Influence of the Acetification Process on Phenolic Compounds

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Little is known about the change of phenolic compounds and total phenolic content by the acetification process. The aim of this study was to assess the contents of selected phenolic compounds of cider and red and white wines in comparison to phenolic profiles in corresponding vinegars by using a new HPLC method for the simultaneous separation and quantification of polar phenolic acids and less polar flavonoids. Identifications were made by retention times and by means of mass spectra. Additionally, total phenolic contents of wines and vinegars were determined photometrically. The decrease in total phenol content by the acetification process was highest for cider vinegars (40%) and lower for red and white wine vinegars (13 and 8%, respectively). A decrease in the contents of individual phenolic compounds of vinegars from white white and ciders was not observed. In contrast, the contents of individual phenolic compounds in red wine vinegar decreased ∼50%.

Keywords: *Wine; vinegar; phenolic compounds; HPLC; mass spectrometry*

INTRODUCTION

Phenolic compounds are secondary plant metabolites that have long been associated with flavor and color characteristics of fruits, juices, and wines. These phenolic compounds attract great interest due to their postulated health-protecting properties (Carbo et al., 1999). Foremost is their antioxidative effect, manifested by the ability to scavenge free radicals (Frankel et al., 1995; Soleas et al., 1997b) or to prevent oxidation of low-density lipoproteins (Rifici et al., 1999). Although phenolic contents of wines and juices have been investigated in numerous studies (Frankel et al., 1995; Mangas et al., 1997; Simonetti et al., 1997; Soleas et al., 1997a; Suárez et al., 1996), little is known about alterations of phenolic profiles and total phenolic content by the acetification process. Previous characterizations of vinegar are devoted to the investigation of its origin, method of elaboration, and age (Galvez et al., 1994; Garcia-Parilla et al., 1997, 1998). Only a few studies compare the influence of different acetification processes on the phenolic composition of wine vinegars (Mangas et al., 1997), whereas controlled investigations comparing the phenolic profiles of wines and their corresponding vinegars are lacking. The aim of the present study was to assess the content of selected phenolic compounds of cider and red and white wines and to compare these phenolic profiles with those of the corresponding vinegars. For this purpose, we established a new HPLC method for the simultaneous separation and quantification of polar phenolic acids and less polar flavonoids. Additionally, we assessed total phenolic contents to verify changes in total phenolic content during the acetification process.

MATERIALS AND METHODS

Samples. Wine and vinegar samples were supplied by Hengstenberg (Esslingen, Germany) and Kühne (Hamburg, Germany). Vinegar processing was carried out in bioreactors with submerged culture. In this study, a total of two ciders (C), two white wines (W), and one red wine (R) with their corresponding vinegars (CV, WV, RV) were analyzed. The samples were stored at 4 °C. Before analysis, samples were passed through a 0.45 μm syringe filter (Gelman Sciences, Rossdorf, Germany), and R, RV, C, and CV were diluted with ultrapure water (1 + 1 and 1 + 4, respectively). W and WV were analyzed without additional dilution. Samples were analyzed in duplicate, and data were expressed as means. The concentrations were calculated on the basis of an area normalization method, using external calibration curves of the standard compounds.

Gradient HPLC System with UV—Vis Detection. The HPLC system (Sykam, Gilching, Germany) consisted of a solvent delivery system S 1100, an HPLC controller S 2000, a low-pressure gradient mixer S 8110, and an autosampler Marathon Basic$^+$ (Spark, Emmen, Netherlands) with a 100 μL fixed loop (injection volume = 20 μL). Separation of the phenolic compounds was carried out on a Grom-Sil 120 ODS-4 HE column (125 mm × 4 mm, 5 μm; Grom Analytik + HPLC GmbH, Herrrenberg, Germany) at room temperature. The analytes were monitored with a Spectroflow 757 variable wavelength detector (Kratos GmbH, Karlsruhe, Germany) at 280 and 520 nm, respectively, with a flow cell of 12 μL (8 mm). Data quantitation was performed with a Chromatopac C-R6A data processor (Shimadzu, Kyoto, Japan). Identification of phenolic compounds was carried out by comparing retention times with those of commercially available standards and by spiking the sample with standard solutions.

