A new process for the combined recovery of pectin and phenolic compounds from apple pomace☆

Andreas Schieber[a,*], Petra Hilt[a], Petra Streker[a], Hans-Ulrich Endreß[b], Christine Rentschler[b], Reinhold Carle[a]

[a]*Hohenheim University, Institute of Food Technology, Section Plant Foodstuff Technology, Garbenstrasse 25, D-70599 Stuttgart, Germany*
[b]*Herbstreith & Fox KG, Pektinfabrik Neuenbürg, Turnstrasse 37, D-75305, Neuenbürg, Germany*

Abstract

A process for the combined recovery of pectin and phenolic compounds from apple pomace, the primary by-product of apple juice production, was developed. The process includes extraction of dried apple pomace with diluted mineral acid and adsorption of phenolic constituents by a hydrophobic styrene–divinylbenzene copolymerisate. After elution with methanol, the polyphenolics were concentrated in vacuo, stabilised by lyophilisation, and characterised by high-performance liquid chromatography. The predominant compounds were phloridzin, chlorogenic acid and quercetin glycosides. Adsorptive removal especially of oxidised phenolic compounds led to a considerable decolourisation of the pomace extracts, as revealed by determination of $L*a*b*$ values, hue angle and chroma. Gelling properties of pectin were not adversely affected. While the polyphenolics recovered from apple pomace may be used as natural antioxidants or as functional food ingredients, extended fields of application may be obtained for decolorised, refined apple pectins. Furthermore, investigations on the phenolic composition of several New Zealand apple cultivars, of apple seeds, and on the effects of pomace drying on the stability of polyphenolics were carried out.
© 2002 Elsevier Science Ltd. All rights reserved.

Keywords: Apple pomace; Pectin; Phenolic Acids; Flavonoids; Adsorption

Industrial relevance: Apple pomace is the primary by-product of apple juice production. Its use for further product recovery is an appropriate measure for economic reasons as well as from an environmental point of view. Apple pomace is being used successfully for pectin production and recent work regards it as rich source of polyphenols. Therefore, the main objective of this study was to establish a combined recovery process for pectins and polyphenols. A successful recovery process has been developed which can easily be integrated into existing pectin production processes. Scale-up has already been initiated. Further, purification of the phenolic fraction can lead to valuable natural antioxidants and antimicrobial food ingredients.

1. Introduction

Apple juice is the most popular fruit juice in Germany with a per capita consumption of approximately 12 l annually (VdF, 2000). Approximately 700 000 t of apples are produced into juice, resulting in 250 000 t per year of wet apple pomace as the primary by-product (Endreß, 2000). The numerous ways of utilising apple pomace have recently been reviewed by Kennedy et al. (1999). According to these authors, the uses of apple pomace can be classified either as a waste reduction strategy, or obtaining a high value product, or preferably both. The production of pectin is considered the most reasonable way of utilisation both from an economic and from an ecological point of view (Fox, Asmussen, Fischer & Endreß, 1991; Endreß, 2000). Pectins are used in foods as a gelling agent, thickener, texturiser, emulsifier and stabiliser (Thakur, Singh & Handa, 1997). Furthermore, non-food applications were also reported (Endreß, 1991).

Wet apple pomace consists of approximately 10–15% pectin on a dry weight basis (Endreß, 2000). For the recovery of pectin, dried apple pomace is usually extracted with diluted mineral acids at elevated temperatures to solubilise the protopectin localised in the cell wall and middle lamella, respectively. After concentration, the pectin is precipitated by addition of alcohol, dried, ground and finally sieved (May, 1990). In many applications, apple pectins are characterised by superior gelling properties in comparison to citrus pectins. However, their brown hue may lead to limitations with respect to their use in very light-coloured products. The colour of apple pomace and of the pectins recovered is caused by oxidation of phenolic compounds, which are co-extracted from the pomace and only partially removed by the precipitation step. Approaches at bleaching apple pomace with alkaline peroxide resulted in loss of the polyphenolics and in degradation of the major part of the pectins, and efficient bleaching could only be obtained at the cost of low yields (Renard et al., 1996).

Recently, apple pomace including seeds has been demonstrated to be a rich source of polyphenolics (Lu & Foo, 1997, 1998; Foo & Lu, 1999; Schieber, Keller & Carle, 2001), and some phenolic constituents, especially the procyanidins and quercetin glycosides, have been shown to exert strong antioxidant activity in vitro (Lu & Foo, 2000). Furthermore, tumour-cell proliferation has been strongly inhibited in vitro by apple extracts, and these effects have been attributed to phenolic acids and flavonoids (Eberhardt, Lee & Liu, 2000). Thus, polyphenolics from apple pomace are considered highly valuable compounds which may be used as functional food ingredients and as natural antioxidants to replace their synthetic equivalents which have experienced growing rejection. The studies reported so far on the recovery of phenolic compounds were restricted to laboratory scale and did not provide a perspective for a complete utilisation of apple pomace.

Therefore, the main objective of the present study was to establish a process for the combined recovery of pectin and polyphenolics. Furthermore, it should be investigated whether colour characteristics of apple pectins may be improved by removal of phenolic compounds. Since quantitative data on apple seed phenolics are scarce and reports on the phenolic profile of seeds are contradictory (Lu & Foo, 1998; Awad, de Jager & van Westing, 2000), investigations on apple seeds as a potential source of polyphenolics were also included.

