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A novel approach to quality and authenticity control of fruit products using fractionation and characterisation of cell wall polysaccharides

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Dedicated to Prof. Dr. R. Wild (Heidelberg) on the occation of his 60th birthday

Abstract

Cell wall polysaccharides were investigated for their suitability as markers for quality and authenticity control of fruit products. For this purpose, the alcohol-insoluble residue (AIR) was fractionated into water-, oxalate-, acid-, and alkali-soluble pectins, hemicellulose and cellulose. Neutral sugars of each fraction were determined by capillary zone electrophoresis. Particular attention was given to strawberries and cherries of different cultivars, proveniences, and stages of ripening, whereas preliminary investigations were also conducted on apples. Within the respective fruit species, characteristic neutral sugar profiles of the AIR fractions were found, which may be used for the differentiation of fruit products devoid of carbohydrate-based hydrocolloids, e.g. purees. Furthermore, the isolated hemicellulose fraction not only allowed the detection of admixtures of non-declared fruits on the basis of the monosaccharide composition. Even more important, since the content of the hemicellulose fraction proved to be constant in the AIR, its determination provided promising perspectives for calculating the fruit content of complex products such as fruit preparations, jams and spreads.

Keywords: Strawberry; Cherry; Apple; AIR; Authenticity; Fruit content; Monosaccharide composition; Fractional isolation

1. Introduction

Adulteration of foods is a serious economic problem and may occur during the complete production process. Since high-priced fruits command premium prices, producers of fruit-based products such as juices, jams, jellies, purees and fruit preparations might be tempted to blend these products with cheaper fruits. In addition to admixtures of adulterants, the claimed fruit contents are frequently not met. Both types of adulteration are difficult to detect and lead to a deterioration of product quality. For consumer protection, it is of essential importance to guarantee food authenticity combined with quality and to avoid unfair competition.

The determination of characteristic phenolic compounds is a common tool for the differentiation of fruit species (Silva, Andrade, Valentao, Mendes, Seabra, & Ferreira, 2000; Wald & Galensa, 1989), but these constituents have also been shown to undergo great variations with different cultivars, stages of ripeness and geographical origins. Furthermore, using improved analytical methods, isorhamnetin glycosides and phloridzin so far considered typical of pears and apples, respectively, have recently been detected in other fruits (Hvattum, 2002; Hilt et al., 2003; Schieber, Keller, Streker, Klaiber, & Carle, 2002). Anthocyanins have been demonstrated to be degradated to a large extent during processing of jams (García-Viguera, Zafrilla, & Tomas-Barberán, 1997). NMR spectroscopy and IRMS have also been used for the control of food authenticity (Colquhoun, 1998; González, Remaud, Jamin, Naulet, & Martin, 1999). However, these methods are highly

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sophisticated and require experienced users. As shown for tomato ketchup, PCR techniques are not applicable to the analysis of heated products since the DNA is degradated in acidic milieu (Adam & Zimm, 1977; Hupfer, Hotzel, Sachse, & Engel, 1998).

Most methods for the determination of the fruit content are based on the quantification of low-molecular compounds such as amino acids, organic acids, minerals and sugars (Nehring, Prehn, & Skott, 1978; Pilando & Wrolstad, 1992; Wallrauch, 1995). However, owing to fruit ripeness and different cultivars, these parameters are subject to considerable variations and can easily be manipulated. Chemometric methods such as FTIR spectroscopy, which have been suggested to be suitable both for the detection of fruit adulteration and the determination of the fruit content (Holland, Kemsley, & Wilson, 1998; Wilson, Slack, Appleton, Sun, & Belton, 1993), require a large number of well-defined samples of known cultivar and origin for extensive calibration.

The composition of the plant cell wall has been a matter of extensive investigations during the past decades. Most studies conducted so far have focused on the analysis of its structure and organisation and on the characterisation of changes during ripening and textural improvement of fruits (Femenia, Waldron, Robertson, & Selvendran, 1999; Hegde & Maness, 1996; Legentil, Guichard, Piffaut, & Haluk, 1995; Renard, Voragen, Thibault, & Pilnik, 1991; Carle, Borzych, Dubb, Siliha, & Maier, 2001). However, only little attention has been paid to the role of cell wall constituents and their neutral saccharide composition in quality control of food. Therefore, the objective of the present study was to develop an innovative approach for the control of the authenticity by characterisation of fruit-specific cell wall saccharides. Furthermore, it should be investigated whether the cell wall fractions obtained could also be used for the determination of the fruit content of fruit preparations. Owing to their economic relevance, particular attention was given to strawberries and cherries of different cultivars, proveniences, and stages of ripeness. Preliminary investigations were also conducted on apple fruits.