Gradient HPLC System with Mass Spectrometric Detection. To confirm the identity of phenolic compounds with similar retention times and to identify unknown compounds, we used a gradient HP HPLC system series 1100 (Hewlett-Packard, Böblingen, Germany) combined with an autosampler ALS G1313A, a quat pump G1311A, a degasser G1322A, and a column oven ColComp G1316A at 30 °C. The mass spectrometric detector was a Micro Mass Platform II (Mass Lynx 4.0, Manchester, U.K.) equipped with a cross-flow interface. We used electrospray ionization. Source temperature was maintained at 120 °C, cone voltage at 50 V, and acceleration lens potential at 0.5 kV. Negative ion characterization was performed in the *m*/*e* range of 140−800 and positive ion characterization in the *m*/*e* range of 200−800 at a scan rate of 2 scans/s and a multiplier voltage of 650 V. Capillary voltage

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Table 1. Content of Individual Phenolic Compounds (Milligrams per Liter) of C and Corresponding CV

	C1	CV1	C2	CV2
phenolic acids				
protocatechuic acid	27	20	7,0	18
chlorogenic/caffeic acid	180	180	150	120
p-coumaroylquinic acid	56	51	45	34
flavonoids				
catechin	58	58	44	39
epicatechin	12	11	7.4	5.4
phloretin-xyl-glc	27	30	25	23
phloridzin	37	41	20	20
quercitrin	15	20	<0.1	<0.1

was 3.5 kV for positive and 3.0 kV for negative ionization, respectively.

HPLC Conditions. All solvents were of HPLC grade and degassed with helium before use. Solvent A consisted of acetonitrile/water/formic acid (3:87:10, v/v/v), and solvent B was composed of acetonitrile/waterr/formic acid (50:40:10, v/v/v). Elution was performed at a flow rate of 0.9 mL/min using the following gradient conditions: 0−6 min, 0% B; 6−14 min, 0−12% B; 14−23 min, 12−17% B; 23−34 min, 17−50% B; 34−41 min, 50−70% B.

Reagents and Standards. Ultrapure water was generated with an Elga Maxima water purification system, including reverse osmosis, activated carbon, and ion-exchange cartridges (Elga, Lane End, U.K.). Protocatechuic acid and quercetin 3-rhamnoside (= quercitrin) were provided from Sigma (Deisenhofen, Germany); gallic acid, (+)-catechin, and (−)-epicatechin were from Aldrich (Steinheim, Germany); phloridzin was from Fluka (Buchs, Switzerland); chlorogenic acid, sinapic acid, caffeic acid, and malvidin 3,5-diglucoside chloride (= malvin chloride) were purchased from Roth (Karlsruhe, Germany); and malvidin 3-glucoside chloride (= oenin chloride) was from Extrasynthèse (Genay, France). Folin−Ciocalteu phenol reagent and anhydrous sodium carbonate were obtained from Merck (Darmstadt, Germany). All chemicals used were of analytical or HPLC grade.

Standard Solutions for HPLC. For stock solutions, 1−2 mg of the standard compound was dissolved in 1 mL of methanol (mobile phase solvent A, in the case of the anthocyanins). Stock solutions were stored at 4 °C. Fifty microliters of each stock solution was used for preparation of a standard mixture. This mixture was further diluted with ultrapure water to yield concentrations in the range of 1−43 mg/L (1−10 mg/L in the case of malvidin 3-glucoside). Calibration curves were obtained from triplicate injections of four concentrations. Phloretin−xyloglucoside (phloretin-xyl-glc) was quantified as phloridzin and *p*-coumaroylquinic acid as chlorogenic acid; the anthocyanins were quantified as malvidin 3-glucoside.

Total Phenol Content. Total phenol content was measured photometrically at 675 nm (Uvicon 930, Kontron Instruments, München, Germany) after reaction with Folin−Ciocalteu phenol reagent according to the method described by Singleton and Rossi (Singleton et al., 1965) using gallic acid as a calibration standard.

