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Article

Effect of the Roast Level on Lipophilic and Hydrophilic Compounds in Pot and Filter Coffee Beverages

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ABSTRACT: Roast levels may affect the profile and content of phytochemicals in coffee. The composition of potentially bioactive compounds was investigated in pot coffee (PC) and filter coffee (FC) beverages prepared from light, medium, medium-dark, and dark roasted coffee using UHPLC-QTOF/MS. The metabolites affected by roast level (ANOVA, $p < 0.05$) included deoxycarnitine, tyrosine, and sn-glycero-3-phosphocholine. Compounds such as dehydrosalsolidine, deoxycarnitine, and adenosine 3,5-cyclic monophosphate were identified in coffee for the first time. The intensities of chlorogenic acid, trigonelline, choline, and rosmarinic acid in the beverages decreased with a higher roast level. In contrast, the level of quinic acid, theobromine, and *N,N*-dimethylpiperidinium, a Maillard reaction product, increased. The caffeine content was relatively stable from light to dark roast, while cholesterol-elevating diterpenes decreased. This study provides information on the optimal coffee roast level for several potentially bioactive compounds and aroma precursors to maximize coffee quality, especially in terms of its potential health benefits.

KEYWORDS: *coffee, metabolomics, roast level, cafestol, kahweol, beverage*

1. INTRODUCTION

Coffee is a popular beverage with *Coffea arabica* and *Coffea canephora* (*robusta*) as the two coffee species with economic value. *Coffea arabica* covers about 60% of the world's coffee production[1] and it has a milder flavor and aroma,[2] higher sugar, diterpene and lower caffeine content than *robusta*.[3,4]

Coffee contains 10−15% (w/w) of oil with triglycerides as the predominant lipid fraction followed by diterpenes, sterols, free fatty acids and other polar lipids.[5] The other nutrients present in coffee include proteins, carbohydrates, minerals and vitamins.[6] Bioactive compounds present in coffee often reported for their health effect include caffeine, theobromine, and trigonelline (alkaloids), cafestol and kahweol (diterpenes), chlorogenic acid and quinic acid (polyphenols), and niacin (a vitamin).[7,8] Specifically, chlorogenic acid contributes to the regulation of blood glucose and lipid metabolism,[9,10] and caffeine may lower the incidence of Parkinson's disease through the mechanism of antagonizing adenosine A_{2A} receptors in the brain.[11] Additionally, trigonelline may enhance memory,[12] while diterpenes, reported for both their positive and negative effect, could protect against cancer and improve liver health[8,13] or increase serum cholesterol.[14]

The composition of a coffee beverage is mostly influenced by coffee species, coffee composition, roasting degree, and brewing technique.[15,16] Our previous study clearly demonstrated differences in metabolite profiles of coffee beverages of different types.[16] The preferred roasting level of coffee varies on an individual and a cultural basis. However, in the Nordic countries, there seems to be a transition from traditional light roasts to darker roast levels. Although the transition seems to result from a change in the preferred taste, it is possible that the roasting level also affects the composition and content of bioactive compounds in the coffee beverage.

Roasting as such, is essential for the characteristic flavor of coffee. The coffee roasting process is divided into drying, roasting green beans at temperatures between 180 and 250 °C, and cooling.[17] The degree of roasting is dependent on the temperature and time combination. According to the Agtron classification of roast level, roasted coffee is typically categorized as light, medium-light, medium, medium-dark, dark, very dark, or extreme dark within a numeric scale of about 95 to 30 from lightest to darkest roast ground coffee, respectively.[18]

In addition to sensory characteristics, roasting significantly alters the composition of coffee.[19] For instance, the high temperatures employed during roasting promote complex chemical reactions like Maillard reaction, caramelization, pyrolysis and Strecker degradation, which produce nonvolatile compounds such as *N*-acetyl-2-methylpyrrole, 3-ethylpyridine and aroma-active compounds including 2-methylpyrazine, 2,2′-bifuran, guaiacol, and furfuryl acetate.[20−22] Roasting time and temperature, the variety of coffee beans, defective coffee beans and brewing method[23] affect the content of undesirable, even potentially carcinogenic or neurotoxic[24] compounds such as acrylamide, hydroxymethylfurfural, furfuryl alcohol, furan, and polycyclic aromatic hydrocarbons,[22,23] which are simultane-

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ously produced from precursors like simple sugars, amino acids, and fatty acids during roasting.[15]

The degree of roasting is likely to affect physical and chemical reactions in coffee such as the degradation or conversion of certain compounds, the destruction of the cell wall, and water evaporation which results in a reduction in bean weight.[25] For example, previously, the oil and cafestol,[26] as well as the chlorogenic acid, choline, and trigonelline contents have been detected to decrease as roasting progresses.[6,19] To date, the trend in caffeine content during coffee roasting is inconclusive since some authors have reported either an increase,[27] a decrease,[28] or a stable trend,[19] and thus more in-depth studies are needed. The content of acrylamide, a potentially carcinogenic compound formed at high temperatures, has been reported to increase when coffee is roasted between 60 and 180 °C and decrease with further roasting.[17,28] The temperature range of 180−223 °C for 20−27 min has been observed as the roast parameters for preserving the phenolic content of coffee.[29,30]

The composition and content of bioactive compounds in coffee beverages influence the sensory characteristics and health implications of coffee. Hence, it is important to explore how roasting affects these compounds in the consumed form, namely, ready-to-drink coffee beverages. Although a few studies[17,19] on the effect of roast level on certain compounds in coffee beans exist, extensive metabolomics analysis on how roasting affects a wider range of potentially bioactive lipophilic and hydrophilic compounds in both unfiltered and filtered coffee beverages is still needed. For these reasons, this study aimed at in-depth investigations, on a molecular level, of the effect of roast level on the profile of lipophilic and hydrophilic compounds in pot coffee (PC) and filter coffee (FC) beverages prepared from light, medium, medium-dark, and dark roasted coffee grounds of *Coffea arabica* using both targeted and untargeted metabolomics approaches. This study also aimed to quantify the levels of caffeine, cafestol, and kahweol palmitate, peculiar to coffee, because of their diverse health benefits connected to coffee consumption.[8,11,13,14] This study utilized both unfiltered (PC) and filtered (FC) brewing techniques.

2. MATERIALS AND METHODS

2.1. Coffee Samples. Roasted and ground coffee beans (100% *Coffea arabica*, origin: Brazil, Colombia, Central America, and Africa) were prepared on a laboratory scale under the following conditions to reach manufacturers' grading of roast levels light, medium, medium-dark, and dark. The roasting temperatures and times were typically within the range of 210−215 °C for 5 min (roast level 1: light), 215−220 °C for 5−7 min (roast level 2.5: medium), 225−235 °C for 5−7 min (roast level 4: medium-dark), and 235−245 °C for 7 min (roast level 5: dark). Coffees were roasted on a Probat Probatino 1 batch roaster (PROBAT-WERKE von Gimborn Maschinenfabrik GmbH, Emmerich am Rhein, Germany). The batch size was 1 kg, and biogas was used to heat the burner. The coffee beans were ground to coarseness typical for commercial coffee products on the Finnish market, i.e., 1040 μm (PC) and 800 (FC), with Mahlkönig EK43 (Hemro International AG, Zürich, Switzerland). The color of the roasted coffee beans was measured with a HunterLab Aeros spectrophotometer (HunterLab, Reston, USA). The L* values were 21.9 for roast level 1; 18.5 for roast level 2.5; 16.1 for roast level 4 and 13.2 for roast level 5. The ground coffee was packaged in vacuum-packed plastic bags and stored at −20 °C until brewing.

2.2. Chemicals and Reagents. Acetone, acetonitrile, methanol, 2-propanol of LC-MS grade were purchased from Honeywell/Riedel de Haën (Seelze, Germany). Formic acid was purchased from VWR (Leuven, Belgium) and ammonium acetate from Sigma-Aldrich (Steinheim, Germany). All standards were of purity ≥ 90%. Kahweol palmitate standard was purchased from phytoLab (phytoLab GmbH & Co. KG, Vestenbergsgreuth, Germany). Cafestol, caffeine and kahweol standards were purchased from ChemCruz (Santa Cruz Biotechnology, Inc., Dallas, Texas, USA).

2.3. Preparation of External Standard Solutions. The stock solution of kahweol, cafestol, kahweol palmitate, and caffeine standards were prepared by weighing 0.2 mg of standard into a 2 mL volumetric flask. The weighed standards were dissolved to the mark with methyl *tert*-butyl eter (MTBE): methanol (10:3, v/v) except for caffeine which was dissolved with acetonitrile.

2.4. Calibration Curve. From the stock solutions, standard solutions of cafestol, caffeine, kahweol, and kahweol palmitate within a range of 0.001−100 μg/mL were prepared for the calibration curves. All the calibration curves were linear with the coefficient of determination (R^2) greater than 0.99 (Supporting Information, Figure S1).

2.5. Preparation of Coffee Beverages. FC was prepared from 60 g of coffee grounds and 1 L of fresh cold water using a coffee making machine (Moccamaster, Technivorm, Netherlands) (Supporting Information, Figure S2). The average brew volume was 895 mL. PC was prepared by adding 60 g of coffee grounds to 1 L of water already brought to a boil in a decanter kettle and stirred. The kettle was put back on the stove, brought to a boil, lifted immediately off the stove, kept for 5 min and decanted. The average brew volume was 885 mL. All coffee beverages were allowed to cool to room temperature (RT) and stored immediately at −80 °C until analysis. Sample names were abbreviated according to brewing method and roast level (PC_r1; light roast pot coffee, PC_r2.5; medium roast pot coffee, PC_r4; medium-dark roast pot coffee, PC_r5; dark roast pot coffee, FC_r1; light roast filter coffee, FC_r2.5; medium roast filter coffee, FC_r4; medium-dark roast filter coffee, and FC_r5; dark roast filter coffee). Triplicates of each coffee roast level were prepared for both beverage types. A total of 24 samples were analyzed.