2. Materials and methods

2.1. Sources of apple pomace extracts and apple seeds

Acidic pomace extracts (pH 2.8; 3.1 °Bx) and dried apple seeds were provided by Herbstreith & Fox KG

Pektinfabrik Neuenbürg (Neuenbürg, Germany). The extracts were immediately frozen at −20 °C until use.

2.2. Chemicals and solvents

All reagents and solvents used were obtained from Merck (Darmstadt, Germany) and were of HPLC or analytical grade. Sources of reference compounds: Flavonol glycosides, protocatechuic acid, p-hydroxybenzoic acid, caffeic acid, p-coumaric acid, ferulic acid, phloretin, catechin and epicatechin (Roth, Karlsruhe, Germany); chlorogenic acid (Sigma, St. Louis, MO, USA); flavonols, procyanidin B1 and procyanidin B2 (Extrasynthese, Lyon, France); phloridzin (Fluka, Buchs, Switzerland); quercetin 3-xyloside and quercetin 3-arabinoside (Plantech, Reading, UK).

2.3. Adsorption of phenolic compounds

Amberlite XAD 16HP (Rohm & Haas, Frankfurt a.M., Germany) was used for the adsorptive removal of phenolic compounds. Amounts of 1.77 l of the resin were filled into a glass column XK 50/100 (90×5 cm i.d., Amersham Pharmacia Biotech, Uppsala, Sweden) and washed with five volumes of distilled water to remove impurities using a Mohno pump (Netzsch, Waldkraiburg, Germany). Since preliminary studies had shown that adsorption of polyphenolics was most efficient at elevated temperatures (data not given), the acidic pomace extract was preheated at 60 °C in a water bath MP-19 (Julabo, Seelbach, Germany) and then applied to the column at a flow rate of approximately 10 bed volumes per hour. For in-line control of the adsorption capacity, the extinction of the eluate was monitored spectrophotometrically at 420 nm using a Lambda 20 spectrophotometer (Perkin Elmer, Dreieich, Germany). The pectin-containing effluent was collected, and residues of pectin were removed from the column with distilled water until no pectin could be detected by alcohol precipitation. Phenolic compounds were eluted with 2 bed volumes of methanol. The solvent was evaporated in vacuo, and residual water was removed by lyophilisation (Lyvac GT2, Finn-Aqua, Munich, Germany) at 0.075 mbar for 80 h. The lyophilisate was triturated and stored in amber glass bottles in a desiccator. The complete process is shown in Fig. 1.

2.4. Characterisation of the lyophilisate

2.4.1. Determination and quantification of phenolic compounds

Twenty milligrams of the lyophilisate were suspended in 5 ml of methanol, sonicated and finally membrane-filtered (0.45 μm). The separation of phenolic compounds was performed on an HPLC series 1100 (Hewlett Packard, Waldbronn, Germany) equipped with
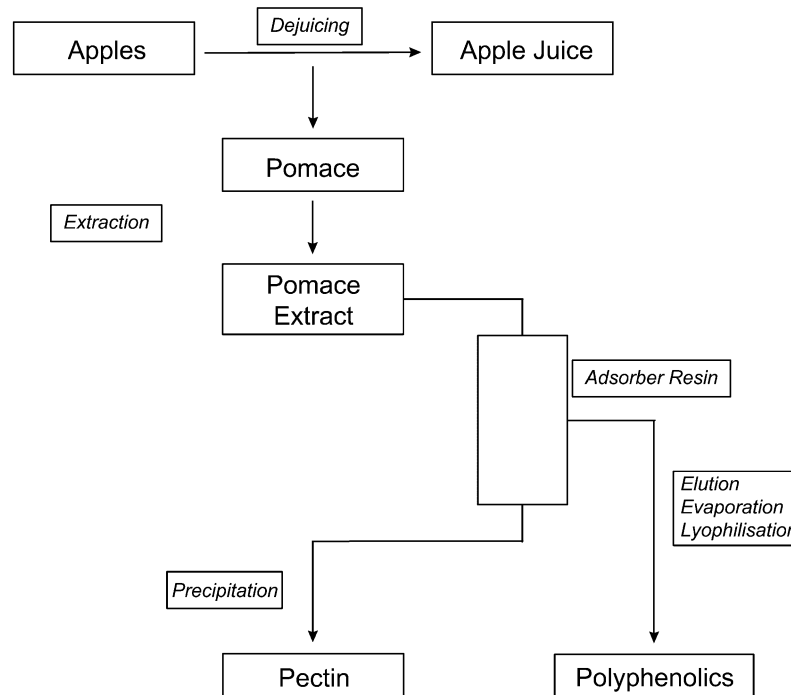
Fig. 1. Process for the combined recovery of pectin and phenolic compounds from apple pomace.

ChemStation software, a degasser G1322A, a binary gradient pump G1312A, a thermoautosampler G1329/1330A, a column oven G1316A and a diode array detector G1315A. The column used was an Aqua 5 μm C18, 125 Ü (250×4.6 mm i.d.) from Phenomenex (Torrance, CA, USA), and a security guard C18 ODS (4.0×3.0 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: 10–55% B (50 min), 55–100% B (10 min), 100–10% B (5 min). The injection volume for all samples was 10 μl. Simultaneous monitoring was performed at 280 nm for catechins, proanthocyanidins, dihydrochalcones and benzoic acids, at 320 nm for hydroxycinnamic acids, and at 370

nm for flavonols at a flow rate of 1 ml/min. Spectra were recorded from 200–600 nm.

