ELSEVIER

Journal of Chromatography A, 910 (2001) 265–273

JOURNAL OF CHROMATOGRAPHY A

www.elsevier.com/locate/chroma

Determination of phenolic acids and flavonoids of apple and pear by high-performance liquid chromatography

Andreas Schieber*, Petra Keller, Reinhold Carle

Hohenheim University, Institute of Food Technology, Section Plant Foodstuff Technology, Garbenstrasse 25, D-70599 Stuttgart, Germany

Received 20 March 2000; received in revised form 29 November 2000; accepted 29 November 2000

Abstract

A new HPLC stationary phase has been applied to the analysis of phenolic acids and flavonoids with diode array and mass spectrometric detection. The separation of 26 standard compounds was achieved within 1 h. The stationary phase displayed excellent resolution especially of flavonol glycosides. The analytical system has been used for the determination of phenolic compounds in apple pomace and apple juice, and in extracts of pear fruits of different cultivars. Apple pomace was found to be a promising source of phenolics. However, yields are affected by the drying conditions applied. Furthermore, the applicability of the analytical system for the authenticity control of apple and pear juice was demonstrated by determination of characteristic quercetin and isorhamnetin glycosides, and dihydrochalcones, respectively. Since isorhamnetin-3-glucoside was present in all pear cultivars investigated, the usefulness of arbutin as a specific marker of pear products appears to be doubtful.

Keywords: Phenolic acids; Flavonoids

1. Introduction

The main phenolic compounds of apples are esters of caffeic and *p*-coumaric acids with quinic acid, flavanol monomers, di- and oligomers, quercetin glycosides, and dihydrochalcones [1–8]. In pear, the predominant phenolic constituents are chlorogenic, caffeic, *p*-coumaroyl quinic and *p*-coumaric acids, arbutin, and a number of procyanidins and flavonol glycosides [9–12]. According to epidemiological studies the intake of flavonoids is inversely correlated with the risk of coronary heart disease and cancer [13]. Polyphenols, as well as carotenoids and other secondary metabolites, have been demonstrated to act as antioxidants and are assumed to contribute to the beneficial health effects of fruits and vegetables. Apple pomace has recently been described as a source of polyphenols [14,15], and some phenolic constituents of apple pomace, especially the procyanidins and quercetin glycosides, have been shown to exert strong antioxidant activity in vitro [16].

Phenolic compounds have been widely recognized as indicators of adulteration of fruit juices and other products such as jams and puree [17–24]. However, there is still disagreement as to which polyphenols

*Corresponding author. Tel.: +49-711-459-3125; fax: +49-711-459-4110.

E-mail address: schieber@uni-hohenheim.de (A. Schieber).

0021-9673/01/$ – see front matter
PII: S0021-9673(00)01217-6

should be taken as a marker. While isorhamnetin-3-glucoside has been described to be indicative of pear [19,22], this flavonol has not been detected by others [24]. Recently, arbutin has been suggested as a marker to detect adulterations of apple juice with pear juice, however, isorhamnetin-3-glucoside has not been considered [25].

The diversity of apple phenolics is still an analytical challenge. Despite a great number of investigations, their separation and quantification proves difficult. Especially the simultaneous determination of quercetin glycosides together with benzoic and cinnamic acid derivatives and dihydrochalcones, represents a problem [5,7,26]. Furthermore, colorimetrical methods, e.g. Folin–Ciocalteu, do not allow the differentiation of individual compounds and tend to overestimate total phenol contents due to interference of reducing substances [27].

The main objective of our study was to establish an analytical system for the separation of phenolic acids and flavonoids of apple and pear. This method was applied to the characterization and quantification of phenolic constituents in apple juice and apple pomace, and in extracts of pear fruits, by HPLC and HPLC–MS. Furthermore, blends of apple juice with pear juice and vice versa were analyzed to evaluate the applicability of the system for authenticity control. The method presented in this paper is based on a stationary phase with polar endcapping newly available on the market.

2. Experimental

2.1. Reagents and solvents

Reagents and solvents were purchased from Merck (Darmstadt, Germany), and were of analytical or HPLC grade. Solid-phase extraction cartridges (Chromabond, 1 g) were obtained from Macherey-Nagel (Düren, Germany).

Flavonol glycosides, protocatechuic acid, catechin, epicatechin, caffeic acid, *p*-cumaric acid, ferulic acid, quercetin, arbutin, and phloretin were from Roth (Karlsruhe, Germany); chlorogenic acid was obtained from Sigma (St. Louis, MO, USA); 5-hydroxymethylfurfural and phloridzin were purchased from Fluka (Buchs, Switzerland); procyanidin B1 and procyanidin B2 were from Extrasynthese (Lyon, France). Quercetin-3-xyloside was obtained from Plantech (Reading, UK).