2.6. Compound Extraction for UHPLC- QTOF/MS Analysis.

2.6.1. Oil Extraction. Oil was extracted from coffee beverages as reported previously[16,31] with slight modification of the solvent-to-sample ratio. Briefly, 5 mL of extraction solvent (MTBE/methanol (10:3, v/v)) was added to 5 mL of coffee beverage, mixed (20 min; Stuart roller mixer STR1, power 50 W, Bibby Sterilin LTD, UK) and centrifuged (5 min; 700*g*; RT). The supernatant was transferred to a separate tube, and the residue was extracted with 2 mL of extraction solvent mixture (MTBE/methanol/water (10:3:2.5, v/v)), mixed (1 min), and centrifuged (5 min; 700*g*; RT). The supernatant from the residue was collected and added to the previous supernatant. 1.5 mL of ultrapurified water was added to the combined supernatant, mixed (1 min), and centrifuged (5 min; 700*g*; RT) for phase separation. The organic phase (upper layer) containing the extracted oil was collected, evaporated under nitrogen flow at 40 °C to complete dryness, weighed, dissolved in 10 mL of MTBE/methanol (10:3, v/v) and kept at −20 °C until analysis.

2.6.2. Extraction of Hydrophilic Compounds. Hydrophilic compounds were extracted from coffee beverages based on a previous method.[32] Briefly, 100 μL of coffee beverage was added to 400 μL of cold acetonitrile, mixed for 10 s, and centrifuged (7000*g*, 4 °C; VWR, Micro Star 17R, Germany) for 5 min. The supernatant was filtered through a 0.2 μm PTFE filter into an autosampler vial and kept at −20 °C until analysis. Sample tubes were kept on ice during the preparation period.

2.7. Compound Analysis Using UHPLC-QTOF/MS. The separation methods used during UHPLC-QTOF/MS analysis (Elute UHPLC, mass spectrometer: Impact II QTOF, Bruker Daltonic, Bremen, Germany) of compounds in the PC and FC beverages were analyzed by hydrophilic interaction liquid chromatography (HILIC) and Reversed-Phase (RP). An external pump (New Era Pump Systems, Inc., model number: 300, USA) connected to the LC machine was utilized to pump the calibrant (sodium formate) into the UHPLC machine during analysis. Prior to analysis of the coffee beverages, the column was conditioned by running the eluents through it for 15 min followed by injecting triplicates of extraction

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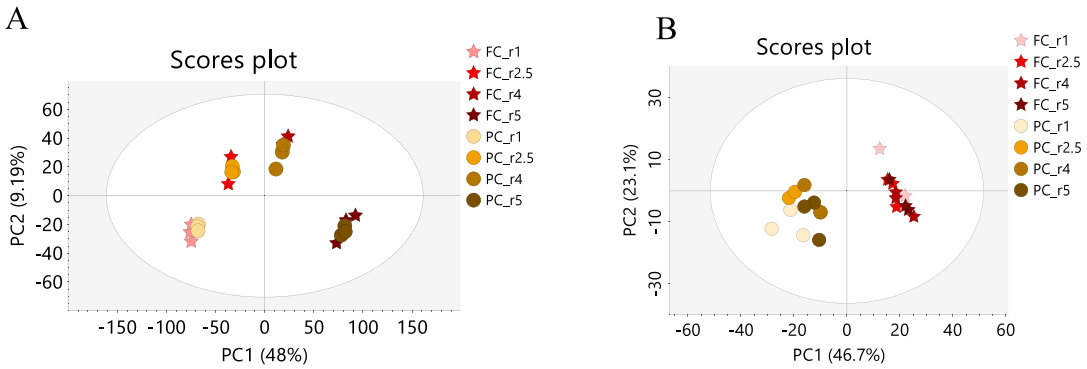

Figure 1. PCA scores plot of (A) combined intensities of hydrophilic compounds analyzed in the positive and negative ionization modes for pot coffee (PC) and filter coffee (FC) beverages and (B) intensities of lipophilic compounds analyzed in the positive ionization mode for the lipid extracts of pot coffee (PC) and filter coffee (FC) beverages of light (r1), medium (r2.5), medium-dark (r4), and dark (r5) roast levels.

solvents as blanks. A blank was also injected after analysis of every group of FC and PC beverage samples. FC and PC beverage groups were randomized during analysis.

2.7.1. Analysis of Lipophilic Compounds. Lipophilic compounds were analyzed based on a slight modification of the previous method described by Ollennu-Chuasam et al.[16] with respect to the flow rate used in the LC method. RP column (Waters Acquity UHPLC column, BEH C18, 2.1 × 100 mm, 1.7 μm particle size) was utilized for the analysis of oil extracts (0.25 mg) from the coffee beverages and standards (cafestol, kahweol, and kahweol palmitate standard solutions). The solvents used were water with 1% ammonium acetate (1 M) and 0.1% formic acid added (solvent A), and acetonitrile and isopropanol (1:1 v/v) with 1% ammonium acetate (1 M) and 0.1% formic acid added (solvent B). The flow rate was 0.3 mL/min at injection volume of 2 μL. The LC gradient was 0−35% B from 0 to 2 min, increased to 100% B from 2 to 7 min, kept at 100% B from 7 to 14 min, and decreased to 35% B for 7 min. The total runtime was 21 min, and the acquisition time was 16.5 min. The autosampler and column temperatures were 15 and 50 °C, respectively. The positive analysis mode was employed with the ESI end plate offset and capillary at 500 and 4500 V, respectively. The nebulizer pressure was set at 1.8 bar, the dry gas (N_2) flow was 8.0 L/min, and the dry temperature was 220 °C. An Auto MS scanning mode range of 50−1300 m/z was used.

2.7.2. Analysis of Hydrophilic Compounds. Hydrophilic compounds were analyzed with both RP and HILIC separation methods.[32] The solvents utilized for the RP column (Waters Acquity UHPLC column, BEH C18, 2.1 mm × 100 mm, 1.7 μm particle size) were water (solvent A) and methanol (solvent B) with 0.1% of formic acid added to both solvents. The solvents used for the HILIC column (Acquity UHPLC HILIC column BEH Amide, 2.1 mm × 100 mm, 1.7 μm particle size) were acetonitrile and water with 20 mM ammonium formate (1:1, v/v) (solvent A) and acetonitrile and water with 20 mM ammonium formate (9:1, v/v) (solvent B). The LC methods used were based on previous methods.[32] Briefly, the HILIC column used the starting solvent ratio of 0% A and 100% B. The gradient was continued at 100% B for 2.5 min, decreased to 0% B at 10 min, increased to 100% B, and kept at 100% B for 2.5 min. The flow rate was 0.6 mL/min, the injection volume was 2 μL and the total run time was 12.5 min. The RP column used the start solvent ratio of 98% A and 2.0% B. The gradient was increased to 100% B at 10 min, continued at 100% B for 4.5 min, decreased to 2.0% B and kept at 2.0% B for 2 min. The flow rate was 0.4 mL/min, the injection volume was 2 μL and the total run time was 16.5 min. The positive and negative ionization modes were used with the ESI source end plate offset and capillary voltage set at 500 and 3500 V, respectively. The autosampler and column temperatures were 4 and 50 °C, respectively. The dry temperature was 325 °C, the nebulizer pressure was 3.1 bar, and the dry gas (N_2) flow was 10.0 L/min. For structural confirmation, MS/MS analysis of one sample in every coffee beverage group was carried out. CID energies of 10, 20, and 40 eV were applied over the mass range of 50−1600 m/z for MS/MS scanning mode.

2.8. Data Processing. Bruker Compass DataAnalysis 5.1 (Bruker Daltonic GmbH, Bremen, Germany) was used for data analysis of the hydrophilic and lipophilic compounds. UHPLC-QTOF/MS files were afterward uploaded to MS-DIAL (version 4.80) for data processing and feature extraction. For data processing, the minimum peak height of 3000 A was used, and the peak picking parameters were MS^1 and MS^2 with mass tolerance of 0.01 and 0.025 Da, respectively. The ratio of sample maximum to blank average was set at ≥ 5 and the gap filling by compulsion feature was used. For feature reduction, adducts for positive and negative modes were manually selected accordingly: positive mode ($[2M + H]^+$, $[M + H]^+$, $[M + Na]^+$, $[M + ACN + H]^+$, $[M + CH_3OH + H)]^+$, $[M + NH_4]^+$, $[M + K]^+$, $[M + H\text{-}H_2O]^+$) and negative mode ($[M + Cl]^-$, $[M + FA\text{-}H]^-$, $[M - H]^-$, $[2M - H]^-$, and $[M - H_2O\text{-}H]^-$). The peak spots were selected for viewing based on MS^2 acquired, reference matched, blank filtering, unknown, and suggested compounds. The peak shapes of compounds were manually inspected, and compound identification was achieved by (i) precursor ion mass-to-charge ratio (m/z); (ii) pattern of mass spectra; (iii) retention time; (iv) intense feature if the compound was present in both the positive and negative modes; (v) matching the spectra with an in-house library consisting of pure reference standards analyzed previously with an identical method and equivalent LC-MS instrument, and publicly available databases (MS-DIAL Web site (https://systemsomicslab.github.io/compms/msdial/main.html); Human Metabolome Database (https://hmdb.ca); Lipid maps (https://www.lipidmaps.org/)). In the case where several adducts originated from the same metabolite, the most abundant adduct was chosen as the representative adduct for reporting.

Kahweol, kahweol palmitate, cafestol, and caffeine in the coffee beverages were identified by matching their spectra and retention times to standards. The feature list after MS-DIAL processing was exported to excel and filtered to remove redundant isomers and compounds that have less than 5-fold average intensities in the coffee beverages compared to those in the blanks.

