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Pilot-Scale Resin Adsorption as a Means To Recover and Fractionate Apple Polyphenols†

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The purification and fractionation of phenolic compounds from crude plant extracts using a food-grade acrylic adsorbent were studied at pilot-plant scale. A diluted apple juice concentrate served as a model phenolic solution for column adsorption and desorption trials. Phenolic concentrations were evaluated photometrically using the Folin−Ciocalteu assay and by HPLC-DAD. Recovery rates were significantly affected by increasing phenolic concentrations of the feed solutions applied to the column. In contrast, the flow rate during column loading hardly influenced adsorption efficiency, whereas the temperature and pH value were shown to be crucial parameters determining both total phenolic recovery rates and the adsorption behavior of individual polyphenols. As expected, the eluent composition had the greatest impact on the desorption characteristics of both total and individual phenolic compounds. HPLC analyses revealed significantly different elution profiles of individual polyphenols depending on lipophilicity. This technique allows fractionation of crude plant phenolic extracts, thus providing the opportunity to design the functional properties of the resulting phenolic fractions selectively, and the present study delivers valuable information with regard to the adjustment of individual process parameters.

KEYWORDS: Apple polyphenols; adsorption; desorption; adsorbent resin; recovery; fractionation

INTRODUCTION

Beginning with the "French paradox" observations in the 1990s (*1*), interest in plant secondary metabolites has significantly increased because of their putative health benefits. A number of epidemiological studies have revealed inverse correlations between diets rich in these phytochemicals and the incidence of diseases associated with oxidative stress, such as cardiovascular diseases, strokes, and certain forms of cancer. Numerous in vitro studies have revealed cellular mechanisms that may explain such beneficial effects. Apple polyphenols, which are the focus of the present study, have been shown to affect the apoptosis of human cancer cells, the metabolic activation of certain chemical carcinogens (*2*, *3*), and the phosphorylation of epidermal growth factor receptors in cancer cells (*4*). These effects might be responsible for the inhibition of human cancer cell growth (*5*). Furthermore, the reduction of triglyceride and cholesterol absorption has been reported, as well as of the absorption of cholesterol oxidation products in vivo, caused by apple polyphenols (*6*−*8*). For this reason, plant foods particularly rich in phenolic compounds, such as cloudy apple juices compared with clear juices, are supposed to be particularly valuable in a healthy diet. The polyphenol contents of apple juices strongly depend on the cultivar and the process technology applied, and concentrations of total phenols above 1 g/L may be found (*9*). Thus, polyphenols are the most important plant secondary metabolites found in apples and apple-derived products besides vitamins, provitamins, and sterols, which occur in only the micrograms−milligrams per kilogram range in fresh apples.

Besides health-related aspects, the recovery of phenolic compounds as functional ingredients appears to be increasingly attractive. Plant extracts or purified phenolics might be useful as alternatives to synthetic antioxidants and food colorants, for the mitigation of lipid oxidation and the control of nonenzymatic browning during food processing (*10*−*14*).

Consequently, there is an increasing demand for phenolic compounds and their industrial recovery from waste material (*15*) to produce preparations with tailor-made functional properties, that is, to fractionate crude plant extracts into different polyphenol subclasses which vary in their pharmacological effects and fields of application with respect to their technological properties. Typically, apple polyphenols are recovered from pomace and pectin extracts, which are readily available, inexpensive, and usually characterized

†Dedicated to Prof. Dr. Hans Becker, Saarbrücken, on the occasion of his 70th birthday.

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by high polyphenol contents (*16*). The crude extracts are commonly purified and concentrated by applying, for example, ultrafiltration or resin adsorption, without, however, systematically optimizing such advanced technologies.

Resin adsorption has proven to be one of the most efficient techniques for the selective enrichment and recovery of polyphenolic plant secondary metabolites. Adsorption with styrene−divinylbenzene or acrylic polymers is currently performed on an industrial scale, for example, to debitter citrus products (*17*, *18*), or to standardize and stabilize juices and juice concentrates, by removing browning reaction products or compounds that may cause precipitation during storage (*19*, *20*). Recent studies have shown that mainly food-grade styrene−divinylbenzene and acrylic resins may also be used for the recovery of valuable compounds from citrus juices and byproducts thereof (*21*−*24*) and other plant sources by eluting the adsorbed compounds with alcoholic or hydroalcoholic solutions. Adsorption technology can be easily integrated into existing production lines and allows semicontinuous operation (*16*). Although widely applied in the food industry, there are few studies dealing with the systematic evaluation of process parameters for the adsorption and desorption of individual phenolic compounds using polymeric adsorbents, thus rendering industrial process development mostly empirical (*25*−*29*).

As a continuation of our work on the adsorption of phenolic compounds using polymeric resins (*25*, *28*, *29*), in this study an acrylic adsorbent was applied to investigate the influence of process parameters on the adsorption. For this purpose, a diluted apple juice concentrate was used as a model for column adsorption experiments at pilot scale, because apples and apple-derived products significantly contribute to the uptake of phenolic compounds via the human diet. Furthermore, apples and apple juices are characterized by a wide diversity of phenolics, including monomeric and polymeric flavan 3-ols, hydroxycinnamic acids, dihydrochalcones, and flavonols (*30*), thus rendering apple juices an ideal model for studying the behavior of different phenolic compounds upon their contact with polymeric resins. The aim of the present study was to optimize efficiency and selectivity of polyphenol adsorption and elution by systematically varying process parameters and, thus, to study whether the enrichment of individual compounds or compound classes by systematic adjustment of process parameters is possible. These included temperature, pH value, flow rate, and concentration of the solute. Furthermore, desorption of polyphenols should be studied at different temperatures by variation of solvents (ethanol, methanol, water) and by alkaline elution of the phenolics.

MATERIALS AND METHODS

Materials. All reagents and chemicals were of analytical or HPLC grade and were purchased from Ajax Finechem (Auckland, New Zealand) except for sodium carbonate, which was obtained from Merck (Darmstadt, Germany), and Folin−Ciocalteu reagent, which was obtained from Sigma (St. Louis, MO). Solutions and eluents were prepared with deionized water. For apple polyphenol recovery experiments on a pilot-plant scale, Alimentech P-495, an ethylene glycol cross-linked polymethylmethacrylate resin, with lipophilic properties was used (Bucher-Alimentech Ltd., Auckland, New Zealand). According to the manufacturer, the resin material has a particle size of 0.35−0.85 mm, a surface area of 450 m^2/g, a pore radius of 200−300 Å, a porosity of 1.1 mL/g, a density of 1.09 g/mL, and a moisture content of 58−65%.

Individual phenolic compounds were quantified by external calibration. Reference compounds were obtained from Sigma [caffeic acid, (+)-catechin, chlorogenic acid, *p*-coumaric acid, phloretin, phloretin 2-*O*-glucoside (phloridzin), and quercetin 3-*O*-rutinoside)], Extrasynthèse (Genay, France) (quercetin 3-*O*-glucoside and quercetin 3-*O*-rhamnoside), and Acros Organics (Geel, Belgium) (quercetin).