2.2. Specification of samples

Apple juices were produced by milling apples of a single cultivar (*Malus sylvestris* cv. Jonagold, and cv. Elstar, respectively) and pressing the mash obtained in a rack and cloth press. To remove coarse particles, the juice was centrifuged in a chamber separator. For pasteurization, the juice was heated at 85–90°C in a plate heat exchanger, the hot juice was bottled, sealed with steam injection, and cooled to ambient temperature. Commercial apple and pear juices and pear fruits (*Pyrus communis* cv. Alexander Lucas, Anjou and Red Williams) were purchased from the local market. The pomace obtained from apple juice production was either air dried (110°C) or lyophilized. Dried apple pomace was also supplied by a local fruit juice producer.

2.3. Instrumentation and chromatographic conditions

The separation of phenolic compounds was performed on a HP HPLC series 1100 (Hewlett Packard, Waldbronn, Germany) equipped with CHEMSTATION software, a degasser G1322A, a binary gradient pump G1312A, a thermoautosampler G1329/1330A, a column oven G1316A and a diode array detection system G1315A. The column used was an Aqua 5 μm C_{18} (250×4.6 mm I.D.) from Phenomenex (Torrance, CA, USA), and a security guard C_{18} ODS (4×3.0 mm I.D.). The column was 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% B to 55% B (50 min), 55% B to 100% B (10 min), 100% B to 10% B (5 min). The injection volume for all samples was 10 μl. Simultaneous monitoring was performed at 280 nm (catechins, proanthocyanidins and benzoic acids), 320 nm (hydroxycinnamic acids) and 370 nm (flavonols) at a flow-rate of 1 ml/min. Spectra were recorded from 200 to 600 nm.

HPLC mass spectrometry was carried out on a HP

HPLC series 1100 (Hewlett-Packard, Waldbronn, Germany) combined with a mass spectrometric detector Platform II (Micro Mass, Manchester, UK). Conditions: electrospray ionization (negative mode); source temperature 120°C; cone voltage ramp 10–50 V; acceleration lens potential 0.5 kV; m/z range 100–800; scan rate 0.4 scan/s, multiplier voltage 650 V.

2.4. Sample preparation

Pomace and pear fruits were extracted by stirring with aqueous acetone (70%, v/v) for 1 h at ambient temperature. After filtration, acetone was removed in vacuo (280 mbar, 30°C). For the determination of catechins and procyanidins, the aqueous solution was adjusted to pH 7.0 and extracted three times with ethyl acetate (50 ml each). The combined extracts were evaporated to dryness in vacuo. The residue was dissolved in methanol (10 ml), membrane filtered (0.2 μm) and used for HPLC. For the isolation of all other polyphenols, the aqueous phase was adjusted to pH 1.5 and extracted as described above.

Apple juice (50 ml) was adjusted to pH 7.0 and pH 1.5, respectively, and extracted three times with ethyl acetate (50 ml). The combined extracts were evaporated to dryness, and the residue was dissolved in 10 ml of water. The solution was passed through a C_{18} solid-phase extraction cartridge which had been preconditioned with methanol and 0.1% HCl, respectively. After washing with water, the analytes were eluted with methanol (5 ml) and used for chromatographic analyses. Alternatively, the combined ethyl