2.4.2. Determination of neutral saccharides and galacturonic acid

After dissolving 500 mg of the lyophilisate in 10 ml of water, the solution was adjusted to pH 2 by addition of HCl. Phenolic compounds were removed by extraction with ethyl acetate (3×15 ml). After phase separation, the organic layer was discarded, and the aqueous solution was concentrated in vacuo to a volume of 10 ml. Aliquots of 3 ml were used for acid hydrolysis which was performed after addition of 400 μl of HCl (37%) at 100 °C for 1 h. For purification of the hydrolysate, Polyamide MN SC6, particle size 0.05–0.16 mm (Macherey Nagel, Düren, Germany), was filled into a glass column (volume 14 ml) and conditioned with 30 ml of methanol/conc. aqueous ammonia (99.5:0.5, v/v), 60 ml of distilled water, 30 ml of water/glacial acetic acid (99.8:0.2, v/v), and 60 ml of distilled water (Wald & Galensa, 1989). The hydrolysate was adjusted to pH 3 and applied to the resin. Elution was performed with 25 ml of water. The effluent was collected and evaporated to dryness. The residue was dissolved in water and used for further analyses.

The chromatographic determination of sugars was performed on a Waters Alliance 2690 Separations Module (Waters, Milford, MA, USA) equipped with an RI Detector 2410 (Waters) which was operated at 30 °C.

Table 1
$L*a*b*$ values, chroma and hue angle of pomace extracts before (−) and after (+) adsorptive removal of phenolic compounds

Sample	Adsorption	L^*	a^*	b^*	Chroma	Hue Angle
1	−	61.4	5.7	27.9	28.5	78.5°
	+	65.0	2.7	16.0	16.2	80.4°
2	−	55.5	6.6	30.4	31.1	77.8°
	+	65.7	2.6	16.9	17.1	81.3°
3	−	69.6	5.6	32.0	32.5	80.1°
	+	79.8	1.7	14.6	14.7	83.4°

The column used was a Polyamin II, 250×4.6 mm i.d. (YMC Europe, Schermbeck, Germany) with a guard column (10×4 mm i.d.) of the same material. The sugars were eluted isocratically using acetonitrile–water (75:25, v/v) at a flow rate of 1 ml/min. The column temperature was 25 °C (YMC Application Note).

Free galacturonic acid was determined by high-performance anion exchange chromatography using the same chromatographic system as described for neutral saccharides except for the stationary phase which was a Nucleosil 100–10 SB (250×4 mm i.d.), particle size 10 μm (Macherey-Nagel, Düren, Germany) and a guard column (10×4 mm i.d.) of the same material. 20 mg of the lyophilisate were suspended in 4 ml of 0.3 M ammonium formate buffer (pH 5.4) and membrane-filtered. After injection of 50 μl of the sample, isocratic elution was performed at 1 ml/min.

2.4.3. Protein

Total nitrogen was determined by the Kjeldahl method (Büchi, Konstanz, Germany) using 1.2 g of the lyophilisate. Crude protein was calculated by multiplication of the total nitrogen content by 6.25.

2.4.4. Lipophilic compounds

Lipophilic compounds were determined gravimetrically after Soxhlet extraction of 2.5 g of the lyophilisate with *n*-hexane for 5 h.

2.5. Extraction and purification of polyphenolics from apple seeds

Apple seeds were ground in a laboratory sieving machine model ZM1 (Retsch, Haan, Germany), mesh size 0.2 mm. Amounts of 20 g were extracted with acetone/water (70:30, v/v) for 1 h. After centrifugation (4000 rpm, 15 min), acetone was evaporated in vacuo, and the aqueous solution was sequentially extracted at pH 1.5 and pH 7 with ethyl acetate (3×50 ml) as described previously (Schieber et al., 2001). The combined extracts were dried over sodium sulfate. The organic solvent was evaporated to dryness, and the residue was dissolved in methanol for HPLC analysis.

2.6. Extraction and purification of polyphenolics from apples

Polyphenolics from apple fruits (cvs. 'Pacific Rose', 'Sunrise', 'Braeburn', 'Royal Gala') obtained from New Zealand were extracted and purified as described previously (Schieber et al., 2001).

2.7. Colour measurement

Colour parameters (*L*a*b**) of three pomace extract samples before and after adsorptive removal of phenolic compounds were recorded on a photometer Lambda 20 equipped with an integration sphere RSA-PE-20 and WinCol software (all from Perkin Elmer, Dreieich, Germany). The samples were placed into a 1 cm cell (Ratiolab, Dreieich, Germany), and *L*a*b** values were determined using Illuminant D65 and 10 ° observer angle. Chroma (*C*) and hue angle (H) were calculated as

$$C = \sqrt{a^{*2} + b^{*2}}; \ \ H = \arctan\left(\frac{b^*}{a^*}\right)$$

2.8. Industrial drying of apple pomace

Wet apple pomace (25–30% dry matter) of industrial juice production (Streker, Aspach, Germany) was dried in a three-stage drum dryer (Swiss-Combi, Switzerland) within 5–8 min by hot air (300–700 °C). The temperature of the pomace did not exceed 50–60 °C during the drying process. An aliquot of the same lot was collected prior to industrial drying and stored frozen at −20 °C. For the determination of polyphenolics, amounts of 50 g of dry pomace were weighed into a beaker. To inhibit polyphenol oxidation, 100 ml of a solution of ascorbic acid (10 g/l) and sodium chloride (0.5 g/l) was added (Pizzocaro, Torreggiani & Gilardi, 1993). After stirring, the sample was allowed to stand for 10 min. Extraction and purification of polyphenolics were carried out as described previously (Schieber et al., 2001).