Model solutions for adsorption experiments were prepared using a clear apple juice concentrate [72.2% total soluble solids (TSS)], which was kindly supplied by ENZAFOODS New Zealand Ltd. (Hastings, New Zealand).

Methods. *Polyphenol Adsorption Studies on Pilot-Plant Scale*. To simulate industrial-scale adsorption processes, a heat-insulated stainless steel adsorption column (l = 150 cm, i.d. = 3.81 cm) provided by Bucher-Alimentech Ltd. (Auckland, New Zealand) was used. The column was filled with 1 L of Alimentech P-495 acrylic resin, which is commercially used for food processing and thus should be systematically evaluated with respect to its potential to recover and fractionate polyphenols. Temperature control was achieved by conditioning the resin with thermostated water and adjusting the temperature of the feed solutions, rinse water, and eluents using a 3 kW process heater. The solutions were delivered with a ProMinent Beta solenoid metering pump (type BT5a; ProMinent Dosiertechnik GmbH, Heidelberg, Germany).

A diluted apple juice concentrate was used as a model polyphenol solution. The concentrate was diluted with deionized water to yield feed solutions of 15, 25, 35, and 45% TSS, respectively, to approximate TSS values of apple juices and to further study the effects of feed stream concentration on the adsorption behavior of polyphenols. Furthermore, the effects of flow rate [4, 6, and 8 bed volumes (BV)/h] during the adsorption step and the pH value of the model solutions were studied. The latter parameter significantly affects the lipophilicity of phenolic compounds due to its influence on their protonation/deprotonation equilibria. To evaluate pH, the feed stream pH was either kept unchanged (pH ∼4.0−4.1) or adjusted to pH 3.0 and 2.5 using phosphoric acid (85%). The solutions were applied to the column either at 20 °C or at 40 and 60 °C, respectively, to study temperature effects on the interaction of the target compounds with the resin material. In total, 30 L of the feed solution was applied to the column for each experiment. During application of the test solutions, 2 L fractions were collected and subsampled for photometric and HPLC determination. The resin was subsequently washed with deionized water (1.5 BV as recommended by the manufacturer; same flow rate as during the adsorption step), with two samples of 1.0 and 0.5 L collected for quantifying polyphenol losses during this step. In the first series of single experiments the elution of phenolic compounds was performed with 96% ethanol, collecting samples of 0.5 and 0.25 L. The amounts of total and individual phenolic compounds adsorbed to the resin were calculated from the difference between the feed solution contents and those of the column effluents during the adsorption step.

Desorption Experiments: Effects of Eluents and Temperature. Thirty liters of a 25% TSS apple juice model solution was applied to the column at a flow rate of 6 BV/h and a temperature of 20 °C. For polyphenol elution at 20 °C, ethanol/water mixtures (96, 48, and 24% ethanol; v/v) and methanol/water mixtures (100, 50, and 25% methanol; v/v) were compared with water (elution at 20 and 80 °C), because hydroalcoholic solutions are commonly applied for desorption. Furthermore, elution trials were performed with NaOH solutions [0.5, 1.0, and 2.0% (w/v)] to study organic solvent-free polyphenol elution. To avoid polyphenol loss due to degradation or oxidation at high pH values, the column effluents were eluted into diluted phosphoric acid solutions to reach pH values of ∼3.5−5.1. To achieve this, polyphenols were eluted into 500 and 1000 mL volumetric flasks, which were filled with 250 and 500 mL of phosphoric acid solutions, respectively, and the solutions were made up to the mark with the eluates. All single elution trials were performed at a flow rate of 2 BV/h.

Resin Regeneration. After each adsorption/desorption experiment, the resin was backwashed with deionized water at a flow rate of 8 BV/h to reclassify the polymer bed. Subsequently, the resin was regenerated according to the manufacturer's recommendations by successively flushing with 4 L of a 2% (w/v) NaOH solution, 2 L of deionized water, and 2 L of phosphoric acid (2%, w/v) to neutralize the polymer and finally rinsing with 6 L of deionized water. Each step was performed at a flow rate of 4 BV/h. After regeneration and conditioning, the resin was reused for the subsequent adsorption experiments.

Quantification of Individual Phenolic Compounds by HPLC. Separation and quantification of individual phenolic compounds were performed using a Shimadzu HPLC system (Kyoto, Japan) with a Phenomenex C18 Synergi Hydro-RP column (Torrance, CA; 250 × 4.6 mm i.d., 4 μm particle size) and a C18 ODS guard column (4.0 × 3.0 mm i.d.), operated at 35 °C. Compound elution was performed with eluent A (0.1% formic acid

Table 1. Process Parameters and Adsorption Rates of Pilot-Plant Scale Adsorption Experiments (BV = 1 L)[a]

					absolute amount of total phenolics in model solution (g of CE)						absolute amount of total adsorbed phenolics (g of CE)						adsorption rate (%)					
expt	pH value of feed solution	flow rate (BV/h)	feed concn (% TSS)	temp (°C)	mean	SD	*b*	*c*	*d*	*e*	mean	SD	*b*	*c*	*d*	*e*	mean	SD	*b*	*c*	*d*	*e*
1	uc	6.0	25.0	20	40.89	0.42	a	A	γ	I	27.33	0.09	b	A	β	II	66.84	0.22	a	A	β	III
2	uc	6.0	25.0	40	41.99	0.17	a				27.85	0.06	a				66.33	0.14	a			
3	uc	6.0	25.0	60	40.93	0.70	a				24.95	0.13	c				60.95	0.31	b			
4	uc	8.0	25.0	20	39.66	0.35		AB			25.07	0.14		B			63.20	0.36		B		
5	uc	4.0	25.0	20	39.34	0.03		B			26.79	0.15		A			68.10	0.37		A		
6	uc	6.0	15.0	20	21.66	0.21			δ		16.37	0.10			γ		75.54	0.44			α	
7	uc	6.0	35.0	20	56.73	0.14			β		28.79	0.31			$\alpha\beta$		50.75	0.55			γ	
8	uc	6.0	45.0	20	78.72	0.51			α		30.21	0.75			α		38.38	0.95			δ	
9	3.0	6.0	25.0	20	40.66	1.28				I	29.03	0.00				I	71.39	0.01				I
10	2.5	6.0	25.0	20	37.69	0.31				I	26.48	0.02				III	70.25	0.06				II

[a] BV, bed volume; CE, catechin equivalents; SD, standard deviation; uc, unchanged (pH ~4.0−4.1). [b] Different lower case letters illustrate significant differences in experiments applying different temperatures as deduced from Tukey test. [c] Different upper case letters illustrate significant differences in experiments applying different flow rates as deduced from Tukey test. [d] Different Greek letters illustrate significant differences in experiments applying different feed solution concentrations as deduced from Tukey test. [e] Different Roman numerals illustrate significant differences in experiments applying different pH values of the feed solutions as deduced from Tukey test.

in acetonitrile, v/v) and eluent B [0.1% formic acid in water and acetonitrile (95: 5, v/v)] at a flow rate of 1.0 mL/min using the following gradient program: from 0% A to 8.7% A (5 min), 8.7% A isocratic (10 min), from 8.7% A to 17% A (10 min), from 17% A to 20% A (5 min), from 20% A to 30% A (9 min), from 30% A to 50% A (4 min), from 50% A to 95% A (5 min), 95% A isocratic (5 min), and from 95% A to 0% A (2 min). The total run time was 65 min. Peak identification was achieved by comparing retention times and UV spectra with those of reference compounds and by LC-MS analysis. Quantification of individual phenolics was performed at 280 nm (dihydrochalcones, flavanols), 320 nm (hydroxycinnamic acids), and 370 nm (flavonols). Polyphenols were quantified using a calibration curve of the respective reference compound or a structurally related compound with a molecular weight correction factor if standards were not available (*31*). All HPLC determinations were performed in duplicate.