2.9. Statistical Analysis. Triplicates of each roast level (prepared from different coffee brews) were analyzed for PC and FC beverages ($n = 3$), and the results were presented as mean ± standard deviation. The data on all the compounds in excel after filtering were submitted to SIMCA 18 (Multivariate Data Analysis Solution), where the data were $\log_{10}$ transformed, the outliers checked, and missing values excluded. The data standardization was achieved with Unit Variance (UV) scaling, the data was normalized by median, and the data linearity was checked using scatter plot. Data on the lipophilic and hydrophilic compounds were investigated separately to explore possible differences in the coffee beverages with respect to roast level using Principal Component Analysis (PCA). PCA was performed to identify sample grouping between the coffee beverages prepared from the different roast levels. ANOVA and posthoc (Tukey's test) were used to analyze statistical differences ($p < 0.05$) in

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Table 1. Hydrophilic Metabolites, Diterpenes and Statistically Significant Lipids (ANOVA, $p < 0.05$) Identified in the FC and PC Beverages Prepared from the Different Roast Levels

Ionization mode	ID level	compound name	compound class	metabolite ID	adduct	[a] t_R	formula	observed[b] m/z	mass difference (mDa)	MS/MS fragments (relative intensity %)
					hydrophilic metabolites					
RP positive										
	1	caffeine	alkaloids	HMDB0001847	$[M + H]^+$	4.839	$C_8H_{10}N_4O_2$	195.0878	0.19	195.088 (100), 138.8659 (50), 110.1712 (5)
	2	chlorogenic acid	(poly)phenols	HMDB0003164	$[M + H]^+$	4.349	$C_{16}H_{18}O_9$	355.1021	0.31	163.0387 (100), 164.042 (9), 145.0283 (2), 135.044 (1)
	1	trigonelline	alkaloids	HMDB0000875	$[M + H]^+$	1.3527	$C_7H_7NO_2$	138.055	0.03	138.551 (100), 94.0654 (10), 92.0498 (8), 78.034 (10), 110.0604 (2)
	1	niacin	vitamins	HMDB0001488	$[M + H]^+$	1.711	$C_6H_5NO_2$	124.0393	0.01	124.0397 (100), 80.0495 (15), 78.0339 (12), 122.0968 (8), 106.029 (4)
	2	choline	cholines	HMDB0000097	$[M]^+$	6.385	$C_5H_{14}NO$	104.1065	1	104.1072 (100), 68.9823 (5), 60.0821 (2)
	1	pyroglutamic acid	amino acids	HMDB0000267	$[M + H]^+$	4.606	$C_5H_7NO_3$	130.0491	1.33	84.0444 (100), 68.997 (15), 130.0497 (13), 85.0472 (2)
	2	dehydrosalsolidine	alkaloids	[c]22652	$[M + H]^+$	3.492	$C_{12}H_{15}NO_2$	206.1175	0.01	206.1182 (100), 205.0983 (12), 134.0604 (5), 163.0405 (3), 162.0911 (2)
	2	methyl chlorogenate	(poly)phenols	[c]6476139	$[M + Na]^+$	5.317	$C_{17}H_{20}O_9$	391.0996	0.49	391.0998 (100), 199.0367 (10), 177.0543 (7), 215.0527 (7), 373.0891 (6)
	2	cynarin	(poly)phenols	[c]6124212	$[M + Na]^+$	5.996	$C_{25}H_{24}O_{12}$	539.1158	4.22	539.1163 (100), 377.0837 (21), 359.0735 (16), 163.0399 (8)
	2	*N*-acetyltryptophan	amino acids	HMDB0255052	$[M + H]^+$	6.248	$C_{13}H_{14}N_2O_3$	247.108	0.68	188.0712 (100), 159.0919 (99), 210.1033 (50), 246.113 (16), 146.0598 (15)
	2	adenosine 3′,5′-cyclic monophosphate	purines	[c]7059571	$[M + H]^+$	1.791	$C_{10}H_{12}N_5O_6P$	330.0595	0.49	136.0614 (100), 330.0588 (25), 137.0634 (7), 98.9841 (2)
	1	theobromine	alkaloids	HMDB0002825	$[M + H]^+$	3.495	$C_7H_8N_4O_2$	181.0721	0.04	181.0719 (100), 138.0658 (45), 121.008 (20), 163.0611 (15), 116.763 (15), 110.713 (12), 137.0814 (10), 93.0128 (7)
	1	rosmarinic acid	(poly)phenols	HMDB0003572	$[M + H - C_9H_{10}O_5]^+$	4.34	$C_{18}H_{16}O_8$	163.0388	0.88	135.0439 (100), 163.0391 (80), 117.0335 (55), 145.0285 (50), 89.0386 (32), 107.0492 (12), 136.0475 (8), 79.0543 (6)
HILIC positive										
	1	adenosine	purines	HMDB0000050	$[M + H]^+$	1.658	$C_{10}H_{13}N_5O_4$	268.1024	1.64	136.0611 (100), 268.1011 (20), 137.0638 (7), 115.0398 (2)
	1	*trans*-zeatin	amino purines	HMDB0012204	$[M + H]^+$	2.038	$C_{10}H_{13}N_5O$	220.1177	1.63	220.1179 (100), 110.0594 (30), 148.0609 (17), 148.0609 (12), 202.1075 (10), 136.061 (2)
	2	tyrosine	amino acids	[c]6057	$[M + H]^+$	2.868	$C_9H_{11}NO_3$	182.0801	1.61	182.0801 (100), 124.0387 (20), 136.0751 (18), 164.0693 (3), 122.0233 (2)
	2	3-hydroxypyridine	pyridines	[c]7971	$[M + H]^+$	4.3	C_5H_5NO	96.04404	0.87	96.044 (100), 68.0492 (4), 78.033 (3)

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Table 1. continued

Ionization mode	ID level	compound name	compound class	metabolite ID	adduct	[a] t_R	formula	observed[b] m/z	mass difference (mDa)	MS/MS fragments (relative intensity %)
					hydrophilic metabolites					
	2	ferulic acid	(poly)phenols	HMDB0000954	$[M + H - H_2O]^+$	2.105	$C_{10}H_{10}O_4$	177.0537	0.3	145.0275 (100), 117.0325 (55), 177.0535 (40), 149.059 (35), 89.038 (15),130.1029 (2)
	2	4-caffeoylquinic acid	(poly)phenols	[c]58427569	$[M + H]^+$	4.171	$C_{16}H_{18}O_9$	355.1	0.06	163.0381 (100), 164.0412 (12), 181.0481 (3)
	1	caffeic acid	(poly)phenols	HMDB0001964	$[M + H - H_2O]^+$	4.17	$C_9H_8O_4$	163.0381	0.08	135.0435 (100), 163.038 (75), 145.0278 (42), 117.0329 (41), 89.0384 (28), 136.0467 (7), 146.0307 (7), 107.0486 (6), 77.0379 (4)
	1	sucrose	disaccharide	HMDB0000258	$[M + Na]^+$	11.234	$C_{12}H_{22}O_{11}$	365.1037	1.28	365.1031 (100), 203.0515 (35), 185.0409 (18), 204.0545 (2)
	2	4-aminophenol	anilines	HMDB0001169	$[M + H]^+$	1.98	C_6H_7NO	110.0593	0.79	110.0597 (100), 82.0652 (5), 94.0287 (2)
	2	*p*-phenetidine	aminophenols	HMDB0246417	$[M + H]^+$	1.262	$C_8H_{11}NO$	138.0907	0.64	138.0906 (100), 110.0594 (14), 123.0671 (4)
	2	*sn*-glycero-3-phosphocholine	cholines	HMDB0000086	$[M]^+$	6.385	$C_8H_{21}NO_6P$	258.1083	2.38	104.1065 (100), 258.1089 (42), 184.0726 (18), 124.9994 (15), 86.0963 (7), 105.11 (6), 166.0622 (2)
	2	*N*,*N*-dimethylpiperidinium	amines	[c]18743	$[M + H]^+$	1.163	$C_7H_{16}N$	114.1272	N/A	114.1275 (100), 112.1125 (13), 113.1147 (4), 98.0956 (3), 84.0811 (2), 72.0799 (2)
	2	4-isopropylaniline	amines	[c]7464	$[M + H]^+$	0.938	$C_9H_{13}N$	136.1116	0.55	136.1115 (100), 107.0726 (10), 84.0805 (7), 121.0882 (5), 91.0538 (2)
	2	pipecolinic acid	amino acids	HMDB0000716	$[M + H]^+$	4.23	$C_6H_{11}NO_2$	130.0856	1.18	84.0805 (100), 130.0856 (40), 85.083 (4), 87.0546 (3)
	2	3-hydroxy-1,2-dimethylpyridin-4(1H)-one	pyridines	[c]2972	$[M + H]^+$	2.461	$C_7H_9NO_2$	140.0699	0.11	140.0698 (100), 123.067 (5), 110.0589 (5), 123.067 (5), 94.0646 (3)
	2	*N*-(2,4-dimethylphenyl) formamide	amides	[c]92363	$[M + H]^+$	1.17	$C_9H_{11}NO$	150.0906	0.74	150.0904 (100), 107.0723 (55), 149.1066 (10), 108.076 (4), 150.0768 (6), 96.044 (5)
	2	*N*-methylcorydaldine	alkaloids	[c]303906	$[M + H]^+$	4.632	$C_{12}H_{15}NO_3$	222.1113	1.25	222.1118 (100), 176.1067 (7), 194.1166 (6), 178.1221 (4),
	2	deoxycarnitine	amino acids	HMDB0001161	$[M + H]^+$	3.948	$C_7H_{15}NO_2$	146.1171	0.95	87.0438 (100), 146.1172 (80), 131.0615 (20), 133.9598 (14), 88.0462 (6), 86.0581 (4), 123.9621 (3), 60.081 (2)
RP-negative	1	quinic acid	(poly)phenols	HMDB0003072	$[M - H]^-$	1.608	$C_7H_{12}O_6$	191.0398	N/A	191.0401 (100), 85.0227 (5), 111.0397 (4), 87.0041 (3), 109.0208 (2)
					lipophilic metabolites					
	1	cafestol	diterpenes	[c]108052	$[M + H]^+$	2.854	$C_{20}H_{28}O_3$	317.2149	N/A	271.2081 (100), 299.2035 (55), 316.158 (40), 253.196 (38), 91.0556 (32), 317.2157 (30), 165.0713 (25), 81.0342 (28), 145.0694 (24), 105.0715 (22)
	1	cafestol palmitate	diterpenes	[c]77014447	$[M + H - H_2O]^+$	8.27	$C_{36}H_{58}O_4$	537.4346	N/A	281.1934 (100), 537.436 (40), 131.0509 (25), 282.1968 (22),

