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Quantitative Studies on Roast Kinetics for Bioactives in Coffee

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ABSTRACT: Quantitative analysis of the bioactives trigonelline (**1**), *N*-methylpyridinium (**2**), caffeine (**3**), and caffeoylquinic acids (**4**) in a large set of roasted Arabica (total sample size $n = 113$) and Robusta coffees (total sample size $n = 38$) revealed that the concentrations of **1** and **4** significantly correlated with the roasting color ($P < 0.001$, two tailed), whereas that of **2** significantly correlated inversely with the color ($P < 0.001$, two tailed). As dark-roasted coffees were rich in *N*-methylpyridinium whereas light-roasted coffees were rich in trigonelline and caffeoylquinic acids, manufacturing of roast coffees rich in all four bioactives would therefore necessitate blending of two or even more coffees of different roasting colors. Additional experiments on the migration rates during coffee brewing showed that all four bioactives were nearly quantitatively extracted in the brew (>90%) when a water volume/coffee powder ratio of >16 was used.

KEYWORDS: *coffee, N-methylpyridinium, trigonelline, caffeoylquinic acids, caffeine, roasting color*

INTRODUCTION

Coffee's popularity in the world is unbroken, and the production of coffee is still increasing, reaching values of ~7.14×10^6 t in 2011 with Brazil and Vietnam as the most important exporting countries. Besides the United States and Brazil consuming 18.5 and 16.6% of the total volume of raw coffee, respectively, roughly 34.4% was consumed by the European Union. Germany (7.9%) followed closely the coffee-hungriest countries of Scandinavia, Austria, and Switzerland. In 2011, Germans consumed 6.9 kg of coffee per capita, corresponding to 150 L of coffee beverage, making the dark brew even more popular than beer.[1] Coffee has advanced into a lifestyle product with putatively promising effects on health, leaving behind the pure pleasure of the alluring aroma and fancy flavor. Modest consumption of the dark brew has been associated with reduced risks to develop diabetes type 2, coronary heart disease or various cancers in later life.[2−5]

The coffee brew supplies numerous bioactive compounds to the human body, among which caffeine is the most thoroughly investigated. Besides the well-known stimulating effect on the central nervous system, caffeine has recently been suggested to be possibly involved in the prevention of Alzheimer's disease due to its ability to disrupt the amyloid precursor-protein-cleaving activity of γ-secretases in the brain, thus inhibiting the formation of aggregated plaques of amyloid-Aβ.[6] In contrast to caffeine, the alkaloid trigonelline is degraded by decarboxylation during the roasting process, generating the cation *N*-methylpyridinium (NMP).[7−9] An increasing body of scientific data suggests that this compound induces enzyme systems involved in detoxification of xenobiotics[10] and activates the Nrf2/ARE pathway, inducing cellular defense mechanisms.[11,12] In human cervical cancer cells, NMP affected the early multiplication of herpes simplex type 1 and polio virus and inhibited influenza in dog kidney cells.[13] On the basis of animal data, its precursor, trigonelline, is discussed to be involved in delaying insulin response to oral glucose uptake, however, with yet unknown contribution to the putative antidiabetogenous effects of coffee.[14,15]

In contrast to NMP, chlorogenic acids, known as the quantitatively predominating phenols in raw coffee, are reported to exhibit antioxidant properties in in vitro test systems[10,16] and are gradually degraded upon bean roasting to give chlorogenic acid lactones[17,18] as well as breakdown products such as catechol and guaiacol.[19−21] Although these polyphenols are strongly metabolized after consumption, they are believed to be involved in scavenging processes of free radicals generated within cell metabolism.[22,23] Moreover, some neuroprotective activities of the metabolites dihydroferulic acid and feruloylglycine have been reported recently.[24]

As the roasting process is the most important factor determining the chemical composition of a final coffee product and the concentrations of trigonelline (**1**), *N*-methylpyridinium (**2**), caffeine (**3**), and caffeoyl quinic acids (**4**, Figure 1), the aim of this study was to investigate the relationship between the roasting color and the quantity of these bioactive target compounds in a large set of industrially roasted coffee samples and to develop basic principles for the blending of differently roasted Arabica and Robusta coffees to obtain a product rich in compounds **1**−**4**. Moreover, quantitative investigation of the extraction yield should be conducted to obtain first insights into the migration rates of the bioactives and to assess whether the