Mass spectrometric determinations were carried out using an LCQ Deca ion trap mass spectrometer equipped with an ESI interface (ThermoQuest, Finnigan, San Jose, CA) coupled to a Surveyor HPLC. For further details see ref *25*.

Total Phenol Determination (*Folin−Ciocalteu*). Phenolic compounds were quantified spectrophotometrically according to the method of Singleton et al. (*32*). Aliquots of 1 mL of the samples were combined with 5 mL of the Folin−Ciocalteu reagent. Five minutes after mixing, 4 mL of a Na_2CO_3 solution (75 g/L) was added and incubated after thorough stirring at 20 °C for 90 min in the dark. Aliquots of 200 μL were transferred to microplates for spectrophotometric determinations (absorbance at 760 nm) using a SpectraMax Plus^{384} photometer controlled by SOFTmax Pro software (version 3.1.2; Molecular Devices Corp., Sunnyvale, CA). Calibration curves were established using a (+)-catechin standard in the range of 25−125 mg/L. All analyses were performed in duplicate.

Statistical Analysis. Significance of differences between the analytical data of independent experiments ($\alpha = 0.05$) was determined using the Tukey test. Data evaluation was performed with the SAS software package (SAS Institute, Cary, NC; software version 9.1).

RESULTS AND DISCUSSION

Adsorption. *Determination of Overall Phenolic Adsorption Efficiency and Comparison of Individual Compounds*. In the first series of experiments the effects of temperature, feed concentration, flow rate, and pH value of the feed solution on the adsorption of total and individual phenolic compounds were investigated. The process conditions applied in the present study, the amount of phenolics in the feed solutions, the resulting amounts of phenolics adsorbed by the acrylic resin, the adsorption rates, and the results of statistical analyses are given in **Table 1**. The latter two values were calculated by difference, taking into account the total phenolic contents of the feed solutions and fractions collected during column loading and deionized water rinsing of the resin.

(*a*) *Adsorption Behavior As Evaluated by Total Phenolic Contents* (*Folin−Ciocalteu*). A typical profile of the total phenolic contents [mg of catechin equivalents (CE)] of the fractions collected during sample application (Fr.1−15), the deionized water rinse step (R.1−2), and elution with 96% ethanol (El.1−14) is exemplified in **Figure 1** (experiment 1, see **Table 1** for parameters). As can be deduced from the phenolic amounts in the fractions collected during sample application, increasing amounts of polyphenols were lost as column loading progressed, which is typical for pilot- and industrial-scale sorption processes. In the experiment presented in **Figure 1**, approximately 23% of the phenolics were not adsorbed after the application of 10 L of the model solution, with the leakage increasing to 43% after 20 L and to around 54% after the application of 30 L (data not shown). The overall loss of total phenolics during column loading was 30.5%, and another 2.7% was lost during column rinsing. Adsorption rates depend on the resin capacity and the phenolic content of the feed solution; thus, the service life of the adsorption column must be thoroughly predetermined to guarantee cost-efficient polyphenol recovery. Therefore, polyphenol binding onto adsorbent resins on industrial scale always remains incomplete (*23*, *24*), and the volume of feed solution applied and the resulting leakage rates determine the cost-efficiency of the process. Thirty bed volumes of sample solution was applied to the column in all further experiments, irrespective of incomplete polyphenol adsorption, to standardize the sample loading procedure.

(*b*) *Adsorption Behavior As Evaluated by Individual Phenolic Compounds* (*HPLC-DAD*). Individual phenolic compounds were quantified in each fraction collected during the adsorption step of all experiments. Twelve phenolic compounds, comprising hydroxycinnamic acids, dihydrochalcones, flavanols, and flavonols, were identified and quantified by HPLC-MS and HPLC-DAD. In **Figure 2** the relative leakages of these compounds in each fraction collected during adsorption are exemplified for experiment 1 (refer to **Table 1**). The data clearly demonstrate that adsorption is strongly dependent on hydrophobicity, which has also been revealed in our previous studies on the adsorption of phenolics from model solutions and crude plant extracts (*25*, *33*). Highly hydrophilic compounds, such as 4-caffeoylquinic, 5-caffeoylquinic, and *p*-coumaroylquinic acids, exhibited the highest leakages, amounting to 7.7% for 4-caffeoylquinic acid in the final fraction (Fr.15). This resulted in total leakage rates of 56.3, 37.5, and 32.1%, respectively, of the aforementioned