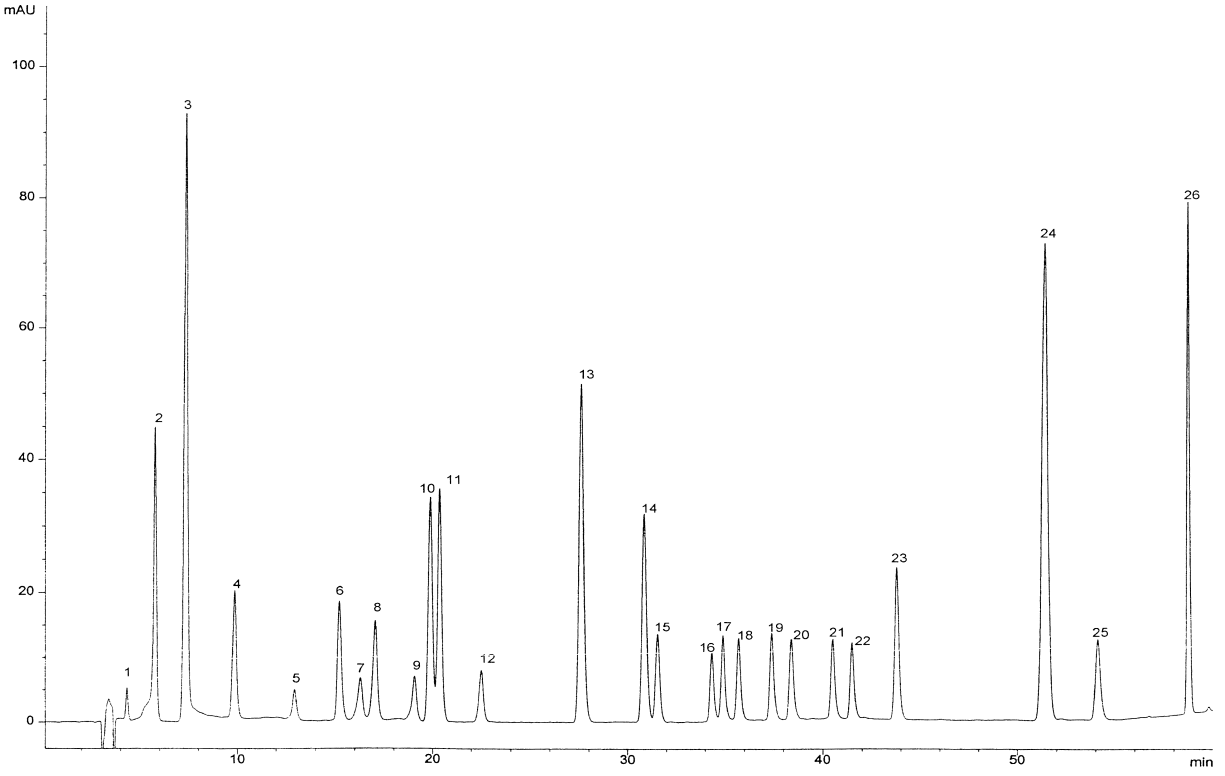

Fig. 1. Separation of a standard mixture of phenolic compounds (and 5-hydroxymethyl furfural and cinnamic acid) by RP-HPLC (280 nm). 1 = Arbutin, 2 = gallic acid, 3 = 5-hydroxymethyl furfural, 4 = protocatechuic acid, 5 = procyanidin B1, 6 = 4-hydroxybenzoic acid, 7 = catechin, 8 = chlorogenic acid, 9 = procyanidin B2, 10 = caffeic acid, 11 = syringic acid, 12 = epicatechin, 13 = *p*-coumaric acid, 14 = ferulic acid, 15 = sinapic acid, 16 = quercetin-3-rutinoside, 17 = quercetin-3-galactoside, 18 = quercetin-3-glucoside, 19 = quercetin-3-xyloside, 20 = quercetin-3-arabinoside, 21 = quercetin-3-rhamnoside, 22 = isorhamnetin-3-glucoside, 23 = phloridzin, 24 = cinnamic acid, 25 = quercetin, 26 = phloretin.

acetate extracts were dried over sodium sulfate without using solid-phase extraction cartridges.

3. Results and discussion

3.1. Methodology

In this study a stationary phase newly available on the market was used for the determination of polyphenols in apple juice and pomace, in pear extracts, and in mixtures of apple and pear juice. The column was specifically developed for the separation of very polar analytes which are usually not sufficiently retained on common reversed phases. To the best of our knowledge, the application of this stationary phase to the analysis of phenolic compounds has not yet been described. In comparison to stationary phases hitherto used in our laboratory and by other research groups [28,29], improved resolution especially of the quercetin glycosides is achieved.

The separation of a standard mixture of 26 phenolic compounds and 5-HMF is shown in Fig. 1. As can be seen, baseline separation was achieved for virtually all components including hydroxybenzoic and hydroxycinnamic acids, flavanol monomers and dimers, and the dihydrochalcone derivative phloridzin. Remarkably, the present method allows the separation of six quercetin glycosides and of isorhamnetin-3-glucoside. The elution order of the quercetin glycosides investigated is 3-rutinoside, 3-galactoside, 3-glucoside, 3-xyloside, 3-arabinoside and 3-rhamnoside. This is in agreement with previous studies using conventional reversed-phase systems [26].

ESI mass spectra were recorded in the negative ion mode. Due to the ionization conditions applied, $[M-H]^-$ ions were obtained, and only minimum fragmentation was observed. Characteristic ions were used for peak assignment.