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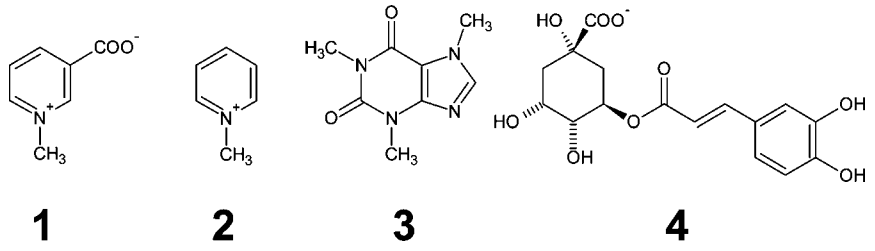

Figure 1. Chemical structures of the bioactive coffee ingredients trigonelline (**1**), *N*-methylpyridinium (**2**), caffeine (**3**), and 5-*O*-caffeoylquinic acid (**4**).

analytical fingerprint generated through roasting is conveyed to the coffee brew.

MATERIALS AND METHODS

Coffee Samples. Roasting of coffee and characterization of the roast color was done by the Technical University Hamburg (roast matrix) and by Tchibo GmbH, Hamburg, respectively (industrial roasts). All coffee samples were kindly provided by Tchibo GmbH, Hamburg.

Chemicals. Solvents and reagents for quantitative analysis were purchased from Sigma-Aldrich (Steinheim, Germany). Ninety-six-well plates (RP18, 100 mg per well), manifold, and collection plates were from Phenomenex (Darmstadt, Germany). Water was prepared by a Milli-Q apparatus (Millipore, Bedford, MA, USA). Synthetic d_3-trigonelline hydroiodide, d_3-NMP iodide, and NMP iodide were synthesized as reported earlier.[7,8,26]

Determination of Roast Color. The determination of the roast color was done with a Color Tester LFM 1 Dr Lange type using an OG 539 yellow light filter and a photocell analyzing the diffuse light reflected from a sample of ground roast coffee. Data are given as nondimensional scale parts ("Skalenteile", skt). The system is calibrated to a scale from 110 to 35 on the basis of different reference roasts; a light roast is represented by values between 90 and 110 and a dark roast by values between 35 and 60 scale parts.

Roast Coffee Matrix. Raw coffee samples (Arabica Brazil, 300 g per experiment) were roasted in a laboratory-scale fluidized bed roaster (Novopak, Germany) with roasting gas (air) flowing rate of 150 $m^3\ h^{-1}$. Roasting was performed for 2, 5, and 15 min, respectively, with adjusted roasting temperature between 200 and 290 °C to meet a color value of 35, 50, 65, 80, 90, and 110 scale parts. The matrix therefore comprised 6 (color) × 3 (time) coffee samples (n = 18 samples in total). At the end of each roasting procedure, the coffee beans were quenched with air (150 $m^3\ h^{-1}$) to cool the coffee beans to room temperature within 2 min. Roastings were prepared in three repetitions; the resulting roast coffees were pooled to produce one sample characterized by roast color and roast time. Coffee beans were frozen in liquid nitrogen and milled to a fine powder, which was kept tightly closed in glass bottles to avoid uptake of moisture.

Formation and Degradation of Bioactives in Industrially Roasted Coffee Samples. Coffee samples roasted on an industrial scale were provided by Tchibo GmbH. The samples originated from industrial roast trials with three different Arabica proveniences (Brazil, Guatemala, and Colombia) and one Robusta (Vietnam). Roastings were performed on different industrial roasters (Probat Neptune E 1500, Probat Jupiter 750, Neuhaus Neotec RFB) with roasting times between 5 and 20 min at temperatures between 200 and 330 °C. Samples of the roasted coffee beans were frozen in liquid nitrogen and milled to a fine powder, which was kept tightly closed in glass bottles to avoid uptake of moisture.

