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Investigating the Chemical Changes of Chlorogenic Acids during Coffee Brewing: Conjugate Addition of Water to the Olefinic Moiety of Chlorogenic Acids and Their Quinides

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Supporting Information

ABSTRACT: Coffee is one of the most popular and consumed beverages in the world and is associated with a series of benefits for human health. In this study we focus on the reactivity of chlorogenic acids, the most abundant secondary metabolites in coffee, during the coffee brewing process. We report on the hydroxylation of the chlorogenic acid cinnamoyl substituent by conjugate addition of water to form 3-hydroxydihydrocaffeic acid derivatives using a series of model compounds including monocaffeoyl and dicaffeoylquinic acids and quinic acid lactones. The regiochemistry of conjugate addition was established based on targeted tandem MS experiments. Following conjugate addition of water a reversible water elimination yielding *cis*-cinnamoyl derivatives accompanied by acyl migration products was observed in model systems. We also report the formation of all of these derivatives during the coffee brewing process.

KEYWORDS: *chlorogenic acids, roasted coffee, caffeoylquinic acids, chlorogenic acid lactones, diacyl chlorogenic acids, caffeoyl-γ-quinides, antioxidant, LC−MS^n*

INTRODUCTION

Classically, chlorogenic acids are a family of esters formed between quinic acid and certain *trans*-cinnamic acids, most commonly caffeic, *p*-coumaric, and ferulic acids[1−3] and sometimes dimethoxycinnamic, trimethoxycinnamic, and sinapic acids.[4−6] In the IUPAC system (−)-quinic acid is defined as 1L-1(OH),3,4/5-tetrahydroxycyclohexane carboxylic acid, but Eliel and Ramirez[7] recommend 1*α*,3*R*,4*α*,5*R*-tetrahydroxycyclohexane carboxylic acid. Chlorogenic acids are widely distributed in plants,[2,3] but the coffee bean is remarkably rich, containing at least 72 chlorogenic acids that are not acylated at the C1 of the quinic acid moiety.[4−6,8−10] These have been subdivided into 13 classes according to the type of ester substituent (e.g., caffeoyl, feruloyl, sinapoyl, etc.) and the number of ester substituents (monoacylquinic acids, diacylquinic acids, or triacylquinic acids).[4−6,8−10]

Several pharmacological activities of chlorogenic acids including antioxidant activity,[11] ability to increase hepatic glucose utilization,[12] inhibition of the HIV-1 integrase,[13] antispasmodic activity,[14] and inhibition of the mutagenicity of carcinogenic compounds[15] have been reported so far. Coffee is considered the major source of chlorogenic acids in a typical human diet with an estimated 200 mg of total chlorogenic acids present in a 200 mL cup. Coffee, after water and black tea, is the third most consumed beverage globally and the second most traded commodity after crude oil, accounting for exports worth an estimated U.S. $15.4 billion in 2010 (International Coffee Organization (ICO), 2011). Its estimated annual retail value exceeded $70 billion[16,17] in 2010 with employment in the coffee sector estimated at about 26 million people worldwide in 52 producing countries (ICO, 2011).

To obtain the popular coffee beverage, the coffee cherry undergoes three important processing steps. First the flesh is mechanically removed from the cherry, which after washing and drying yields the green coffee bean. Second, the green coffee beans are roasted at temperatures ranging from 180 to 220 °C for 8−15 min, producing the desired aroma and taste of coffee.[18,19] Finally, the roasted beans are ground into a powder and infused with hot water, at times like in espresso brewing under pressure, to yield after filtration the coffee beverages. At each of these three processing steps it must be expected that the chemical content of the coffee bean changes. For the first step it is reported that residual enzymatic activity in the harvested beans leads to some chemical changes of coffee components.[20,21] During roasting a myriad of chemical changes take place producing thousands of novel products from the main phytochemical constituents of the green coffee bean including chlorogenic acids as their main secondary metabolites, carbohydrates, and proteins in thermal dehydration reactions and Maillard type reactions. Chemical changes reported for chlorogenic acids include chlorogenic acid lactones formation through the loss of a water molecule from the quinic acid moiety and formation of an intramolecular ester bond.[22] Furthermore, acyl migration has been reported in model systems forming C1-substituted chlorogenic acids. Along with chlorogenic acids, their lactones also contribute to coffee flavor and, despite their low concentrations, their impact on the final cup quality may be significant. Chlorogenic acids lactones have also been studied for their potential hypoglycemic effects[22] and for their actions at opioid and adenosine brain receptors.[23]

To the best of our knowledge the last processing steps involving the brewing of coffee powder with boiling water have

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never been investigated, although chemical changes of chlorogenic acids can be anticipated at this level as well. In this contribution we report on our investigation of novel compounds formed from chlorogenic acids during this brewing process. In order to characterize novel chlorogenic acids derivatives, we take advantage of LC−MS^n methods developed to characterize hydroxycinnamoyl quinic acids.[4−6,9−11] The MS fragmentation patterns in tandem MS spectra, UV spectrum, retention time, relative hydrophobicity, and synthetic standards have been utilized to develop structure-diagnostic hierarchical keys for the identification of chlorogenic acids and shikimates. In the present study we applied these methods to the qualitative profiling of novel chlorogenic acids derivatives formed during coffee brewing.

MATERIALS AND METHODS

All the chemicals (Analytical grade) were purchased from Sigma-Aldrich (Bremen, Germany). Ground coffee (Robusta) was purchased from a supermarket in Bremen (Germany).

Brewing of Coffee. Ground coffee (3 g) was infused in 100 mL of hot water and stirred for 10 min. The prepared brew was cooled to room temperature, filtered through a membrane filter, and directly used for LC−MS.

Brewing of CGAs and Derivatives. Commercially available chlorogenic acids standards together with synthesized chlorogenic acids derivatives (each sample 500 μg) were infused in 3 mL of hot water each and stirred for 5 h under reflux. The solvent was removed under low pressure, and the samples were dissolved in MeOH and used for LC−MS^n.

LC−MS^n. The 1100 series LC equipment (Agillent, Bremen, Germany) comprised a binary pump, an autosampler with a 100 μL loop, and a DAD detector with a light-pipe flow cell (recording at 320 and 254 nm and scanning from 200 to 600 nm). This was interfaced with an ion-trap mass spectrometer fitted with an ESI source (Bruker Daltonics HCT Ultra, Bremen, Germany) operating in full scan, auto MS^n mode to obtain fragmentation. As necessary, MS^2, MS^3, and MS^4 fragment-targeted experiments were performed to focus only on compounds producing a parent ion at m/z 335, 353, 367, 371, 515, and 533. Tandem mass spectra were acquired in auto-MS^n mode (smart fragmentation) using a ramping of the collision energy. Maximum fragmentation amplitude was set to 1 V, starting at 30% and ending at 200%. MS operating conditions (negative mode) had been optimized using 5-caffeoylquinic acid with a capillary temperature of 365 °C, a dry gas flow rate of 10 L/min, and a nebulizer pressure of 10 psi.

HPLC. Separation was achieved on a 250 mm × 3 mm i.d. column containing C18-amide 5 μm, with a 5 mm × 3 mm i.d. guard column of the same material (Varian, Darmstadt, Germany). Alternatively, separation was also achieved on a 150 mm × 3 mm i.d. column containing diphenyl 5 μm, with a 5 mm × 3 mm i.d. guard column of the same material (Varian, Darmstadt, Germany). The data presented in this paper were with few exceptions generated with the C18-amide column. Solvent A was water/formic acid (1000:0.005 v/v), and solvent B was methanol. Solvents were delivered at a total flow rate of 500 μL/min. The gradient profile was linear from 10 to 70% B in 60 min followed by 10 min isocratic and a return to 10% B at 90 min and 10 min isocratic to re-equilibrate.