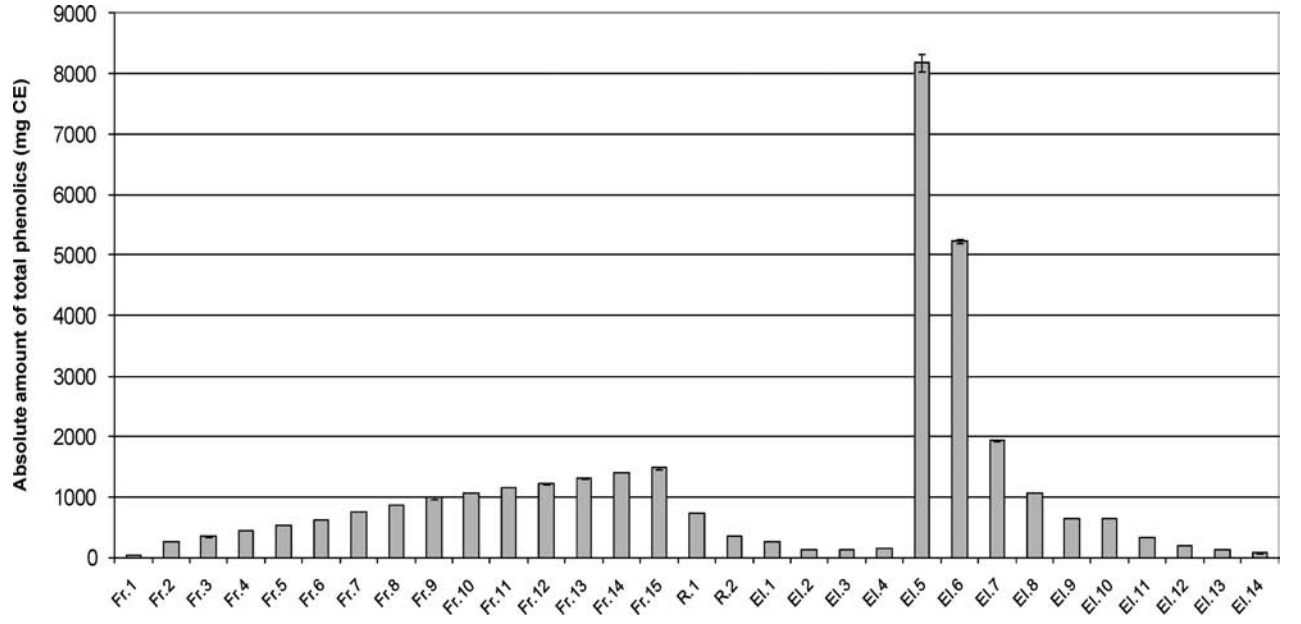

Figure 1. Total phenolic contents of the fractions collected during the adsorption, rinsing, and elution steps [model solution, 25% TSS; flow rate, 6 BV/h (adsorption), 2 BV/h (desorption); temperature, 20 °C; elution with 96% ethanol]; mean ± standard deviation of the mean. Fr.1−15, fractions 1−15 (2 L each); R.1−2, rinsing step (1.0 and 0.5 L); El.1−14, elution 1−14 (El.1, 10−14, 500 mL each; El.2−9, 250 mL each); CE, catechin equivalents.

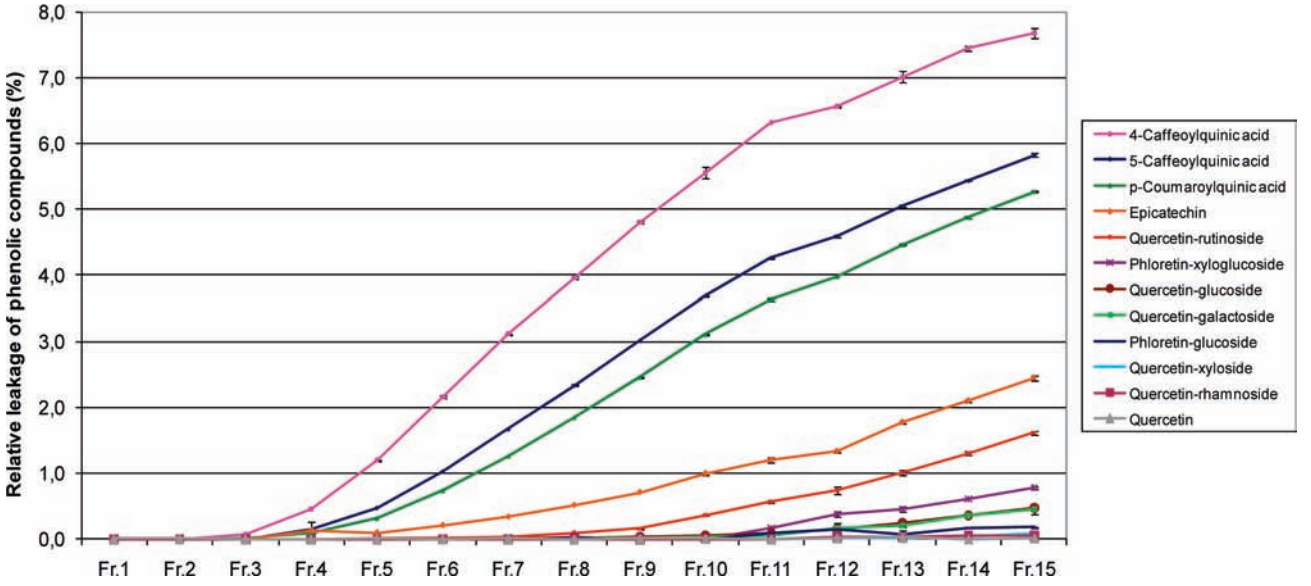

Figure 2. Relative leakage of individual phenolic compounds quantified in each fraction collected during adsorption (adsorption experiment 1; cf. **Table 1**); mean ± standard deviation of the mean. Fr.1−15, fractions collected during adsorption (V = 2 L each).

hydroxycinnamates when 30 L of the model solution was applied to the adsorption column. Intermediate leakages were observed for epicatechin (11.9%) and quercetin-rutinoside (5.9%), the latter being the most hydrophilic quercetin glycoside present. All other flavonol glycosides, which are more lipophilic, showed only minor leakages, as demonstrated by the values for quercetin glucoside (1.4%), galactoside (1.3%), xyloside (0.2%), and rhamnoside (0.2%). The very hydrophobic quercetin aglycone was almost quantitatively (99.9%) bound by the resin. The dihydrochalcones phloretin-xyloglucoside and phloretin-glucoside were also effectively adsorbed to the resin (97.6 and 99.3%, respectively). The total leakage rates of these phenolic compounds during the application of 30 BV of the model solutions for all adsorption experiments are shown in **Table 2** and further discussed below.

(*1*) *Effects of Solute Concentration on the Adsorption of Phenolic Compounds*. As expected, the adsorption efficiency markedly increased when a diluted model solution (15% TSS; 722 mg of CE/L, expt 6) was used for the adsorption experiment (**Table 1**). Accordingly, total phenolic adsorption rates increased compared with the previous experiment (*1*) using a 25% TSS model solution. Around 20% of the phenolics were not adsorbed after the application of 10 L of the model solution, and the leakage increased to 28 and 38% after the application of 20 and 30 L, respectively (data not shown). Increasing the TSS contents of the model solutions to 35 and 45% (expts 7, 8) led to a significant reduction in the total adsorption rate (49.2 and 61.6% leakage, respectively).

As can be seen from the absolute amounts of polyphenols adsorbed to the resin (**Table 1**), even 30 BV of highly concentrated feed solutions (25 or 35% TSS) did not result in saturation of the adsorbent resin, because applying a 45% TSS model solution allowed greater total polyphenol adsorption. The determination

Table 2. Total Leakage Rate of Individual Phenolic Compounds (Percent) during the Application of 30 BV of the Apple Phenolic Model Solutions to the Adsorption Column (for Process Parameters of Experiments 1−10, cf. **Table** 1; Mean ± Standard Deviation)[a]