Pomace samples were extracted with 70% aqueous acetone as described previously [14]. For further purification of the extract, the aqueous phase was separated against ethyl acetate at pH 7.0 and pH 1.5. This fractionation was required since catechin, epicatechin, and the procyanidins are not detectable following acidic extraction.

Juice samples were also extracted with ethyl acetate at pH 7.0 and pH 1.5 according to a modified method of Delage et al. [30]. HPLC analysis without

Table 1
Contents (mg/kg dry matter) of phenolic compounds isolated from apple pomace samples[a]

Compound	Commercial pomace	Apple pomace cv. Jonagold		Apple pomace cv. Elstar	
		Air dried (110°C)	Lyophilized	Air dried (110°C)	Lyophilized
5-HMF	220	27	–	21	–
Procyanidin B1	Trace	–	–	–	–
Catechin	14	7.8	9.4	12	14
p-Coumaroyl glucose[b]	10	–	–	–	–
Chlorogenic acid	450	79	54	40	33
p-Coumaroyl quinic acid[b]	34	6.2	4.7	9.0	7.9
Procyanidin B2	47	120	160	93	130
Caffeic acid	8.2	–	–	–	–
Epicatechin	77	160	190	140	160
p-Coumaric acid	3.0	–	–	–	–
Quercetin-3-galactoside	360	200	210	260	280
Quercetin-3-glucoside	130	31	31	75	81
Quercetin-3-xyloside	160	99	100	76	93
Quercetin-3-rhamnoside	230	350	340	110	120
Phloridzin	910	390	350	400	390
Quercetin	67	17	19	23	3.5
Phloretin	Trace	0.7	1.0	1.4	1.1

[a] Sample preparation and HPLC determination were performed in duplicate; –, not detectable.
[b] Tentatively identified; calculated as coumaric acid.

further purification resulted in some peak impurities. Since the accurate quantification, especially of the flavonol glycosides, was impossible, solid-phase extraction had to be applied. As an alternative, the ethyl acetate extract was dried over sodium sulfate. The results obtained by both procedures were comparable, however, drying with sodium sulfate proved to be a faster and cheaper method.

3.2. Apple pomace

As shown in Table 1 and in Fig. 2, the predominant phenolic constituents in the commercial apple pomace were phloridzin and chlorogenic acid, followed by quercetin glycosides. Consistent with the results described by Lu and Foo [14], the predominant flavonol glycoside was quercetin-3-galactoside. In contrast to a previous study where high quantities of quercetin-3-arabinoside were reported [14], we only found trace amounts. Interestingly, a compound displaying the characteristic UV spectrum of a quercetin glycoside was detected. Mass spectrometric characterization revealed a m/z ratio of 433, corresponding to a quercetin pentoside. However, it could not be assigned to one of the reference substances available. It is unclear whether this pentoside is an artefact resulting from the drying procedure, or whether it is split off the cell wall by thermal treatment.