RESULTS AND DISCUSSION

Food processing changes dramatically the chemical composition of a food. It is well established that new products arising from food processing are responsible for desirable sensory and organoleptic properties of food; it is less established but highly probable that these products are responsible for health benefits of numerous foods. Therefore, elucidating structures of processing products and their mechanism of formation constitutes an important area of food analysis. Coffee processing consists of four steps: removal of flesh, drying, roasting, and brewing. While the first three have been investigated in some detail the last step has received no attention from a chemical composition perspective. During analysis of roasted coffee we noted that samples obtained from methanol extraction if compared to samples obtained from hot water extraction displayed notable differences in their chemical profile. Hot water extracts contained a significantly larger number of chromatographically resolvable caffeoyl and dicaffeoylquinic acid derivatives along with a series of further previously unidentified components clearly produced in the brewing process. Hence, we decided to investigate the products formed in coffee brewing from chlorogenic acid derivatives in more detail. Surprisingly, hot water is not just a simple solvent in food chemistry but can on occasions act as a reactive reagent, as presented here and in previous work on tea fermentation, where water was shown to be the key reagent in thearubigin formation.[24] For this study a total of three monoacylated and three diacylated chlorogenic acids, all purchased as reference standards, were used individually as model systems along with established food processing products in roasted coffee 1-*O*-caffeoyl-1,5-quinide and 1-*O*-feruloyl-1,5-quinide, obtained by total synthesis; for the selective synthesis[25−27] of 1-*O*-caffeoyl-1,5-quinide and 1-*O*-feruloyl-1,5-quinide, methods reported in literature were followed with minor modifications. Additionally, four different commercial roasted Robusta coffee samples were prepared by brewing of coffee powder with boiling water and the compounds identified in the model systems were compared to those observed in the real brew.

For the synthetic part, the allyl substituent for the phenolic OH is in our experience the preferred protecting group over acetyl protection; syntheses with both protecting groups were presented in this work. Acid removal of the acetyl protecting group seemed to generate a multitude of unidentified side products that made purification of the desired product rather tedious or even impossible. Base removal of the acetyl protection opens the 1,5-lactone, thus generating an additional step (closing the lactone) in the synthesis; in addition, after the deprotection step, the compounds were still of higher purity when the allyl protection was used. 1-*O*-Caffeoyl-1,5-quinide was synthesized using allyl protection for caffeic acid phenols, while 1-*O*-feruloyl-1,5-quinide was made available using acetyl protection. To generate 1-*O*-caffeoyl-1,5-quinide, the commercially available quinic acid and caffeic acid were used as starting material. After selective protection of the reactive moieties, an esterification step generated the main intermediate in good yield. The desired product was then obtained after the final two deprotections. At temperatures higher than 70 °C in the allyl deprotection step, the isopropylidene protection is also removed making the synthesis shorter by one step; however, the yield of the product obtained after purification by column chromatography was not satisfactory, and we preferred an additional step in the synthesis with better yields and higher purities.

Preliminary Assessment of Data. All data for the chlorogenic acids presented in this paper use the recommended IUPAC numbering system;[1] the same numbering system was adopted for water addition products of chlorogenic acids, their *cis*-isomers, and their acyl-migration isomers.

Investigation of Model Compounds. First, we subjected three monocaffeoylquinic acids, 3-*O*-caffeoylquinic acid, 4-*O*-caffeoylquinic acid, and 5-*O*-caffeoylquinic acid, to model brewing conditions using 10 min of hot water (100 °C) treatment followed by HPLC−MS analysis. The HPLC chromatograms

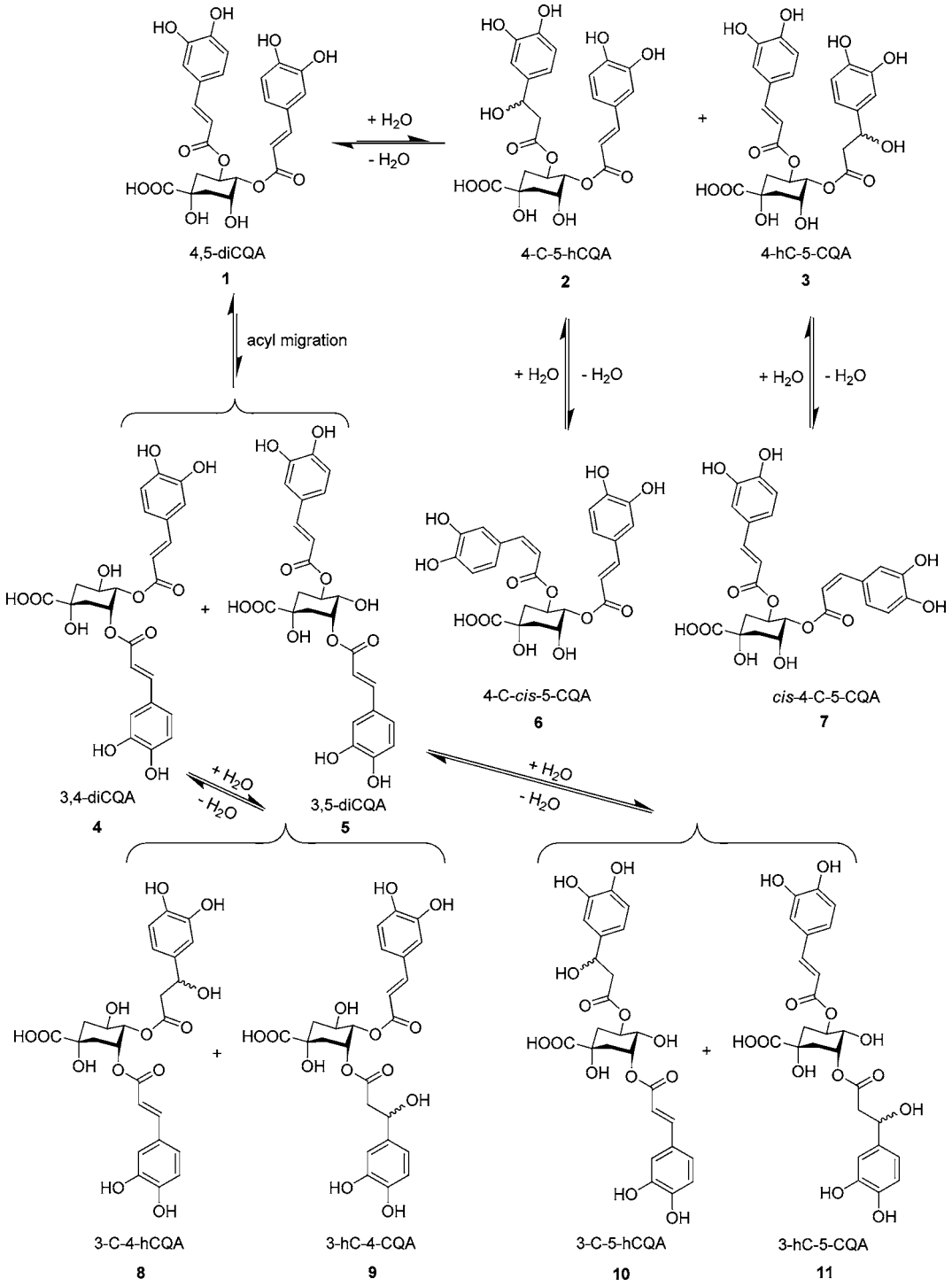

Figure 1. Formation of chlorogenic acid derivatives during the brewing of coffee (Tables 1 and 2).

showed between 4 and 12 distinct peaks corresponding to the products formed. The main focus was on the water addition products, a reaction mechanism leading to water addition and elimination being proposed in Figure 1. The products observed can be categorized into three types of chlorogenic acids derivatives: first, hydroxydihydrocaffeoylquinic acids arising through conjugate addition of water to the olefinic cinnamoyl moiety (Figure 2); second, acyl-migration products, including a selection of different caffeoylquinic acid regioisomers; and finally, trans−cis isomerization (*cis*-caffeoylquinic acids) products, presumably obtained by reversible β-elimination of water from hydroxydihydrocaffeoylquinic acids. The last two classes of derivatives have been analyzed and identified on previous occasions using authentic reference compounds in conjunction with LC−MS/MS.[28,29] In this study they were assigned based on retention times and tandem MS data and listed whenever observed (Table 1). Further unidentified minor products were observed in the chromatograms.