2. Materials and methods

2.1. Plant material

Frozen strawberries (*Fragaria x ananassa* Duch. cv. 'Senga Sengana', 'Camarosa'; *Fragaria vesca* L.) and cherries (*Prunus cerasus* L. cv. 'Schattenmorelle', 'Oblacinska', 'Lutowka') of different proveniences representing the most frequently used raw materials for food processing were obtained from Zentis (Aachen, Germany), Schwartau (Bad Schwartau, Germany) and Wild (Berlin, Germany). Strawberries (cv. 'Elsanta') were collected from a farm of professional growers (Stuttgart, Germany). Apples (*Malus domestica* Borkh. cv. 'Boskop', 'Glockenapfel') were purchased from the local market (Stuttgart, Germany). All fruits were harvested in 2001 (Table 1).

2.2. Alcohol-insoluble residue

Strawberries and cherries were freeze-dried and roughly ground. Apples were cored, peeled, sliced and also lyophylised. The lyophylisate (30 g) was homogenised in boiling ethanol (300 mL, 80% v/v) using an Ultra-Turrax blender. After boiling for 1 h, the insoluble solids were collected on a Büchner funnel. This procedure was continued five times until a clear extract was obtained. The residue was stirred overnight in pure acetone, passed through a Gl glass sinter filter and air-dried at 40 °C for 24 h.

2.3. Sequential extraction of the alcohol-insoluble residue

The alcohol-insoluble residue (AIR) (1.5 g) was suspended in 100 mL of distilled water and stirred at 40 °C for 30 min. After centrifugation at 15,000 *g* for 25 min the cake was resuspended in distilled water (100 mL), extracted at 40 °C for 1 h under stirrring and centrifuged again. The combined supernatants were dialysed exhaustively against distilled water for two days using dialytic membranes (type 36/32, pore size 25–50 Å, Roth, Karlsruhe, Germany). The water-soluble pectin (WSP) extract was then freeze-dried. The pellet from water extraction was suspended and stirred in 100 mL of ammonium oxalate solution (0.5% w/v) at 40 °C for 90 min. The suspension was centrifuged for 25 min at 15,000 *g* and the pellet was washed twice with 100 mL of distilled water. The supernatants were pooled, dialysed for two days against distilled water and freeze-dried to yield the oxalate-soluble pectin (OXP) fraction. Dilute hydrochlorid acid (0.05 M) was used for further extraction of the residue at 60 °C for 90 min. The homogenates were centrifuged at 15,000 *g* for 25 min. The remaining pellet was washed twice with 100 mL of distilled water. The supernatants were pooled and referred to as HCl-soluble pectin (HSP) fraction. The HSP extract was finally treated as described for the WSP and OXP fractions. The residue was then extracted with 100 mL of aqueous sodium hydroxide (0.05 M) at 4 °C for 3 h. After centrifugation at 15,000 *g* for 20 min, the pellet was rinsed twice. The supernatants were pooled and the pH adjusted to 6.5 with HCl, followed by the treatment according to the previous fractions in order to produce the NaOH-soluble pectin (OHP) fraction. The final extraction was carried out using 100 mL of aqueous sodium hydroxide (16% w/w) at 30 °C for 5 h. After centrifugation and rinsing, the supernatants were combined and the pH adjusted to 6.5. This hemicellulose

Table 1
Specifications of fruit samples

Fruit species	Cultivar	Stage of ripeness	Provenience	Sample code
Strawberry	Camarosa	ripe/turning, pink/red	Spain	FC
	Senga sengana	ripe/turning, pink/red	Germany	FS I
	Senga sengana	ripe/turning, pink/red	Poland	FS II
	Senga sengana	ripe/turning, pink/red	China	FS III
	Elsanta	ripe, red	Germany	FE I
	Elsanta	turning, white-pink	Germany	FE II
Wild strawberry	Unknown	ripe/turning, pink/red	Morocco	FW
Cherry	Lutowka	ripe, red	Poland	CL
	Schattenmorelle	ripe, red	Turkey	CS
	Oblacinska	ripe, red	Serbia	CO
Apple	Glockenapfel	ripe, firm	Germany	AG
	Boskop	ripe, firm	Germany	AB

(HC) fraction was then treated as described above. The remaining pellet consisted of insoluble solids such as lignin and cellulose (CL fraction). The CL fraction was finally suspended in 100 mL of distilled water, dialysed and lyophilised.