Preparation of Coffee Brew. For the investigation of the extraction yields of compounds **1**–**4**, coffee brews were prepared by percolation of finely powdered roast coffee with freshly boiled tap water at ratios of water volume/coffee powder of 7.5, 19, 30, and 50, respectively, followed by filtration. The volume of the resulting filtrates was measured using a graduated cylinder. The concentrations of **1**–**4** were determined as given below using aliquots (100 μL) of the filtrates.

Quantitative Analysis of Bioactives in Coffee. Quantitation of trigonelline and *N*-methylpyridinium was done using a stable isotope dilution based HILIC-LC-MS/MS method;[25] caffeine and caffeoylquinic acids (as the sum of 3-, 4-, and 5-*O*-caffeoylquinic acids) were analyzed with HPLC-DAD.[18] Briefly, an aliquot (200–400 mg) of coffee powder was mixed with aqueous ethanol (50:50, v/v, 5 mL) and heated in a closed reaction vessel with stirring (1 h, 100 °C). The suspension was centrifuged and the supernatant decanted into a volumetric flask (25 mL). The residue was resuspended twice in water and centrifuged, and the supernatants were added to the flask, which was finally made up to 25 mL with water.

Aliquots of the extract (100 μL) or coffee brew (100 μL, see above), respectively, were added to the internal standard solution (100 μL) containing d_3-trigonelline (1 mM in water) and d_3-NMP (0.25 mM in water) and equilibrated for 15 min with light shaking. Aliquots (100 μL) of the solutions were applied on a 96-well plate SPE (100 mg, RP18 preconditioned with acetonitrile, followed by water, 200 μL each) and sucked through the material. The target compounds were eluted with water (2 × 100 μL), the combined eluates were diluted with acetonitrile (10:90, v/v), and an aliquot (1 μL) was injected into the HPLC-MS/MS system. The system was an Agilent 1200 HPLC (Agilent, Waldbronn, Germany) connected to an API 3200 triple-quadrupole mass spectrometer (Applied Biosystems/MDS Sciex, Darmstadt, Germany). Chromatography was performed isocratically at a flow of 400 μL min^{-1} with a mixture of water/acetonitrile/ammonium acetate (5 M in water)/formic acid (100:885:0.1:15) on a 50 × 2 mm, 2.1 μm, Kinetex HILIC column (Phenomenex, Aschaffenburg, Germany) protected by a Krud Catcher (Phenomenex). The run length was 5 min. Curtain gas, nebulizer gas, and heater gas were set to 35, 50, and 60, respectively. Ionization was in positive electrospray mode with an ion spray voltage of 5.5 kV; the temperature of the heater gas was 550 °C. Optimization of ionization and fragmentation for sensitive MRM analysis was obtained by software-assisted ramping of the respective ion source and collision cell parameters. The mass transitions monitored were m/z 141→97 (d_3-trigonelline, quantifier), 141→95 (d_3-trigonelline, qualifier), 97→79 (d_3-NMP, quantifier), 97→78 (d_3-NMP, qualifier), 138→94 (trigonelline, quantifier), 138→92 (trigonelline, qualifier), 94→79 (NMP, quantifier), and 94→78 (NMP, qualifier). Standards were prepared in 95% acetonitrile containing the analytes trigonelline and *N*-methylpyridinium (0.1, 0.2, 0.5, 1, 2, 5, and 10 relative to the respective internal standard) and internal standards (fixed concentration). Calibration curves were obtained by plotting the acquired data (triplicate injection) of area ratios of analyte/internal standard versus concentration ratios of analyte/internal standards followed by linear regression ($\mathbf{1}/\mathbf{d_3 1}$: (area **1**/area $\mathbf{d_3 1}$) = 1.230 × ($c\mathbf{1}/c\mathbf{d_3 1}$) − 0.059, R^2 = 0.9998; $\mathbf{2}/\mathbf{d_3 2}$: (area **2**/area $\mathbf{d_3 2}$) = 0.759 × ($c\mathbf{2}/c\mathbf{d_3 2}$) − 0.013, R^2 = 0.9998).