3. Results and discussion

Apple polyphenolics are mainly localised in the peel (Teuber & Herrmann, 1978; Dick, Redden, DeMarco, Lidster & Grindley, 1987; Lommen, Godejohann, Venema, Hollman & Spraul, 2000) and in the seeds (Lu & Foo, 1998; Awad et al., 2000) of apple fruits. Due to the low extraction yields during juice production (Price, Prosser, Richetin & Rhodes, 1999), most of the phenolics are retained in the pomace and contribute to its brown colour after oxidation. Consequently, the pectin recovered from apple pomace is also adversely affected.

Recently, pectolytic and cellulolytic enzymes have been used for apple pomace liquefaction (Will, Bauckhage & Dietrich, 2000). The resulting juices contained significantly higher contents of polyphenolics, especially phloridzin, phloretin xyloglucoside and quercetin glycosides, than control samples produced by mash maceration with pectolytic enzymes. However, in the European Union the use of cellulolytic enzymes for fruit juice production is still prohibited by law, and elevated levels of polyphenolics may lead to astringency and even bitterness (Haug & Gierschner, 1979). Furthermore, apple pomace resulting from mash enzyming

Table 2
Composition of the lyophilisate recovered from apple pomace

Compound	%
Neutral saccharides	60.0
Arabinose	53.1
Glucose	4.9
Rhamnose	2.0
Galacturonic acid	5.0
Lipophilic compounds	1.0
Proteins	4.0
Polyphenolics[a]	11.8
Total	81.8

[a] For composition of the polyphenol fraction, see Table 3.

cannot be exploited for the recovery of pectin since the polysaccharides are partly depolymerised (Schols, in't Veld, van Deelen & Voragen, 1991; Dongowski & Sembries, 2001).

In contrast to the various approaches mentioned above, the process described in the present study (Fig. 1) allows the combined recovery of pectin and polyphenolics from apple pomace (Carle et al., 2001). The resin used consists of a styrene–divinylbenzene copolymerisate which has been approved for food use by the FDA and is widely used for debittering of citrus juices (Shaw, Baines, Milnes & Agmon, 2000). Phenolic compounds are adsorbed mainly through $\pi – \pi$ interactions of their aromatic nuclei with the stationary phase (León-González & Pérez-Arribas, 2000), while pectins pass the resin without significant retention. Due to the low pH of the pomace extract, the hydrophobicity of phenolic acids and thus their propensity to adsorption is enhanced.

During the process, the adsorption capacity was monitored spectrophotometrically at 420 nm. After application of three bed volumes to the resin, the initial absorbance of 0.698 of the pomace extract decreased to 0.149 and increased slowly but steadily to 0.226 after 42 bed volumes. Concomitant HPLC analyses revealed that up to approximately 40 bed volumes, only very low levels (0.5 mg/l) of chlorogenic acid were co-eluted with the decolourised pomace extract. These findings are indicative of a high binding capacity of the resin and a very efficient retention of phenolic compounds. In addition to the spectrophotometrical determination of the degree of browning, $L*a*b*$ values of the pomace extracts were recorded before and after adsorptive separation of polyphenolics. The results shown for three samples are exemplified in Table 1. $L*$ values, representing the lightness of the pomace extracts, of all samples increased considerably. A striking decrease in chroma and an increase in hue angle was observed, which meant that the samples were becoming more yellow. From these findings it can be deduced that most of the compounds responsible for the brown colour of

the pectin extracts were adsorbed by the resin. Further investigations revealed that the degree of esterification and the galacturonic acid content of the pectin recovered from pomace extracts after decolourisation slightly increased (75.1 vs. 73.4%, and 51.8 vs. 51.7%, respectively). In addition, a moderately improved breaking strength of gels produced from these pectins was observed. Therefore, extended fields of application may be developed for these refined apple pectins.

Since residues of high-molecular compounds, e.g. pectins, proteins and starch, would precipitate on the column after addition of alcohol, the resin was washed with water prior to elution of the polyphenolics. For stabilisation, the eluate was concentrated in vacuo and finally lyophilised. The average yield was 1.2 g lyophilisate per liter pomace extract. The chemical composition of the lyophilisate is shown in Table 2. Since preliminary investigations by thin-layer chromatography had confirmed that free sugars were only present in negligable amounts, aliquots of the lyophilisate were hydrolysed to obtain monosaccharides which were characterised and quantified by HPLC. The main constituents were neutral saccharides (60%), with arabinose being the predominant sugar. It is well known that arabinose is a major constituent both of the pectin and of the hemicellulose fractions of apple cell walls (Voragen, Timmers, Linssen, Schols & Pilnik, 1983; Voragen, Schols & Pilnik, 1986; Schols et al., 1991). It is therefore concluded that arabinose-rich pectin fragments possibly attached to polyphenolics could not be removed by washing with water and were eluted with alcohol.

The presence of free galacturonic acid may be attributed to partial acid hydrolysis during the initial extraction of pectin. Lipophilic compounds amounted to 1% of the lyophilisate and were not further characterised. It is assumed that they mainly consist of waxes which are known to occur in the pomace (Ramm, Baumann & Gierschner, 1994). Although waxes are extracted to a very limited extent, due to their pronounced hydrophobic properties, they may be accumulated by the adsorber resin. The average crude protein content of dried apple pomace amounts to 4.5%. Since a protein conversion number of 6.25 may lead to an under reporting error of approximately 3% (Kennedy et al., 1999), the real protein content of the lyophilisate may be slightly higher than determined.