For the class of hydroxydihydrocaffeoylquinic acids, a detailed individual tandem MS study was conducted for each novel derivative reported. The substrates tested successfully for water addition at the C=C of the cinnamoyl moiety of

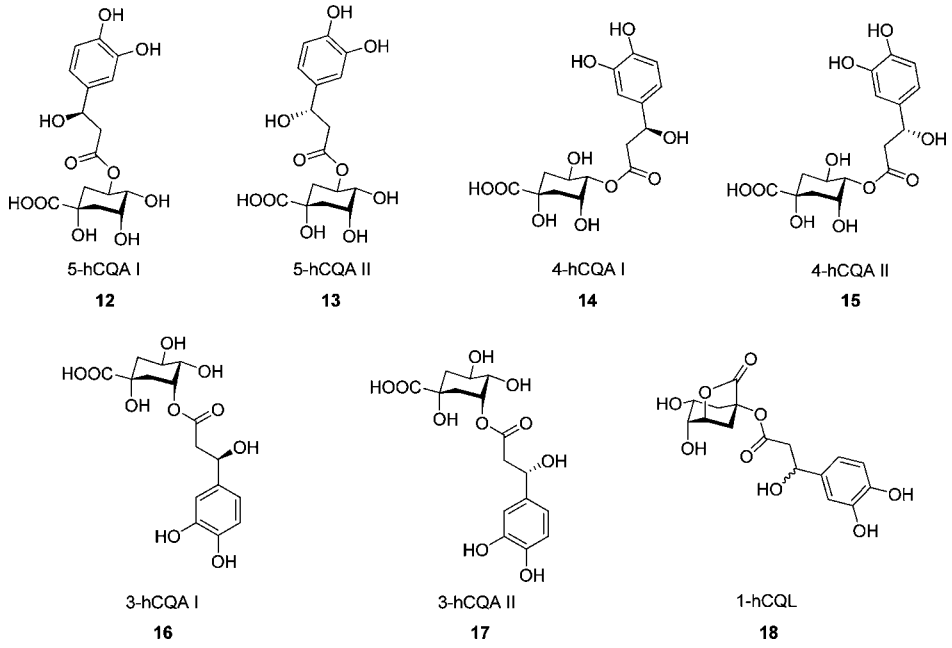

Figure 2. Monoacylated water addition derivatives of chlorogenic acids and lactones formed during brewing (Tables 1 and 2).

Table 1. Retention Times of Chlorogenic Acids and Their Derivatives

starting[a]	product name[a]	t_R [min]	m/z [M − H]
5-CQA	4-CQA	20.6	353
	5-CQA	20.1	353
	cis-5-CQA	23.0	353
	5-hCQA I	7.3	371
	5-hCQA II	7.9	371
4-CQA	3-CQA	13.1	353
	4-CQA	20.6	353
	5-CQA	20.1	353
	cis-3-CQA	11.9	353
	cis-4-CQA	16.5	353
	cis-5-CQA	23.0	353
	4-hCQA I	6.9	371
	4-hCQA II	7.9	371
3-CQA	3-CQA	13.1	353
	4-CQA	20.6	353
	cis-3-CQA	11.9	353
	3-hCQA I + II	5.6	371
3,4-diCQA	3,4-diCQA	36.7	515
	3,5-diCQA	37.4	515
	4,5-diCQA	41.4	515
	cis-3,4-diCQA I	35.9	515
	cis-3,4-diCQA II	38.5	515
	3-CQA	13.1	353
	4-CQA	20.6	353
	5-CQA	20.1	353
	3-hC-4-CQA	27.9	533
	3-C-4-hCQA	27.4	533
3,5-diCQA	3,4-diCQA	36.7	515
	3,5-diCQA	37.4	515
	4,5-diCQA	41.4	515
	cis-3,5-diCQA I	36.1	515
	cis-3,5-diCQA II	38.5	515
	3-C-5-hCQAI	24.1	533
	3-hC-5-CQA I	27.6	533
	3-hC-*cis*-5-CQA	28.4	533
	3-hC-5-CQA II	30.9	533
	3-CQA	13.1	353
	4-CQA	20.6	353
	5-CQA	20.1	353
4,5-diCQA	3,4-diCQA	36.7	515
	3,5-diCQA	37.4	515
	4,5-diCQA	41.4	515
	cis-4,5-diCQA I	39.5	515
	cis-4,5-diCQA II	45.8	515
	3-CQA	13.1	353
	4-CQA	20.6	353
	5-CQA	20.1	353
	4-hC-5-CQA	30.4	533
	3-hC-5-CQA II	30.9	533
	3-C-5-hCQA II	31.8	533
1-CQL (diphenyl column)	1-CQL	31.1	335
	3-CQL	26.7	353
	4-CQL	28.2	353
	1-CQA	10.9	353
	3-CQA	13.1	353
	4-CQA	20.6	353
	5-CQA	20.1	353
	1-hCQL	18.3	353
1-FQL (diphenyl column)	1-FQA	18.7	367
	3-FQA	19.2	367
	4-FQA	26.7	367
	5-FQA	27.1	367
	cis-1-FQA	16.1	367
	cis-4-FQA	24.1	367
	cis-5-FQA	29.9	367
caffeic acid	no water addition derivatives observed		
ferulic acid	no water addition derivatives observed		

[a]C, caffeoyl; F, feruloyl; hC, 3-hydroxydihydrocaffeoyl; QA, quinic acid; QL, quinic acid lactone.