2.4. Hydrolysis of the cell wall fractions

The cell wall fractions were hydrolysed using sulphuric acid. For this purpose, 300 μL of isopropanol and then 300 μL of H_2SO_4 (72% w/w) were added to 30 mg of the AIS. After a reaction time of 1 h at room temperature, the suspension was diluted with 5 mL of distilled water and heated at 121 °C for 1 h. The hydrolysate was neutralised with 750 μL of aqueous ammonia (25% w/w). The volume was made up to 10 mL and an aliquot was centrifuged for 2 min at 10,000 *g*. Some modifications were necessary for the hydrolysis of the insoluble residue (C-fraction). The quantity of H_2SO_4 (72% w/w) was increased to 500 μL and 5.4 mL of distilled water were used for dilution. Additionally, the cold hydrolysis was carried out in an ultrasonic bath for 2 h.

2.5. Analysis of neutral sugars by capillary zone electrophoresis

Individual neutral sugars were derivatised with 4-aminobenzoic acid ethyl ester and subsequently separated by capillary zone electrophoresis based on two methods described previously (Dahlman, Jacobs, Liljenberg, & Olsson, 2000; Huber, Grill, Oefner, & Bobleter, 1994). For derivatisation, 200 μL of the hydrolysed sample, 200 μL of the internal standard solution (0.2 g/L 2-deoxy-D-ribose) and 240 μL of the derivatisation reagent (100 mg/mL 4-aminobenzoic acid ethyl ester, 100 mg/mL glacial acid and 10 mg/mL sodium cyanoborhydride in methanol) were mixed in a glass tube with a plastic screw cap. The tube was placed in a heating block at 80 °C for 1 h. Immediately after the derivatisation, 800 μL of the dilution buffer (125 mmol/L sodium hydroxide and 438 mmol/L boric acid, pH 8.5) were added and mixed, followed by cooling at 4 °C for 15 min. The precipitated excess of ABEE was removed by membrane filtration. The filtrate was analysed using a Biorad Biofocus 2000 capillary electrophoresis system equipped with a UV–Vis detector (Biorad, USA). The hydrodynamic mode was used at 30 p.s.i. for injection. Separation of the analytes was performed at 25 kV and 20 °C in a bare-fused-silica capillary (70 cm × 50 μm). An aqueous solution of sodium hydroxide (300 mmol/L) and boric acid (438 mmol/L) served as the running electrolyte buffer. Absorption of the derivatised saccharides was recorded at 306 nm. Individual sugars were identified by comparison with authentic standards and quantified using 2-deoxy-D-ribose as an internal standard. Data analysis was carried out with the Biofocus 2000 integration software.

3. Results and discussion

3.1. Sequential extraction of the alcohol-insoluble residue

The contents and composition of the AIR determined after extraction of fruit cell walls are shown in Table 2. It can be seen that considerable differences were found for the genera with respect to the AIR contents, ranging from 6.7 g/kg (cherry cv. 'Oblacinska') to 25.4 g/kg (strawberry cv. 'Senga Sengana'). Even within individual strawberry species and cultivars the AIR contents differed in a wide range, which may be attributed to the different stages of ripeness. While commercial IQF fruits were of heterogenous ripeness, self-harvested strawberries (FE I, FE II) exclusively consisted of full-ripe and turning fruits, respectively.

Whereas no differences in the AIR content were found between the cherry cultivars 'Schattenmorelle' and 'Lutowka' (10.3 g/kg), cv. 'Oblacinska' contained only 6.7 g/kg. Apple samples, which were of similar ripeness, showed comparable AIR contents. These findings are in general agreement with the results of previous

Table 2
Amount and composition of AIR extracted from cell walls of strawberries, cherries and apples

	AIR[a] (g/100 g DM)	Composition of AIR (g/100 g AIR)						Recovery (%)
		WSP	OXP	HSP	OHP	HC	C	
FC	19.3 ± 0.3	13.2	9.8	14.0	9.5	13.3	30.4	90.2
FS I	25.4 ± 0.9	21.8	9.0	9.2	11.5	13.4	23.2	88.0
FS II	19.5 ± 0.1	17.1	9.1	10.1	9.1	13.8	29.9	89.2
FS III	21.1 ± 0.3	14.0	4.2	8.2	11.1	13.7	33.1	84.3
FE I	15.6 ± 0.1	18.7	8.5	9.7	11.4	14.6	26.4	89.4
FE II	23.3 ± 0.2	18.9	6.9	11.8	14.2	12.7	23.9	88.5
FW	24.0 ± 0.2	20.8	8.7	9.9	8.4	14.1	24.5	86.4
CL	10.3 ± 0.4	14.3	19.0	11.7	20.7	16.1	10.3	92.1
CS	10.3 ± 0.4	18.0	15.2	11.3	17.7	15.1	13.8	91.0
CO	6.7 ± 0.2	18.4	17.4	11.7	18.7	16.7	9.3	92.2
AG	13.3 ± 0.04	5.6	4.4	12.3	12.6	16.8	37.2	88.9
AB	14.4 ± 0.1	5.9	6.6	10.5	15.0	15.2	35.6	88.9