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Table 1. continued

Ionization mode	ID level	compound name	compound class	metabolite ID	adduct	[a] t_R	formula	observed [b] m/z	mass difference (mDa)	MS/MS fragments (relative intensity %)
					lipophilic metabolites					
										147.1118 (20), 277.1776 (20), 239.2399 (10), 105.0715 (8), 173.0983 (7)
	2	cafestol oleate	diterpenes	[c]102305903	$[M + H - H_2O]^+$	8.259	$C_{38}H_{60}O_4$	563.4511	N/A	281.1929 (100), 563.4517 (28), 147.1185 (22), 282. 1965 (22), 131.0509 (13), 173.0981 (8), 105.0713 (7), 279.1778 (6), 119.0873 (5)
	2	cafestol linoleate	diterpenes	[c]102305902	$[M + H - H_2O]^+$	7.849	$C_{38}H_{58}O_4$	561.4348	N/A	228.1933 (100), 561.4359 (30), 131.0511 (28), 279.1777 (22), 147.1189 (16), 263.2402 (8), 157.067 (8), 105.7015 (6), 95.087 (5)
	2	cafestol arachidate	diterpenes	[c]102305907	$[M + H - H_2O]^+$	9.08	$C_{40}H_{66}O_4$	593.4966	N/A	281.1928 (100), 593.4982 (38), 282.196 (20), 279.177 (16), 131.0587 (15), 147.1183 (14), 173.0976 (7), 157.0552 (6), 183. 0824 (4)
	2	cafestol stearate	diterpenes	[c]102305906	$[M + H - H_2O]^+$	8.696	$C_{38}H_{62}O_4$	565.466	N/A	281.1932 (100), 565.4664 (38), 282.1965 (20), 279.1772 (18), 131.0509 (14), 147.1186 (13), 173.098 (6), 157.0668 (5)
	2	cafestol lignocerate	diterpenes	[c]102305911	$[M + H - H_2O]^+$	9.775	$C_{44}H_{74}O_4$	649.561	N/A	281.1917 (100), 649.5591 (40), 279.1917 (40), 282. 1955 (35), 131.0508 (23), 147.1187 (15), 173.0981 (10), 108.1016 (8), 145.0662 (7)
	2	cafestol behenate	diterpenes	[c]102305909	$[M + H - H_2O]^+$	9.42	$C_{42}H_{70}O_4$	621.529	N/A	281.1927 (100), 621.5295 (40), 297.1775 (40), 131.0507 (22), 282.1956 (19), 619.5143 (17), 147.1183 (12), 157.0664 (7), 183.0824 (6), 223.1142 (6)
	1	kahweol	diterpenes	[c]114778	$[M + H]^+$	3.862	$C_{20}H_{26}O_3$	315.1989	N/A	315.1991 (100), 313.325 (50), 314.3284 (32), 172.172 (28), 142.1611 (25), 279.1758 (18), 105.0719 (13), 145.0668 (10), 173.1751 (7), 133.0667 (7)
	1	kahweol palmitate	diterpenes	[c]101046637	$[M + H - H_2O]^+$	8.212	$C_{36}H_{56}O_4$	535.4191	N/A	131.0509 (100), 279.1773 (99), 535.4196 (40), 281.193 (38), 157.0667 (30), 109.1027 (23), 183.0826 (23), 145.0666 (20), 233.1142 (20)
	2	kahweol oleate	diterpenes	[c]98053268	$[M + H - H_2O]^+$	8.205	$C_{38}H_{58}O_4$	561.4352	N/A	131.0509 (100), 279.1774 (55), 561.4353 (21), 183.0824 (20), 109.1026 (21), 145.0667 (20), 157.0667 (18), 171.0828 (16), 135.1183 (15)
	2	kahweol linoleate	diterpenes	[c]98464936	$[M + H - H_2O]^+$	7.788	$C_{20}H_{26}O_3$	559.4197	N/A	279.1772 (100), 131.0508 (85), 559.4197 (50), 281.1926 (50), 157.0667 (29), 109.1025 (28), 183.01826 (27), 145.0667 (22)
	2	kahweol arachidate	diterpenes	[c]102305918	$[M + H]^+$	9.045	$C_{40}H_{64}O_4$	609.4934	N/A	313.2769 (100), 337.378 (79), 575.5094 (70), 609.4935 (60),

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Table 1. continued

Ionization mode	ID level	compound name	compound class	metabolite ID	adduct	[a] t_R	formula	observed[b] m/z	mass difference (mDa)	MS/MS fragments (relative intensity %)
				lipophilic metabolites						
										95.0873 (11), 279.1776 (9), 133.0663 (9), 109.1026 (9), 81.0709 (8), 145.0672 (8)
	2	kahweol stearate	diterpenes	[c]98464941	$[M + H]^+$	8.646	$C_{38}H_{60}O_4$	581.4609	N/A	581.4621 (100), 279.1774 (11), 145.0668 (7), 133.0663 (6), 563.4511 (5), 251.1458 (4), 143.0873 (2), 91.055 (2)
	2	kahweol lignocerate	diterpenes	[c]102305922	$[M + H - H_2O]^+$	9.708	$C_{44}H_{72}O_4$	647.5458	N/A	647.4643 (100), 347.18 (18), 441. 2954 (12), 291.1175 (10), 147.1185 (6), 189.1654 (4) ,348.1833 (4), 385.2313 (2)
	2	kahweol gadoleinate	diterpenes	[c]102305916	$[M + H - H_2O]^+$	8.635	$C_{40}H_{62}O_4$	589.4676	N/A	279.178 (100), 131.0507 (58), 589.4643 (40), 157.0667 (36), 223. 1135 (23), 135.1181 (18), 95.0869 (14), 171.0809 (13), 145.0662 (11)
	2	TG 50:2	triglycerols	N/A	$[M + NH_4]^+$	10.228	$C_{53}H_{98}O_6$	864.7714	6.34	591.5037 (100), 551.5094 (60), 864.7739 (59), 592.5079 (42), 847.7468 (20), 552. 5136 (19), 279.2365 (17), 261.2233 (13), 239.2391 (12)
	2	DG 32:0	diglycerols	N/A	$[M + NH_4]^+$	9.22	$C_{35}H_{68}O_5$	586.5466	6.04	551.5079 (100), 313.277 (50), 552.512 (32), 314.2794 (10), 95.0865 (5), 109.1036 (4), 83.0872 (3)
	2	phosphatidylcholine 34:2	phosphatidylcholines	N/A	$[M + H]^+$	8.353	$C_{42}H_{80}NO_8P$	758.5761	6.71	184.0757 (100), 758.5759 (80), 185.0784 (5), 86.0976 (3), 125.002 (2)
	2	phosphatidylcholine 36:4	phosphatidylcholines	N/A	$[M + H]^+$	7.948	$C_{44}H_{80}NO_8P$	782.5785	7.14	184.0755 (100), 782.5763 (82), 185.0792 (5), 125.0019 (3), 86.0982 (2)
	2	phosphatidylcholine 38:2	phosphatidylcholines	N/A	$[M + H]^+$	9.133	$C_{46}H_{88}NO_8P$	814.6393	7.32	184.0754 (100), 814.6384 (90), 654.6068 (25), 185.0788 (10), 262.2565 (6), 121.1026 (4), 298.2796 (4), 282. 2842 (3.5), 95.0877 (3)

[a] t_R = retention time (min), [b] m/z = mass-to-charge ratio, [c] = PubChem, HMDB = Human Metabolome Database, RP = reversed-phase, HILIC = hydrophilic interaction liquid chromatography, ID level; 1 = in-house database, 2 = public database.

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Table 2. Oil, Cafestol, Kahweol and Caffeine Contents of the FC and PC Beverages Prepared from Different Roast Levels of Coffee[a]

		concentration of metabolites (μg/mL)		
coffee beverage	oil content (g/100 mL)	cafestol	kahweol palmitate	caffeine
FC				
light	0.05 ± 0.002	1.38 ± 0.07	11.13 ± 0.35	346.2 ± 3.7
medium	0.06 ± 0.002	0.84 ± 0.09	7.33 ± 1.28	348.0 ± 3.4
medium-dark	0.05 ± 0.002	0.34 ± 0.06	5.92 ± 0.74	347.7 ± 2.8
dark	0.05 ± 0.006	0.17 ± 0.02	5.93 ± 0.61	354.1 ± 4.3
PC				
light	0.09 ± 0.006	1.52 ± 0.05	326.84 ± 67.19	392.4 ± 7.8
medium	0.08 ± 0.001	0.80 ± 0.08	216.28 ± 12.85	401.2 ± 24.6
medium-dark	0.07 ± 0.006	0.35 ± 0.06	144.88 ± 22.15	411.8 ± 34.6
dark	0.08 ± 0.004	0.18 ± 0.01	155.15 ± 24.76	416.2 ± 19.5

[a]Data is provided as mean ± SD, n = 3. Free kahweol was observed in trace amounts hence was not presented in Table 2.

the intensities of diterpenes and other lipid classes in the coffee beverages using MetaboAnlayst ver. 6.0 (https://www.metaboanalyst.ca/). ANOVA and Welch's t test were employed to investigate the differences in the oil contents and statistically significant metabolites in the coffee beverages with respect to roast level (R studio, ver. 4.4.1). Benjamini–Hochberg false discovery rate (FDR) was used to correct the p-values for multiple testing.

3. RESULTS AND DISCUSSION

3.1. Hydrophilic Analysis - PCA of the FC and PC Beverages of Varying Roast Levels. All coffee beverages were prepared from the same ground coffee blend (100% *Coffea arabica*) using UHPLC-QTOF/MS. More than 7500 lipophilic and 7000 hydrophilic molecular features were obtained in the positive ionization mode for lipophilic compounds and in both the positive and negative ionization modes for hydrophilic compounds, respectively.

For hydrophilic compound analysis, the PCA scores plot of the first principal component (PC1) explained 48.0%, while the second principal component (PC2) explained 9.19% of the variation between the beverages prepared from the different coffee roast levels (Figure 1 A). The level of fit (R^2) and predictive ability (Q^2) for the PCA model were 0.572 and 0.459, respectively, for the first 2 components. However, the addition of the third component increased the values slightly to 0.651 (R^2) and 0.508 (Q^2). PC1 and PC2 explained over 55% of the variation in the data for hydrophilic compounds in coffee beverages.