RESULTS AND DISCUSSION

Ciders and Cider Vinegars. The analysis of C and the corresponding CV confirmed the presence of the phenolic acids protocatechuic acid, chlorogenic/caffeic acid, and *p*-coumaroylquinic acid besides the flavonoids catechin, epicatechin, phloretin-xyl-glc and phloridzin (Table 1; Figure 1). In C1 and CV1 we additionally identified quercitrin. Characteristic ions in the LC-ESI$^-$ mass spectra enabled peak assignment for *p*-coumaroylquinic acid and phloretin-xyl-glc, for which we had no standards. The deprotonated molecular ion [M − H]$^-$ of phloretin-xyl-glc acid was observed as base peak at *m/e* 567, whereas the aglycon fragment [M − (xyl-glc) − H]$^-$ was observed at *m/e* 273 (Figure 2). Identification of *p*-coumaroylquinic acid was achieved by the molecular ion [M − H]$^-$ at *m/e* 337 and the signal for quinic acid [M − coumaroyl − H]$^-$ at *m/e* 191 (Figure 3). The dominating signal in the chromatograms of cider and apple vinegar was the peak of chlorogenic/caffeic acid. Chlorogenic and caffeic acid were not separated, although we varied the polarity of the eluents. Identification of caffeic acid with the simultaneous presence of chlorogenic acid by mass spectroscopy was not possible because chlorogenic acid, as a caffeic acid ester of quinic acid, showed the same mass signals as caffeic acid itself (Figure 4). The contents of phloridzin (20−41 mg/L), chlorogenic/caffeic acid (120−180 mg/L), *p*-coumaroylquinic acid (34−56 mg/L), and catechin/epicatechin (44−70 mg/L) were in good agreement with

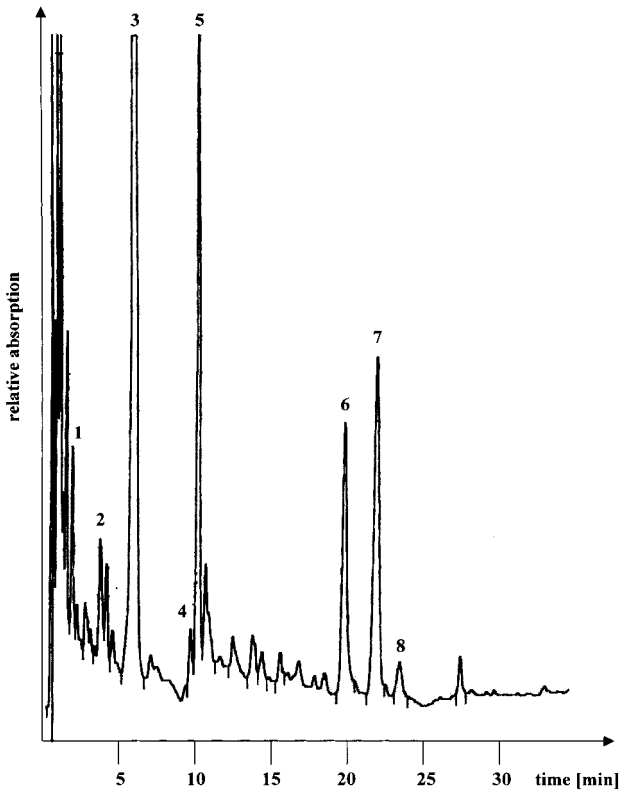

Figure 1. Typical HPLC chromatogram corresponding to a cider vinegar obtained with UV detection at 280 nm (diluted 1 + 4). Peak assignment: 1, protocatechuic acid; 2, catechin; 3, chlorogenic/caffeic acid; 4, epicatechin; 5, *p*-coumaroylquinic acid; 6, phloretin-xyl-glc; 7, phloridzin; 8, quercitrin.

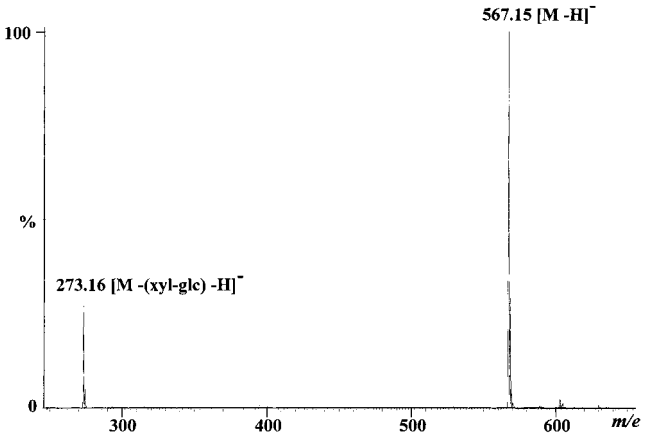

Figure 2. LC-ESI$^-$ mass spectrum of phloretin-xyl-glc from a cider vinegar: [M − H]$^-$, deprotonated molecular ion at *m/e* 567; [M − (xyl-glc) − H]$^-$, phloretin fragment ion at *m/e* 273.