[a] mean ± SD of $n = 3$ replicate determinations.

studies in which considerable variation of the AIR contents of strawberries (Legentil et al., 1995; Voragen, Timmers, Linssen, Schols, & Pilnik, 1983), cherries (Batisse, Fils-Lyacon, & Burett, 1994), and apples (Massiot, Baron, & Drilleau, 1994) were reported.

The AIR compositions of strawberries were found to be very inhomogenous with respect to the pectin fractions WSP, OXP and HSP (Table 2). This is not surprising since these fractions are particularly affected by the stage of fruit ripeness, as reported by several authors (Batisse et al., 1994; Percy, Melton, & Jameson, 1997; Vierhuis, Schols, Beldman, & Voragen, 2000). In the order cherries, strawberries and apples, decreasing amounts of pectic compounds were observed, which is in agreement with the results published by Voragen et al. (1983). In contrast to the WSP, OXP, and HSP fractions, a more constant composition was found for the OHP, HC, and C fractions. It is particularly noteworthy that especially the HC fractions showed a significant constancy within individual genera. Even for the full ripe and turning strawberries (FE I, FE II) only a slight difference of the hemicellulose content was found. As expected, the HC content of the heterogeneous strawberry raw material ranged between the values obtained for FE I and FE II.

3.2. Analysis of neutral sugars by capillary zone electrophoresis

In most studies previously dealing with cell wall analysis, GC and HPLC techniques were applied for the determination of neutral sugars. However, gas chromatographic analysis requires tedious and time-consuming conversion of sugars into volatile compounds, usually into their alditol acetates or trimethylsilyl derivatives (Albersheim, Nevins, English, & Karr, 1967; Morvai & Molnár-Perl, 1991). On the other hand, HPLC techniques often suffer from low resolution of analytes and poor sensitivity. Therefore, in the present study neutral sugars were separated and quantified by means of capillary zone electrophoresis which has been demonstrated to be sufficiently sensitive (Dahlman et al., 2000). The method allowed the simultaneous determination of rhamnose, xylose, ribose, glucose, mannose, arabinose, fucose, and galactose within less than 25 min, as can be seen from Fig. 1(a). 2-Deoxy-D-ribose was used as an internal standard (Huber et al., 1994). The separation of neutral sugars from the hydrolysed HC and HSP fractions of strawberry AIR is exemplified in Fig. 1(b) and (c), respectively. Peaks were assigned by comparing the migration times of sample and standard electropherograms. Since the derivatisation is very specific to aldoses and ketoses and the hydrolysate contains exclusively monosaccharides from plant cell wall polysaccharides without interferring compounds, an unambiguos assignment of the peaks was accomplished. Quantification was carried out by dividing peak area by migration time and relating this quotient to that of the internal standard. These relations were plotted as a function of the concentration resulting in a calibration curve for the ABEE derivative of each monosaccharide. Linear calibration curves were obtained within a concentration range of 0.01–0.2 g/L with regression coefficients of $0.995 < r^2 < 0.998$.

3.3. Monosaccharide composition of the fractions

After isolation, the AIR was sequentially fractionated and the neutral sugar composition of each fraction was determined. Pectins are generally extracted with water, chelating agents, hot dilute acid and cold diluted alkali. Water solubilises free pectins with a high degree of methoxylation which are mainly located in the middle lamella of the cell wall, whereas oxalate leads to solubilisation of pectins with free carboxylic acid groups by chelating calcium ions. Treatment with hydrochloric acid causes cleavage of glycosidic bonds, and dilute al-