Minor compounds found in apple pomace were

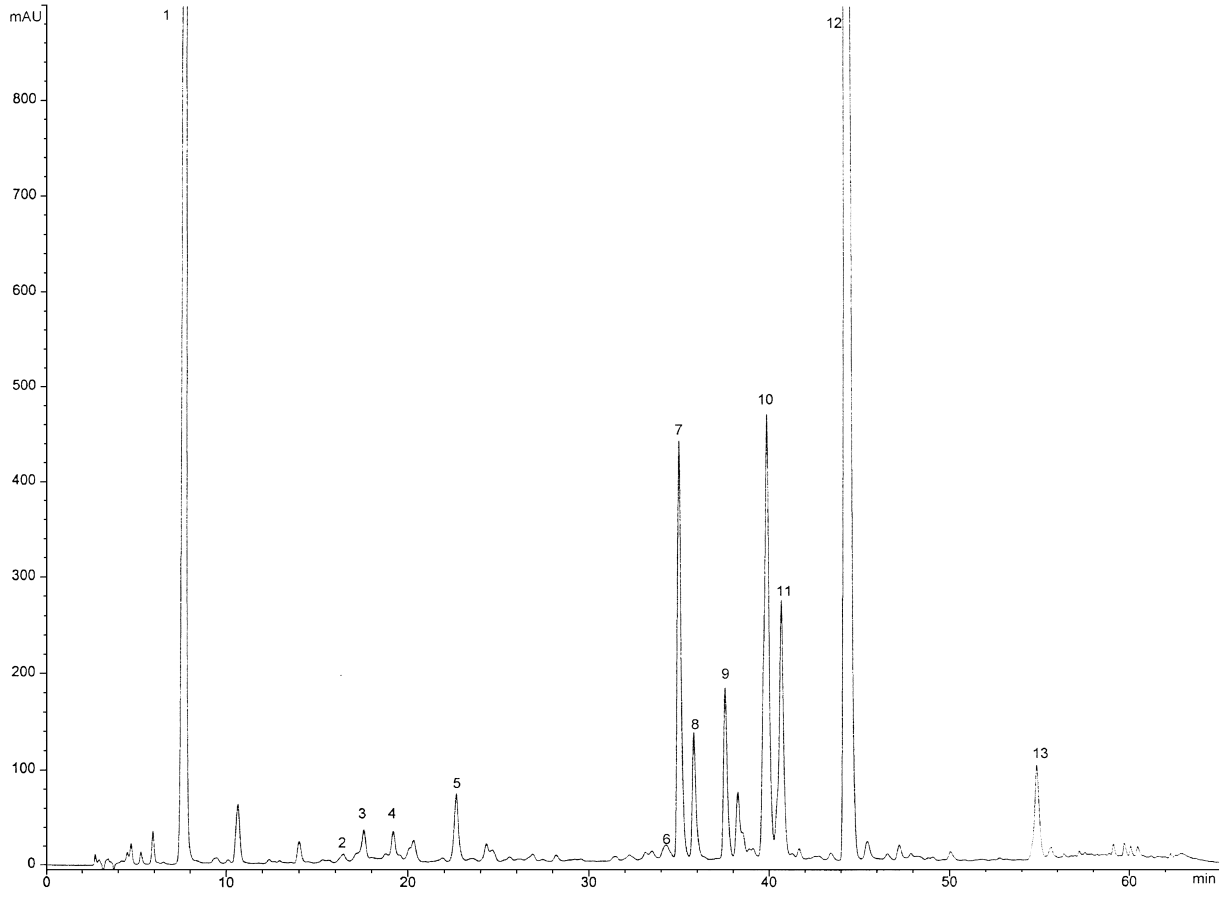

Fig. 2. HPLC of phenolic compounds (280 nm) isolated from a commercial apple pomace sample (neutral fraction). 1=5-HMF, 2=catechin, 3=tentatively identified as *p*-coumaroyl glucose 4=procyanidin B2, 5=epicatechin, 6=quercetin-3-rutinoside, 7=quercetin-3-galactoside, 8=quercetin-3-glucoside, 9=quercetin-3-xyloside, 10=m/z=433, 11=quercetin-3-rhamnoside, 12=phloridzin, 13=quercetin.

catechin and epicatechin, *p*-coumaric and caffeic acids and quercetin. Whereas several procyanidins from apple pomace were characterized by NMR spectroscopy and mass spectrometry [15], in our samples only procyanidin B2 was present in significant amounts. Traces of procyanidin B1 and B5 were found in apple pomace and juice by mass spectrometry, but quantification of procyanidin B5 was impossible because of coelution with quercetin-3-rutinoside.

Total amounts of phenolics are substantially lower than described elsewhere [14]. This may be due to differences in the raw material used, i.e. different apple cultivars, or to the analytical methodology applied. A more likely explanation is that commercial pomace used in our study was subjected to harsh drying conditions, leading to partial degradation of phenolics, whereas freeze-dried apple pomace was used by others [14]. This is also reflected by the relatively high content of 5-hydroxymethylfurfural. Drying temperatures exceeding 100°C led to a significant reduction of extractable polyphenols and condensed tannins, and to a decrease of the antioxidant activity of grape pomace; however, individual polyphenols were not determined [31].

Therefore, the effects of drying conditions on the phenolic constitutents were investigated using fresh pomace of two different cultivars (Jonagold, Elstar). An aliquot of pomace was air dried at 110°C, another portion was subjected to lyophilization. The results are also presented in Table 1. As can be seen, 5-HMF was not detected in lyophilized samples. In most cases, amounts of phenolic compounds were higher in extracts of lyophilized apple pomace, although differences were not as pronounced as expected. Obviously, air drying at 110°C is still a relatively gentle method compared to the procedures used on an industrial scale. The results show that apple pomace is a rich source of polyphenols. However, there may be substantial differences in amounts, depending on the raw material used and the treatment of the pomace prior to extraction.