	adsorption expt																					
compound	1		2		3		4		5		6		7		8		9		10			
5-caffeoylquinic acid	37.5 ± 0.0	cBγI	47.5 ± 0.0	b	63.2 ± 0.1	a	40.6 ± 0.3	A	34.5 ± 0.4	C	16.4 ± 0.1	δ	51.5 ± 0.1	β	61.9 ± 0.2	α	20.2 ± 0.1	II	19.0 ± 0.0	III
4-caffeoylquinic acid	56.3 ± 0.2	cAγI	67.8 ± 0.1	b	75.5 ± 0.8	a	50.5 ± 4.4	A	50.6 ± 0.3	A	32.0 ± 0.1	δ	62.9 ± 0.6	β	67.9 ± 0.4	α	30.3 ± 0.4	II	29.8 ± 0.2	II
p-coumaroylquinic acid	32.1 ± 0.0	cBγI	42.1 ± 0.0	b	59.3 ± 0.1	a	36.7 ± 0.9	A	29.1 ± 0.1	C	13.4 ± 1.4	δ	45.5 ± 0.2	β	56.3 ± 0.0	α	14.8 ± 0.1	II	13.7 ± 0.0	III
phloretin-glucoside	0.7 ± 0.0	aAγI	0.4 ± 0.0	a	1.1 ± 0.3	a	0.9 ± 0.1	A	0.2 ± 0.1	B	0.0 ± 0.0	δ	4.7 ± 0.0	β	19.7 ± 0.0	α	0.8 ± 0.1	I	0.9 ± 0.1	I
phloretin-xyloglucoside	2.4 ± 0.2	aBγII	0.8 ± 0.0	b	2.5 ± 0.1	a	4.4 ± 0.1	A	0.3 ± 0.0	C	0.1 ± 0.1	δ	14.8 ± 0.0	β	34.5 ± 0.0	α	4.5 ± 0.0	I	4.7 ± 0.0	I
epicatechin	11.9 ± 0.0	cBγII	13.3 ± 0.0	b	22.7 ± 0.3	a	15.4 ± 0.1	A	9.0 ± 0.0	C	2.8 ± 0.1	δ	27.2 ± 0.1	β	44.5 ± 0.5	α	16.5 ± 0.1	I	16.5 ± 0.0	I
quercetin	0.1 ± 0.0	aAβI	0.3 ± 0.3	a	0.3 ± 0.2	a	0.1 ± 0.0	A	0.1 ± 0.0	A	0.1 ± 0.0	β	0.1 ± 0.0	β	0.8 ± 0.1	α	0.1 ± 0.0	I	0.1 ± 0.0	I
quercetin-rhamnoside	0.2 ± 0.1	aAγI	0.0 ± 0.0	a	0.1 ± 0.0	a	0.2 ± 0.0	A	0.0 ± 0.0	A	0.0 ± 0.0	γ	5.1 ± 0.1	β	21.9 ± 0.0	α	0.3 ± 0.1	I	0.4 ± 0.0	I
quercetin-xyloside	0.2 ± 0.1	aABγI	0.1 ± 0.0	a	0.0 ± 0.0	a	0.3 ± 0.0	A	0.0 ± 0.0	B	0.0 ± 0.0	γ	5.4 ± 0.0	β	23.5 ± 0.2	α	0.2 ± 0.1	I	0.3 ± 0.0	I
quercetin-glucoside	1.4 ± 0.0	aBγIII	0.5 ± 0.0	c	0.9 ± 0.0	b	3.0 ± 0.1	A	0.4 ± 0.0	C	0.0 ± 0.0	δ	12.6 ± 0.0	β	33.3 ± 0.1	α	2.7 ± 0.0	II	2.8 ± 0.0	I
quercetin-galactoside	1.3 ± 0.2	aBγII	0.4 ± 0.1	b	0.7 ± 0.0	ab	2.8 ± 0.1	A	0.3 ± 0.0	C	0.0 ± 0.0	δ	11.4 ± 0.2	β	31.6 ± 0.2	α	2.0 ± 0.1	I	2.4 ± 0.2	I
quercetin-rutinoside	5.9 ± 0.2	aBγII	3.7 ± 0.1	c	5.3 ± 0.1	b	10.4 ± 0.2	A	3.0 ± 0.1	C	0.1 ± 0.0	δ	24.5 ± 0.3	β	45.4 ± 0.2	α	10.2 ± 0.1	I	10.2 ± 0.0	I

[a] Different lower case letters, upper case letters, Greek letters, and Roman numerals (horizontal) illustrate significant differences in leakage rates of one phenolic compound in experiments applying different temperatures (expts 1−3), different flow rates (expts 1, 4, 5), different feed solution concentrations (expts 1, 6−8), and different pH values of the feed solutions (expts 1, 9, 10), respectively, as deduced from Tukey test.

of equilibrium constants and maximum sorption capacities using well-established adsorption theories, such as the Langmuir and Freundlich isotherms, is applicable for comparing various resins with regard to their potential to adsorb hydrophobic compounds (*25*−*29*). However, equilibrium is not achieved under conditions typically applied in industrial processes such as in the present study. Thus, the Langmuir and Freundlich parameters could not be calculated with the data obtained in the present study.

The major concentration-dependent effects observed in total phenolic contents were also seen for the individual phenolics. Adsorption rates of the hydroxycinnamates and epicatechin were significantly affected with increasing concentration of the model solutions, resulting in leakage rates up to 67.9% for 4-caffeoylquinic acid when a 45 °Brix solution was applied to the adsorbent. Interestingly, adsorption of the dihydrochalcones and the flavonols was strongly reduced with increasing concentration. For example, quercetin-rutinoside and the hexosides quercetin-glucoside and quercetin-galactoside, which were quantitatively adsorbed using a 15% TSS model solution, showed leakages of 45.4, 33.3, and 31.6%, respectively, at 3-fold TSS concentration. This might be due to the interference of other phenolic compounds such as oligomeric and polymeric components, mainly flavan 3-ols (*8*, *9*), which were not quantified in the present study and may compete with low molecular weight compounds for adsorption sites, thus reducing adsorption of the phenolics monitored in this study. Low molecular weight phenolics may also compete for sorption sites on the resin surface, thus affecting recovery rates of individual compounds in solutions with different phenolic composition (*33*), and even nonphenolic compounds such as saccharides, which were not expected to interact with the resin, have been shown to affect polyphenol recovery rates (*34*). This may explain the aforementioned findings using various feed concentrations.

(*2*) *Flow Rate Effects on Polyphenol Binding in Column Adsorption Experiments*. The applicability of industrial adsorption processes depends not only on the service life of adsorption columns, and thus on the need to desorb the phenolics and regenerate the resin, but also on the volume of the fluid stream that can be continuously processed within a given time. Therefore, the effects of flow rate on polyphenol recovery rates were studied. In addition to the flow rate of 6 BV/h recommended by the resin manufacturer, flow rates of 4 and 8 BV/h were also tested. Lower adsorption efficiency was expected from the shorter contact times between the model solution and the resin at increased flow rates, whereas the opposite effect should apply for lower flow rates. This was confirmed in our experiments (**Table 1**). However, flow rate-dependent effects were comparatively small, and only at 8 BV/h was a significantly lower adsorption rate observed (63.2%), whereas 68.1 and 66.8% of total phenolics were bound to the resin at 4 and 6 BV/h, respectively.

Analogous to total phenolic contents, a change in flow rate also resulted only in minor changes of individual polyphenol adsorption rates. Again, maximal effects were observed for the hydroxycinnamic acids and epicatechin, where 5-caffeoylquinic acid showed relative leakages of 34.5, 37.5, and 40.6% at flow rates of 4, 6, and 8 BV/h, respectively. With the exception of quercetin-rutinoside, which showed a leakage of 10.4% at 8 BV/h, the adsorption rate of all other components remained virtually unchanged.