Approximately 12% of the lyophilisate consisted of phenolic compounds. Considering the average yield of 1.2 g lyophilisate, in total 0.14 g of polyphenolics may be recovered from 1 l pomace extract. The phenolic fraction was characterised by HPLC using a stationary phase with hydrophilic endcapping. This stationary phase displayed excellent resolution properties especially for apple and pear phenolics and was therefore applied to issues of authenticity control of fruit juices (Schieber et al., 2001; Schieber, Hilt, Streker, Klaiber & Carle,

Table 3
Composition of the phenolic fraction of the lyophilisate

Phenolic Compound	mg/g
Epicatechin	9.3
Procyanidin B2	9.3
Catechin	2.4
Chlorogenic acid	14.3
p-Coumaroylquinic acid[a]	1.8
p-Coumaric acid	0.5
Ferulic acid	0.4
Quercetin 3-galactoside	11.4
Quercetin 3-rhamnoside	4.7
Quercetin 3-glucoside	3.9
Quercetin 3-xyloside	1.8
Quercetin 3-rutinoside	1.3
Quercetin 3-arabinoside	1.1
Phloridzin	40.4
Phloretin xyloglucoside[b]	8.0
Quercetin	6.5
Phloretin	0.5
Total	117.6

[a] Calculated as p-coumaric acid.
[b] Calculated as phloridzin; data given in Table 3 are mean values of two replicate determinations.

2002) and for the determination of phenolic acids and flavonoids from apple cultivars (Keller, Streker, Arnold, Schieber & Carle, 2001). As can be seen from Table 3, the main phenolic compounds detected in the lyophilisate were phloridzin, chlorogenic acid and a number of quercetin glycosides, with quercetin 3-galactoside being the predominant flavonol. Flavanol derivatives (catechins, procyanidin) were also found in appreciable quantities, whereas the amounts of hydroxycinnamates other than chlorogenic acid were considerably lower. The presence of the aglyca quercetin and phloretin can be attributed to hydrolysis during pectin extraction.

Since the phenolic compounds detected in the lyophilisate were also found in dried apple pomace samples (Schieber et al., 2001), it becomes evident that the genuine phenolic profile of apples is not considerably changed by the process. In contrast to Lu and Foo (1997) who did not report the presence of chlorogenic acid in apple pomace, this compound was recovered in high yields. However, evidence is given that some procyanidins are lost since epicatechin oligomers were shown to occur in apple pomace (Foo & Lu, 1999). Hydrolytic cleavage of procyanidins under acidic conditions of pectin extraction, and oxidation of catechins to procyanidin oligomers which were strongly retained by the adsorber resin and could therefore not be detected by our analytical system, respectively, may explain these losses. The latter assumption is supported by the recent findings that apple procyanidins, especially those of a higher degree of polymerisation, rapidly associate with cell wall material (Renard, Baron, Guyot & Drilleau, 2001). The formation of covalent bonds between qui-

nones and cell wall polymers including proteins also cannot be ruled out. Investigations on the fate of procyanidin oligomers and polymers are currently under way, especially since complete elucidation of all constituents of the lyophilisate has not yet been achieved.

In our previous studies on the phenolic composition of apple pomace (Schieber et al., 2001) only approximately one third of the amounts reported by Lu and Foo (1997) were found. Since their results were based on lyophilised pomace samples from New Zealand apples, whereas we investigated pomace from the South of Germany which has been subjected to industrial drying, it was assumed that either differences of the raw material or harsh drying conditions were responsible for differing polyphenol contents.

Therefore, the effect of industrial drying of apple pomace on the stability of phenolic compounds was also investigated. It can be seen from Table 4 that in total more than 2 g/kg of polyphenolics on a dry matter basis were found in apple pomace. These amounts are in excellent agreement with those reported earlier (Schieber et al., 2001) although the pomace samples were from quite different sources. Instead of listing all compounds that were individually quantified, the amounts of the polyphenol subclasses have been summed up. Among these fractions, only the flavanols (catechin, epicatechin, procyanidins) have been adversely affected by the industrial drying process, as revealed by a decline from 406 to 318 mg/kg. In contrast, contents of flavonols, hydroxycinnamates and dihydrochalcones slightly increased and remained unchanged, respectively. The fact that even an increase was observed for flavonols and hydroxycinnamates may be ascribed to the inhomogeneity of pomace samples rather than to analytical problems. These results clearly reveal that industrial apple pomace drying is not as deleterious as previously expected (Schieber et al., 2001), and that the differences observed between our study and the reports of Lu and Foo (1997) may more likely be attributed to differences in the raw material. Since high incident light with a high UV intensity may lead to increased levels of flavonoids in apples (Lister, Lancaster, Sutton & Walker, 1994), considerably higher contents of poly-

Table 4
Contents (mg/kg dry matter) of phenolic fractions of apple pomace before and after drying in a three-stage drum dryer

Phenolic fraction	Wet pomace[a]	Dried pomace
Flavonols	633	673
Flavanols	406	318
Dihydrochalcones	867	861
Hydroxycinnamates	502	562
Total	2408	2414

[a] Values calculated after determination of dry matter by infrared drying.