The coffee beverages separated into four different groups based on roast level, namely: (i) light roast FC and PC beverages, (ii) medium roast FC and PC beverages, (iii) medium-dark roast FC and PC beverages, and (iv) dark roast FC and PC beverages. The third replicate of medium roast FC beverage (FC_r2.5.3) was identified as an outlier and excluded from the figure for the PCA scores plot (Figure 1A). As shown in Figure 1A, coffee beverages of the same roast level clustered together, irrespective of the brewing method. The results revealed that PC and FC beverages prepared from the same roast level were similar in their hydrophilic profiles. This finding suggests that the roast level of coffee has a greater impact on the hydrophilic compounds in PC and FC beverages than the preparation method.

Statistical analysis (ANOVA, $p < 0.05$) revealed deoxycarnitine, tyrosine, *sn*-glycero-3-phosphocholine, 4-caffeoylquinic acid, trigonelline, pyroglutamic acid, and methyl chlorogenate in both FC and PC beverages as the most statistically significant hydrophilic metabolites that contributed to the separation of coffee beverages prepared from the same method but different coffee roast. Information on the 15 most statistically significant metabolites which influenced the profile of the FC, and PC beverages is provided as Supporting Information Table S1.

3.2. Hydrophilic Metabolites Identified in FC and PC Beverages of Different Coffee Roasts. In this study, a total of 32 hydrophilic compounds belonging to 13 compound classes were identified in the coffee beverages: polyphenols (7), alkaloids (6), amino acids (5), cholines (2), purines (2), pyridines (2), amines (2), anilines (1), vitamins (1), amino purines (1), aminophenyls (1), disaccharides (1) and amides (1) (Table 1).

3.3. Statistically Significant Hydrophilic Metabolites Affected by Roast Level. Coffee beverages were compared for statistically significant hydrophilic metabolites between light and dark roast levels (Welch's t.test, roast 1vs 5, $p < 0.05$) (Supporting Information Table S2). For FC beverages, the content of tyrosine, 3-hydroxypyridine, choline, caffeic acid, trigonelline, adenosine, ferulic acid, pyroglutamic acid, *trans*-zeatin and methyl chlorogenate decreased from light to dark roast while 4-aminophenol and 4-isopropylaniline increased from light to dark roast (Supporting Information Table S2). Regarding PC beverages, the content of 4-caffeoylquinic acid, chlorogenic acid, *sn*-glycero-3-phosphocholine, 3-hydroxypyridine, ferulic acid, sucrose, caffeic acid, choline, trigonelline, and deoxycarnitine decreased from light to dark roast whereas 4-aminophenol, pyroglutamic acid, 4-isopropylaniline, and pipecolinic acid increased from light to dark roast (Supporting Information Table S2).

Furthermore, in-depth analysis of the untargeted metabolomics data revealed some statistically significant tentatively identified hydrophilic metabolites not commonly associated with coffee beverages. For both FC and PC beverages, *N*-methylcorydaldine, dehydrosalsolidine, 3-hydroxy-1,2-dimethylpyridin-4(1H)-one increased with at higher coffee roast level while *N*-(2,4 -dimethylphenyl) formamide increased from light to medium followed by a decrease to medium-dark and an increase to dark roast. Additionally, 3,5-cyclic monophosphate increased from light to medium roast followed by a decrease from medium to dark roast, while rosmarinic acid, pyridoxine, and cynarin decreased with increased coffee roast level. To the best of the authors' knowledge, this is the first time dehydrosalsolidine, deoxycarnitine, and adenosine 3,5-cyclic monophosphate have been detected in coffee. The MS/MS

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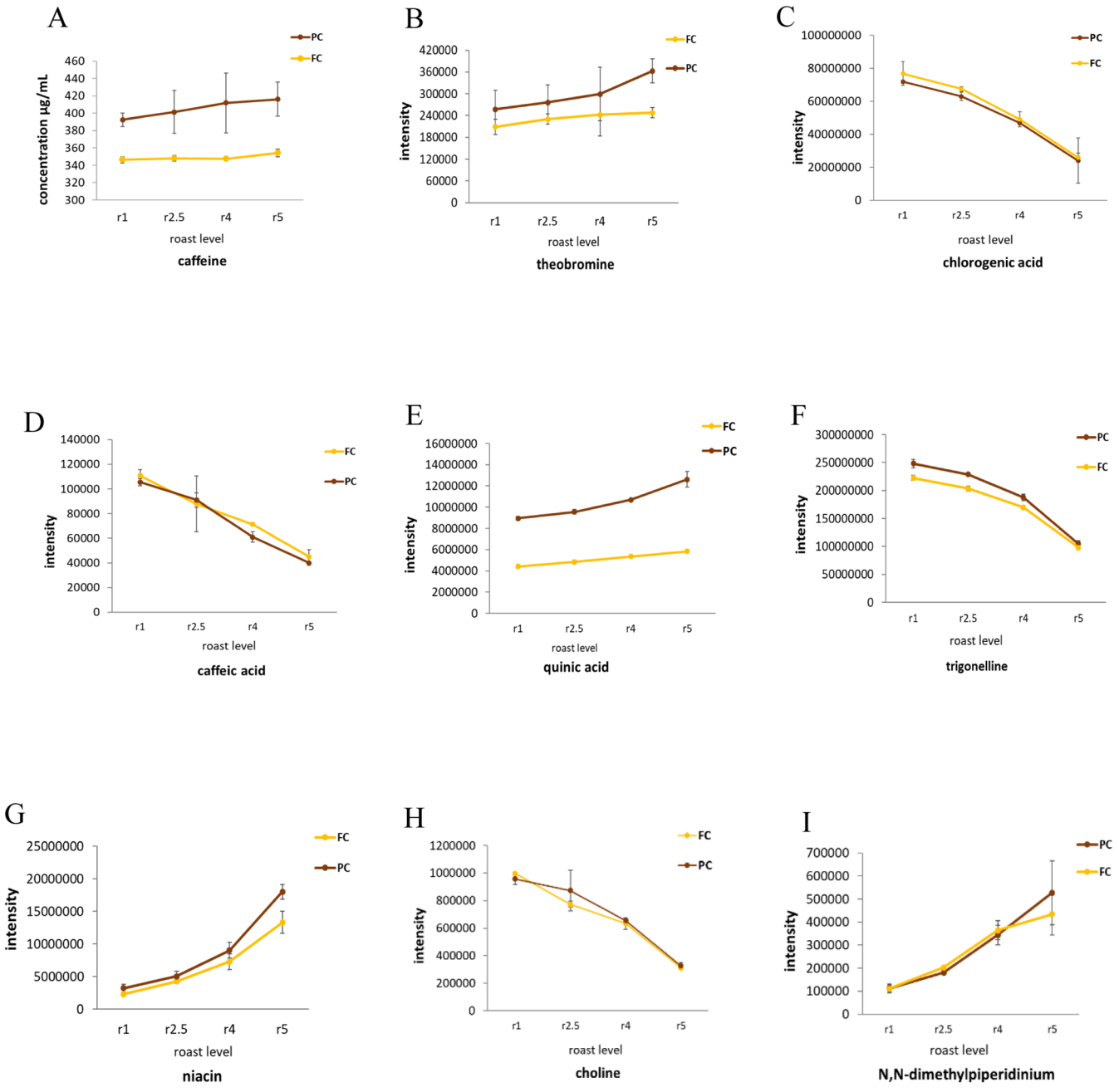

Figure 2. Trend of the intensities of (A) caffeine, (B) theobromine, (C) chlorogenic acid, (D) caffeic acid, (E) quinic acid, (F) trigonelline, (G) niacin, (H) choline, and (I) *N, N*-dimethylpiperidinium in filter coffee (FC) and pot coffee (PC) beverages prepared from light (r1), medium (r2.5), medium-dark (r4), and dark (r5) roast levels of coffee. All of the graphs were plotted with intensity values except for caffeine, which was plotted with concentration values (μg/mL). Data is provided as mean ± SD, n = 3.

spectra for these newly detected compounds have been provided as Supporting Information Figure S3.

3.3.1. The Trend of Selected Hydrophilic Bioactive Metabolites in FC, and PC Beverages of Different Coffee Roasts. Based on the widely reported health effects of caffeine, theobromine, chlorogenic acid, caffeic acid, quinic acid, trigonelline, niacin, and choline in coffee,[7−12] these metabolites were selected for further discussion.

3.3.1.1. Caffeine and Theobromine. The caffeine concentrations (μg/mL) were 346.2 ± 3.7 (light), 348.0 ± 3.4 (medium), 347.7 ± 2.8 (medium-dark), and 354.1 ± 4.3 (dark) for FC beverages and 392.4 ± 7.8 (light), 401.2 ± 24.6 (medium), 411.8 ± 34.6 (medium-dark), and 416.2 ± 19.5 (dark) for PC beverages (Table 2). The concentration of caffeine, the main stimulant in coffee, remained relatively stable in the FC and PC beverages regardless of the roasting conditions (Figure 2A and Table 2). This occurrence could be attributed to the thermal stability of caffeine during roasting as reported by Wei et al.[19] The relative stable content of caffeine observed in our study agrees with findings from Wei et al.[19] but disagrees with that of Lindsey et al.[27] and Hečimović et al.[28] who reported either an increase[27] or a decrease[28] in caffeine content as roasting progresses. Regarding the changes in caffeine content during coffee roasting, the disparity could be explained from the viewpoint that coffee beans lose moisture, expand, and decrease in weight during roasting;[25]

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therefore, compared with light-roasted coffee, more coffee beans of dark roast are required per unit weight of ground coffee for brewing. Due to this, ground coffee beverages of darker roast level may have slightly higher caffeine content compared to lighter roasted ones. Irrespective, the trend of the caffeine content from light to dark roast FC, and PC beverages could be considered relatively stable in this study (Figure 2A).

Theobromine, a compound that differs from caffeine by an extra methyl group, showed a relatively slight increase in intensity with an increased roast level (Figure 2B). Moisture loss during roasting[25] could have resulted in the minor increase of theobromine per unit weight with increased roast level of coffee beans as previously stated in the case of caffeine. The intensity of theobromine in the FC and PC beverages was highest in dark roast coffee (Figure 2B).