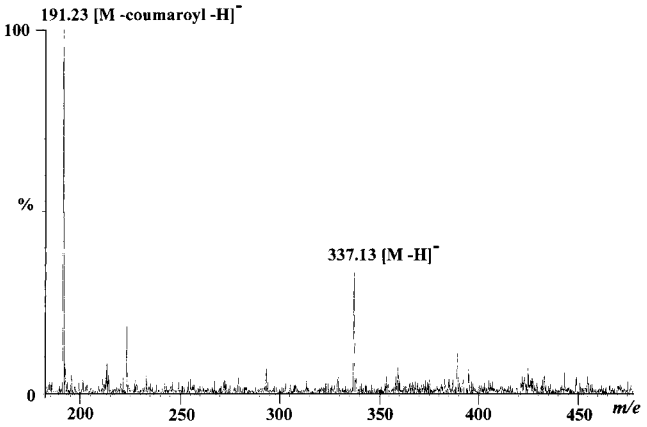

Figure 3. LC-ESI⁻ mass spectrum of *p*-coumaroylquinic acid from a cider vinegar: [M − H]⁻, deprotonated molecular ion at *m/e* 337; [M − coumaroyl − H]⁻, quinic acid fragment ion at *m/e* 191.

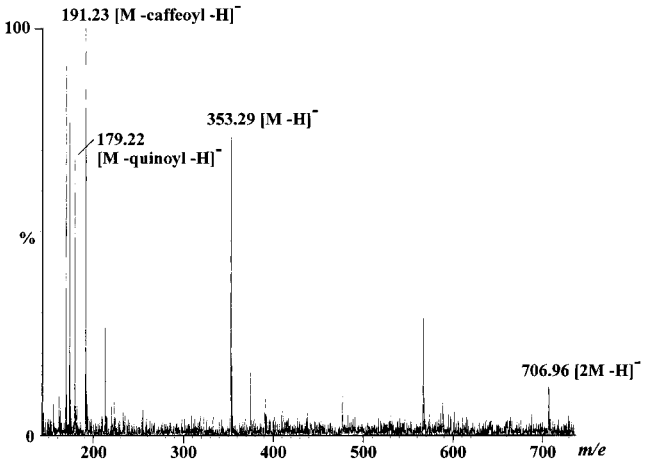

Figure 4. LC-ESI⁻ mass spectrum of chlorogenic acid from a cider vinegar: [M − H]⁻, deprotonated molecular ion at *m/e* 353; [M − quinoyl − H]⁻, caffeic acid fragment ion at *m/e* 179; [M − caffeoyl − H]⁻, quinic acid fragment ion at *m/e* 191; [2M − H]⁻, signal of adduct of two chlorogenic acid molecules at *m/e* 707.

contents reported in the literature (Lee et al., 1988; Pearson et al., 1999; Spanos et al., 1990). The concentrations of the individual phenolic compounds did not seriously change after the acetification process. Surprisingly, the concentration of the protocatechuic acid in CV2 was higher than in the precursor product C2. Protocatechuic acid might be a microbial ring fission product of flavonoids (Schneider et al., 1998; Tsuda et al., 1999) or some contamination from another acetification batch.

For C1 and CV1, the total phenolic contents by the Folin−Ciocalteu method were calculated to yield 609 and 416 mg/L, respectively, and for C2 and CV2 814 and 462 mg/L, respectively, which is in the range for apple juice [401−990 mg/L (Pearson et al., 1999)]. During the acetification process, the content of total phenols decreased ∼40% (Table 4), indicating a strong phenol degradation or transformation.

Phloridzin and phloretin-xyl-glc, characteristic phenolic compounds of the C, could also be detected in considerable amounts in the corresponding vinegar.

White Wines and White Wine Vinegars. In the W1 and WV1 we identified catechin and epicatechin, whereas in the W2 and WV2 either epicatechin or catechin was identified (Table 2). The W and the WV contained many

Table 2. Content of Individual Phenolic Compounds (Milligrams per Liter) of W and Corresponding WV

	W1	WV1	W2	WV2
phenolic acids				
protocatechuic acid	<0.1	<0.1	1.1	4.1
caffeic acid	3.1	3.1	1.6	1.1
flavonoids				
catechin	59	48	<0.1	24
epicatechin	11	12	2.1	<0.1

Table 3. Content of Individual Phenolic Compounds (Milligrams per Liter) of R and Corresponding RV