3.3. Apple juice and pear extracts

As expected, chlorogenic acid, phloridzin, and epicatechin were the main phenolic compounds in apple juice (Table 2). However, depending on the raw material, significant differences in the total amounts of phenolics are evident. When we investigated a number of commercial pear juices and nectars for their polyphenol pattern, phloridzin and

Table 2
Contents (mg/l) of phenolic compounds of apple juice samples[a]

Compound	Commercial juice	Apple juice cv. Jonagold	Apple juice cv. Elstar
5-HMF	0.9	–	–
Procyanidin B1	2.3	–	–
Catechin	3.5	–	2.8
p-Coumaroyl glucose[b]	1.7	–	–
Chlorogenic acid	130	32	10
p-Coumaroylquinic acid[b]	23	3.8	5.0
Procyanidin B2	9.1	0.6	3.2
Caffeic acid	5.1	–	–
Epicatechin	12	5.4	15
p-Coumaric acid	0.6	–	–
Quercetin-3-galactoside	1.5	0.7	1.0
Quercetin-3-glucoside	1.0	0.3	0.6
Quercetin-3-xyloside	0.9	0.4	0.1
Quercetin-3-rhamnoside	3.4	3.5	1.1
Phloretin-2′-xyloglucoside[c]	11	1.4	1.6
Phloridzin	24	4.9	5.9
Quercetin	1.2	–	–

[a] Sample preparation and HPLC determination were performed in duplicate; –, not detectable.
[b] Tentatively identified; calculated as coumaric acid.
[c] Tentatively identified; calculated as phloridzin.

phloretin xyloglucoside were found in most samples, indicating the presence of apple juice. This is in agreement with a previous study where the admixture of apple was also suspected [22]. To obtain an authentic phenolic profile, pear fruits of three different cultivars (Alexander Lucas, Anjou and Red Williams) were extracted as described above. A typical HPLC separation of pear flavonol glycosides is shown in Fig. 3. As can be seen, seven compounds were detected at a wavelength of 370 nm, three of which were quercetin glycosides (3-rutinoside, 3-galactoside and 3-glucoside). Isorhamnetin-3-glucoside could be easily identified by comparison with the standard. Further isorhamnetin glycosides could be tentatively identified as isorhamnetin-3-rhamnogalactoside and 3-rutinoside, respectively, by their UV spectral and mass spectrometric data, and by comparison with literature data [9–12].

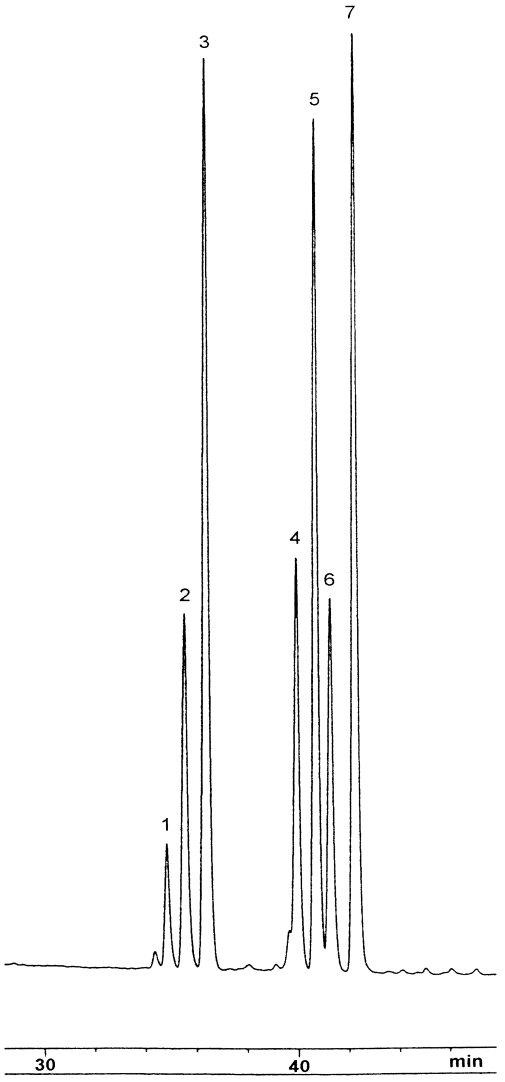

Fig. 3. Separation of flavonol glycosides isolated from pear fruit cv. Alexander Lucas (370 nm). 1=Quercetin-3-rutinoside, 2=quercetin-3-galactoside, 3=quercetin-3-glucoside, 4=m/z=623, 5=m/z=623, 6=m/z=477, isorhamnetin-3-galactoside, 7=isorhamnetin-3-glucoside; 4, 5 and 6 were tentatively identified as isorhamnetin-3-rhamnogalactoside, isorhamnetin-3-rhamnoglucoside and isorhamnetin-3-galactoside.