The analysis of caffeine and caffeoylquinic acids, determined as the total amount of 3-*O*-, 4-*O*-, and 5-*O*-caffeoylquinic acids, was performed as reported earlier.[18] An aliquot (100 μL) of the extract solution or coffee brew, respectively, was diluted with water (900 μL) and membrane-filtered (0.45 μm), and 10 μL was injected into the HPLC-DAD system (Jasco, Groß-Umstadt, Germany). Separation was performed on a 250 × 4.6 mm, 5 μm, Luna phenylhexyl column

Table 1. Trigonelline (1), *N*-Methylpyridinium (2), Caffeine (3), and Caffeoylquinic Acids (4) in a Light-Roasted and a Dark-Roasted Coffee Sample

	color	concentration (μmol/g; RSD, %)[a] of			
sample	scale parts[b]	1[c]	2[c]	3[d]	4[d]
light coffee	84	51.09 (1.8)	5.36 (2.9)	68.94 (2.2)	53.88 (0.1)
dark coffee	54	14.42 (5.7)	21.49 (2.6)	68.58 (1.7)	13.40 (5.6)

[a]Data are means of individual extractions (n = 6) of sample aliquots. [b]See Materials and Methods for measurement of scale parts. [c]Quantified by SIDA-LC/MS/MS. [d]Quantified by HPLC-DAD as the sum of 3-*O*-caffeoyl-, 5-*O*-caffeoyl-, and 4-*O*-caffeoylquinic acid.