Table 5
Contents (mg/kg fresh weight) and distribution of phenolic compounds in skin, flesh and core of four New Zealand apple cultivars

Apple cultivar	cv. 'Pacific Rose'		cv. 'Sunrise'		cv. 'Braeburn'		cv. 'Royal Gala'	
	mg/kg	%	mg/kg	%	mg/kg	%	mg/kg	%
Skin								
Flavonol glycosides	188.8	41.3	205.6	49.6	79.9	31.5	367.6	60.4
Dihydrochalcones	26.7	5.8	36.6	8.8	21.6	8.5	16.5	2.7
Flavanols	153.9	33.7	133.5	32.2	127.1	50.1	166.8	27.4
Hydroxycinnamates	87.7	19.2	38.7	9.3	25.2	9.9	58.2	9.5
Sum	457.1		414.4		253.8		609.1	
Flesh								
Flavonol Glycosides	–	–	1.7	1.5	–	–	1.7	1.6
Dihydrochalcones	4.0	8.2	5.8	5.0	5.1	4.8	10.0	9.4
Flavanols	11.3	23.3	69.8	60.1	58.7	54.9	44.4	41.8
Hydroxycinnamates	33.2	68.5	38.9	33.4	43.1	40.3	50.2	47.2
Sum	48.5		116.2		106.9		106.3	
Core								
Flavonol glycosides	12.0	4.8	14.8	4.8	3.7	1.9	20.5	6.3
Dihydrochalcones	87.4	34.6	140.6	45.4	76.4	38.1	160.1	49.0
Flavanols	35.5	14.1	44.0	14.2	25.3	12.6	36.0	11.0
Hydroxycinnamates	117.4	46.5	110.4	35.6	95.1	47.4	109.9	33.7
Sum	252.3		309.8		200.5		326.5	

phenolics might be a consequence of the light conditions prevailing in the Southern hemisphere. In a preliminary study, four New Zealand apple cultivars ('Pacific Rose', 'Sunrise', 'Braeburn', 'Royal Gala') were investigated for their polyphenolic profile. The contents and distribution of phenolic compounds in skin, flesh and core of these cultivars are presented in Table 5. Whereas in the skins flavonol glycosides and flavanols were the predominant constituents, dihydrochalcones and hydroxycinnamates were only present at low levels. As expected, the flesh was a comparatively poor source of phenolic acids and flavonoids, with only hydroxycinnamates and flavanols being present in appreciable amounts. In contrast, the core contained large amounts of dihydrochalcones (phloridzin and phloretin xyloglucoside) and hydroxycinnamates which mainly originate from the seeds.

Although these limited data indicate that skins and core are rich sources of polyphenolics, a comparison with the relevant literature revealed that the polyphenol contents of New Zealand apples are, if at all, not considerably higher than in apples from other regions (Pérez-Ilzarbe, Hernández & Estrella, 1991; Golding, McGlasson, Wyllie & Leach, 2001; Van der Sluis, Dekker, de Jager & Jongen, 2001). However, results are difficult to compare due to differences in the analytical methods that have been applied for the determination of phenolic compounds. It should further be taken into account that polyphenol levels do not only depend on the intensity and quality of light exposure but also on cultivar, maturity, temperature, soil nutrients, storage conditions, etc. (Escarpa & Gonzalez, 1998; Lancaster, Reay, Norris & Butler, 2000; van der Sluis et al., 2001).

Therefore, extended screening of apple cultivars and more detailed studies on the factors affecting polyphenol composition are still necessary.

Interestingly, isorhamnetin 3-glucoside was detected in skin and core extracts of the cultivar 'Sunrise' at a level of 12.4 and 0.2 mg/kg, respectively, on a fresh weight basis. This flavonol glycoside has so far been considered typical of pear fruits and has therefore been used as a marker for the detection of fraudulent admixtures of pear juice to apple juice (Wald & Galensa, 1989). Very recently, however, isorhamnetin 3-glucoside and a second isorhamnetin glycoside have been detected in extracts of 'Brettacher' apples by LC-MS for the first time (Schieber et al., 2002). The contents found in skins and core were in excellent agreement with those determined in the cultivar 'Sunrise' in the present study. This result confirms that isorhamnetin glycosides are more widespread in apple than previously assumed, and demonstrates the need of establishing more reliable markers for the control of the authenticity of fruit juices.

Seeds constitute approximately 2–3% of the total weight of apple pomace (Carson, Collins & Penfield, 1994). While they are not considered part of the edible portion, they are used for the recovery of apple seed oil. Owing to its high content of linoleic and oleic acids, apple seed oil may be used for food or in cosmetics (Kennedy et al., 1999). However, its limited availability is still a problem, and recovery of valuable by-products would make seed exploitation more profitable. Apple seeds have been shown to be a source of phenolic antioxidants, with phloridzin being the most important compound, while procyanidins and flavonol glycosides were absent or present at low levels (Jham, 1996; Lu &

Table 6
Phenolic composition (mg/kg) of dried apple seeds

Phenolic Compound	mg/kg
Epicatechin	9.6
Procyanidin B2	17.0
Catechin	n.d.
Chlorogenic acid	119.8
p-Coumaroylquinic acid[a]	9.4
Quercetin 3-galactoside	12.9
Quercetin 3-rhamnoside	25.0
Quercetin 3-glucoside	5.9
Phloridzin	1915.0
Phloretin xyloglucoside[b]	47.4
Phloretin	6.3
Total	2168.3

[a] Calculated as p-coumaric acid.

[b] Calculated as phloridzin; the data given in Table 6 are mean values of three replicate determinations.