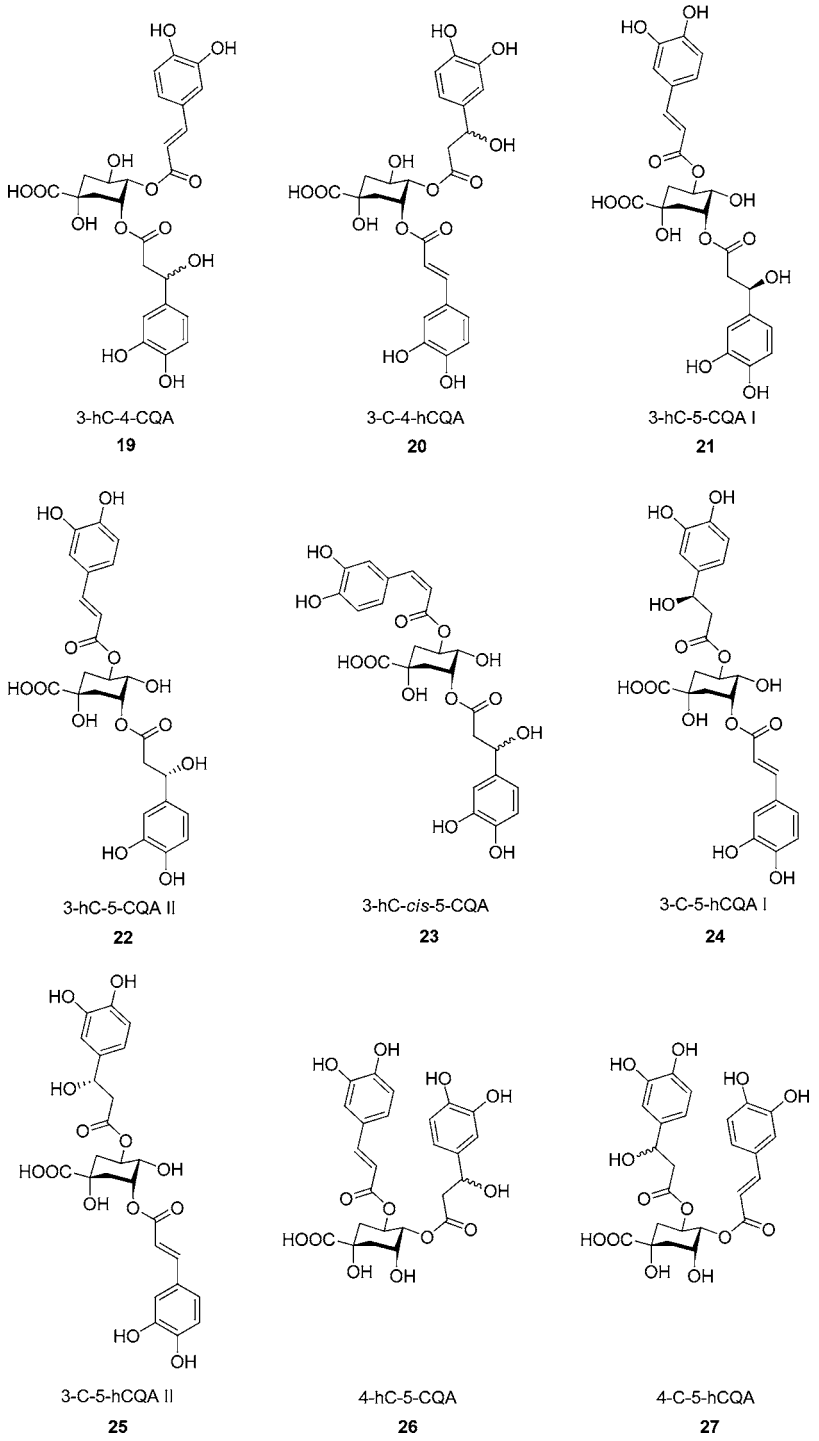

Figure 3. Diacylated water addition derivatives of chlorogenic acids formed during brewing (Tables 1 and 2).

chlorogenic acids (hydroxydihydrocaffeoylquinic acids are the products of this process) were 3,4-di-*O*-caffeoylquinic acid, 3,5-di-*O*-caffeoylquinic acid, and 4,5-di-*O*-caffeoylquinic acid. All novel diacylated hydroxydihydrocaffeoylquinic acids observed in the present work are shown in Figure 3.

Tandem MS Characterization of Monoacylated 3′-Hydroxydihydrocaffeoylquinic Acids (M_r = 371). Conjugate water addition to the olefinic cinnamoyl moiety of monoacylated chlorogenic acids was observed for three caffeoyl-containing substrates (3-*O*-caffeoylquinic acid, 4-*O*-caffeoylquinic acid, and 5-*O*-caffeoylquinic acid). For each of the three investigated monoacylated chlorogenic acids two corresponding hydroxydihydrocaffeoylquinic acids resulting from water addition could be distinguished as two chromatographically resolved peaks, appearing as pseudomolecular ions in the negative ion mode at m/z 371($[M - H^+]^-$) (e.g., 5-*O*-caffeoylquinic acid, Figure 4); for 3-*O*-caffeoylquinic acid they virtually coeluted, but an early shoulder in the chromatographic peak can be observed. The MS^3 fragmentation patterns of the precursor ion at m/z 353 ($[M - H^+ - H_2O]^-$) of hydroxydihydrocaffeoylquinic acids are similar or even identical to the MS^2 fragmentation of the corresponding chlorogenic acids, based on the

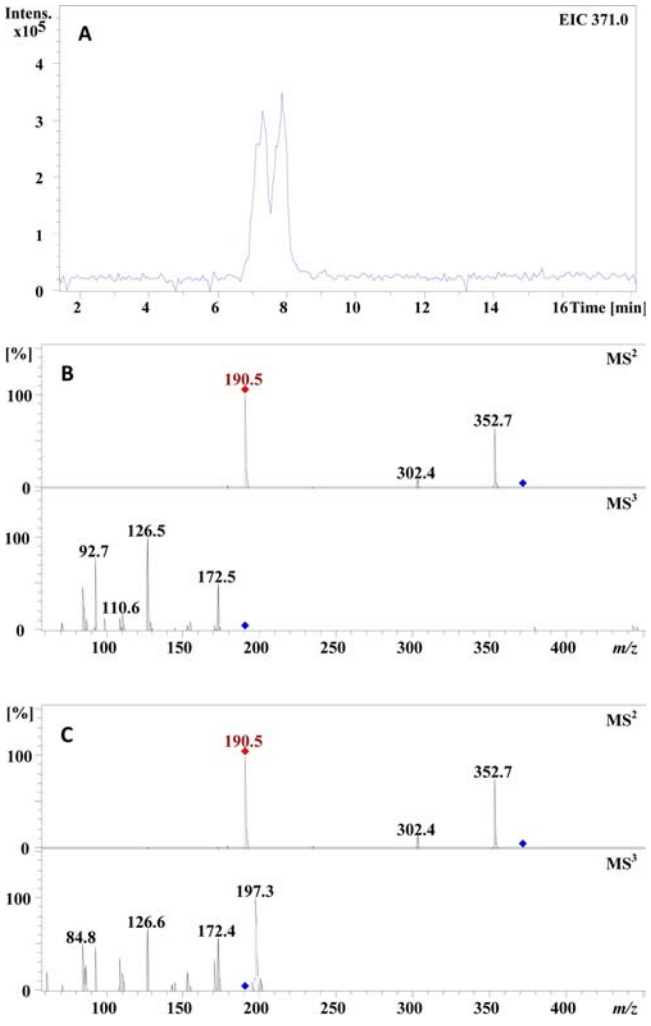

Figure 4. A) EIC of ion at m/z 371 showing two diastereomeric water addition compounds 12 and 13; B) Tandem MS spectrum in the negative ion mode of early eluting peak in a of 12, 13 with precursor ion at m/z 371; C)Tandem MS spectrum in the negative ion mode of later eluting peak in a of 12, 13 with precursor ion at m/z 371.

structural identity of the precursor ions, and therefore allow unambiguous assignment of acyl regiochemistry. Equally, the MS^4 fragment spectra of hydroxydihydrocaffeoylquinic acids are similar to the MS^3 spectra of the corresponding chlorogenic acids, as expected. In all six cases the hydroxyl moiety was confirmed by the MS^n fragmentation to be located at the β-posititon in the dihydrocinnamoyl residue, and on no occasion could an α-hydroxyl be detected (Table 2). This assignment of regiochemistry is based on a characteristic fragment ion at m/z 233 ($C_9H_{13}O_7$) showing a neutral loss of 138 Da corresponding to $C_7H_6O_3$. The fragmentation mechanism observed here can be classified as a retro-aldol type fragmentation, indicative of the regiochemistry of water addition. We have previously shown for malate esters of quinic acid that this fragmentation pathway can be used to unambiguously establish alcohol regiochemistry in chlorogenic acids chemistry.[30]

This finding implies that water addition to the double bond of the cinnamoyl residue of chlorogenic acids takes place in a regiospecific manner. The result is in contrast to the finding by Dawidowicz et al.[31] who attributed their observations to the presence of both β- and α-hydroxylated 5-*O*-caffeoylquinic acid, in a similar experiment; a more recent publication by the same group is in agreement with our finding.[29] In our work the two very closely eluting peaks with pseudomolecular ions at m/z 371 observed after brewing of 5-*O*-caffeoylquinic acid are clearly the two diastereoisomers of 5-*O*-(3′-hydroxydihydrocaffeoyl)quinic acid, **12** and **13** (Figure 2), as confirmed by the characteristic retro-aldol fragment ion in the fragment spectra. The same is true for 3-*O*-caffeoylquinic acid and 4-*O*-caffeoylquinic acid (two diastereisomers for each), but in the case of 3-*O*-caffeoylquinic acid they were virtually coeluting and could not be chromatographically resolved well with the method we used. Tandem MS is isomer blind with respect to stereochemistry, but since the water molecule is added regiospecifically to the cinnamoyl residue, the two chromatographic peaks observed for each β-hydroxylated chlorogenic acid giving identical tandem MS data could only be the two diastereoisomers.

