JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Identification and Quantification of Caffeoylquinic Acids and Flavonoids from Artichoke (*Cynara scolymus* L.) Heads, Juice, and Pomace by HPLC-DAD-ESI/MS^n

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A method for the identification and quantification of phenolic compounds from artichoke (*Cynara scolymus* L.) heads, juice, and pomace by HPLC with diode array and mass spectrometric detection was developed. Among the 22 major compounds, 11 caffeoylquinic acids and 8 flavonoids were detected. Quantification of individual compounds was carried out by external calibration. Apigenin 7-*O*-glucuronide was found to be the major flavonoid in all samples investigated. 1,5-Di-*O*-caffeoylquinic acid represented the major hydroxycinnamic acid, with 3890 mg/kg in artichoke heads and 3269 mg/kg in the pomace, whereas in the juice 1,3-di-*O*-caffeoylquinic acid (cynarin) was predominant, due to the isomerization during processing. Total phenolic contents of ~12 g/kg on a dry matter basis revealed that artichoke pomace is a promising source of phenolic compounds that might be recovered and used as natural antioxidants or functional food ingredients.

KEYWORDS: Artichoke; *Cynara scolymus* L.; flavonoids; caffeoylquinic acids; HPLC-DAD-ESI/MS^n

INTRODUCTION

Artichokes (*Cynara scolymus* L.) have been known since the 4th century B.C. as a food and remedy (*1*) and were appreciated by the ancient Romans as a tasty vegetable with beneficial effects on digestion (*2*, *3*). Artichokes still play an important role in human nutrition, especially in the Mediterranean diet. In 2002, annual world production amounted to 1.2 million tons, with Italy being the most important producer (*4*). Apart from being consumed as fresh and canned vegetables, artichoke heads are also processed into pharmaceutical preparations.

Various studies have demonstrated the health-promoting potential of artichokes, especially their hepatoprotective (*5*, *6*), anticarcinogenic (*7*), antioxidative (*8*−*11*), and hypocholesterolemic (*12*, *13*) activities. Inhibition of cholesterol biosynthesis and a dose-dependent reduction of low-density lipoprotein oxidation were observed for luteolin and, to a lesser extent, for its 7-*O*-glucoside (*8*, *14*). These mechanisms may contribute to the reduction of atherosclerosis (*15*) by preventing oxidative modification of blood lipoproteins and reducing blood cholesterol levels (*16*) through choleretically induced elimination and inhibition of hepatic cholesterol biosynthesis (*17*, *18*).

In view of these diverse effects, more sophisticated techniques are required for assessing the contribution of individual compounds to their health-promoting potential. Although a number of methods for the determination of polyphenolics in artichoke products have been reported (*19*−*22*), the simultaneous determination of all caffeoylquinic acids and flavonoids has not yet been described. Because the separation was carried out with eluents containing phosphoric acid or trifluoroacetic acid (TFA) (*23*−*27*), these methods do not allow on-line coupling of HPLC to a mass spectrometer, due to the nonvolatility of phosphoric acid or possible memory effects caused by TFA. In other studies samples were fractionated using Sephadex LH-20 before HPLC analysis (*28*, *29*). However, these procedures proved to be time-consuming, tedious, and not suitable for routine analysis of a great number of samples.

Very recently the utilization of byproducts of artichoke processing, which may represent up to 50−60% of the fresh weight (*30*), has been taken into consideration. Studies so far conducted investigated the suitability of artichoke byproducts as animal feedstuff (*31*), for fiber production (*32*), and for the recovery of functional food ingredients (*33*). However, quantitative data on the contents of individual phenolic compounds in these matrices are still lacking. Therefore, the main objective of the present study was to establish a suitable HPLC method for the simultaneous determination of phenolic acids and flavonoids in artichokes. Because the availability of reference substances is limited and the identification of structurally related compounds solely on the basis of UV spectra may prove to be difficult, particular attention was given to compatibility of the eluents with mass spectrometric detection. The method was applied to the characterization and quantification of phenolic constituents of artichoke heads, juice, and pomace.

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10.1021/jf049625x CCC: $27.50
Published on Web 06/04/2004

MATERIALS AND METHODS

Plant Material. Artichoke heads (*Cynara scolymus* L. cv. Green Globe), artichoke pomace, and artichoke juice were provided by Walther Schoenenberger Pflanzensaftwerk GmbH & Co. KG (Magstadt, Germany). Artichoke heads and artichoke pomace from juice production were lyophilized. The commercially available juice was stored at ambient temperature until analysis.