Foo, 1998). Since reports on the phenolic profile are contradictory and quantitative data are limited, studies on the phenolic composition of apple seeds were also included. The results are shown in Table 6. Consistent with the findings of previous investigations (Jham, 1996; Lu & Foo, 1998; Awad et al., 2000), phloridzin was the predominant compound, amounting to almost 90% of total polyphenolics. Chlorogenic acid, phloretin xyloglucoside, epicatechin, procyanidin B2, and a number of quercetin glycosides were also present in appreciable amounts. While quercetin glycosides were not detected by Awad et al. (2000), they found low levels of cyanidin 3-galactoside. In conclusion, although the phenolic profile of the seeds seems to be more limited compared to the skins, the seeds are rich in polyphenolics, yielding more than 2 g per kg in total. Therefore, apple seeds are a promising source of valuable compounds which may be used in health food. An economically feasible way of utilisation would be the recovery of cold-pressed seed oil with subsequent extraction of the polyphenolics.

4. Conclusions

A new process for the combined recovery of pectin and phenolic compounds from apple pomace is described. It is particular noteworthy that the process could be easiliy integrated in the pectin production. Therefore, the industrial scale-up has already been commenced, allowing the isolation of large amounts of polyphenolics. Future investigations will be directed at further purification of the phenolic fraction and will include studies on their stability and their application as natural antioxidants and functional food ingredients.

Acknowledgments

References

Awad, M. A., de Jager, A., & van Westing, L. M. (2000). Flavonoid and chlorogenic acid levels in apple fruit: characterisation of variation. *Scientia Horticulturae, 83,* 249–263.

Carle, R., Keller, P., Schieber, A. L., Rentschler, C., Katzschner, T., Rauch, D., Fox, G. F. & Endreß, H.-U. (2001). Method for obtaining useful materials from the by-products of fruit and vegetable processing. Patent Application WO 01/78859.

Carson, K. J., Collins, J. L., & Penfield, M. P. (1994). Unrefined, dried apple pomace as a potential food ingredient. *Journal of Food Science, 59,* 1213–1215.

Dick, A. J., Redden, P. R., DeMarco, A. C., Lidster, P. D., & Grindley, T. B. (1987). Flavonoid glycosides of Spartan apple peel. *Journal of Agricultural and Food Chemistry, 35,* 529–531.

Dongowski, G., & Sembries, S. (2001). Effects of commercial pectolytic and cellulolytic enzyme preparations on the apple cell wall. *Journal of Agricultural and Food Chemistry, 49,* 4236–4242.

Eberhardt, M. V., Lee, C. Y., & Liu, R. H. (2000). Antioxidant activity of fresh apples. *Nature, 405,* 903–904.

Endreß, H.-U. (1991). Non-food uses of pectin. In R. H. Walter, *The Chemistry and Technology of Pectin* (pp. 251–268). San Diego: Academic Press.

Endreß, H.-U. (2000). High quality resulting from product integrated environment protection-PIUS. *Fruit Processing, 10,* 273–276.

Escarpa, A., & Gonzalez, M. C. (1998). High-performance liquid chromatography with diode-array detection for the determination of phenolic compounds in peel and pulp from different apple varieties. *Journal of Chromatography A, 823,* 331–337.

Foo, L. Y., & Lu, Y. (1999). Isolation and identification of procyanidins in apple pomace. *Food Chemistry, 64,* 511–518.

Fox, G. F., Asmussen, R., Fischer, K., & Endreß, H.-U. (1991). Aufwand und Nutzen der Apfeltresterverwertung. *Flüssiges Obstet., 58,* 492–499.

Golding, J. B., McGlasson, W. B., Wyllie, S. G., & Leach, D. N. (2001). Fate of apple peel phenolics during cold storage. *Journal of Agricultural and Food Chemistry, 49,* 2283–2289.

Haug, M., & Gierschner, K. (1979). Auswirkung unterschiedlicher industrieller Herstellungsverfahren (Auspressen, Extraktion) auf die phenolischen Verbindungen in Apfelsäften. *Deutsche Lebensmittel-Rundschau, 75,* 248–253.

Jham, G. N. (1996). High-performance liquid chromatographic quantitation of phloridzin in apple seed, leaf and callus. *Journal of Chromatography A, 719,* 444–449.

Keller, P., Streker, P., Arnold, G., Schieber, A., & Carle, R. (2001). Bestimmung phenolischer Verbindungen in Tafel- und Mostäpfeln mittels HPLC. *Flüssiges Obstetrics, 68,* 480–483.

Kennedy, M., List, D., Lu, Y., Foo, L. Y., Newman, R. H., Sims, I. M., Bain, P. J. S., Hamilton, B., & Fenton, G. (1999). Apple pomace and products derived from apple pomace: Uses, composition and analysis. In H. -F. Linskens, J. F. Jackson, (pp. 76–119) *Modern methods of plant analysis*, vol. 20. Berlin, Heidelberg: Springer.

Lancaster, J. E., Reay, P. F., Norris, J., & Butler, R. C. (2000). Induction of flavonoids and phenolic acids in apple by UV-B and temperature. *Journal of Horticultural Science & Biotechnology, 75,* 142–148.

León-González, M. E., & Pérez-Arribas, L. V. (2000). Chemically modified polymeric sorbents for sample preconcentration. *Journal of Chromatography A, 902,* 3–16.

Lister, C. E., Lancaster, J. E., Sutton, K. H., & Walker, J. R. L. (1994). Developmental changes in the concentration and compo-

sition of flavonoids in skin of a red and a green apple cultivar. *Journal of the Science of Food and Agriculture, 64*, 155–161.

Lommen, A., Godejohann, M., Venema, D. P., Hollman, P. C. H., & Spraul, M. (2000). Application of directly coupled HPLC-NMR-MS to the identification and confirmation of quercetin glycosides and phloretin glycosides in apple peel. *Analytical Chemistry, 72*, 1793–1797.