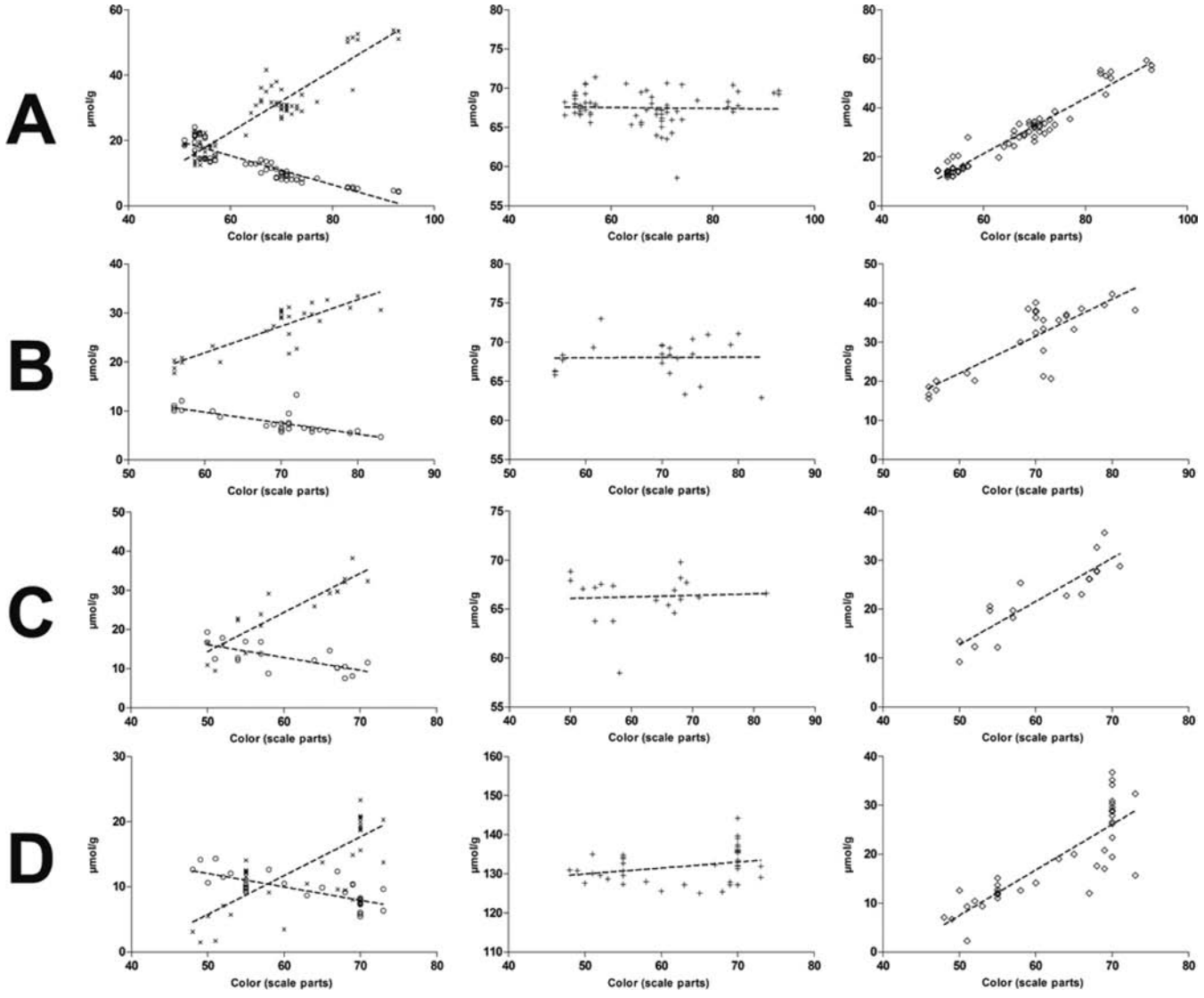

Figure 2. Concentration (μmol/g) of trigonelline (**1**, ×, left column) and NMP (**2**, ○, left column), caffeine (**3**, +, middle column) and the sum of 3-*O*-caffeoyl-, 5-*O*-caffeoyl-, and 4-*O*-caffeoylquinic acids (**4**, ◇, right column) versus roast color (scale parts) in Arabica (A, Brazil, n = 69; B, Guatemala, n = 27; C, Colombia, n = 17); and D, Robusta (n = 38). Note that the color is given on the x-axis as scale parts ("Skalenteile", skt).

(Phenomenex, Darmstadt, Germany) with ammonium formate buffer (eluent A, 0.25 M, pH 3.5) and methanol (eluent B). Chromatography was performed at a flow rate of 0.8 μL min^{-1} starting at 25% B and increasing to 28% within 38 min and, then, to 100% within 6.5 min. Quantitative data were obtained using 5-point calibration curves (5–500 μM) of caffeine and 5-chlorogenic acid, monitored at 272 and 324 nm, respectively.

Statistical Analysis. Graphpad prism 5.04 was used for data processing, correlation analysis, regression calculations, and preparation of graphs.

RESULTS AND DISCUSSION

The first aim of the study was to investigate the formation and degradation of the coffee bioactives trigonelline (**1**), *N*-methylpyridinium (NMP, **2**), caffeine (**3**), and *O*-caffeoylquinic acids (**4**) as a function of roast color. In an initial experiment, Arabica Brazil raw coffee was roasted to a light (84 skt) and a dark color (54), and the analytes **1**–**4** were quantified (Table 1). With the exception of caffeine, the analytes were affected by the roasting color. Trigonelline and *O*-caffeoylquinic acids were strongly diminished in the dark-roasted sample, whereas *N*-

Table 2. Linear Regression Data, R^2, and Spearman Correlation Data (ρ) of Trigonelline (1), *N*-Methylpyridinium (2), Caffeine (3), and the Sum of 3-*O*-Caffeoyl-, 5-*O*-Caffeoyl-, and 4-*O*-Caffeoylquinic Acids (4) versus Roast Color (Scale Parts)