Solvents and Reagents. Reagents and solvents were purchased from VWR (Darmstadt, Germany) and were of analytical or HPLC grade. Deionized water was used throughout. Chromabond, 1000 mg, solid-phase extraction cartridges were obtained from Macherey & Nagel (Düren, Germany).

Apigenin 7-*O*-glucoside and 1,3-di-*O*-caffeoylquinic acid (cynarin) were from Roth (Karlsruhe, Germany); 5-*O*-caffeoylquinic acid (chlorogenic acid), luteolin 7-*O*-glucoside, and narirutin were obtained from Extrasynthese (Lyon, France); caffeic acid was purchased from Fluka (Buchs, Switzerland).

HPLC Analysis. Polyphenol analyses were carried out using a series 1100 HPLC (Hewlett-Packard, Waldbronn, Germany) equipped with ChemStation software, a model G1322A degasser, a model G1312A binary gradient pump, a model G1329/1330A thermoautosampler, a model G1316A column oven, and a model G1315A diode array detection system. The column used was a 150 × 3.0 mm i.d., 4 μm particle size, C18 Hydro-Synergi from Phenomenex (Torrance, CA), with a security guard 4 × 3.0 mm i.d. C_{18} ODS column, operated at a temperature of 25 °C. The mobile phase consisted of 2% (v/v) acetic acid in water (eluent A) and of 0.5% acetic acid in water and acetonitrile (50:50, v/v; eluent B). The gradient program was as follows: 10−18% B (20 min), 18−24% B (10 min), 24−30% B (15 min), 30% B isocratic (20 min), 30−55% B (5 min), 55−100% B (5 min), 100% B isocratic (8 min), 100−10% B (2 min). Total run time was 90 min. The injection volume for all samples was 5 μL. Simultaneous monitoring was performed at 280 nm (narirutin), 320 nm (hydroxycinnamic acids), 330 nm (apigenin derivatives), and 350 nm (luteolin derivatives) at a flow rate of 0.4 mL/min. Spectra were recorded from 200 to 600 nm.

For calibration curves, appropriate volumes of the standard stock solutions (1000 mg/L) were diluted with methanol, and nine concentration levels (0.5, 2, 5, 10, 20, 40, 100, 200, and 500 mg/L) were analyzed. For quantification, peak areas were correlated with the concentrations according to the calibration curve. Monocaffeoylquinic acids were calculated as chlorogenic acid, and dicaffeoylquinic acids were quantified as cynarin. Apigenin and luteolin derivatives were calculated as apigenin 7-*O*-glucoside and luteolin 7-*O*-glucoside, respectively. Naringenin 7-*O*-glucoside was calculated as narirutin. All data presented are mean ± standard deviation of four independent experiments ($n = 4$).

LC-MS Analysis. Analyses were performed with the HPLC system described above coupled on-line with a Bruker (Bremen, Germany) model Esquire 3000+ ion trap mass spectrometer fitted with an ESI source. Data acquisition and processing were performed using Esquire Control software. Negative ion mass spectra of the column eluate were recorded in the range *m*/*z* 50−1000. Nitrogen was used both as drying gas at a flow rate of 9.0 L/min and as nebulizing gas at a pressure of 45.0 psi. The nebulizer temperature was set at 365 °C, and a potential of 2287 V was used on the capillary. 5-*O*-Caffeoylquinic acid was used for the optimization of ionization and fragmentation parameters.

Sample Preparation. Amounts of 4 g of freeze-dried artichoke heads and pomace were homogenized with a Grindomix GM 200 knife mill (Retsch, Haan, Germany) and extracted by stirring with aqueous methanol (60%, v/v) for 1 h at ambient temperature. After filtration through a filter paper, the extracts were evaporated to dryness in vacuo at 30 °C, and the residue was dissolved in water. After the pH had been adjusted to 7.0, the volume was made up to 25 mL with deionized water. Juice samples were adjusted to pH 7.0 before solid-phase extraction.

Purification and fractionation were carried out using C_{18}-reversed phase cartridges, which were activated with 5 mL of methanol and then rinsed with 10 mL of water. Aliquots of 2 mL of juice or 4 mL of the extracts were applied to the sorbent. Hydroxycinnamic acid derivatives were subsequently eluted with 50 mL of 10% aqueous methanol (fraction I). Rinsing with 50 mL of pure methanol eluted neutral compounds (fraction II). The eluates were evaporated to dryness in vacuo, and the residues obtained were dissolved in 4 mL (fraction I) and 1 mL (fraction II) of 50% aqueous methanol, respectively.

