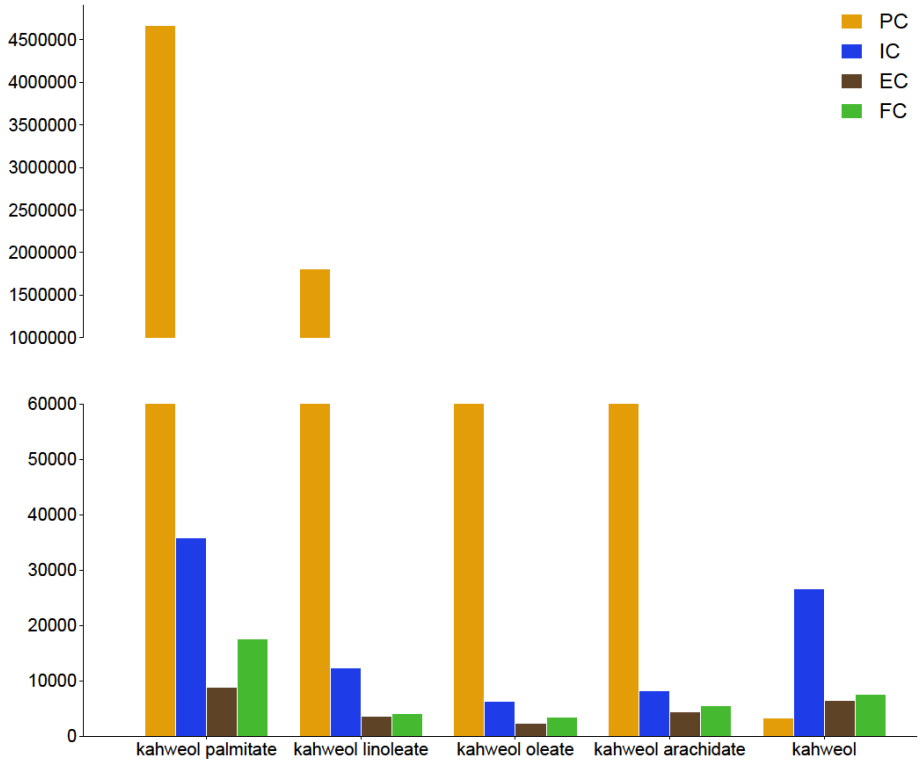

752

753



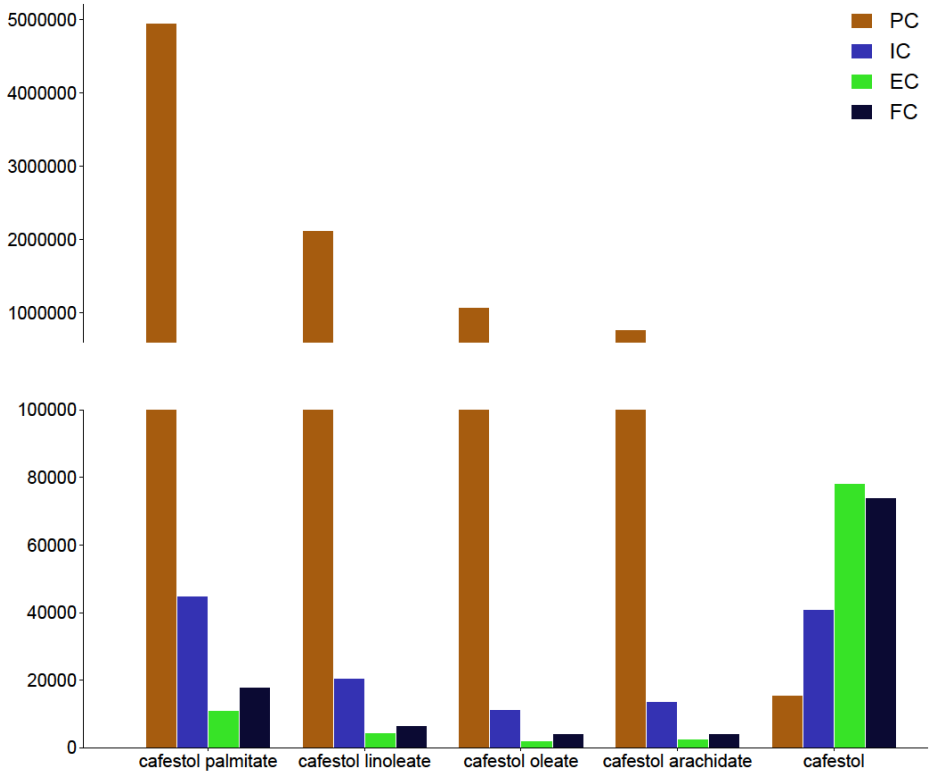

754

755




756

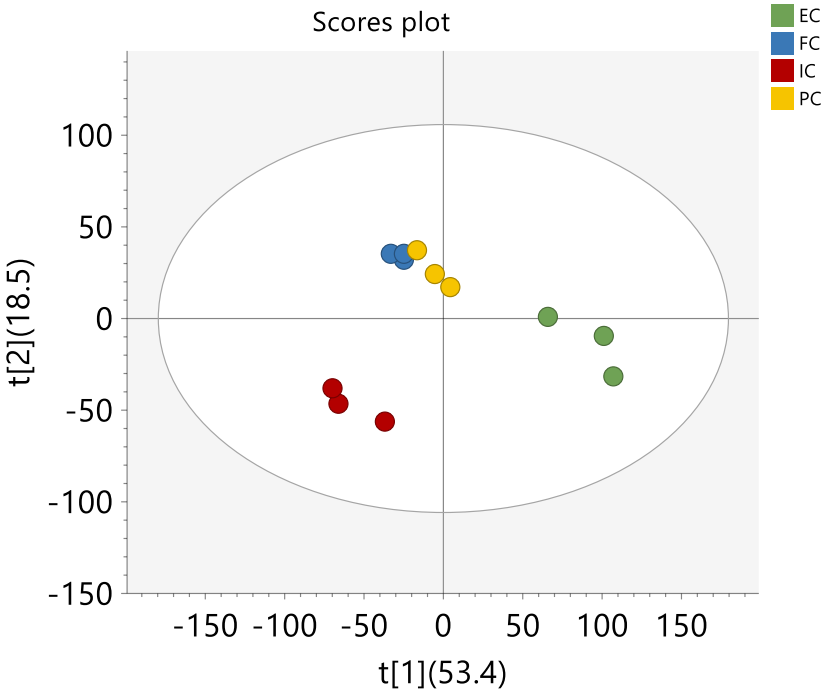
757




758

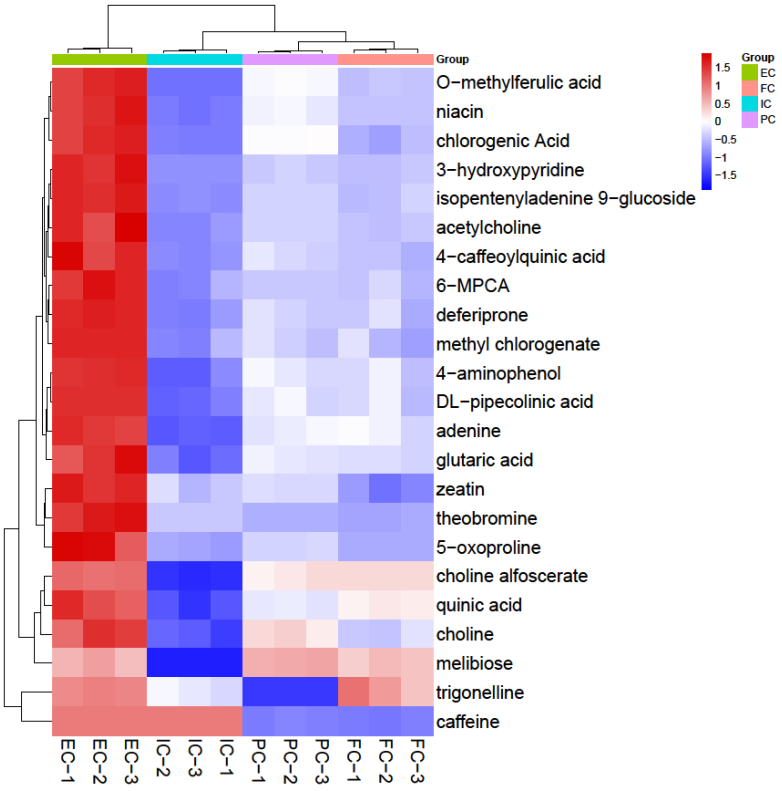
759

760 Hydrophilic compounds




761

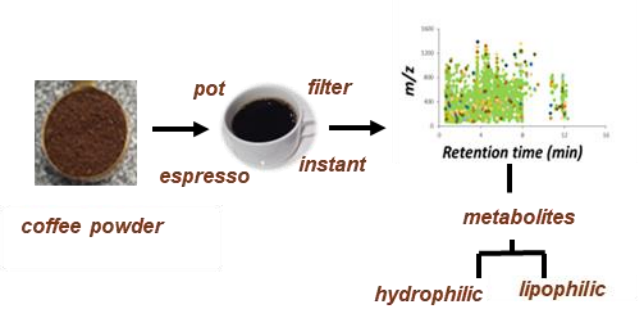
762


Group
O−methylferulic acid
niacin
chlorogenic Acid
3−hydroxypyridine
isopentenyladenine 9−glucoside
acetylcholine
4−caffeoylquinic acid
6−MPCA
deferiprone
methyl chlorogenate
4−aminophenol
DL−pipecolinic acid
adenine
glutaric acid
zeatin
theobromine
5−oxoproline
choline alfoscerate
quinic acid
choline
melibiose
trigonelline
caffeine
EC−1
EC−2
EC−3
IC−2
IC−3
IC−1
PC−1
PC−2
PC−3
FC−1
FC−2
FC−3
1.5
1
0.5
0
−0.5
−1
−1.5
Group
EC
FC
IC
PC

763

764 **Graphical Table of Contents (TOC)**

765




766