		compounds			
	no. of samples, *n*	1	2	3	4
Brazil	69	$y = 0.9464x - 34.21$, $R^2 = 0.8587$[a] $\rho = 0.8159$[b]	$y = -0.4436x + 41.92$, $R^2 = 0.8133$[a] $\rho = -0.9513$[b]	$y = -0.0053x + 67.86$, $R^2 = 0.0008$ $\rho = -0.0641$[d]	$y = 1.1340x - 46.80$, $R^2 = 0.9411$[a] $\rho = 0.9296$[b]
Guatemala	27	$y = 0.5420x - 10.62$, $R^2 = 0.7228$[a] $\rho = 0.7643$[b]	$y = -0.2223x + 23.07$, $R^2 = 0.5590$[a] $\rho = -0.7276$[b]	$y = -0.0010x + 68.20$, $R^2 < 0.0001$ $\rho = 0.1099$[d]	$y = 0.9465x - 34.75$, $R^2 = 0.6790$[a] $\rho = 0.6860$[b]
Colombia	17	$y = 0.9364x - 31.46$, $R^2 = 0.8116$[a] $\rho = 0.9416$[b]	$y = -0.3601x + 34.68$, $R^2 = 0.5424$[b] $\rho = -0.7794$[c]	$y = -0.0153x + 66.98$, $R^2 = 0.0021$ $\rho = -0.1759$[d]	$y = 0.8940x - 32.05$, $R^2 = 0.7830$[a] $\rho = 0.9262$[b]
Robusta India	38	$y = 0.6235x$-25.93, $R^2 = 0.6399$[a] $\rho = 0.8360$[b]	$y = -0.2052x$+22.31, $R^2 = 0.5867$[a] $\rho = -0.8280$[b]	$y = 0.1515x$+122.4 $R^2 = 0.0832$ $\rho = 0.3509$ ($P = 0.0308$)	$y = 0.9300x$-39.04 $R^2 = 0.7066$[a] $\rho = 0.8836$[b]

[a]Slope is significantly different from zero. [b]$P < 0.0001$. [c]$P < 0.001$. [d]Not significant.

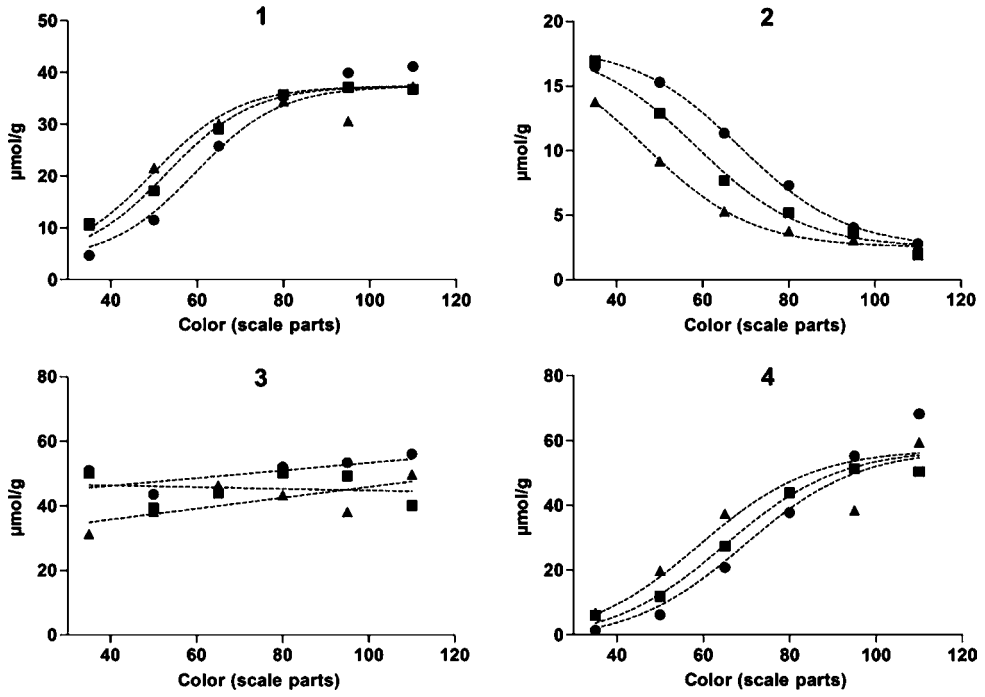

Figure 3. Concentrations (μmol/g) of trigonelline (**1**), *N*-methylpyridinium (**2**), caffeine (**3**), and the sum of 3-*O*-caffeoyl-, 5-*O*-caffeoyl-, and 4-*O*-caffeoylquinic acids (**4**) in Arabica Brazil roasted to a defined color (35−110 scale parts) within 2 (triangles), 5 (squares), and 15 min (dots). Note that the color is given on the *x*-axis as scale parts ("Skalenteile", skt).