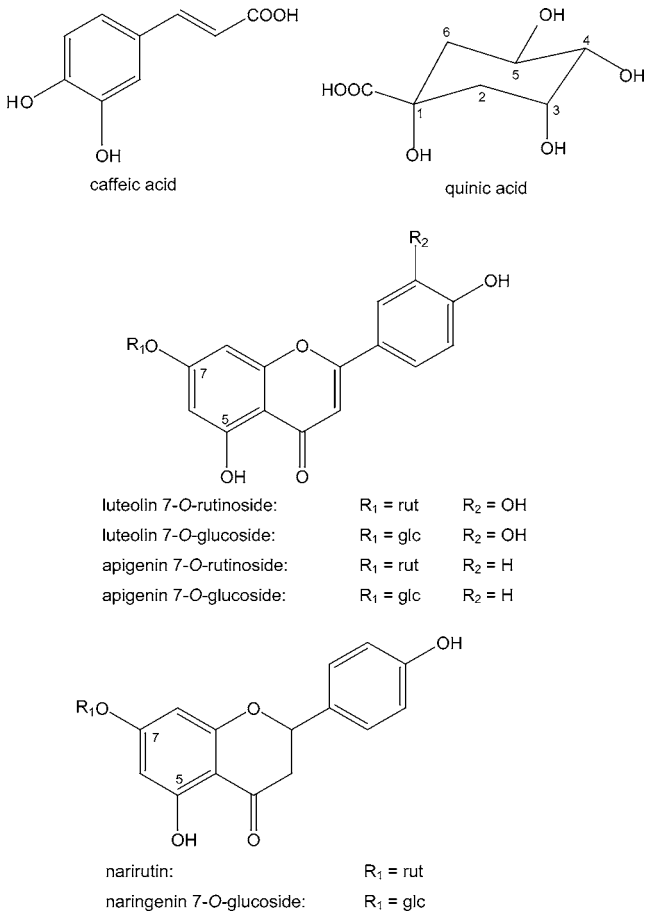

Figure 1. General structures and substitution patterns of caffeoylquinic acids and flavonoids detected in artichokes.

Recovery Studies. Recovery studies were performed by adding suitable amounts of standard stock solutions to artichoke heads or juice samples. Luteolin 7-*O*-glucoside, apigenin 7-*O*-glucoside, and narirutin were added prior to extraction of artichoke heads. Chlorogenic acid, caffeic acid, and cynarin were added to the juice. The samples were treated as described above. Determinations for recovery studies were performed in duplicate.

RESULTS AND DISCUSSION

Methodology. In this study a stationary phase with hydrophilic endcapping, which has been demonstrated to be suitable for the determination of phenolic compounds from different matrices such as apple (*34*), mango (*35*), and black carrots (*36*), was used for the analysis of caffeoylquinic acids and flavonoids (**Figure 1**). As can been seen from **Figures 2** and **3**, baseline separation was achieved for twenty major compounds.

Ground samples of artichoke heads and pomace were extracted with 60% aqueous methanol as described previously without further modification (*23*). The use of a magnetic stirrer proved to be more effective than ultrasonication for the extraction of phenolic compounds due to a better wettability of the lyophilized samples. Solid-phase extraction was employed for further purification of the crude extracts. Best results for the fractionation of the phenolic compounds were obtained by successive elution with 10% aqueous methanol (fraction I) and with pure methanol (fraction II). Thus, problems resulting from large differences in the contents of hydroxycinnamic acids and