Arbutin, which has been described as a marker of pear juice in apple juice [25], showed a very low retention in the chromatographic system used in our study (Fig. 1) and in the system employed by others [24]. Therefore, interpretation of analytical results may prove difficult. In addition, arbutin could only be detected in very low amounts whereas contents of isorhamnetin-3-glucoside were considerably higher in all cultivars investigated (Table 3). With our method the detection of pear in apple products can be easily performed by determination of the flavonol glycoside pattern. To demonstrate the suitability of the analytical system, apple juice and pear juice were blended at a ratio of 90:10 and 10:90 (v/v), respectively, and analyzed for their flavonol glycosides. The results are presented in Fig. 4. Isorhamnetin-3-glucoside is well separated from the quercetin glycosides and phloretin glycosides at a column temperature of 25°C, whereas others had to raise the column temperature and increase the concentration of acetic acid to improve resolution of the respective phenolic compounds [19]. On the other hand, the method is also suitable for the detection of apple juice in pear juice by using the dihydrochalcone derivatives phloridzin and phloretin xyloglucoside as characteristic markers (chromatogram not shown).

4. Conclusion

The stationary phase used in this study displays excellent separation of the phenolic constituents isolated from apple juice and pomace, respectively. Remarkably, all quercetin glycosides are baseline separated and do not interfere with phloretin glycosides. It may also be applied to the determination of

Table 3
Contents (mg/kg fresh weight) of phenolic compounds in pear fruit extracts of three different cultivars[a]

Compound	cv. Alexander Lucas	cv. Anjou	cv. Red Williams
Arbutin	1.0	Trace	0.4
Chlorogenic acid	15	17	21
Epicatechin	2.0	1.6	6.1
Quercetin-3-rutinoside	0.5	0.6	0.2
Quercetin-3-galactoside	1.1	Trace	1.0
Quercetin-3-glucoside	3.5	0.6	2.1
Isorhamnetin-3-glucoside	3.6	0.9	9.1

[a] Sample preparation and HPLC determination were performed in duplicate.

phenolics in other fruits or fruit products, as exemplified for pear. The chromatographic system may be employed either to characterize the genuine phenolic compounds (fingerprint), to investigate the influence of technological treatment on phenolic constituents of fruits and vegetables, or for authenticity control. As volatile eluents were used, coupling to a mass spectrometer is possible without changing chromatographic conditions.

Since arbutin could only be quantified in two of the three pear cultivars studied, its significance as a possible marker for pear is under question. Extension of the studies to further cultivars should clarify whether isorhamnetin-3-glucoside is a more suitable indicator for the detection of pear admixtures in apple products.

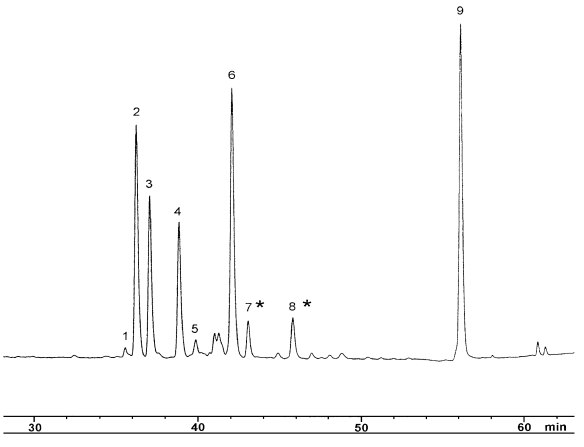

Fig. 4. Detection of flavonol glycosides (370 nm) in a mixture of apple juice and pear juice (90:10, v/v). 1=Quercetin-3-rutinoside, 2=quercetin-3-galactoside, 3=quercetin-3-glucoside, 4=quercetin-3-xyloside, 5=quercetin-3-arabinoside, 6=quercetin-3-rhamnoside, 7=isorhamnetin-3-glucoside (indicator of pear), 8=phloridzin (indicator of apple), 9=quercetin.

Apple pomace represents a promising source of phenolic constituents, especially of chlorogenic acid, phloridzin and quercetin glycosides, which may be used as natural antioxidants or as ingredients of functional foods. Since harsh drying conditions have been shown to decrease yields, investigations of the effects of different time–temperature regimes on extractable polyphenols have been commenced.