5-*O*-(3′-Hydroxydihydrocaffeoyl)quinic acid I, **12**, and 5-*O*-(3′-hydroxydihydrocaffeoyl)quinic acid II, **13**, were identified by their m/z 371 ($[M - H^+]^-$) parent ion, and they both produced the MS^2 base peak at m/z 191 ($[\text{quinic acid} - H^+]^-$) and a secondary peak at m/z 353 ($[M - H_2O - H^+]^-$). 5-Acylation was confirmed by the low intensity MS^2 secondary peak at m/z 179 ($[\text{caffeic acid} - H^+]^-$), the MS^3 base peak at m/z 85, and the MS^3 secondary peak at m/z 173 ($[\text{quinic acid} - H_2O - H^+]^-$), as detailed in previous studies.[5,9] The presence of the hydroxyl group at the β-position was confirmed by the very low intensity MS^2 peak at m/z 233 (Table 2). A nonregiospecific water addition to the originally trans double bond of the cinnamoyl residue should have generated an additional MS^2 fragment at either m/z 249 (quinic acid moiety) or m/z 123 (caffeoyl moiety), neither being detected. This observation is consistent with the findings for all the mono- and diacylated chlorogenic acids tested for water addition in the present study; the specific MS^2 fragment always points toward the hydroxyl at the β-position and never at α-position.

4-*O*-(3′-Hydroxydihydrocaffeoyl)quinic acid I, **14**, and 4-*O*-(3′-hydroxydihydrocaffeoyl)quinic acid II, **15**, were preliminarily identified by their m/z 371 and produced the MS^2 base peak at m/z 353 ($[M - H_2O - H^+]^-$) and secondary peaks at m/z 191 ($[\text{quinic acid} - H^+]^-$), 179 ($[\text{caffeic acid} - H^+]^-$), 173 (most intense, $[\text{quinic acid} - H_2O - H^+]^-$), and 135 ($[\text{caffeic acid} - CO_2 - H^+]^-$). The MS^3 spectrum of m/z 353 revealed the base peak at m/z 173 and secondary peaks at m/z 191, 179, and 135 (low intensity), specific to acylation at C4 of quinic acid. The MS^4 spectrum produced the base peak at m/z 85 and secondary peaks at m/z 111 and m/z 93, fragments whose structure was proposed before.[9] The water addition specific fragment, though of very low intensity, appeared as expected at m/z 233 in the MS^2 spectrum.

3-*O*-(3′-Hydroxydihydrocaffeoyl)quinic acid I, **16**, and 3-*O*-(3′-hydroxydihydrocaffeoyl)quinic acid II, **17**, were preliminarily identified by their m/z 371 and produced the MS^2 base peak at m/z 353 ($[M - H_2O - H^+]^-$) and secondary peaks at m/z 191 ($[\text{quinic acid} - H^+]^-$), 179 ($[\text{caffeic acid} - H^+]^-$), 173 ($[\text{quinic acid} - H_2O - H^+]^-$), 135 ($[\text{caffeic acid} - CO_2 - H^+]^-$). The MS^3 spectrum of m/z 353 revealed the base peak at m/z 191 and secondary peaks at m/z 179, 173, and 135, with the last two of low intensity, specific to acylation at C3 of quinic acid. The water addition specific fragment, though of very low intensity, appeared as expected at m/z 233 in the MS^2 spectrum. Assignment of acyl regiochemistry in these cases is only possible in a targeted MS^3 experiment of the precursor

Table 2. Negative Ion MS^4 Data for Detected Hydroxydihydrocaffeoylquinic Acids and Hydroxydihydrocaffeoylquinide

			MS^2					MS^3			MS^4		
				secondary peak		β-OH peak			secondary peak			secondary peak	
	compd[a]	MS^1 parent ion m/z	base peak m/z	m/z	int	m/z	int	base peak m/z	m/z	int	base peak m/z	m/z	int
1	5-hCQA	370.9	190.5	352.7	86	233.4 (MS^2)	0.2	126.5	172.5	60			
									110.6	18			
									92.7	79			
									84.8	55			
2	4-hCQA	371.0	352.7	190.5	8	233.4 (MS^2)	1.7	172.5	190.5	35	110.6	92.7	83
				178.5	13				178.8	52			
				172.5	94				134.5	6			
				134.6	16								
3	3-hCQA	370.9	352.7	190.5	30	232.5 (MS^2)	1.5	190.5	178.8	44	126.5	172.4	27
				178.6	16				172.8	13		85.1	37
				172.6	16				134.6	11			
				134.6	66								
4	1-hCQL (diphenyl column)	352.9	334.8	172.6	8	214.6 (MS^2)	0.1	160.6	178.6	6	132.7		
				160.6	6				172.8	50			
				136.6	11				132.7	8			
5	3-hC-4-CQA	533.0	514.9	352.8	95	394.8 (MS^2) 232.6	6.3	352.7	334.8	12	172.5	191.0	47
				462.5	9	(MS^2)	2		298.7	2		178.9	93
				370.7	27				254.6	6		134.5	12
				334.8	46				191.0	6			
				298.7	4				178.9	15			
				254.6	6				172.5	21			
				191.0	5								
				178.9	8								
				172.5	18								
6	3-C-4-hCQA	533.0	370.8	514.8	72	394.8 (MS^2)	1.6	352.8	190.6	10	172.5	190.6	23
				462.5	45		1.2		178.9	14		178.9	54
				352.8	95	232.5 (MS^2)	3.3		172.5	69			
				334.9	34				134.5	15			
				190.9	7	232.5 (MS^3)							
				178.8	9								
				172.5	33								
7	3-C-5-hCQA	533.1	352.7	514.8	13	394.5 (MS^2)	1.1	190.5	334.7	13	85.0	126.8	37
				462.4	62				178.8	68			
				396.8	15				172.8	20			
				371.2	37				134.5	12			
				335.1	12								
				190.5	16								
				178.8	8								
				173.0	8								
8	3-hC-5-CQA	533.1	370.7	514.9	2	394.8 (MS^2)	0.4	352.7	190.5	30	190.4	178.8	44
				462.5	45		0.1		178.8	21		172.8	23
				396.5	12	232.5 (MS^2)	1.7		172.8	25		160.5	6
				352.7	11				134.5	64		134.5	18
				335.1	2	232.5 (MS^3)							
				190.5	3								
				178.8	1								
				173.0	2								
9	4-C-5-hCQA (diphenyl column)	532.9	352.9	514.9	90	394.8 (MS^2)	5.4	172.6	334.8	29	154.5	110.8	71
				462.6	27		1.4		190.6	38		92.8	55
				396.6	6	232.8 (MS^2)			178.6	63			
				370.8	16				134.6	11			
				335.0	33								
				190.6	5								
				178.8	8								

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Table 2. continued

			MS^2					MS^3				MS^4		
				secondary peak		β-OH peak			secondary peak				secondary peak	
	compd[a]	MS^1 parent ion m/z	base peak m/z	m/z	int	m/z	int	base peak m/z	m/z	int	base peak m/z	m/z	int
				172.6	16								
10	4-hC-5-CQA	533.0	370.8	514.8	3	394.8 (MS^2)	0.5	352.7	190.5	13	172.5	190.5	81
				462.5	24		0.3		178.5	8		178.5	53
				396.6	90	232.5 (MS^2)	0.9		172.5	58		134.5	10
				352.7	24				134.5	18			
				334.9	2	232.5 (MS^3)							
				190.9	2								
				178.8	2								
				172.5	9								

[a]C, caffeoyl; F, feruloyl; hC, 3-hydroxydihydrocaffeoyl; QA, quinic acid; QL, quinic acid lactone.

fragment ion at m/z 353, since only this ion is structurally identical to the parent ion in the chlorogenic acid reference mass spectra.

Characterization of Monoacylated 3′-Hydroxydihydrocaffeoylquinic Acid Lactone (M_r = 353). 1-*O*-(3′-Hydroxydihydrocaffeoyl)quinic acid lactone, **18** (Figure 2), or 1-*O*-(3′-hydroxydihydrocaffeoyl)-1,5-quinide was observed as a derivative of the parent 1-*O*-caffeoyl-1,5-quinide, which was made available through total synthesis. The corresponding water-addition derivative containing a feruloyl moiety was not observed for the synthetic 1-*O*-feruloyl-1,5-quinide substrate, but it cannot be concluded at the moment (and it is unlikely) that water addition to the C=C of the cinnamoyl residue is peculiar to caffeoyl-containing substrates only.