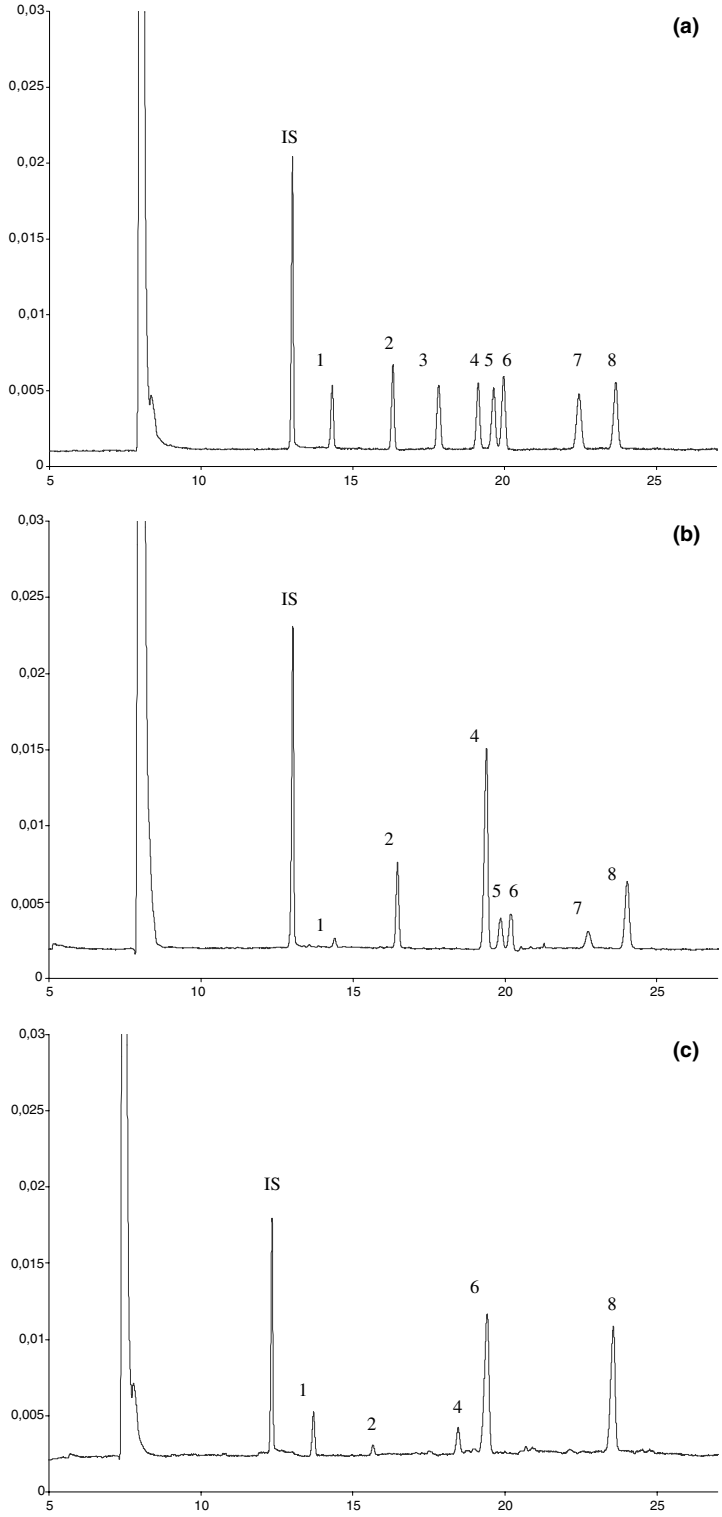

Fig. 1. Separation of monosaccharides by capillary zone electrophoresis (a) standard mixture (0.5 mg/mL) (b) hydrolysed HC fraction from strawberry fruits (c) hydrolysed HSP fraction from strawberry fruits. Identification: IS, Internal Standard (2-deoxy-ribose); 1, rhamnose; 2, xylose; 3, ribose; 4, glucose; 5, mannose; 6, arabinose; 7, fucose; 8, galactose.

kali hydrolyses esters with pectic compounds and phenolic acids. Increasing concentrations of alkali release hemicellulose-associated polysaccharides, while the remaining alkali-insoluble residue mainly consists of lignin and cellulose (Åman & Westerlund, 1996; Selvendran & Robertson, 1990).

The neutral saccharide compositions of the WSP, OXP, HSP, and OHP fractions of strawberries, cherries

and apples are summarised in Tables 3–6. As can be seen, arabinose and galactose were the predominant saccharides of all strawberry pectin fractions. Consistent with the results reported by Barbier and Thibault (1982), these monosaccharides were also found in highest amounts in cherry pectin fractions, however, compared to strawberries, the arabinose-galactose ratio was remarkably shifted towards arabinose. Galactose was found to be predominant in the WSP and OXP fractions of apples, whereas in the HSP and OHP fractions a reverse order for galactose and arabinose was observed. Rhamnose and xylose were present in lower quantities in the pectin fractions of all fruits investigated, while fucose, ribose, and mannose proved to be very minor constituents.