(*3*) *Temperature Effects on the Adsorption of Total and Individual Phenolics*. Although adsorption phenomena are temperature-dependent (*28*, *29*), inconsistent results on the optimum conditions for the adsorptive recovery of plant secondary metabolites have been reported (*35*). Therefore, the effects of different temperatures on adsorption efficiency were also considered in this study. Compared with 20 °C, adsorption at 40 °C was insignificantly different (66.3 and 66.8%, respectively), whereas a further increase in temperature (60 °C) significantly decreased adsorption to 61.0% (**Table 1**). This indicates that the interaction between the sorbent and the target compounds is weakened at increased temperatures, which is in accordance with the results of previous laboratory-scale experiments (*25*). Therefore, the temperature during adsorption should be kept as low as possible to maximize binding strength.

In contrast to the small decrease in overall phenolic adsorption at 60 °C, hydroxycinnamate adsorption was strongly affected by temperature even as low as 40 °C, at which significantly lower amounts were adsorbed onto the acrylic resin. The same trend was observed for epicatechin; however, the effects were not as pronounced as was seen for the quinic acid esters. Unexpectedly, dihydrochalcone and flavonol adsorption was hardly affected by temperature (**Table 2**, expts 1−3).

(*4*) *pH Effects on the Interaction of Polyphenols with the Acrylic Resin*. In our previous study the pH value was shown to affect polyphenol adsorption significantly. This was attributed to an increase in acidity, rendering acidic compounds such as hydroxybenzoic and hydroxycinnamic acids more lipophilic than their deprotonated forms (*25*). Because all experiments described so far were performed using feed solutions at the natural pH value of the juice (pH ∼4.0−4.1), further trials were carried out with acidified samples. For this purpose, apple juice was adjusted to pH 3.0 and 2.5 using phosphoric acid. In both experiments acidification significantly increased adsorption rates compared to the adsorption trial performed without pH adjustment (**Table 1**).

HPLC analyses revealed major pH-dependent effects on individual compounds. As expected from the pK_a value of 5-caffeoylquinic acid (3.95) (*34*), hydroxycinnamic acid adsorption was markedly improved with increasing feed solution acidity. Thus, at the natural pH value of apple juice concentrate (pH 4.0−4.1), around 50% of the molecules were deprotonated, rendering them highly hydrophilic. Lowering the pH to 3.0 and 2.5 increased protonation of these polyphenols, thus favoring their adsorption due to higher lipophilicity. Whereas the adsorption of most other compounds was not affected by higher acidity, epicatechin and quercetin-rutinoside showed higher leakage under acidic conditions (**Table 2**). This may be attributed to the protonation of the molecules, giving them a positive charge and thus also rendering them more hydrophilic, and this phenomenon has also been observed in our previous studies for some compounds (*34*).

Desorption. *Elution of Total Phenolics and Individual Phenolic Compounds*. In a second series of experiments, various solvent systems were compared for ability to elute apple phenolics from the adsorbent resin. Furthermore, fractionation of phenolic compounds during elution was also studied to make use of such chromatographic effects. For these experiments, phenolic compounds were brought into contact with the acrylic resin under the conditions of experiment 1 (25% TSS; 30 BV; flow rate during adsorption, 6 BV/h; temperature during adsorption, 20 °C). Three fractions of 10 L and two fractions of 1 and 0.5 L were collected during sample application and column rinsing, respectively, to calculate the absolute amount of phenolics bound by the adsorbent.

(*a*) *Effects of Organic Solvent and Aqueous Elution on the Recovery of Phenolic Compounds*. (*1*) *Ethanol*. The elution profile of an experiment performed with 96% ethanol is illustrated in **Figure 1**. The first four fractions collected during elution (total volume = 1.25 L) contained negligible amounts of phenolic compounds because these mainly consisted of the rinsing water. Subsequently, highly concentrated fractions were eluted. Their concentrations and the number of fractions containing large amounts of phenolics depended on the alcohol concentration used for elution. Comparison of these findings with literature data proves difficult due to the lack of comparable studies taking into consideration both the effects of different solvents and the recovery in different fractions collected upon elution. Using 96% ethanol for the elution step, 69.6% of the total phenolics bound by the resin were recovered. Even higher recovery rates (76.9%) were obtained with 48% ethanol. This correlated with our previous laboratory elution experiments using an accelerated solvent extraction (ASE) system, with which maximum recovery rates were achieved with ∼60 vol % ethanol. However, lowering the ethanol concentration to 25% markedly decreased total polyphenol recovery rate (32.4%, **Figure 3a**).

(*2*) *Methanol*. Using undiluted methanol, recovery was almost the same as for 96% ethanol. However, differences between elution with aqueous ethanol and methanol were observed. Elution with 50 and 25% methanol lowered recovery rates (58.4 and 17.5%, respectively) compared with the respective hydroethanolic solutions (76.9 and 32.4%, respectively), which is in agreement with the results of our previously published ASE experiments, by which maximum recovery rates were obtained with ∼60 vol % ethanol and 70−80 vol % methanol (*25*).

The type of eluent and the concentration of the organic solvents affected not only the total yield of polyphenols in the eluates but also the polyphenol concentrations of the fractions and, thus, the elution profile (**Figure 3a**). With 96% ethanol and 100% methanol, only three fractions contained notable amounts of polyphenols, with the remaining fractions containing < 3% of the total amount of phenolics bound to the resin. Decreasing the alcohol concentration resulted in lower total phenolic contents in the major polyphenol fractions. However, total phenolics exceeded 5% in up to six fractions.

Photometric determinations of total phenolic contents revealed recovery rates below 80% under any experimental condition. Therefore, it appears that oligomeric and polymeric phenolics, which should have been detected photometrically, but not by HPLC, exert stronger binding to the resin and were thus only partially eluted.

The elution profiles of individual phenolic compounds were also monitored by HPLC. The application of aqueous alcoholic solutions improved recovery rates of low molecular weight phenolics. Complete recovery of all monomeric polyphenols quantified in the present study was achieved with undiluted methanol and ethanol (data not shown). At lower alcohol concentrations the elution profiles of individual phenolic compounds differed. Desorption with methanol/water (50:50, v/v) is exemplified in **Figure 4a**. Each of the fractions El.2-El.9 consisted of 250 mL column effluent, whereas fractions El.1 and El.10−14 were obtained by collecting 500 mL each, thus explaining the second elution maximum seen in **Figure 4a**. The hydroxycinnamates, epicatechin and phloretin-xyloglucoside, were quantitatively recovered from the resin, with maximum amounts being eluted in fractions El.6 and E1.7. The elution behavior of quercetin (Q) and its glycosides was determined by their hydrophilicity, as evidenced by the minimal elution of the aglycone (0.2%) with 50% methanol, compared with 49.2 and 56.2% of Q-rhamnoside and Q-xyloside, respectively, in fractions El.1−14. A further slight increase in hydrophilicity led to higher recovery rates, amounting to 81.1 and 78.6% for Q-glucoside and Q-galactoside, respectively. The more hydrophilic disaccharide Q-rutinoside was quantitatively recovered from the resin. This again corroborates the findings of previous studies, revealing that the binding strength between phenolics and adsorbent resins is affected by the lipophilicity of the target compounds (*25*, *33*, *34*). Consequently, selective recovery of polyphenols according to their polarity is possible because of their different elution behaviors with aqueous alcoholic solutions, thus allowing fractionation. This is achieved by making use of the structural properties of specific polyphenol subclasses to selectively enrich and fractionate them (*21*).