1-*O*-(3′-Hydroxydihydrocaffeoyl)-1,5-quinide, **18**, was preliminarily identified by its m/z 353 ($[M - H^+]^-$) parent ion. Compound **18** can be easily mistaken as a caffeoylquinic acid because of the identical MS m/z value (formally 1-*O*-caffeoylquinic acid $- H_2O + H_2O$, only that the water elimination and addition take place at different moieties within the molecule), but tandem MS can easily discriminate between 1-*O*-caffeoylquinic acid[10] and **18**. 1-*O*-(3′-hydroxydihydrocaffeoyl)-1,5-quinide, **18**, produced the MS^2 base peak at m/z 335 ($[M - H_2O - H^+]^-$) and secondary peaks with different intensities at m/z 173 ($[\text{quinic acid} - H_2O - H^+]^-$), 161, 137, and 111. The MS^3 spectrum produced the base peak at m/z 161 and secondary peaks with different intensities at m/z 179, 173, 155, 133, 111, and 93. The structure of these tandem MS fragments was previously proposed.[32] In the MS^4 spectrum the base and only peak was observed at m/z 133. The specific fragment confirming the β-hydroxyl position was detected in MS^2 at m/z 215. An α-hydroxyl would be expected to generate fragments at most likely m/z 231 or m/z 123, but neither was detected. It is interesting to note that compound **18** constitutes an isomer of caffeoylquinic acid that could on other occasions easily be mistaken for a diastereoisomer of caffeoylquinic acid observed in roasted coffee.

Characterization of Diacylated Caffeoyl-3′-hydroxydihydrocaffeoylquinic Acids (M_r = 533). When homodicaffeoylquinic acids (3,4-di-*O*-caffeoylquinic acid, 3,5-di-*O*-caffeoylquinic acid, and 4,5-di-*O*-caffeoylquinic acid) were subjected to model coffee brewing conditions, the chromatograms revealed the formation of 6−18 novel products. The products included, similar to the monocaffeoyl derivatives, products of acyl migration, trans−cis isomerization, and conjugate water addition. The conjugate addition of water was always observed only for one of the two caffeoyl moieties showing pseudomolecular ions at m/z 533 in the negative ion mode. Compounds showing conjugate water addition to both cinnamoyl moieties with expected pseudomolecular ions at m/z 551 were on no occasion observed. Interestingly, for some dicaffeoylquinic acids (3,5-di-*O*-caffeoylquinic acid, **5**, and 4,5-di-*O*-caffeoylquinic acid, **1**), both acyl migration and water addition to one acyl moiety were observed in the corresponding product. The order in which the two processes took place could not be established, though we suspect that acyl migration should be the faster process of the two. Acyl migration was observed for all tested mono- and diacylated substrates, but it was only for the diacylated substrates that both acyl migration and water addition could be detected in a product in our study. Other work confirms that the two processes take place jointly for monoacylated substrates as well, and conjugate water addition to an acyl-migration monoacylated product was reported.[29] Products arising from acyl migration and *trans−cis* isomerization are summarized in Table 1.

The main analytical challenge for the monohydroxylated dicaffeoylquinic acids structure elucidation constitutes the correct assignment of acyl regiochemistry. In theory regiospecific monohydration of a dicaffeoylquinic acid derivative may result in the formation of four isomeric products. For example, 3,4-di-*O*-caffeoylquinic acid, **4**, may produce one pair of diastereoisomeric monohydroxylated dicaffeoylquinic acids with water being regiospecifically added to the caffeoyl moiety attached to the C4 of quinic acid and a second pair of diastereoisomeric monohydroxylated dicaffeoyl derivatives with water being regiospecifically added to the caffeoyl moiety of the C3 of quinic acid. As suggested earlier, the observation of facile acyl migration under the reaction conditions might complicate the observed product profile with a total of six regioisomeric dicaffeoylquinic acids derivatives able to form in theory a total of 24 isomeric monohydroxylated dicaffeoylquinic acids derivatives. In order to assign the regiochemistry of the caffeoyl moiety on the quinic acid, we have performed multireaction monitoring (MRM) MS^3 experiments for m/z 533 → 353 for all the diacylated water addition derivatives (e.g., 4,5-di-*O*-caffeoylquinic acid, Figure 5).

For 3,4-di-*O*-caffeoylquinic acid, **4** the EIC reveals the presence of only two peaks showing a pseudomolecular ion at

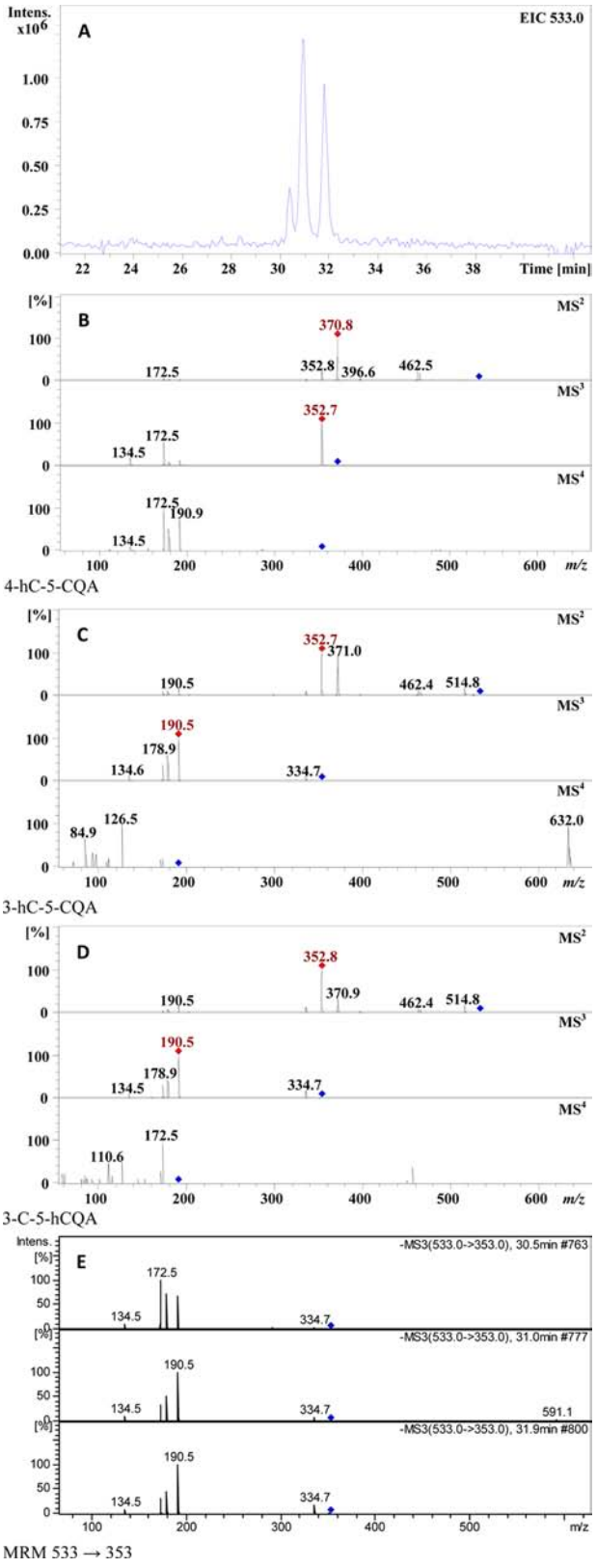

Figure 5. A) EIC of ion at *m/z* 533 showing three regioisomeric water addition compounds to 4, 5 diCQA and 3,5 diCQA; B-D) Tandem MS spectrum in the negative ion mode of hydroxydihydrocaffeoyl quinic acids **7, 8, 10,** with precursor ions at *m/z* 533; E) Multiple reaction monitoring spectra in MS3 of compounds **7, 8 and 9** illustrating fragmentation of precursor ion at *m/z* 353.