The HC fractions showed a neutral composition completely different from those observed in the pectin fractions. From Table 7 it can be seen that variations within the fruit genera occurred to a much lower extent. Glucose was found to be the predominant sugar in the HC fraction of all fruits examined, followed by galactose in cherries and xylose in apples and strawberries (*F. ananassa*), respectively. With the exception of wild strawberry (*F. vesca*), mannose ranged from 10.6% to 15.9%. The arabinose content was highest in the HC fraction of cherries. Fucose, which is a monosaccharide typical of hemicellulose side chains (Voragen, Schols, & Pilnik, 1986), was found in all fruits, while ribose could only be detected in trace amounts.

As expected, glucose was the major compound in the cellulose fraction, ranging from 66% to 81% of total sugars (Table 8). This great variation may be attributed to incomplete hydrolysis by sulphuric acid. Saccharides such as xylose and arabinose typical of hemicellulose could still be detected, indicating its partial retention during sequential fractionation. This is also confirmed by the detection of traces of rhamnose and mannose

Table 3
Neutral sugar composition of the WSP fractions extracted from the cell walls of strawberries, cherries and apples

	Composition of neutral sugars (relative mass%)[c]							
	Rha	Fuc	Rib	Ara	Xyl	Man	Gal	Glc
FC	9.8	1.9	nd[a]	25.9	13.6	2.3	31.5	15.1
FS I	16.4	1.1	tr[b]	35.1	5.9	1.6	28.4	11.2
FS II	12.6	0.9	tr	34.4	5.9	1.7	32.1	12.2
FS III	12.0	nd	3.0	22.9	8.6	2.0	40.1	11.5
FE I	12.6	nd	5.0	28.6	5.9	1.5	37.0	9.3
FE II	12.1	nd	nd	40.7	4.6	1.7	31.3	9.6
FW	9.3	0.5	tr	40.1	7.6	0.9	29.4	12.1
CL	7.3	tr	nd	55.7	2.4	2.1	25.8	6.3
CS	7.7	0.6	nd	60.9	2.4	2.2	26.2	5.8
CO	5.8	0.7	nd	52.2	2.5	2.3	27.4	9.2
AG	4.8	nd	2.2	32.3	2.1	1.2	47.7	9.6
AB	8.8	tr	nd	33.2	3.2	2.2	42.6	9.8

[a] nd: not detectable.
[b] tr: <0.5%.
[c] Results were obtained from duplicates, std error < 10%.

Table 4
Neutral sugar composition of the OXP fractions extracted from the cell walls of strawberries, cherries and apples

	Composition of neutral sugars (relative mass%)[c]							
	Rha	Fuc	Rib	Ara	Xyl	Man	Gal	Glc
FC	8.3	2.8	5.0	27.9	5.7	3.2	38.0	9.1
FS I	24.1	0.7	0.9	32.0	3.6	2.6	23.7	12.3
FS II	11.0	2.5	3.8	37.3	3.4	3.2	29.7	9.1
FS III	22.1	nd[a]	nd	20.8	5.2	4.3	34.7	13.0
FE I	18.9	nd	7.8	25.6	3.1	5.2	31.3	8.1
FE II	15.8	1.2	1.3	36.3	2.0	tr[b]	33.8	9.3
FW	14.7	1.2	1.8	32.3	7.7	1.6	32.5	16.4
CL	13.6	0.6	1.2	61.7	2.0	3.9	14.0	3.0
CS	14.4	0.7	nd	60.5	2.0	1.7	15.9	4.7
CO	12.1	nd	0.6	60.5	3.7	2.5	15.8	4.9
AG	7.5	nd	0.8	28.2	4.6	tr	39.8	18.7
AB	10.9	nd	7.6	32.6	2.8	0.8	30.9	14.4

[a] nd: not detectable.
[b] tr: <0.5%.
[c] Results were obtained from duplicates, std error < 10%.

Table 5
Neutral sugar composition of the HSP fractions extracted from the cell walls of strawberries, cherries and apples

	Composition of neutral sugars (relative mass%)[c]							
	Rha	Fuc	Rib	Ara	Xyl	Man	Gal	Glc
FC	11.3	tr[b]	tr	32.5	2.4	1.1	47.9	4.6
FS I	12.7	1.1	0.5	46.0	3.2	2.0	27.2	7.3
FS II	12.6	tr	tr	44.7	3.4	2.3	28.0	8.5
FS III	13.9	nd[a]	nd	24.5	2.0	nd	54.8	4.7
FE I	12.4	nd	1.5	36.3	2.1	nd	41.3	6.5
FE II	12.1	nd	nd	47.1	2.0	nd	32.3	6.5
FW	12.3	0.6	tr	37.4	4.1	1.2	30.5	9.0
CL	8.4	nd	nd	77.0	1.7	0.9	10.3	1.8
CS	9.1	nd	nd	76.7	1.7	nd	10.9	1.6
CO	8.1	0.6	2.4	72.6	1.4	0.8	11.8	2.2
AG	7.9	tr	nd	66.0	2.0	nd	16.0	7.7
AB	11.1	0.5	nd	63.4	3.4	nd	14.7	6.9

[a] nd: not detectable.
[b] tr: <0.5%.
[c] Results were obtained from duplicates, std error < 10%.