3.3.2. Chlorogenic Acid, Caffeic Acid, and Quinic Acid. Chlorogenic acid is the most predominant polyphenol in coffee beans. However, it easily degrades into quinic and caffeic acids at high temperatures.[30] The thermal decomposition of chlorogenic acid could explain the decrease in its intensity from light to dark roast in coffee beverages (Figure 2C). Chlorogenic acid was highest in light roast FC beverages and lowest in dark roast PC and FC beverages (Figure 2C). A high chlorogenic acid content in coffee beverages could be an indicator of high antioxidant and anti-inflammatory properties.[33] Nevertheless, it is noteworthy that chlorogenic acid contributes to the astringent taste in coffee beverages;[34] hence, a decrease in its intensity could present a more pleasant mouth feel. Overall, the highest chlorogenic acid content was observed in light roast, and the lowest chlorogenic acid content was observed in dark roast coffee beverages.

Figure 2D shows a decrease in the intensity of caffeic acid with an increased roast level in FC and PC beverages, although caffeic acid is a degradative product of chlorogenic acid.[30] On the other hand, quinic acid, another degradative compound of chlorogenic acid,[30] increased with increased roast level from light to dark roast in PC, and FC beverages (Figure 2). The degradation of chlorogenic acid into quinic acid at high temperatures[19] could have contributed to this increase. Dark roast coffee beverages recorded the highest intensity of quinic acid for the different coffee types. Sensory-wise, quinic acid contributes to the bitterness in roasted coffee beans;[19] therefore, an increase in its content during roasting could contribute to the bitter taste of dark roast coffee.

3.3.3. Trigonelline, Niacin, and Choline. Trigonelline contributes to the positive health effect of coffee such as improved memory and dendrites' regeneration;[12] nevertheless, it is thermally unstable.[35] Figure 2F shows a steady decrease in the intensity of trigonelline from light to dark roast PC, and FC beverages. According to the literature, trigonelline decomposes into nonvolatile compounds such as niacin and other pyridine derivatives at high temperatures during coffee roasting[35] resulting in a decrease in its content with increased roast level.[19] Thus, the intensity of niacin increased from light to dark roast FC, and PC beverages (Figure 2G). The highest content of trigonelline was observed in light roast PC beverages, and the lowest content was observed in dark roast FC beverages.

The steady decrease in choline intensity from light to dark roast FC, and PC beverages (Figure 2H) agrees with the literature.[36] The reduction in choline content can be attributed to the thermal degradation and involvement of choline in chemical reactions to further produce *N,N*-dimethylpiperidinium (mapiquate), a process-induced compound formed during Maillard reaction,[37] and other compounds that contribute to coffee's sensory attributes. In effect, the intensity of *N,N*-dimethylpiperidinium increased from light to dark roast FC, and PC beverages (Figure 2I). The choline content was similar for dark roast PC, and FC beverages (Figure 2H). Light roast FC beverages recorded the highest choline content (Figure 2H).

It is noteworthy that the intensities of chlorogenic acid, trigonelline, caffeic acid, and choline decreased with an increased roast level, but their contents at the different roast levels remained relatively similar between PC and FC beverages (Figure 2C, D, F, H). Likewise, the content of niacin and *N,N*-dimethylpiperidinium showed little variation between PC and FC beverages although an increase in the intensities of these compounds was observed with increased roast level (Figure 2G, I). The results buttress our findings that the roast level of coffee had a greater effect on hydrophilic compounds compared to the brewing method (Figure 1A).

In general, the majority of identified hydrophilic metabolites were highest in light roast PC beverages (Figure 2A,B,E,F). The caffeine, quinic acid, niacin, *N,N*-dimethylpiperidinium, and theobromine contents were highest in dark roast beverages while trigonelline, choline, caffeic acid, and chlorogenic acid were highest in light roast coffee beverages. Overall, the most statistically significant hydrophilic metabolites (ANOVA, $p < 0.05$) (Supporting Information Table S1) with antioxidant properties such as trigonelline, choline, and chlorogenic acid[29] identified in this research were highest in light roast coffee beverages (210−215 °C) (Figure 2C, D, F, and H). This finding agrees with other authors who have reported the temperature range of 180−220 °C as the optimal for the preservation of most phenolic compounds with antioxidant properties.[29,30]

3.4. Oil Content of the FC and PC Beverages Prepared from Different Coffee Roasts. The oil in the FC and PC beverages was extracted and quantified (Table 2). The highest oil contents for the beverages were 0.06 ± 0.002 g/100 mL (medium roast) for FC and 0.09 ± 0.006 g/100 mL (light roast) for PC (Table 2). Statistical analysis (ANOVA, $p < 0.05$) showed no difference in the oil content between roast levels except between medium-dark and light roasts for the PC beverages ($p = 0.03$) as well as between medium roast and light roast ($p = 0.02$) and between medium roast and dark roast ($p = 0.02$) for the FC beverages (Supporting Information Table S3). It is noteworthy that no increasing or decreasing trend was observed for the oil contents of the coffee beverage types with respect to the increased roast level in this study. However, some authors have reported either a continuous increase in the oil content of coffee as roasting progresses[38] or an initial increase in the oil content of coffee followed by a decline with further roasting.[39] The disagreement in the results could be that our study focused on the ready-to- drink coffee beverages while the other studies reported on coffee extracts. With respect to roast level, the differences in the oil contents for the FC and PC beverages of the same roast level could be attributed to brewing method and the particle size of coffee samples (PC: 1040 μm, FC: 800 μm)[16,19] since the coffee blend for FC and PC beverages were the same. Regarding brewing method, the filter paper utilized in the process of FC preparation could have served as a barrier hence retaining some of the coffee's oil resulting in the lower oil contents of FC beverages compared to PC beverages.[16]

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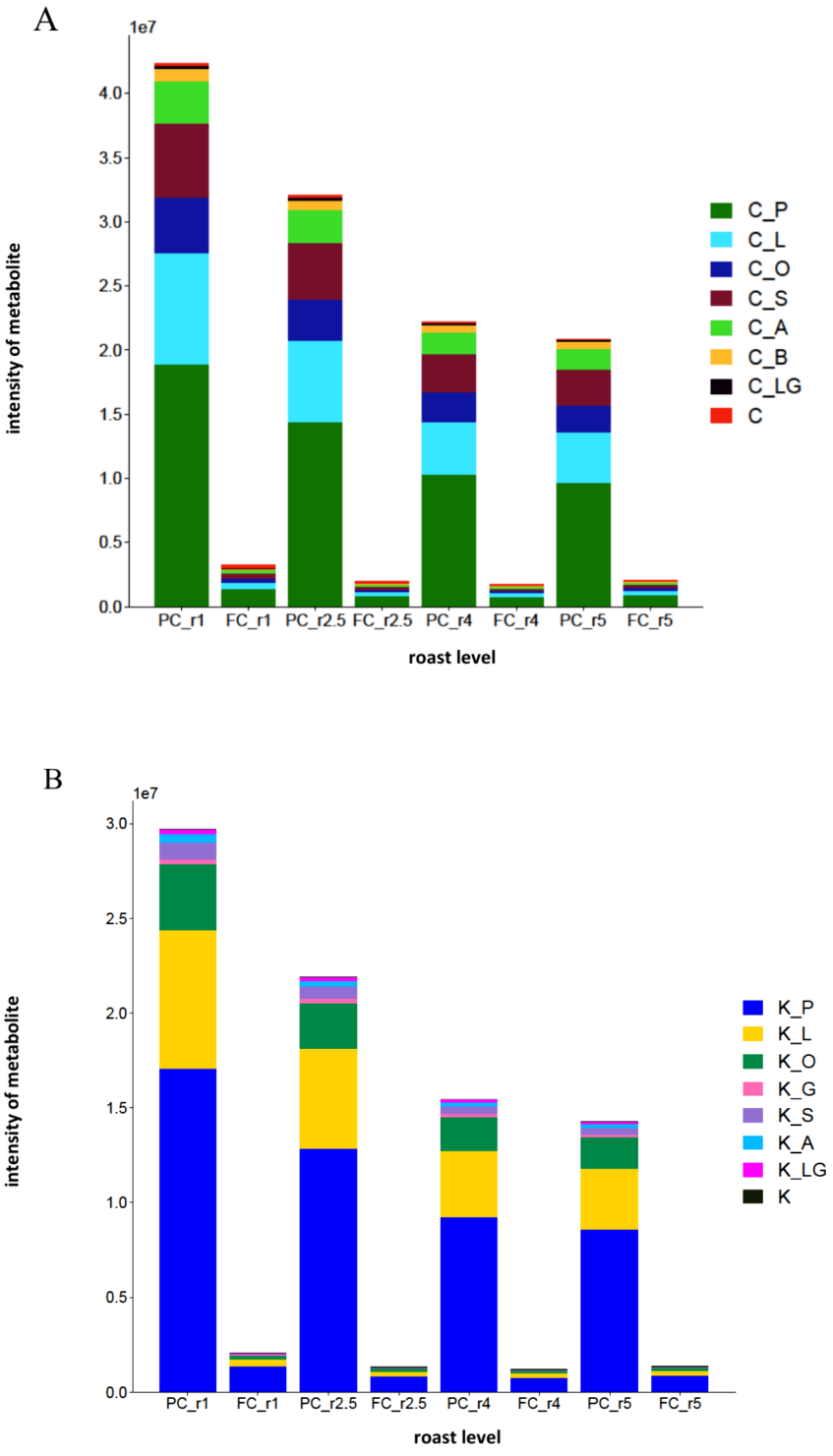

Figure 3. Trend of the intensities of cafestol (C), kahweol (K) and their palmitate (P), linoleate (L), oleate (O), gadoleinate (G), stearate (S), arachidate (A), behenate (B) and lignocerate (LG) esters in the lipid extracts of pot coffee (PC) and filter coffee (FC) beverages prepared from of light (r1), medium (r2.5), medium-dark (r4), and dark (r5) roasted coffee.