methylpyridinium was more prominent in the dark-roasted compared to the light-roasted sample. These results confirmed previous observations.[7−9,17,18,26]

To further substantiate these findings, Arabica (Brazil, Guatemala, Colombia) and Robusta (India) raw coffee seeds were industrially roasted to defined color values between 100 (very light) and 45 (very dark) scale parts within 5−20 min, yielding a set of 151 coffee samples. The samples were characterized by color and were representative of commercially available coffees. Compounds **1**−**4** were quantified in roasted Brazil ($n = 69$), Guatemala ($n = 27$), Colombia ($n = 17$), and Robusta India ($n = 38$), and the quantitative data were plotted against the roasting color followed by linear regression and correlation analysis (Figure 2). Whereas the degradation of **1** during coffee roasting has been investigated in the context of roast gas temperature and roasting time,[26] the data presented here revealed concentration and color significantly correlated in all investigated proveniences (see Table 2 for Spearman ρ). The breakdown of the alkaloid can be approximated as a linear function of the roasting color (see Table 2 for R^2 data). The concentration of **2** significantly correlated negatively with the roasting color, with Spearman's ρ between −0.7276 in the Guatemalan samples and −0.9513 in the Brazilian samples. *N*-Methylpyridinium has been reported as the major reaction product of trigonelline during coffee bean roasting with yields of up to ∼18% (relative to **1**).[8] Our data show that coffee

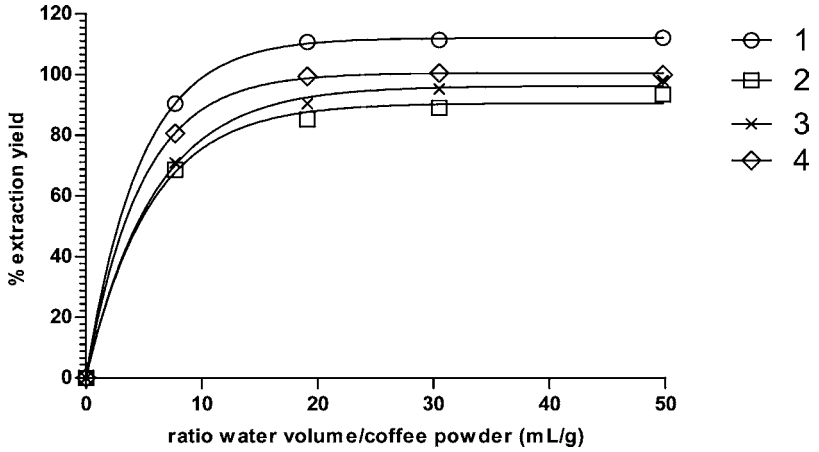

Figure 4. Extraction behavior of trigonelline (**1**, circles), *N*-methylpyridinium (**2**, squares), caffeine (**3**, crosses), and the sum of 3-*O*-caffeoyl-, 5-*O*-caffeoyl-, and 4-*O*-caffeoylquinic acids (**4**, diamonds) during coffee brewing.

roasting in authentic industry scale leads to substantial formation of **2** with yields up to approximately 50% (based on trigonelline) in espresso style roasting (color ∼50 scale parts), exceeding laboratory scale roasting (cf. Figure 2). Within the investigated color range the concentration of NMP approximated a linear function of the color with R^2 between 0.5424 and 0.8133 (cf. Table 2). This is the first time quantitative data on concentrations of compound **2** in industrially roasted coffee are reported. In contrast to **1** and **2**, the alkaloid caffeine (**3**) showed weak correlation with the roast color in the Arabica samples with Spearman's ρ between −0.1759 and 0.1099 (Table 2). The Robusta data suggested a small decline in caffeine concentration the darker the final coffee sample was. Although **3** is thermally stable, it is known that some amounts are lost during roasting due to caffeine's ability to sublime above 178 °C.[28]