Table 6
Neutral sugar composition of the OHP fractions extracted from the cell walls of strawberries, cherries and apples

	Composition of neutral sugars (relative mass%)[c]							
	Rha	Fuc	Rib	Ara	Xyl	Man	Gal	Glc
FC	10.8	tr[b]	4.9	24.7	7.2	1.3	46.7	4.2
FS I	18.6	tr	6.9	33.4	4.5	1.4	29.1	5.9
FS II	9.8	tr	8.4	35.3	5.0	1.0	34.7	5.8
FS III	14.6	nd[a]	7.6	21.1	6.2	nd	47.2	3.4
FE I	12.9	nd	5.9	26.1	3.3	nd	48.9	3.0
FE II	13.2	nd	2.9	33.6	1.3	nd	47.0	1.9
FW	13.7	tr	10.9	27.1	5.0	1.9	36.4	8.2
CL	10.7	1.0	2.3	46.3	5.3	5.3	16.8	12.3
CS	9.5	1.3	3.9	44.6	7.8	6.0	18.8	8.1
CO	9.8	1.2	2.1	46.0	5.8	5.2	17.4	12.5
AG	12.8	tr	nd	48.2	2.7	tr	28.4	7.3
AB	17.5	tr	nd	45.4	4.6	tr	25.2	6.6

[a] nd: not detectable.
[b] tr: <0.5%.
[c] Results were obtained from duplicates, std error < 10%.

Table 7
Neutral sugar composition of the HC fractions extracted from the cell walls of strawberries, cherries and apples

	Composition of neutral sugars (relative mass%)[c]							
	Rha	Fuc	Rib	Ara	Xyl	Man	Gal	Glc
FC	1.2	3.3	nd[a]	5.1	22.0	13.2	13.7	41.5
FS I	1.5	3.2	tr[b]	5.3	22.3	14.5	10.8	42.3
FS II	1.0	2.8	nd	5.0	19.9	15.9	12.5	43.0
FS III	1.3	3.5	nd	4.6	21.0	14.4	11.6	43.6
FE I	1.2	3.6	nd	5.1	18.8	12.2	13.2	45.9
FE II	1.4	3.4	nd	6.2	18.3	13.1	12.6	45.0
FW	1.4	3.5	tr	6.9	20.9	7.9	15.0	44.0
CL	2.8	2.2	tr	18.3	13.2	12.2	20.1	30.9
CS	2.6	2.3	tr	15.9	15.1	12.4	20.5	31.1
CO	3.7	2.3	tr	15.1	13.3	11.6	20.8	33.0
AG	1.3	4.5	nd	6.0	19.4	12.7	12.1	44.1
AB	0.9	4.0	nd	3.6	19.1	13.9	12.0	46.5

[a] nd: not detectable.
[b] tr: <0.5%.
[c] Results were obtained from duplicates, std error < 10%.

Table 8
Neutral sugar composition of the C fractions extracted from the cell walls of strawberries, cherries and apples

	Composition of neutral sugars (relative mass%)[c]							
	Rha	Fuc	Rib	Ara	Xyl	Man	Gal	Glc
FC	1.4	nd[a]	nd	2.7	14.7	nd	5.6	75.6
FS I	1.4	nd	nd	2.9	16.0	1.0	2.7	76.0
FS II	1.3	nd	nd	2.6	16.1	nd	2.6	77.4
FS III	1.4	nd	nd	2.4	16.6	tr	3.2	75.7
FE I	1.6	nd	nd	3.5	19.9	tr	6.4	68.3
FE II	2.1	nd	nd	5.3	21.4	1.0	nd	70.2
FW	1.8	nd	nd	3.7	17.5	nd	4.7	72.3
CL	3.2	nd	nd	13.2	1.4	nd	2.7	79.5
CS	2.6	nd	nd	10.1	4.8	nd	2.3	80.2
CO	3.4	nd	nd	14.2	1.3	nd	2.9	78.2
AG	3.3	tr[b]	nd	10.1	6.4	3.5	10.0	66.4
AB	2.8	tr	nd	5.7	3.4	0.7	6.2	80.8

[a] nd: not detectable.
[b] tr: <0.5%.
[c] Results were obtained from duplicates, std error < 10%.

originating from residual pectic material residue (Renard et al., 1991; Vierhuis et al., 2000).