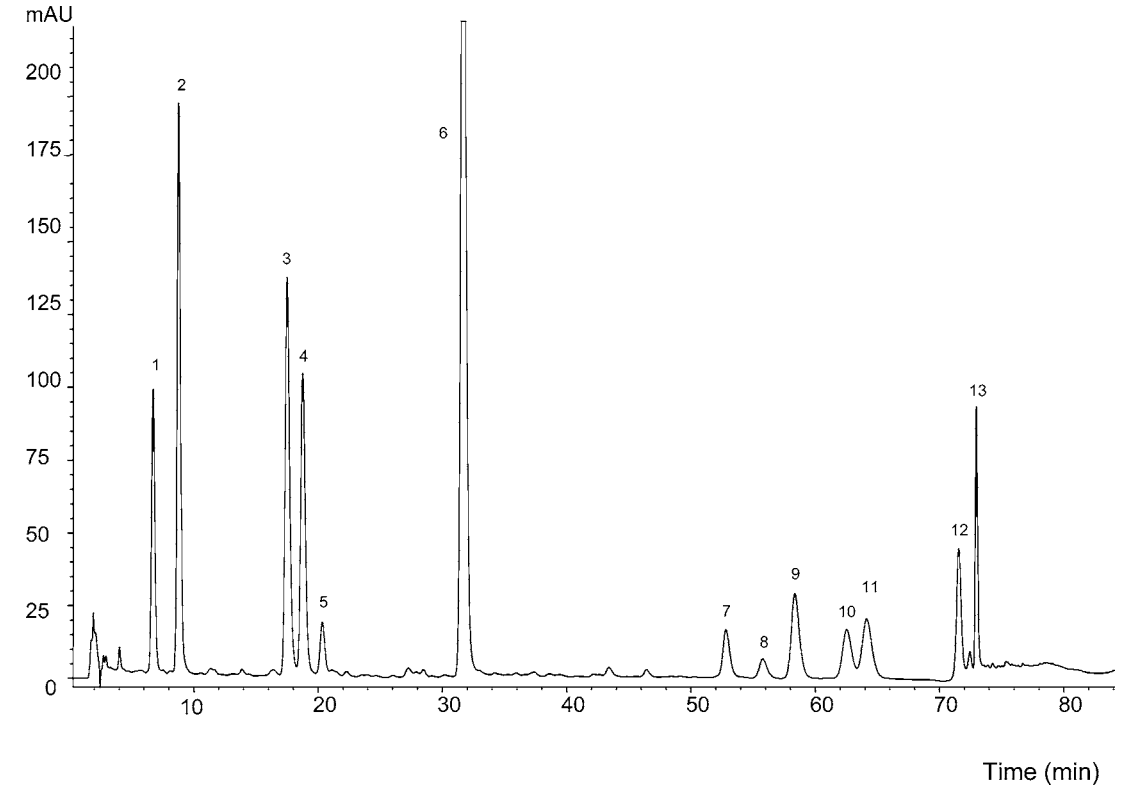

Figure 2. Separation of caffeoylquinic acids and flavones (fraction I) in artichoke juice by HPLC (320 nm). For peak assignment see **Table 1**.

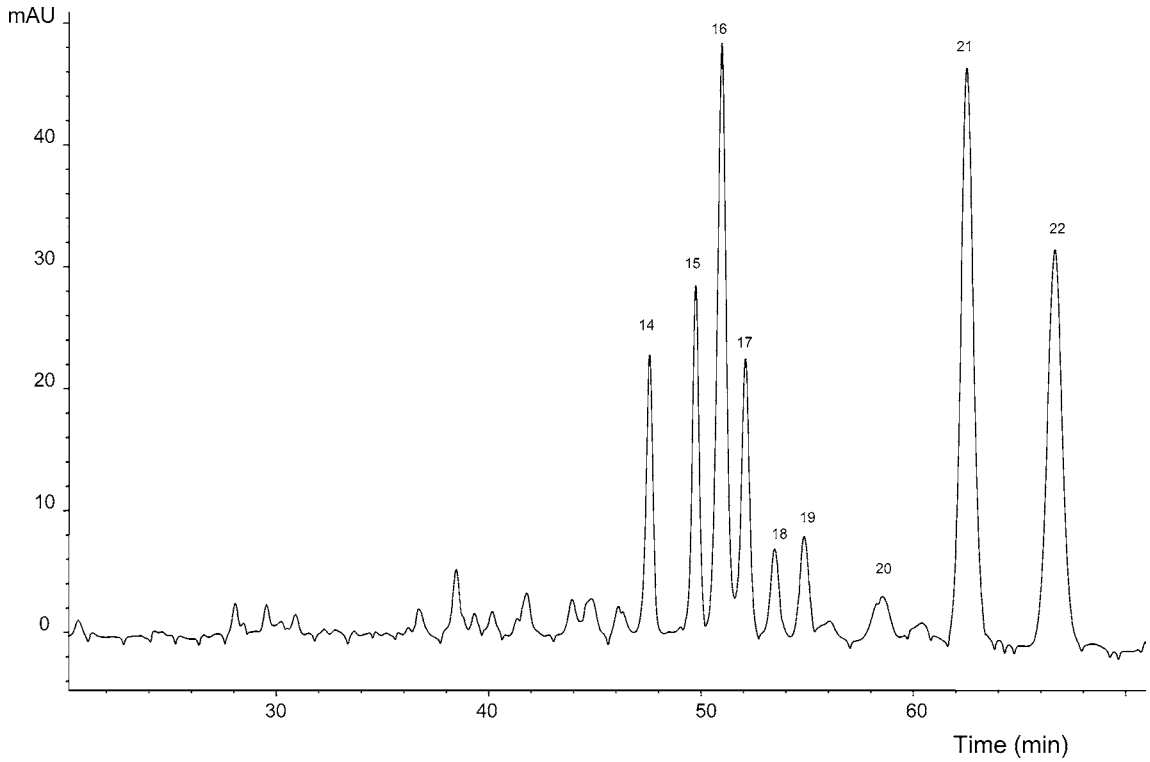

Figure 3. Separation of flavonoids (fraction II) in artichoke juice by HPLC (280 nm). For peak assignment see **Table 1**.