Acknowledgements

The present work was supported by the Federal Department of Education and Research (0339820). The authors wish to thank Dr. W. Armbruster and Dr. M. Lederer (Institute of Food Chemistry, Hohenheim University) for performing mass spectrometric analyses and Mrs G. Arnold for technical assistance.

References

[1] G.A. Spanos, R.E. Wrolstad, D.A. Heatherbell, J. Agric. Food Chem. 38 (1990) 1572.
[2] J. Perez-Ilzarbe, T. Hernandez, I. Estrella, Z. Lebensm. Unters. Forsch. 192 (1991) 551.
[3] F.A. Tomas-Barberan, C. Garcia-Viguera, J.L. Nieto, F. Ferreres, F. Tomas-Lorente, Food Chem. 46 (1993) 33.
[4] B. Suarez Vallez, J. Santamaria Victorero, J.J. Mangas Alonso, D. Blanco Gomis, J. Agric. Food Chem. 42 (1994) 2732.
[5] B. Suarez, A. Picinelli, J.J. Mangas, J. Chromatogr. A 727 (1996) 203.

[6] T. Hernandez, N. Ausin, B. Bartolome, L. Bengoechea, I. Estrella, C. Gomez-Cordoves, Z. Lebensm. Unters. Forsch. A 204 (1997) 151.
[7] A. Escarpa, M.C. Gonzalez, J. Chromatogr. A 823 (1998) 331.
[8] K.R. Price, T. Prosser, A.M.F. Richetin, M.J.C. Rhodes, Food Chem. 66 (1999) 489.
[9] B.K. Nortje, B.H. Koeppen, Biochem. J. 97 (1965) 209.
[10] M.B. Duggan, J. Agric. Food Chem. 17 (1969) 1098.
[11] W. Oleszek, M.J. Amiot, S.Y. Aubert, J. Agric. Food Chem 42 (1994) 1261.
[12] G.A. Spanos, R.E. Wrolstad, J. Agric. Food Chem. 38 (1990) 817.
[13] M.G.L. Hertog, E.J.M. Feskens, P.C.H. Hollman, M.B. Katan, D. Kromhout, Lancet 342 (1993) 1007.
[14] Y. Lu, L.Y. Foo, Food Chem. 59 (1997) 187.
[15] L.Y. Foo, Y. Lu, Food Chem. 64 (1999) 511.
[16] Y. Lu, L.Y. Foo, Food Chem. 68 (2000) 81.
[17] F. Siewek, R. Galensa, K. Herrmann, Z. Lebensm. Unters. Forsch. 179 (1984) 315.
[18] R.L. Rousseff, J. Assoc. Off. Anal. Chem. 71 (1988) 798.
[19] B. Wald, R. Galensa, Z. Lebensm. Unters. Forsch. 188 (1989) 107.
[20] E. Hollborn, B. Wald, R. Galensa, K. Herrmann, Dtsch. Lebensm. Rdsch. 86 (1990) 1.
[21] U. Mareck-Engelke, R. Galensa, K. Herrmann, Flüss. Obst. 58 (1991) 659.
[22] B. Fernandez de Simon, J. Perez-Ilzarbe, T. Hernandez, C. Gomez-Cordoves, I. Estrella, J. Agric. Food Chem. 40 (1992) 1531.
[23] F. Tomas-Lorente, C. Garcia-Viguera, F. Ferreres, F.A. Tomas-Barberan, J. Agric. Food Chem. 40 (1992) 1800.
[24] P.B. Andrade, A.R.F. Carvalho, R.M. Seabra, M.A. Ferreira, J. Agric. Food Chem. 46 (1998) 968.
[25] H.-J. Hofsommer, S. Koswig, Fruit Process. 9 (1999) 471.
[26] W. Oleszek, C.Y. Lee, A.W. Jaworski, K.R. Price, J. Agric. Food Chem. 36 (1988) 430.
[27] K. Herrmann, Flüss. Obst 60 (1993) 7.
[28] A. Rechner, C.-D. Patz, H. Dietrich, Dtsch. Lebensm. Rdsch. 94 (1998) 363.
[29] A. Schieber, W. Ullrich, R. Carle, Inn. Food Sci. Emerg. Technol. 1 (2000) 161.
[30] E. Delage, G. Bohuon, A. Baron, J.-F. Drilleau, J. Chromatogr. 555 (1991) 125.
[31] J.A. Larrauri, P. Ruperez, F. Saura-Calixto, J. Agric. Food Chem. 45 (1997) 1390.