From the results obtained by characterisation of the neutral sugar composition of pectins, hemicellulose and cellulose fractions it became evident that the profile of monosaccharides could serve as a fingerprint. In the case of purees which do not contain hydrocolloids as stabilising ingredients, the neutral sugar profile of the four pectin fractions (WSP, OXP, HSP, OHP) was shown to be useful for the differentiation of fruits and for the detection of adulterants. For example, admixture of apple puree to cherry puree would lead to a decrease of the arabinose content of the cherry WSP and OXP fractions (Tables 3 and 4). From Table 4 it can also be deduced that any adulteration of cherry puree with apples would significantly increase the glucose content of the OXP fraction.

In contrast to purees, most fruit products such as jams, spreads, and fruit preparations are characterised by even more complex compositions. According to the guideline of the German Federation of Food Law and Food Science (BLL), fruit preparations are produced from fruits or fruit constituents, sugars, essences, flavours, colouring foodstuffs, thickening agents and consumable acids. Due to the presence of additional food ingredients, such products are most difficult to analyse with respect to quality and authenticity control. In particular, the determination of their fruit content is still inadequate. According to the BLL guideline, the fruit content of fruit preparations generally amounts to 35%. In the case of raspberry, raspberry–blackberry, red currant, gooseberry, plum and pineapple, the fruit content is at least 30%, and for banana and black currant at least 25%.

While the neutral sugar profile of the pectin fractions may be helpful for the detection of adulterations of fruit purees, it is not suitable for products containing hydrocolloids since these are also precipitated by alcohol during preparation of the AIR, and acidic hydrolysis would release monosaccharides which affect the genuine sugar profile of the fruits. Furthermore, no conclusion can be drawn as to the fruit content.

In contrast to the pectin fractions, the hemicellulose fraction has been found to be particularly suitable for the characterisation of the fruit species in complex matrices, e.g. fruit preparations, jams and spreads. Prior to the isolation of the hemicellulose fraction, hydrocolloids which are present in these products may be extracted together with the pectin originating from the fruit. From Table 7 it becomes evident that the genuine profile of monosaccharides in this fraction is specific enough to serve as a fingerprint. For example, high contents of arabinose and galactose are typical for cherries, while the glucose content is significantly lower than in strawberries and apples. Most interestingly, the adulteration of cherry products with cheaper apple puree would easily be detected by the increase of the arabinose proportions. Our preliminary studies on the composition of the pear hemicellulose fraction revealed that an admixture of pear puree would be detectable in the same way. However, the adulteration of strawberry products by blending with apples cannot be detected, owing to the very similar neutral sugar profiles. In this case, analysis of the polyphenolic compounds would be needed for further differentiation.

The hemicellulose fraction is also of particular interest for the quality control of fruit products, i.e. for the determination of their fruit content. Methods described for the quantification of the fruit content are mostly based on the determination of low molecular compounds such as amino acids, organic acids, and minerals. However, these methods lack in reliability, since low molecular compounds can easily be added in order to feign a higher fruit content. Furthermore, their contents

are largely affected by ingredients such as colouring foodstuffs and consumable acids.

In contrast, hemicellulose as a high molecular compound of the cell wall represents a marker which is much less susceptible to adulterations and is not affected by food ingredients usually added to fruit preparations, spreads and jams. As shown in the present study, the hemicellulose fraction can easily be isolated from the fruit matrix and determined gravimetrically. From Table 2 it becomes evident that within a given fruit species only very minor variations of the hemicellulose content were found. Consistent with the results reported by Hegde and Maness (1996), neither cultivar nor provenience and stage of ripeness affected its content. Our ongoing investigations have shown exceptional stability of the hemicellulose fraction during processing of strawberry fruit preparations (data not shown), which is an indispensible prerequisite for the determination of the fruit content.

4. Conclusion

Sequential fractionation of the AIR and subsequent determination of the neutral sugar composition may serve as a tool for authenticity control of fruit products such as purees which are devoid of carbohydrate-based hydrocolloids. More complex products, e.g., fruit preparations, spreads, and jams may be characterised by the isolation of the hemicellulose fraction and subsequent determination of its neutral saccharide profile, allowing the differentiation of fruit species. Most interestingly, the hemicellulose fraction has also been shown to be a promising marker for the determination of the fruit content. This novel approach to quality and authenticity control has already been applied to strawberry fruit preparations. These results will be presented in a forthcoming communication.

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