flavonoids could be overcome by dissolving the evaporated fractions in appropriate volumes. Furthermore, coelution of compounds **7** and **12** (fraction I) with compounds **16** and **22** (fraction II) could be avoided.

Recovery Studies. Recovery studies for flavonoids were performed by standard addition prior to the extraction of solid samples, whereas stock solutions of the hydroxycinnamates were added to the juice samples because the latter may undergo isomerization during extraction and are therefore hard to quantify (*22*). Recovery rates for phenolic acids were 87% for caffeic acid and 95% for both 5-*O*-caffeoylquinic acid and 1,3-di-*O*-caffeoylquinic acid. For luteolin 7-*O*-glucoside a recovery of 88% was found. Recovery of narirutin and apigenin 7-*O*-glucoside was 91%, respectively.

Identification of Phenolic Compounds by LC-MS. Because only cynarin, chlorogenic acid, caffeic acid, luteolin 7-*O*-

Table 1. UV Spectra and Characteristic Ions of Caffeoylquinic Acids and Flavonoids in Artichoke Juice (*C. scolymus* L.)

compd	retention time (min)	identity	HPLC-DAD λ_{max} (nm)	$[M - H]^-$ *m/z*	HPLC-ESI(–)-MS^n expt *m/z* (% base peak)
			Fraction I		
1	6.7	1-*O*-caffeoylquinic acid	233, 305sh, 328	353	MS^2 [353]: 191 (100), 197 (5), MS^3 [353 → 191]: 127 (100), 172 (79), 85 (79), 93 (56)
2	8.9	3-*O*-caffeoylquinic acid	241, 303sh, 325	353	MS^2 [353]: 191 (100), 179 (49), 135 (13), 173 (3) MS^3 [353 → 191]: 127 (100), 172 (83), 85 (74), 93 (68), 111 (46)
3	17.5	5-*O*-caffeoylquinic acid (chlorogenic acid)	241, 305sh, 326	353	MS^2 [353]: 191 (100), 179 (3) MS^3 [353 → 191]: 127(100), 85 (96), 173 (85), 93 (72), 111 (35)
4	18.8	4-*O*-caffeoylquinic acid	236, 303sh, 326	353	MS^2 [353]: 173 (100), 179 (53), 191 (16), 135 (12) MS^3 [353 → 173]: 93 (100), 111 (80)
5	20.3	caffeic acid	237, 302sh, 323	179	MS^2 [178]: 135 (100)
6	31.7	1,3-di-*O*-caffeoylquinic acid (cynarin)	242, 307sh, 322	515	MS^2 [515]: 353 (100), 179 (35), 335 (34), 191 (20) MS^3 [515 → 353]: 191 (100), 179 (45), 135 (11), 173 (2), 353 (2)
7	52.8	luteolin 7-*O*-glucuronide	254, 266sh, 347	461	MS^2 [461]: 285 (100), 357 (5), 327 (4), 381 (2), 355 (2)