(*3*) *Water*. In another experiment, solvent-free elution of phenolic compounds from the adsorbent resin was studied. Heated water was used for elution, because adsorption experiments revealed lower adsorption rates at increased temperatures.

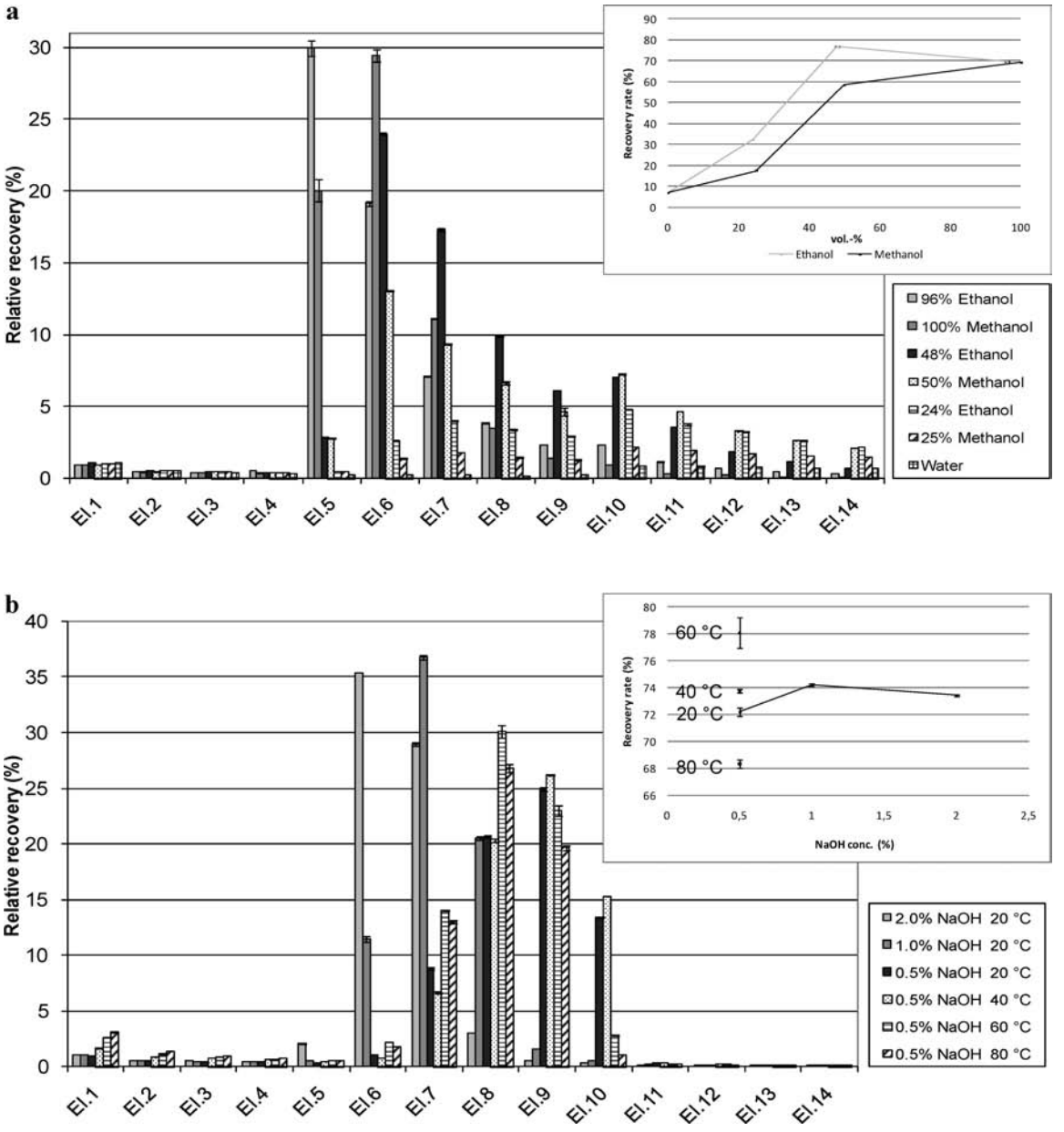

Figure 3. (**a**) Elution profiles using ethanol, methanol, and water at various volume ratios as shown by relative recovery rates of total phenolics (elution, 2 BV/h, 20 °C; column loading, 30 BV/h of a 25% TSS model solution, 6 BV/h, 20 °C); mean ± standard deviation of the mean. Total polyphenol recoveries are illustrated in the inset. El.1−14, elution 1−14 (El.1,10−14, 500 mL each; El.2−9; 250 mL each). (**b**) Elution profiles using sodium hydroxide solutions at various concentrations and temperatures as shown by relative recovery rates of total phenolics (elution, 2 BV/h; column loading, 30 BV/h of a 25% TSS model solution, 6 BV/h, 20 °C); mean ± standard deviation of the mean. Total polyphenol recoveries are illustrated in the inset. El.1−14, elution 1−14 (El.1,10−14, 500 mL each; El.2−9; 250 mL each).

However, even at 80 °C only about 7.0% of the phenolics bound by the resin were eluted, thus demonstrating that solvent-free elution at elevated temperatures cannot be performed under economic conditions (**Figure 3a**). Furthermore, the poor recovery rates may also be due to partial heat-induced degradation of phenolic compounds.

Again, HPLC data revealed that the highly hydrophilic hydroxycinnamates were most easily recovered from the adsorbent resin. Water at 80 °C was able to elute 36.3, 64.9, and 38.9% of 5-caffeoylquinic acid, 4-caffeoylquinic acid, and *p*-coumaroylquinic acid bound to the resin, respectively, whereas all other phenolics were only poorly recovered without organic solvents, underscoring again the aforementioned conclusions.

(*b*) *Alkaline Elution for the Solvent-Free Recovery of Polyphenols*. To test elution without organic solvents, another series of experiments using sodium hydroxide solutions, which are usually applied for resin regeneration, was performed. So far, only experiments demonstrating the elution of hesperidin from adsorbent resins with aqueous or hydroalcoholic NaOH solutions have been reported demonstrating high recoveries (*22*, *23*), whereas the recovery of apple phenolics under these conditions has not been studied. Because phenolic compounds are prone to oxidation and degradation under alkaline conditions, they were eluted into diluted phosphoric acid solutions to minimize losses. Interestingly, recovery rates comparable to or even higher than organic solvent elution were observed. Elution with 0.5, 1.0, and 2.0% NaOH solutions, respectively, yielded 72−74% of the total phenolics adsorbed to the acrylic resin. Alkaline elution may be a practical and safer alternative to inflammable alcoholic solvents, and the low concentrations of NaOH required (0.5%) suggest