	R	RV
phenolic acids		
caffeic acid	7.7	6.7
flavonoids		
epicatechin	44	22
anthocyanins		
delphinidin 3-glucoside	2.8	1.3
cyanidin 3-glucoside	1.2	<0.1
petunidin 3-glucoside	2.9	1.3
peonidin 3-glucoside	1.6	1.2
malvidin 3-glucoside	10	3.8
malvidin 3-glucoside acetate	2.0	1.7
malvidin 3-glucoside coumarate	2.0	0.9

Table 4. Total Phenol Content Measured with Folin−Ciocalteu Phenol Reagent (Calculated as Gallic Acid Equivalents) and the Changes Due to the Acetification Process

sample	total phenol content (mg/L)	change due to acetification (%)
C1	609	
CV1	416	−32
C2	814	
CV2	462	−43
W1	592	
WV1	509	−14
W2	211	
WV2	205	−1.9
R	1448	
RV	1262	−13

polar compounds, making the quantification of catechin difficult. The reported concentrations for catechin, epicatechin, and caffeic acid in white wine were 16−46, 6−60, 1.3−3.6 mg/L, respectively, which correspond well to the amounts calculated in the present study (Frankel et al., 1995; Teissedre et al., 1996). As minor compounds in the W and WV we detected caffeic acid and in the case of W1/WV1 also protocatechuic acid. The concentrations of phenols in the W and WV are low compared to those in the R and RV and are extremely low compared to those in the C and CV. In contrast, the total phenolic contents amounted to 211/592 mg/L for the W and 205/509 mg/L for the WV, whereas previously reported concentrations are in the range of 232−307 mg/L (Munday et al., 1999; Singleton et al., 1965).

The HPLC chromatograms of W and WV showed a number of large signals with short retention times and characteristic mass fragments of catechin and epicatechin, probably corresponding to polymeric phenols, for which we had no standard substances. The presence of polymeric polyphenols might explain the high content of total phenols. The fact that polymeric phenols are oxidized more slowly than monomeric phenols might explain that the acetification process diminished the total phenols only by ∼8%. Concentrations of caffeic acid, catechin, and epicatechin decreased slightly during the acetification process. As was the case for C and CV,

the increase of protocatechuic acid might be explained again by microbial flavonoid degradation or contamination. Concentration of the individual phenolic compounds did not show appreciable changes.

Red Wine and Red Wine Vinegar. In the R and RV, we identified and quantified caffeic acid, epicatechin, and the anthocyanins delphinidin 3-glucoside, cyanidin 3-glucoside, petunidin 3-glucoside, peonidin 3-glucoside, malvidin 3-glucoside, and an acetate and a coumarate of malvidin 3-glucoside (Table 3). Caffeic acid and epicatechin contents are according to published values (Frankel et al., 1995; Goldberg et al., 1996), whereas the anthocyanin contents are only about a third of the earlier reported contents (Mazza, 1995). The total phenolic contents in the R and RV, 1448 and 1262 mg/L, respectively, were actually higher than in all other samples analyzed but are still somewhat lower than previously reported contents in the range of 1416−3197 mg/L (Munday et al., 1999; Singleton et al., 1965). These differences might be due to the various wines used in the studies. The acetification process was associated with a decrease in total phenolic content of 13%. Surprisingly, most of the individual phenolic compounds, mainly the anthocyanins, showed a much higher decrease of ∼50%, probably due to low oxidizable tannins, contributing to the total phenols. Additionally, the concentration of the monomeric phenols might be reduced by oligo- and polymerization (Escribano-Bailón et al., 1996; Saucier et al., 1997), yielding products that still contribute to the total phenol content.

The characteristic anthocyan 3-glucosides of the R also occurred in considerable amounts in the RV.

Conclusion. The results confirm the assumption that the acetification process is accompanied with a decrease in the total phenol content. The decline in total phenol content was highest for CV (40%) and lower for RV and WV (13 and 8%, respectively). A decrease in the individual phenolic compounds iin WV and CV was not observed. In contrast, the individual phenolic compounds in RV decreased ∼50%. The characteristic phenolic compounds of C and R, phloridzin and phloretin-xyl-glc and anthocyan 3-glucosides, were also measurable in considerable amounts in the vinegars.

ABBREVIATIONS USED

W, white wine; R, red wine; C, cider; WV, white wine vinegar; RV, red wine vinegar; CV, cider vinegar; xyl-glc, xyloglucoside.

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