8	55.7	dicaffeoylquinic acid	246, 303sh, 327	515	MS^2 [515]: 353 (100), 203 (61), 299 (37), 255 (28), 173 (19), 317 (17) MS^3 [515 → 353]: 173 (100), 179 (50), 191 (24), 135 (6), 353 (6)
9	58.4	3,4-di-*O*-caffeoylquinic acid	243, 303sh, 325	515	MS^2 [515]: 353 (100), 173 (23), 179 (14), 335 (11), 191 (8), 203 (6) MS^3 [515 → 353]: 173 (100), 179 (68), 191 (46), 135 (11), 353 (2) MS^4 [515 → 353 → 173]: 173 (100), 93 (23), 111 (17)
10	62.5	3,5-di-*O*-caffeoylquinic acid	243, 303sh, 327	515	MS^2 [515]: 353 (100), 191 (12), 179 (5) MS^3 [515 → 353]: 191 (100), 179 (47), 353 (12), 135 (9), 173 (6) MS^4 [515 → 353 → 191]: 191 (100), 173 (43), 127 (26)
11	64.1	1,5-di-*O*-caffeoylquinic acid	243, 303sh, 329	515	MS^2 [515]: 353 (100), 191 (34), 335 (5) MS^3 [515 → 353]: 191 (100), 179 (8) MS^4 [515 → 353 → 191]: 191 (100), 127 (31), 173 (25), 85 (22), 93 (14)
12	71.6	apigenin 7-*O*-glucuronide	231, 267, 338	445	MS^2 [445]: 269 (100), 175 (20) MS^3 [445 → 269]: 269 (100), 175 (29)
13	73.0	4,5-di-*O*-caffeoylquinic acid	243, 303sh, 327	515	MS^2 [515]: 353 (100), 173 (21), 203 (13), 179 (10), 299 (8), 255 (8) MS^3 [515 → 353]: 173 (100), 179 (57), 191 (28), 135 (8) MS^4 [515 → 353 → 173]: 173 (100), 111 (21), 93 (19), 71 (7), 155 (8)
			Fraction II		
14	47.6	ni[a]	252, 267sh, 297, 315, 338	453	MS^2 [453]: 231 (100), 157 (16), 393 (11)
15	49.8	luteolin 7-*O*-rutinoside	255, 266sh, 348	593	MS^2 [593]: 285 (100), 593 (6), 251 (76), 281 (100), 323 (14)
16	51.0	luteolin 7-*O*-glucoside	253, 266sh, 347	447	MS^2 [447]: 285 (100), 447 (4), 327 (3) MS^3 [447 → 285]: 243 (100)
17	52.1	ni[a]	237, 248sh, 263sh, 277, 294, 314	445	MS^2 [445]: 233 (100), 395 (48), 179 (16), 159 (12) MS^3 [445 → 233]: 159 (100)
18	53.5	ni[a]	231, 278	519	MS^2 [519]: 357 (100) MS^3 [519 → 357]: 151 (100), 135 (31), 357 (19), 311 (10)
19	54.9	narirutin	230, 283	579	MS^2 [579]: 271 (100), 177 (2) MS^3 [579 → 271]: 151 (100), 177 (17), 107 (14)
20	58.8	naringenin 7-*O*-glucoside	231, 282	433	MS^2 [433]: 271 (100), 151 (2) MS^3 [433 → 271]: 151 (100), 177 (17), 107 (14)
21	62.3	apigenin 7-*O*-rutinoside	229, 266, 339	577	MS^2 [577]: 269 (100)
22	66.7	apigenin 7-*O*-glucoside	229, 266, 339	431	MS^2 [431]: 269 (100), 431 (8), 311 (4)