Caffeoylquinic acids (**4**) are thermally degraded during coffee roasting.[17,18] Independent of the provenience, the concentration of **4** decreased approximately linearly with decreasing color values (cf. Figure 2 and Table 2). This observation confirms recent reports on the amounts of **4** in commercially available roast coffees, which had been characterized for roast degree on a rough scale from 1 to 5.[28] In a further experiment, raw coffee (Arabica Brazil) was roasted in a laboratory scale roaster to defined color values of 35, 50, 65, 80, 95, and 110 scale parts by variation of the roast gas temperature at a fixed roasting time (2, 5, or 15 min), thus generating a "roast matrix". The quantitative data of **1**−**4** in these samples plotted against the roast color are given in Figure 3. Similar to the first experiments, caffeine did not show a substantial dependency on roast color. For compounds **1**, **2**, and **4** the best-fitting curves were sigmoid shaped. The curves' inflection points obtained from 2, 5, and 15 min roastings were shifted on the color axis (Figure 3). Apparently the concentrations of trigonelline, NMP, and caffeoylquinic acids depended approximately linearly on the roast color only in a range of roughly 35 net scale parts. For example, roasting coffee for 2 min to a final color of 50 generated less NMP than did reaching this color within 15 min (Figure 3, second plot). In the samples roasted for 15 min, the concentrations of trigonelline and caffeoylquinic acids were lower at given color values between 50 and 80 scale parts compared to the samples roasted for 2 or 5 min, suggesting more exhaustive degradation of **1** and **4** during long-term roasting. This observation might be explained by the fact that the internal coffee bean temperature needs to rise above a certain value before degradation reactions can take place. During roasting, the coffee bean browns only after completion of the endothermic phase, in which the temperature stays around 100 °C due to evaporation of water. To obtain a defined roasting color within a narrow time frame of 2 or 5 min, it is therefore necessary to apply a higher roasting temperature as compared to a given time frame of 15 min. In some cases, a high temperature (e.g., >280 °C) may predominately scorch the outer parts of the bean, leading to an early superficial browning, whereas the inner parts stay cooler and are subsequently less affected. In such cases heat-induced degradation reactions can be expected to a lesser extent inside the bean, leading to lower yields of reaction products. As trigonelline, caffeoylquinic acids, and caffeine are supposed to be homogeneously distributed over the coffee seed, it is therefore possible that the conversion of trigonelline to NMP and the degradation of caffeoylquinic acids inside the coffee bean are less efficient when a roast color is reached by a high-temperature/short-time roasting.

A further aim was to investigate whether compounds **1**−**4** are extracted into the coffee brew. A defined amount of coffee powder was percolated with hot water in water volume/coffee powder ratios of 7.5, 19, 30, and 50. The volume of brew was measured in a graduated cylinder, and the concentration of the analytes was quantified by LC-MS/MS and HPLC-DAD. The extraction yields were calculated by 100% × [concentration in brew (μmol/mL) × volume brew (mL)/concentration in powder (μmol/g) × amount powder (g)]. The data were plotted against the ratio of water volume/coffee used for coffee brewing (Figure 4). Whereas a comparably low water/coffee ratio of 7.5 resulted in an extraction yield of the bioactive analytes between 70 and 90%, nearly quantitative extraction of trigonelline (≥100%), caffeoylquinic acids (>99%), caffeine (up to 98%), and *N*-methylpyridinium (up to 95%) was observed at a ratio of at least 16 as typical for a standard filtered coffee.

In conclusion, from the presented data, the degradation of trigonelline and caffeoylquinic acids is inversely correlated with the roasting color, and the generation of *N*-methylpyridinium is positively correlated with the roasting color. The concentrations of **1**, **2**, and **4** can be approximated by a linear function in the color range between ∼90 and 50 scale parts. For a given roast degree, the degradation of trigonelline and caffeoylquinic acids appeared to be more exhaustive in a prolonged roasting

process at lower temperature compared to fast roasting at high temperature. As NMP generation is always associated with degradation of its precursor trigonelline and other thermolabile compounds such as chlorogenic acid,[27] it appears impossible to obtain a product rich in all of these bioactives. A way to design a roast coffee with defined amounts of these bioactives could be blending two or more coffees roasted separately to a defined color, for a desired composition of bioactives in the final product.

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Notes

The authors declare no competing financial interest.

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