3.5. Lipophilic Analysis - PCA of the FC and PC Beverages of Varying Roast Levels. For lipid analysis, an equal amount of oil (0.2 mg/mL) was analyzed for each roast level of FC and PC beverages for the direct comparison of the lipid profiles. The PCA score plot of the lipophilic compounds (Figure 1B) shows the separation of all FC beverages from PC beverages irrespective of the roast level. PC1 explained 46.7% and PC2, 23.1% of the variation between the coffee beverages. The R^2 and Q^2 values for the PCA model were 0.697 and 0.565, respectively, for the first two principal components. The values increased to 0.764 (R^2) and 0.619 (Q^2) when the third component was added. PC1 and PC2 predicted over 69% of the variation in the data. The first replicate of medium roast PC beverages (PC_r2.5.1) and the second replicate of light roast FC beverages (FC_r1.2) were identified as outliers, based on consistently high signal intensities possibly related to an

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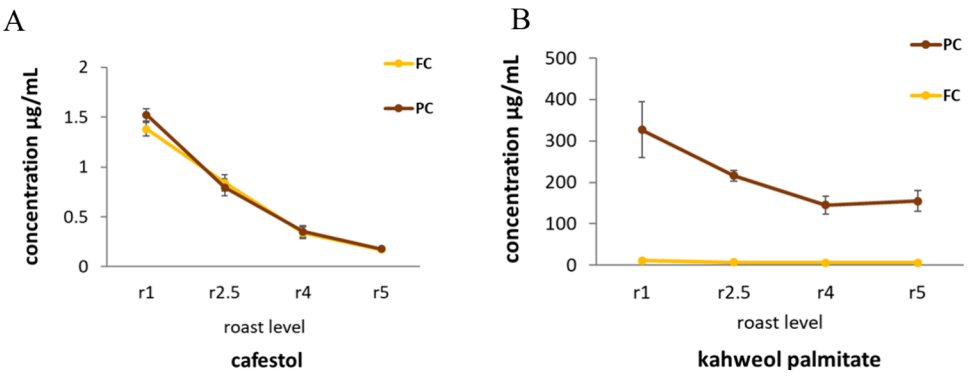

Figure 4. Trend of (A) cafestol and (B) kahweol palmitate concentrations (μg/mL) in the filter coffee (FC) and pot coffee (PC) beverages prepared from light (r1), medium (r2.5), medium-dark (r4), and dark (r5) roast levels of coffee. Data is provided as mean ± SD, n = 3.

error in the sample injection or data acquisition and excluded from the figure for the PCA scores plot (Figure 1B). As seen in Figure 1B, coffee beverages were grouped based on the brewing method, regardless of the coffee roast level, in contrast to what was observed for hydrophilic compounds. The results indicate that the preparation method of coffee has a greater influence on the lipophilic profile of FC, and PC beverages than the roast level of coffee (Figure 1B). The identified metabolites that significantly contributed to the separation of the coffee beverages based on the brewing method include cafestol and phosphatidylcholines 36:4, 32:0, 36:2, 34:1, and 38:2. A list of the first 25 statistically significant compounds that contributed to the separation of PC, and FC beverages are provided as Supporting Information Table S4.

3.6. Lipophilic Compounds in the FC, and PC Beverages of Different Coffee Roasts. A total of 89 lipophilic compounds comprising 9 lipid classes were identified in the coffee beverage types, namely: triglycerols (TG), diterpenes, diglycerols (DG), monoglycerols (MG), phosphatidylcholines, sterols (ST), sterylglycosides (SG), ceramides (Cer), and *N*-acylglycines (NAGly) (Supporting Information, Table S5). Eleven lipophilic compounds with RIKEN ID of known unidentified metabolites were also annotated. TG was the most predominant lipid class with 55 compounds followed by diterpenes (16), phosphatidylcholines (9), DG (2), MG (2), Cer (2), SG (1), ST (1) and NAGly (1). Detailed information on the identified lipophilic compounds has been provided as Supporting Information Table S5.

3.6.1. The Trend and Distribution of Diterpenes in FC, and PC Beverages of Different Coffee Roasts. Kahweol and cafestol, diterpenes mainly present as fatty acid esters in coffee, are reported to exhibit both positive (anticancer and antitumor properties)[8] and negative (cholesterol-elevating properties)[14] health effects. It was of interest to the authors of this study to investigate how the composition and levels of these diterpenes are altered by the roast level of coffee.

In this study, the same amount of lipid extract (0.2 mg/mL) was analyzed for the different roast levels of FC and PC beverages to compare the effect of roast level on cafestol, kahweol, and their esters present in the lipid extracts of the coffee beverages. The diterpenes identified in FC and PC beverages were cafestol (C) and kahweol (K) and their palmitate (P), linoleate (L), oleate (O), gadoleinate (G), stearate (S), and arachidate (A) esters, except for the behenate (B) and lignocerate (LG) esters, which were identified for only cafestol and kahweol, respectively (Figure 3 A) and (Table 1, 2). Apart from kahweol, kahweol stearate, kahweol arachidate, and cafestol, which were present in the $[M + H]^+$ adduct, all other diterpenes were present in the $[M + H - H_2O]^+$ adduct. The information on the respective precursor (m/z) and main fragment ions identified for the diterpenes are reported in Table 1. The results on the precursor and fragmentation ions for the identified diterpenes agree with previous studies.[16,40]

The concentrations of cafestol, kahweol, and kahweol palmitate in the coffee beverages were further quantified in this study using external standards (Figure 4A,B) (Table 2) (Supporting Information Figure S1). Semiquantitative data on the diterpene esters that were not quantified with standards have been provided based on their peak intensities in the lipid extracts of the coffee beverages (Figure 3A).

Unlike the esterified forms, free cafestol had similar concentrations between the brewing types. The average concentrations (μg/mL) of cafestol in the FC beverages were 1.38 ± 0.07 (light), 0.84 ± 0.09 (medium), 0.34 ± 0.06 (medium-dark), 0.17 ± 0.02 (dark), and for PC beverages these were 1.52 ± 0.05 (light), 0.80 ± 0.08 (medium), 0.35 ± 0.06 (medium-dark), and 0.18 ± 0.01 (dark) (Table 2 and Figure 4A). The average concentrations (μg/mL) of kahweol palmitate in the PC beverages were 326.84 ± 67.19 (light), 216.28 ± 12.85 (medium), 144.88 ± 22.15 (medium-dark), and 155.15 ± 24.76 (dark) and for the FC beverages; 11.13 ± 0.35 (light), 7.33 ± 1.28 (medium), 5.92 ± 0.74 (medium-dark), and 5.93 ± 0.61 (dark) (Table 2 and Figure 4B). Kahweol was present in trace amounts in both FC and PC beverages hence its content was not reported. A steady decrease in the cafestol content was observed with increased coffee roast level (Figure 3A) (Figure 4A) which agrees with the literature.[26] The cafestol content was highest in the light roast and lowest in the dark roast FC, and PC beverages. An average decrease in the cafestol content from light to dark roast, 87.5% (FC) and 88.4% (PC), was observed in the beverages. Similar cafestol content was recorded for medium-dark and dark roast FC and PC beverages (Figure 4A, Table 2). The concentration of kahweol palmitate decreased from light to dark roast for the coffee beverage types except for PC which showed a slight increase in kahweol palmitate content from medium-dark to dark (Figure 4B). The concentration of kahweol palmitate in the PC beverages reduced by 55.6% from light to medium-dark roast, followed by an increase of 6.6% from medium-dark to dark roast. Regarding FC beverages, the kahweol palmitate content decreased by 46.7% from light to dark roast. The decrease in the concentration of cafestol[26] and kahweol palmitate in the beverage types could be attributed to the degradation of diterpenes due to pyrolysis that occurs

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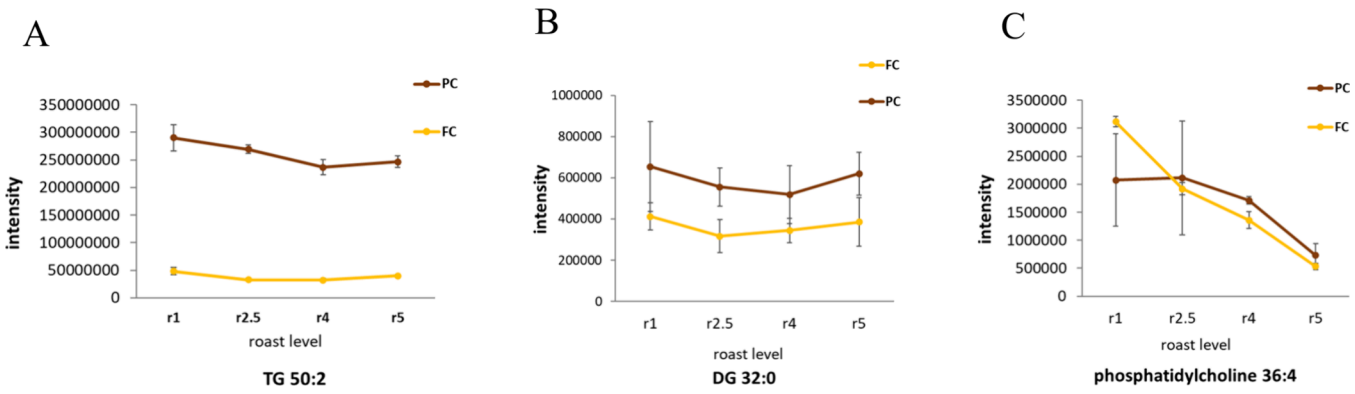

Figure 5. Trend of (A) TG 50:2, (B) DG 32:0 and SE 29:1, (C) phosphatidylcholine 36:4 in filter coffee (FC) and pot coffee (PC) lipid extracts of beverages prepared from light (r1), medium (r2.5), medium-dark (r4), and dark (r5) roast levels of coffee. Data is provided as mean ± SD, n = 3.

during coffee roasting with the reaction intensified as temperature and time increases.[41] Furthermore, research has detected the presence of cafestol, kahweol and their degradative products such as dehydro-cafestol, and dehydro-kahweol, in the gases that evolved during coffee bean roasting.[42] Considering this, the decrease in the content of kahweol palmitate and cafestol from light to dark roast FC, and PC beverages observed in this study could be attributed to the breakdown of diterpenes and their release as gases during roasting.[41,42] In general, PC beverages contained more kahweol palmitate than FC beverages which agrees with other literature.[16,26] It is noteworthy that the lowest concentration of kahweol palmitate (5.93 ± 0.61 μg/mL) was recorded in dark roast FC beverages (Figure 4B).