[a] Not identified.

glucoside, apigenin 7-*O*-glucoside, and narirutin were available as reference compounds, HPLC coupled to mass spectrometry proved to be extremely helpful for peak assignment and further characterization of individual substances. As recently shown for the identification of chlorogenic acids by LC-MS^n, fragmentation patterns obtained by collision-induced dissociation (CID) of isolated pseudomolecular ions provide valuable information for the characterization of isomeric substances (*37*). Identification of compounds **3**, **5**, and **6** (**Figure 2**) and of compounds **16**, **19**, and **22** (**Figure 3**) was based on the comparison of their UV spectra and retention times with those of reference substances. Peak assignment was confirmed by their mass spectrometric behavior (**Table 1**).

Peak 1 (**Figure 2**) was tentatively identified as 1-*O*-caffeoylquinic acid (pseudochlorogenic acid), on the basis of its pseudomolecular ion at *m/z* 353 and by comparing the elution order of the caffeoylquinic acids with that reported in the literature (*24*). Peaks 2 and 4 (**Figure 2**) were identified as 3-*O*-caffeoylquinic acid (neochlorogenic acid) and 4-*O*-caffeoylquinic acid (cryptochlorogenic acid) on the basis of their fragmen-

Table 2. Phenolic Contents of Artichoke Heads, Juice, and Pomace

compd	artichoke heads (mg/kg of DM)	artichoke pomace (mg/kg of DM)	artichoke juice (mg/L)
caffeic acid	nd[a]	11.1 ± 1.5	19.5 ± 2.7
1-*O*-caffeoylquinic acid	758.5 ± 24.8	466.2 ± 13.6	127.9 ± 6.0
3-*O*-caffeoylquinic acid	150.9 ± 5.1	188.1 ± 3.8	263.2 ± 9.5
5-*O*-caffeoylquinic acid (chlorogenic acid)	3143.0 ± 101.4	2033.8 ± 74.6	246.4 ± 12.6
4-*O*-caffeoylquinic acid	109.1 ± 13.2	327.0 ± 8.9	187.6 ± 8.4
1,3-di-*O*-caffeoylquinic acid (cynarin)	95.0 ± 9.3	461.6 ± 14.2	529.5 ± 18.0
3,4-di-*O*-caffeoylquinic acid	172.9 ± 17.3	366.1 ± 7.9	81.8 ± 3.0
3,5-di-*O*-caffeoylquinic acid	762.0 ± 29.3	492.6 ± 25.9	58.3 ± 1.9
1,5-di-*O*-caffeoylquinic acid	3889.9 ± 244.1	3268.8 ± 138.4	72.4 ± 4.2
4,5-di-*O*-caffeoylquinic acid	119.5 ± 16.5	451.7 ± 10.2	72.3 ± 3.1
dicaffeoylquinic acid	239.0 ± 22.3	480.5 ± 5.2	29.2 ± 0.3
luteolin 7-*O*-glucoside	188.4 ± 10.5	319.8 ± 12.5	27.5 ± 1.1
luteolin 7-*O*-glucuronide	363.3 ± 8.2	374.9 ± 10.7	69.2 ± 4.6
apigenin 7-*O*-glucoside	399.7 ± 24.7	558.9 ± 15.7	61.0 ± 0.7
apigenin 7-*O*-glucuronide	1002.8 ± 7.6	1318.6 ± 22.5	159.9 ± 3.4
naringenin 7-*O*-glucoside	54.0 ± 3.9	67.8 ± 2.2	6.2 ± 0.6
luteolin 7-*O*-rutinoside	130.5 ± 5.5	127.1 ± 5.1	14.0 ± 0.2
apigenin 7-*O*-rutinoside	476.4 ± 26.1	554.9 ± 16.4	68.3 ± 1.3
narirutin	56.8 ± 3.9	67.7 ± 2.5	7.4 ± 0.7

[a] Not detected.

tation patterns in the MS^2 and MS^3 experiments. Moreover, their retention times matched those of the compounds prepared by isomerization of 5-*O*-caffeoylquinic acid in a phosphate buffer, which are known to be 3-*O*-caffeoylquinic acid and 4-*O*-caffeoylquinic acid (*38*). Compounds **7** and **12** (**Figure 2**) exhibited pseudomolecular ions at *m*/*z* 461 and 445, respectively. Their product ions at *m*/*z* 285 and 269 were assigned to luteolin and apigenin, corresponding to the loss of a glucuronide moiety (176 Da). Thus, compounds **7** and **12** were characterized as luteolin 7-*O*-glucuronide and apigenin 7-*O*-glucuronide (*26*, *28*), respectively. These findings are also corroborated by their behavior during solid-phase extraction because they were recovered in fraction I and did not show any retention when the pH of the solutions was raised to 7.0. All other flavonoids were eluted into fraction II. Peaks 9, 10, and 13 (**Figure 2**) were tentatively identified as 3,4-di-*O*-caffeoylquinic acid, 3,5-di-*O*-caffeoylquinic acid, and 4,5-di-*O*-caffeoylquinic acid, respectively, on the basis of their fragmentation in the MS^2 and MS^3 experiments, even though the relative intensities of some fragments slightly differed from those described earlier (*37*). However, it should be noted that MS^n spectra are not completely portable between laboratories in the same way as classic EI spectra are. Peak 11, which showed a similar mass spectrometric behavior, was tentatively identified as 1,5-di-*O*-caffeoylquinic acid because its content in the juice was reduced in favor of 1,3-di-*O*-caffeoylquinic acid. This isomerization is well-known to occur during extraction with hot water (*22*). Peaks 15 and 21 (**Figure 3**) were identified, respectively, as luteolin 7-*O*-rutinoside and apigenin 7-*O*-rutinoside, which is in agreement with literature data (*19*, *20*, *22*, *23*, *28*, *29*). Moreover, CID produced prominent fragments of *m*/*z* 285 and 269, which represent the corresponding aglycons, revealing a loss of a rutinose moiety (308 Da). Peak 20 (**Figure 3**) was assigned to naringenin 7-*O*-glucoside (*m*/*z* 433), which showed a loss of the saccharide moiety in the MS^2 experiment (162 Da). This is in accordance with previous studies (*28*). The structures of peaks 14, 17, and 18 could not be elucidated by their fragmentation patterns.