m/z 533 in the negative ion mode. The tandem mass spectra of both isomeric compounds show significant differences (Table 2). The MS^2 spectra of both derivatives show fragment ions with characteristic intensity differences at *m/z* 515 $[M - H^+ - H_2O]^-$, 463 $[M - H^+ - C_7H_6O_3]^-$, 371 $[M - H^+ - C_9H_8O_3]^-$, 353 $[M - H^+ - C_9H_{10}O_4]^-$, 335 $[M - H^+ - C_9H_{12}O_5]^-$, and 173 $[M - H^+ - C_{18}H_{22}O_7]^-$. The fragment ion at 515 yields an ion of 3,4-di-*O*-caffeoylquinic acid and therefore provides no further information- on hydroxylation regiochemistry. Similarly, the ion at 173 is indicative of 4-acylation but reveals no further regiochemical information of the water addition. The fragment ions at 371 and 353 arise from a neutral loss of caffeic acid and hydroxydihydrocaffeic acid, respectively. Further targeted MS^3 of both of these ions provides regiochemical information on which side chain is hydroxylated. For the peak eluting at 27.4 min the MS^3 spectrum of *m/z* 371 results in a base peak at *m/z* 353 $[M - H^+ - H_2O]^-$ and a further fragment ion at 173, indicative of 4-acylation of the hydroxydihydrocaffeic acid side chain. This regiochemical assignment is further supported by an MS^4 experiment of *m/z* 533 to 371 to 353, which shows a base peak at *m/z* 173 and a targeted MS^3 experiment of 353 showing fragment ions consistent with a 3-acylation of the caffeoyl substituent. Therefore, the compound eluting at 27.4 min is assigned as one diastereoisomer of **20**. The second isomer eluting at 27.8 min shows in a targeted MS^3 experiment of *m/z* 533−353 a base peak at *m/z* 173 indicative of a 4-acyl regiochemistry of the caffeoyl substituent. A targeted MS^3 experiment on the fragment ion at *m/z* 371 was not possible because of its low intensity. Therefore, the compound eluting at 27.8 min must be assigned as one diastereoisomer of **19**.

The model brewing of 4,5-di-*O*-caffeoylquinic acid, **1**, resulted in the formation of six compounds with peaks of pseudomolecular ions at *m/z* 533 in the negative ion mode. By application of the same method and arguments as above, the compound eluting at 30.4 min was assigned as one diastereoisomer of **26** and the compound eluting at 30.9 min was assigned as one diastereoisomer of **27**. Two other compounds observed showed identical retention times and tandem MS spectra as the previously assigned **19** and **20**, indicating that acyl migration from the C5 to the C3 position occurred during the model brewing. However, it is unclear whether acyl migration occurred prior to conjugate water addition or after. The remaining two products giving pseudomolecular ions at *m/z* 533 were identified using the same reasoning as mentioned above, the compound eluting at 30.8 min being assigned as one diastereoisomer of **10** while the one eluting at 31.9 min as one diastereoisomer of **11**.

The model brewing of 3,5-di-*O*-caffeoylquinic acid resulted in the formation of two compounds with peaks of pseudomolecular ions at *m/z* 533 in the negative ion mode. The first compound eluting at 24.1 min showed in MS^2 a base peak at *m/z* 371 accompanied by further fragment ions at *m/z* 515, 463, 397, 353, and 191. A targeted MS^3 experiment on the precursor ion at *m/z* 371 resulted in a base peak at *m/z* 353 accompanied by fragment ions at *m/z* 191 and 135. These fragments are consistent with a 5-acyl regiochemistry of the hydroxydihydrocaffeic acid substituent. A targeted MS^3 experiment of the precursor ion at *m/z* 353 confirmed the 3-regiochemistry of the caffeoyl substituent. Therefore, the compound eluting at 24.1 min was assigned as one diasteroisomer of **10**. The second compound eluting at 27.6 min showed in a targeted MS^3 experiment of the precursor ion of 353 a fragment spectrum identical to the MS^2 spectrum of 5-*O*-caffeoylquinic acid and must therefore be assigned as one diastereoisomer of

11. By use of this current method, it remains unclear whether conjugate water addition to dicaffeoylquinic acids is highly stereoselective, since only one chromatographically resolved signal was observed for each pair of diastereoisomers, or whether both pairs of diastereoisomers are present and chromatographically not resolved.

Identification of Hydroxydihydrocaffeic Acids in Brewed Coffee. With the analytical data of the new derivatives in hand we next analyzed four real coffee brews to establish whether conjugate water addition also takes place during coffee brewing. The extracted ion chromatograms of each four coffee brews revealed three to four chromatographic peaks with pseudomolecular ions at m/z 371 and four to six peaks at m/z 533. Molecular formulas of all water addition products were confirmed using high resolution mass spectrometry. By use of retention times and tandem MS data, the hydroxydihydrocaffeic acid derivatives observed in coffee were assigned to regiochemical level. It is noted that the coffee beverage is slightly acidic with pH values for the beverages determined at 5.5; this value represents the low end of the pH interval generally recommended to be safe for beverages, with respect to dental erosion of tooth enamel.[33] It has been reported by Dawidowicz that the thermal decomposition of 5-*O*-caffeoylquinic acid in aqueous solvent is strongly pH-dependent.[29]

By use of relative peak areas of the EICs of (3′-hydroxydihydrocaffeoyl)quinic acids if compared to relative peak areas of monocaffeoyl and dicaffeoylquinic acids, the percent of chlorogenic acids transformed into their hydroxylated derivatives can be tentatively estimated. In the case of monoacylated chlorogenic acids up to 1.5−2% of the chlorogenic acids are transformed into their hydroxylated derivatives, while for the diacylated chlorogenic acids up to 4−4.5% of them suffer this transformation. By extrapolation to the total chlorogenic acids content in a typical 200 mL cup of coffee, it can be tentatively estimated that per cup of coffee a total of up to 8−9 mg of hydroxydihydrocaffeic acids is consumed.

In conclusion, we showed that in model coffee brewing systems mono- and dicaffeoylquinic acids are highly reactive forming acyl migration products, *cis*-caffeoylquinic acids and hydroxydihydrocaffeic acid derivatives. The regiochemistry of the latter compounds was elucidated using advanced tandem MS techniques. We could show that water does not simply act as a solvent and innocent bystander in food processing but acts as a reactive reagent resulting in significant chemical changes of the dietary material. The observation of conjugate addition reactions to the chlorogenic olefinic moiety suggests that nucleophilic thiol and amine functionalities in peptides could undergo this reaction pathway, contributing to many structures in the coffee melanoidine fraction.[34] Compounds identified in the present study might contribute to organoleptic properties and reported health effects of the coffee beverage, but further investigations need to be carried out.

■ ASSOCIATED CONTENT

Supporting Information

High resolution MS data (Table S1); substrates tested for water addition (Figure S1); cis-isomers observed during brewing (Figure S2); acyl migration isomers observed during brewing (Figure S3); water addition to 4-*O*-caffeoylquinic acid (Figure S4); water addition to 3-*O*-caffeoylquinic acid (Figure S5); water addition to 1-*O*-caffeoyl-1,5-quinide (Figure S6); water addition to 3,4-di-*O*-caffeoylquinic acid (Figure S7); water addition to 3,5-di-*O*-caffeoylquinic acid (Figure S8); synthesis of 1-*O*-caffeoyl-1,5-quinide (Figure S9); synthesis of 1-*O*-feruloyl-1,5-quinide (Figure S10). This material is available free of charge via the Internet at http://pubs.acs.org.