As shown in Figure 3A,B and in agreement with other studies,[6,43] the palmitate and linoleate forms were the most predominant diterpene esters identified in the coffee beverages. The free forms of kahweol and cafestol were comparatively low (Figure 3A,B). A decrease in the intensity of diterpenes with increased roast level was observed in the lipid extracts of both FC and PC beverages (Figure 3A,B). Light roast PC beverages recorded the highest intensity of diterpenes. Comparatively, the intensities of cafestol and its esters were higher than that of kahweol and its esters in both FC, and PC beverages (Figure 3A, B). Regardless of the coffee roast level, the total diterpene content was higher in PC beverages compared to FC beverages which agree with literature.[16,43] In effect, the total diterpene content in the lipid extracts of PC beverages would be considerably higher per unit volume of beverage compared with FC beverages because PC beverages contained more oil (Table 2). With respect to the roast level, the most statistically significant diterpenes in FC beverages were cafestol, cafestol arachidate, cafestol palmitate, kahweol stearate, and kahweol palmitate, and those for PC beverages were cafestol, cafestol oleate, cafestol palmitate, kahweol oleate, kahweol gadoleinate, kahweol linoleate, and kahweol palmitate (Supporting Information Table S6). The findings from this study provide vital information for the future development of FC and PC beverages with the desired diterpene contents.

3.6.2. Lipid Classes Significantly Affected by Coffee Roast Level. Triglycerols, diglycerols, and phosphatidylcholines were the first three lipid classes containing the most statistically significant identified lipophilic compounds affected by roast level in the lipid extracts of the coffee beverages (ANOVA, Tukey's test, $p < 0.05$). The topmost statistically significant metabolites identified (ANOVA, all roast levels) for TGs, DGs and phosphatidylcholines in the FC, and PC beverages, (TG 50:2, DG 32:0, phosphatidylcholines 36:4), were selected for further discussion (Supporting Information Table S7 and Figure S4).

3.6.2.1. TGs, DGs and Phosphatidylcholines. An initial decrease in the intensity of the TG 50:2 for PC and FC beverages was observed from light to medium-dark roast, followed by an increase from medium-dark to dark roast (Figure 5A). The decrease in the intensity of TG 50:2 from light to medium-dark roast could be attributed to its thermal degradation and hydrolysis into DGs, MGs, free fatty acids and volatile compounds during coffee roasting.[44] On the other hand, the increase in pressure and temperature during coffee roasting could have resulted in the breakdown of the endosperm of the coffee beans allowing oil to leak to the porous surface hence the increase in TG 50:2 from medium-dark to dark roasted coffee. Zhu et al. have similarly reported an initial decrease in the intensity of TG (16:0) followed by an increase as roasting progressed.[38] It is noteworthy that medium-roast FC beverages recorded the lowest intensity of TG 50:2, and high amounts of TG 50:2 in the plasma can result in a heightened de novo lipogenesis in obese women with gestational diabetes.[45]

DG 32:0 was the most statistically significant DGs identified in the coffee beverage types (Supporting Information, Figure S4). As shown in Figure 5B, the intensity of DG 32:0 decreased from light to medium roast, followed by an increase from medium to dark roast for FC beverages. For PC beverages, the intensity of DG 32:0 decreased from light to medium-dark roast followed by an increase to dark roasted coffee. The initial decrease in the intensity of the DG 32:0 could be attributed to its hydrolysis into MGs. The subsequent increase in DG 32:0 from medium-dark to dark roast coffee beverages could be attributed to the release of oil and the breakdown of TGs associated with high temperatures and prolonged roasting which could have overshadowed the degradation of DG 32:0. Moreover, the increase in the intensity of DG 32:0 was expected in this study since DGs are produced from the hydrolysis of TGs during thermal degradation.[44] It is worth mentioning that medium roast FC beverages recorded the lowest intensity of the DG 32:0 (Figure 5B). According to Furse et al., an elevated amount of DG 32:0 in the body is associated with severe insulin and hyperglycemia in gestational diabetic mothers.[46]

Phosphatidylcholines are a type of phospholipid present in coffee. Figure 5C shows a decrease in the intensity of

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phosphatidylcholine 36:4 from light to dark roast FC, and PC beverages except for PC beverage, which recorded an initial increase from light to medium roast before the decrease. A similar pattern was observed for phosphatidylcholines 34:2, and 38:2. The decrease in the intensity of phosphatidylcholine 36:4 could be attributed to the breakdown of phosphatidylcholines during the coffee roasting into lysophosphatidylcholines, glycerophosphocholines, phosphocholines, fatty acids and other compounds due to their thermal instability.[47]

The knowledge of the potential effect of the roast level on the nutritional properties of coffee beverages and subsequently on the health of coffee consumers is important. Our research findings show that the roast level of coffee affects the intensities of compounds reported to exhibit anti-inflammatory and antioxidant properties, e.g. trigonelline and chlorogenic acid,[7,8] and compounds associated with heightened de novo lipogenesis, severe hyperglycemia, and cholesterol elevation, e.g. TG 50:2, DG 32:0, and diterpenes.[14,45,46] While we observed that most of the bioactive compounds usually associated with coffee, such as chlorogenic acid and trigonelline, were highest in light roast coffee, some of the compounds e.g. niacin and quinic acid, had higher abundance in dark roast coffee. Because the trend in the compound abundances between roast levels was inconclusive and phytochemicals exhibit complex synergistic activity, further research is needed to conclude whether the metabolic differences translate into differences in the health properties of the coffee beverages.

To the best of the authors' knowledge, this is the first study on coffee roast level using untargeted metabolomics to extensively identify molecular-level characteristics in coffee that are significantly impacted by roasting. Furthermore, this study is the first to report dehydrosalsolidine (an alkaloid), deoxycarnitine (an amino acid derivative), and adenosine 3,5-cyclic monophosphate (a purine) as metabolites in *Coffea arabica* and subsequently demonstrates how these metabolites are affected by the roast level. Healthwise, deoxycarnitine may help with gut permeability in the case of environmental enteric dysfunction (EED),[48] while adenosine 5-cyclic monophosphate may promote cell proliferation and safe acceleration of epidermal turnover.[49] Finally, the effect of the coffee roast level on the profile and content of both hydrophilic and lipophilic compounds in ready-to-drink filtered (FC) and unfiltered (PC) coffee beverages is further established in this study.

Since coffee consumption is dependent on consumer preference, the findings from this study could be useful in new product development of coffee focused on health in relation to consumer demands. Additionally, future studies could focus on the optimization of the coffee roasting and brewing processes to attain a cup of coffee with a balance of good nutritional and sensory properties.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsfoodscitech.5c00066.

Calibration curves for caffeine, kahweol, kahweol and cafestol palmitate standards (Figure 1); Images of coffee makers used for the preparation of pot, and filter coffee beverages (Figure 2); One-way ANOVA with Welch's correction of the topmost 15 statistically significant hydrophilic compounds between all the roast levels of filter coffee (FC) and pot coffee (PC) beverages (Table 1); One-way ANOVA with Welch's correction of the statistically significant hydrophilic compounds ($p < 0.05$) between light and dark roast levels of coffee (r1 vs r5) for filter coffee (FC), and pot coffee (PC) beverages (Table 2); Mass spectra of identified metabolites not previously associated with coffee (Figure 3); ANOVA of the oil content in pot coffee (PC) and filter coffee (FC) beverages prepared from light (r1), medium (r2.5), medium-dark (r4) and dark (r5) roast levels of coffee (significance level, $p < 0.05$) (Table 3); ANOVA with Welch's correction of the topmost 25 statistically significant compounds that contributed to the separation of pot coffee (PC) and filter coffee (FC) beverages prepared from the different coffee roasts (significance level, $p < 0.05$) (Table 4); Lipophilic compounds identified in the lipid extracts of pot coffee (PC) and filter coffee (FC) beverages prepared from light (r1), medium (r2.5), medium-dark (r4) and dark (r5) roast levels of coffee (Table 5); Statistical analysis (ANOVA_posthoc (Tukey's test)) of the diterpenes in the lipid extract of pot coffee (PC) and filter coffee (FC) beverages prepared from light (r1), medium (r2.5), medium-dark (r4) and dark (r5) roast levels of coffee (Table 6); ANOVA_posthoc (Tukey's test) showing the metabolites with the most statistical significance in the lipid extracts of pot coffee (PC) and filter coffee (FC) beverages prepared from light (r1), medium (r2.5), medium-dark (r4) and dark (r5) roast levels of coffee (Table 7); Selected lipophilic metabolites of statistical significance for TGs, DGs and phosphatidylcholines in the lipid extracts of pot coffee (PC) and filter coffee (FC) beverages prepared from light (r1), medium (r2.5), medium-dark (r4) and dark (r5) roast levels of coffee (Figure 4) (PDF)

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■ ABBREVIATIONS

PC, pot coffee; FC, filter coffee; r1, light roast; r2.5, medium roast; r4, medium-dark roast; r5, dark roast; C, cafestol; K, kahweol; C_P, cafestol palmitate; K_P, kahweol palmitate; C_L, cafestol linoleate; K_L, kahweol linoleate; C_O, cafestol oleate; K_O, kahweol oleate; C_S, cafestol stearate; K_S, kahweol stearate; C_A, cafestol arachidate; K_A, kahweol arachidate; C_B, cafestol behenate; K_G, kahweol gadoleinate; C_LG, cafestol lignocerate; K_LG, kahweol lignocerate; QTOF, quadrupole time-of-flight; RP, reversed-phase; HILIC, hydrophilic interaction liquid chromatography; MS, mass spectrometry; UHPLC, ultrahigh performance liquid chromatography; PCA, principal component analysis; PC1, first principal component; PC2, second principal component; CID, Collision-induced dissociation; BEH, ethylene bridged hybrid; TG, triglycerols; DG, diglycerols; MGs, monoglycerols; SG, sterylglycosides; ST, sterols,; Cer, ceramides; NAGly, N-acyl ethanolamines; ANOVA, analysis of variance and PTFE, polytetrafluoroethylene.

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