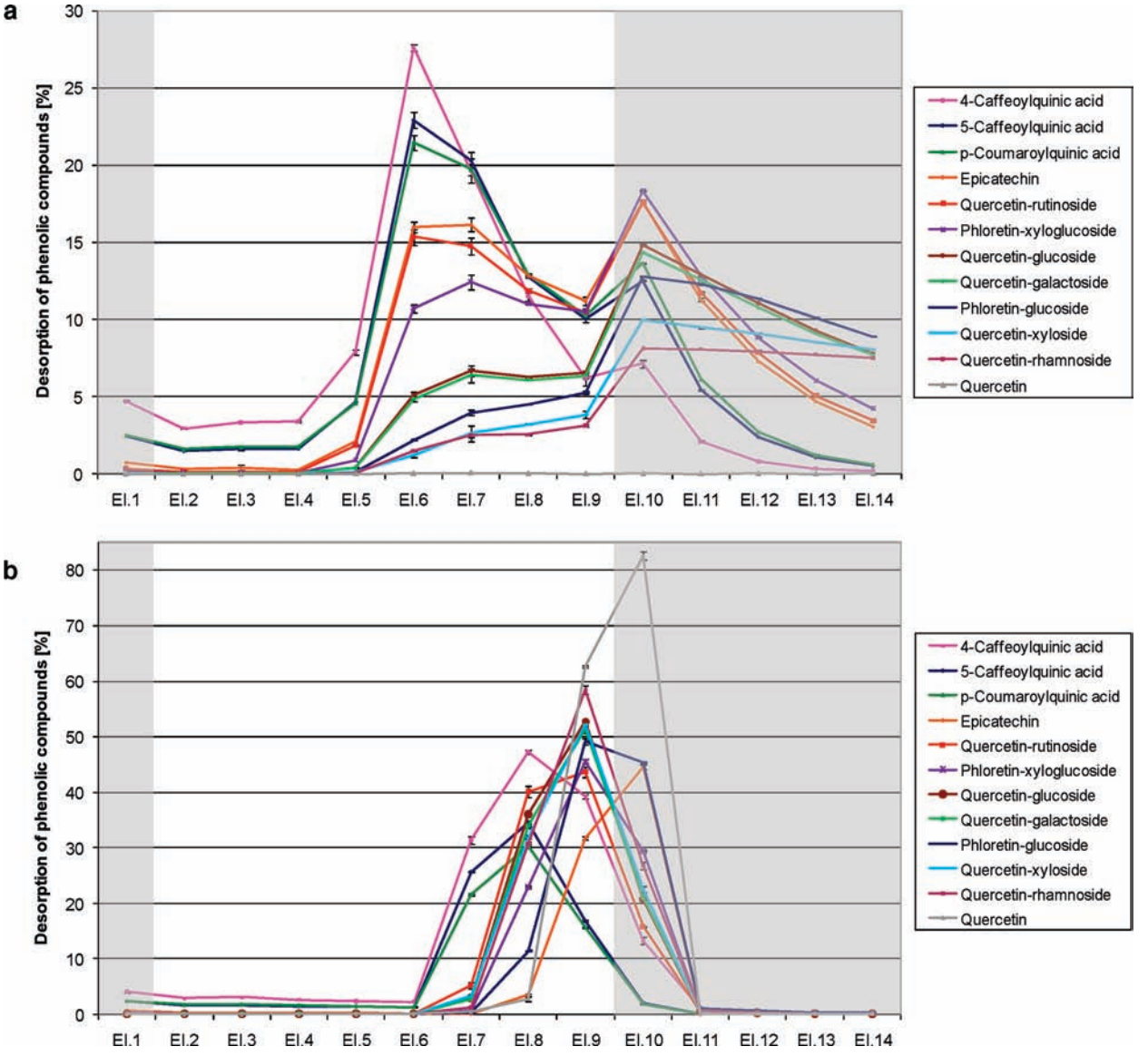

Figure 4. Elution profiles of individual phenolic compounds using (**a**) 50% methanol or (**b**) 0.5% NaOH for desorption as shown by relative recovery rates (elution, 2 BV/h; column loading, 30 BV/h of a 25% TSS model solution, 6 BV/h, 20 °C); mean ± standard deviation of the mean. El.1−14, elution 1−14 (El.1,10−14, 500 mL each (marked in gray); El.2−9, 250 mL each).

that the economics may also be better. However, this procedure may require further purification steps to remove sodium phosphate originating from the neutralization step. Moreover, gentle concentration and drying of hydroalcoholic eluates is easier than concentration and drying of aqueous solutions resulting from nonsolvent elution (*36*).

Elution experiments using 0.5% NaOH solutions were also performed at 20, 40, 60, and 80 °C. The results indicate that an increase in temperature enhanced polyphenol recovery, as can be deduced from the values obtained at 20 °C (72.2%), 40 °C (73.8%), and 60 °C (78.1%), which can be attributed to the weakening of the hydrophobic interactions between the phenolic compounds and the adsorption resin by thermal energy. A further increase in temperature (80 °C) led to a marked drop in the recovery (68.3%), probably due to partial heat-induced degradation of phenolic compounds.

Compared with organic solvent elution, NaOH desorption (**Figure 3b**) produced a different profile, with fractions El.6−10 containing significant amounts of polyphenols, whereas all other fractions revealed negligible total phenolic contents.

As has been deduced from the total phenolic determinations, alkaline elution with subsequent neutralization of the eluate to prevent polyphenol oxidation and degradation might be an alternative to the use of organic solvents. Interestingly, most phenolics were quantitatively recovered using NaOH concentrations as low as 0.5%, with fractions El.7−10 exhibiting highest concentrations (**Figure 4b**). Efficient prevention of phenolic degradation was possible by instant neutralization of the column effluent, as evidenced by the high retention of the phenolics. However, the phenolic profile was slightly changed upon alkaline elution. Whereas only 88.8% of 5-caffeoylquinic acid was eluted, the recovery rate of 4-caffeoylquinic acid amounted to 150.5%, presumably because of isomerization under these pH conditions (*37*). Furthermore, the recovery rate of the quercetin aglycone, present only in low amounts in the feed solutions, amounted to 148.6%, which may be attributed to partial hydrolysis of quercetin glycosides.

The present study demonstrates resin adsorption to be a valuable tool for the recovery and fractionation of phenolic compounds. The process parameters, such as pH, temperature,

flow rate, solute concentration, and type of eluent, were shown to affect the yield and phenolic profile of the purified and concentrated products. Temperature, flow rate, and solute concentration upon adsorption should be kept at an economically justifiable minimum to enhance the cost efficiency of the process. The pH value of the feed stream and the composition of the eluents determine whether the whole phenolic fraction is recovered or specific compounds are able to be selectively enriched. In general, fractionation of crude extracts according to the lipophilicity of the phenolics is possible, and the present study provides useful information to optimize such strategies. The fractionation affects the functional properties of the phenolic preparations, thus making it possible to obtain products with selected characteristics. However, more studies are required to characterize further resin adsorbents with regard to their potential to recover and fractionate phenolic compounds extracted from plant material.

ABBREVIATIONS USED

BV, bed volume; HPLC-DAD, high-performance liquid chromatography−diode array detection; LC-MS, liquid chromatography−mass spectrometry; TSS, total soluble solids.

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