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■ REFERENCES

(1) IUPAC. nomenclature of cyclitols. *Biochem. J.* **1976**, *153*, 23−31.
(2) Clifford, M. N. Chlorogenic acids and other cinnamates—nature, occurrence, dietary burden, absorption and metabolism. *J. Sci. Food Agric.* **2000**, *80*, 1033−1043.
(3) Clifford, M. N. Chlorogenic acids and other cinnamates—nature, occurrence and dietary burden. *J. Sci. Food Agric.* **1999**, *79*, 362−372.
(4) Jaiswal, R.; Patras, M. A.; Eravuchira, P. J.; Kuhnert, N. Profiling and characterization of the chlorogenic acids in green robusta coffee beans by LC-MS^n—Identification of seven new classes. *J. Agric. Food Chem.* **2010**, *54*, 1957−1969.
(5) Jaiswal, R.; Sovdat, T.; Vivan, F.; Kuhnert, N. Profiling and characterization by LC-MS^n of the chlorogenic acids and hydroxycinnamoylshikimate esters in maté (*Ilex paraguariensis*). *J. Agric. Food Chem.* **2010**, *58*, 5471−5484.
(6) Jaiswal, R.; Kuhnert, N. Hierarchical scheme for liquid chromatography/multi-stage spectrometric identification of 3,4,5-triacylchlorogenic acids in green Robusta coffee beans. *Rapid Commun. Mass Spectrom.* **2010**, *24*, 2283−2294.
(7) Eliel, E. L.; Ramirez, M. B. (−)-Quinic acid: configurational (stereochemical) descriptors. *Tetrahedron: Asymmetry* **1997**, *8*, 3551−3554.
(8) Clifford, M. N.; Marks, S.; Knight, S.; Kuhnert, N. Characterization by LC-MS^n of four new classes of *p*-coumaric acid-containing diacylchlorogenic acids in green coffee beans. *J. Agric. Food Chem.* **2006**, *54*, 4095−4101.
(9) Clifford, M. N.; Johnston, K. L.; Knight, S.; Kuhnert, N. Hierarchical scheme for LC-MS^n identification of chlorogenic acids. *J. Agric. Food Chem.* **2003**, *51*, 2900−2911.
(10) Clifford, M. N.; Knight, S.; Kuhnert, N. Discriminating between the six isomers of dicaffeoylquinic acid by LC-MS^n. *J. Agric. Food Chem.* **2005**, *53*, 3821−3832.
(11) del Castillo, M. D.; Ames, J. M.; Gordon, M. H. Effect of roasting on the antioxidant activity of coffee brews. *J. Agric. Food. Chem.* **2002**, *50*, 3698−3703.
(12) Shearer, J.; Farah, A.; de Paulis, T.; Bracy, D. P.; Pencek, R. R.; Graham, T. E.; Wasserman, D. H. Quinides of roasted coffee enhance insulin action in conscious rats. *J. Nutr.* **2003**, *133*, 3529−3532.
(13) McDougall, B.; King, P. J.; Wu, B. W.; Hostomsky, Z.; Reinecke, M. G.; Robinson, W. E., Jr. Dicaffeoylquinic and dicaffeoyltartaric acids are selective inhibitors of human immunodeficiency virus type 1 integrase. *Antimicrob. Agents Chemother.* **1998**, *42*, 140−146.
(14) Trute, A.; Gross, J.; Mutschler, E.; Nahrstedt, A. In vitro antispasmodic compounds of the dry extract obtained from *Hedera helix*. *Planta Med.* **1997**, *63*, 125−129.
(15) Stich, H. F.; Rosin, M. P.; Bryson, L. Inhibition of mutagenicity of a model nitrosation reaction by naturally occurring phenolics, coffee and tea. *Mutat. Res.* **1982**, *95*, 119−128.

(16) Vega, F. E.; Rosenquist, E.; Collins, W. Global project needed to tackle coffee crisis. *Nature* **2003**, *435*, 343.
(17) Lewin, B.; Giovannucci, D.; Varangis, P. *Coffee Markets: New Paradigms in Global Supply and Demand*; The International Bank for Reconstruction and Development, Agriculture and Rural Development, 2004; Discussion Paper 3.
(18) Daglia, M.; Papetti, A.; Gregotti, C.; Berte, F.; Gazzani, G. In vitro antioxidant and ex vivo protective activities of green and roasted coffee. *J. Agric. Food Chem.* **2000**, *48*, 1449−1454.
(19) Nicoli, M. C.; Anese, M.; Manzocco, L.; Lerici, C. R. Antioxidant properties of coffee brews in relation to roasting degree. *Lebensm.−Wiss. Technol.* **1997**, *30*, 292−297.
(20) Kishimoto, N.; Kakino, Y.; Iwai, K.; Fujita, T. Enzymatic synthesis of caffeic acid esters from chlorogenic acid by transesterification and condensation reactions. *Colloq. Sci. Int. Cafe* **2005**, 249−253.
(21) Fujita, T.; Kakino, Y.; Iwai, K.; Mochida, K.; Kishimoto, N. Antiproliferation and anti-influenza viral activities of caffeic acid phenethyl esters synthesized enzymatically from chlorogenic acid and phenethyl alcohol. **2004**, 313−316
(22) Shearer, J.; Farah, A.; de Paulis, T.; Bracy, D. P.; Pencek, R. R.; Graham, T. E. Quinides of roasted coffee enhance insulin action in conscious rats. *J. Nutr.* **2003**, *133*, 3529−3532.
(23) de Paulis, T.; Schmidt, D. E.; Bruchey, A. K.; Kirby, M. T.; McDonald, M. P.; Commers, P. Dicinnamoylquinides in roasted coffee inhibit the human adenosine transporter. *Eur. J. Pharmacol.* **2002**, *442*, 215−223.
(24) Kuhnert, N.; Drynan, J. W.; Obuchowicz, J.; Clifford, M. N.; Witt, M. Mass spectrometric characterization of black tea thearubigins leading to an oxidative cascade hypothesis for thearubigin formation. *Rapid Commun. Mass Spectrom.* **2010**, *24*, 3387−3404.
(25) Rohloff, J. C.; Kent, K. M.; Postich, M. J.; Becker, M. W.; Chapman, H. H.; Kelly, D. E.; Lew, W.; Louie, M. S.; McGee, L. R.; Prisbe, E. J.; Schultze, L. M.; Yu, R. H.; Zhang, L. J. Practical total synthesis of the anti-influenza drug GS-4104. *J. Org. Chem.* **1998**, *63*, 4545−4550.
(26) Barros, A. Synthesis of *N′*-allyl-2-styrylchromones by a Baker Venkataraman transformation. *Heterocycl. Commun.* **2006**, *12*, 141−150.
(27) Boss, R.; Scheffold, R. Cleavage of allyl ethers with Pd/C. *Angew. Chem., Int. Ed. Engl.* **1976**, *15*, 558−55.
(28) Jaiswal, R.; Deshpande, S.; Kuhnert, N. Profiling the chlorogenic acids of *Rudbeckiahirta*, *Helianthus tuberosus*, *Carlinaacaulis* and *Symphyotrichum novae-angliae* leaves by LC-MS^n. *Phytochem. Anal.* **2011**, *22*, 432−441.
(29) Dawidowicz, A. L.; Typek, R. The influence of pH on the thermal stability of 5-*O*-caffeoylquinic acids in aqueous solutions. *Eur. Food Res. Technol.* **2011**, *233*, 223−232.
(30) Jaiswal, R.; Kuhnert, N. Identification and characterization of five new classes of chlorogenic acids in burdock (*Arctium lappa* L.) roots by liquid chromatography/tandem mass spectrometry. *Food Funct.* **2011**, *2*, 63−71.
(31) Dawidowicz, A. L.; Typek, R. Thermal stability of 5-*O*-caffeoylqunic acid in aqueous solutions at different heating conditions. *J. Agric. Food Chem.* **2010**, *58*, 12578−12584.
(32) Jaiswal, R.; Matei, M. F.; Ullrich, F.; Kuhnert, N. How to distinguish between cinnamoylshikimate esters and chlorogenic acid lactones by liquid chromatography−tandem mass spectrometry. *J. Mass Spectrom.* **2011**, *46*, 933−942.
(33) Moynihan, P.; Petersen, P. E. Diet, nutrition and the prevention of dental diseases. *Public Health Nutr.* **2004**, *7*, 201−226.
(34) Jaiswal, R.; Matei, M. F.; Golon, A.; Kuhnert, N. Understanding the fate of chlorogenic acids in coffee roasting using mass spectrometry based targeted and non-targeted analytical strategies. *Food Funct.* **2012**, *3*, 976−984.