Quantification of Individual Compounds in Artichoke Heads, Pomace, and Juice. Despite a great number of studies, comparison of the phenolic contents with literature data is still difficult, due to differences in the analytical methodologies and because the contents may vary considerably with variety and maturity stage of artichokes (*23*). Furthermore, other studies were restricted to pharmaceuticals extracted from artichoke leaves (*21*, *24*), the phenolic profile of which is different from that of artichoke heads (*23*, *28*).

As can be seen from **Table 2**, the caffeoylquinic acids were the predominant phenolic compounds of artichoke heads and in the pomace, with 1,5-di-*O*-caffeoylquinic acid showing the highest amount, followed by 5-*O*-caffeoylquinic acid. Most strikingly, virtually all other phenolic compounds showed higher contents in the pomace compared to the fresh vegetable, which may be due to the inactivation of polyphenol oxidase during blanching (*30*). A more likely explanation is poor extractability of the phenolics during juice production and a changed dry matter composition of the pomace compared to artichoke heads as a consequence of leaching, resulting in a transition of highly soluble compounds such as inulin into the juice. In contrast, a higher amount of 1,5-di-*O*-caffeoylquinic acid was found in artichoke heads compared to the pomace. This may be attributed to the isomerization during juice production to 1,3-di-*O*-caffeoylquinic acid, which is not genuine to artichokes. Consequently, the low content of 1,3-di-*O*-caffeoylquinic acid [95 mg/kg of dry matter (DM)] in fresh artichoke could be due to artifact formation during extraction with aqueous methanol. In contrast, 5-*O*-caffeoylquinic acid content in the pomace was lower, which may be ascribed to its enhanced extractability or to an isomerization to 3-*O*-caffeoylquinic acid and 4-*O*-caffeoylquinic acid (*38*). The presence of caffeic acid, which was not detected in the fresh vegetable, may result from hydrolysis of monocaffeoylquinic acids and dicaffeoylquinic acids during processing. The predominant flavonoids were apigenin 7-*O*-glucuronide and luteolin 7-*O*-glucuronide, followed by apigenin 7-*O*-rutinoside, apigenin 7-*O*-glucoside, luteolin 7-*O*-glucoside, and luteolin 7-*O*-rutinoside. Narirutin and naringenin 7-*O*-glucoside were minor compounds in artichoke heads and pomace. Naringenin 7-*O*-glucoside has only recently been identified in artichoke waste, however, without being quantified (*28*). These results demonstrate that artichoke pomace is characterized by higher polyphenol contents compared to other press residues such as apple pomace (*34*) and, therefore, represents a promising source of phenolic constituents,

which may be used as natural antioxidants or as ingredients of functional foods.

The juice samples showed the same phenolic profile as fresh artichokes and pomace. As expected, 1,3-di-*O*-caffeoylquinic acid was the major phenolic compound in artichoke juice. Due to the heating during blanching and pasteurization, further isomerization of monocaffeoylquinic and dicaffeoylquinic acids occurred. In accordance with a previous study (*22*), a decrease of 5-*O*-caffeoylquinic acid from 33.3% (fresh artichoke) to 14.6% (juice) of total caffeoylquinic acids was accompanied by an increase of 3-*O*-caffeoylquinic acid and 4-*O*-caffeoylquinic acid from 1.6 to 15.6% and from 1.2 to 11.1%, respectively. In contrast, the proportion of the flavonoids remained unchanged, with apigenin 7-*O*-glucuronide and luteolin 7-*O*-glucuronide being the predominant compounds.

The method developed in the present study allows an efficient determination of phenolic compounds from artichoke heads, juice, and pomace with good recovery rates. Baseline separation of 20 polyphenolics was obtained using volatile eluents, which enables coupling of the LC system with a mass spectrometer without change of chromatographic conditions. Fractionation by solid-phase extraction allowed the simultaneous quantitative determination of phenolic acids and flavonoids from different extracts of artichokes. Therefore, the analytical system may be used for screening of raw material and for process and quality control in the pharmaceutical industry. In addition, the method may also find application by food inspection authorities for the evaluation of dietary supplements containing artichoke extracts. Byproducts of artichoke processing have been demonstrated to be a promising source of phenolics that may be used as natural antioxidants or functional food ingredients.

ACKNOWLEDGMENT

We are grateful to Walther Schoenenberger Pflanzensaftwerk GmbH & Co. KG, Magstadt, Germany, for providing artichokes, artichoke juice, and artichoke pomace.

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Received for review March 8, 2004. Revised manuscript received April 30, 2004. Accepted May 3, 2004. We are grateful to Walther Schoenenberger Pflanzensaftwerk GmbH & Co. KG, Magstadt, Germany, for financial support.

JF049625X