Lu, Y., & Foo, L. Y. (1997). Identification and quantification of major polyphenols in apple pomace. *Food Chemistry, 59*, 187–194.

Lu, Y., & Foo, L. Y. (1998). Constitution of some chemical components of apple seed. *Food Chemistry, 61*, 29–33.

Lu, Y., & Foo, L. Y. (2000). Antioxidant and radical scavenging activities of polyphenols from apple pomace. *Food Chemistry, 68*, 81–85.

May, C. D. (1990). Industrial pectins: Sources, production and applications. *Carbohydrate Polymers, 12*, 79–84.

Pérez-Ilzarbe, J., Hernández, T., & Estrella, I. (1991). Phenolic compounds in apples: varietal differences. *Zeitschrift für Lebensmittel-Untersuchung und -Forschung, 192*, 551–554.

Pizzocaro, F., Torreggiani, D., & Gilardi, G (1993). Inhibition of apple polyphenoloxidase by ascorbic acid, citric acid and sodium chloride. *Journal of Food Processing and Preservation, 17*, 21–30.

Price, K. R., Prosser, T., Richetin, A. M. F., & Rhodes, M. J. C. (1999). A comparison of the flavonol content and composition in dessert, cooking and cider-making apples; distribution within the fruit and effect of juicing. *Food Chemistry, 66*, 489–494.

Ramm, A., Baumann, G., & Gierschner, K. (1994). Wachse (einschließlich Triterpene) in Roh- und Reststoffen der Apfelpektingewinnung. *Industrielle Obst- und Gemüseverwertung, 79*, 2–9.

Renard, C. M. G. C., Baron, A., Guyot, S., & Drilleau, J. -F. (2001). Interactions between apple cell walls and native apple polyphenols: quantification and some consequences. *International Journal of Biological Macromolecules, 29*, 115–125.

Renard, C. M. G. C., Rohou, Y., Hubert, C., Della Valle, G., Thibault, J. -F., & Savina, J. -P. (1996). Bleaching of apple pomace by hydrogen peroxide in alkaline conditions: Optimisation and characterisation of the products. *Lebensmittel-Wissenschaft und -Technologie, 30*, 398–405.

Schieber, A., Keller, P., & Carle, R. (2001). Determination of phenolic acids and flavonoids of apple and pear by high-performance liquid chromatography. *Journal of Chromatography A, 910*, 265–273.

Schieber, A., Keller, P., Endreß, H. -U., Rentschler, C., & Carle, R. (2001). Recovery and characterisation of phenolic compounds from by-products of food processing. In W. Pfannhauser, G. R. Fenwick, S. Khokhar, *Proceedings of the EUROFOODCHEM XI. 26–28 September, 2001* (pp. 502–504). Norwich, UK.

Schieber, A., Hilt, P., Streker, P., Klaiber, I., & Carle, R. (2002). Detection of isorhamnetin glycosides in extracts of apples (*Malus domestica* cv. 'Brettacher') by HPLC-DAD and HPLC-APCI-MS/MS. *Phytochemical Analysis, 13*, 87–94.

Schols, H. A., in't Veld, P. H., van Deelen, W., & Voragen, A. G. J. (1991). The effect of the manufacturing method on the characteristics of apple juice. *Zeitschrift für Lebensmittel-Untersuchung und -Forschung, 192*, 142–148.

Shaw, P. E., Baines, L., Milnes, B. A., & Agmon, G. (2000). Commercial debittering processes to upgrade quality of citrus juice products. In M. A. Berhow, S. Hasegawa, G. D. Manners, *Citrus Limonoids: Functional chemicals in agriculture and foods. ACS Symposium Series 758* (pp. 120–131). Washington, DC.

Teuber, H., & Herrmann, K. (1978). Flavonolglykoside der Äpfel (*Malus silvestris* Mill.). *Zeitschrift für Lebensmittel-Untersuchung und -Forschung, 166*, 80–84.

Thakur, B. R., Singh, R. K., & Handa, A. K. (1997). Chemistry and uses of pectin-a review. *Critical Reviews in Food Science and Nutrition, 37*, 47–73.

Van der Sluis, A. A., Dekker, M., de Jager, A., & Jongen, W. M. F. (2001). Activity and concentration of polyphenolic antioxidants in apple: Effect of cultivar, harvest year, and storage conditions. *Journal of Agricultural and Food Chemistry, 49*, 3606–3613.

VdF (Verband der deutschen Fruchtsaftindustrie e.V.) (2000). www.fruchtsaft.de.

Voragen, F. G. J., Schols, H. A., & Pilnik, W. (1986). Structural features of the hemicellulose polymers of apples. *Zeitschrift für Lebensmittel-Untersuchung und -Forschung, 183*, 105–110.

Voragen, F. G. J., Timmers, J. P. J., Linssen, J. P. H., Schols, H. A., & Pilnik, W. (1983). Methods of analysis for cell-wall polysaccharides of fruits and vegetables. *Zeitschrift für Lebensmittel-Untersuchung und -Forschung, 177*, 251–256.

Wald, B., & Galensa, R. (1989). Nachweis von Fruchtsaftmanipulationen bei Apfel- und Birnensaft. *Zeitschrift für Lebensmittel-Untersuchung und -Forschung, 188*, 107–114.

Will, F., Bauckhage, K., & Dietrich, H. (2000). Apple pomace liquefaction with pectinases and cellulases: analytical data of the corresponding juices. *European Food Research and Technology, 211*, 291–297.