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Analytical Methods

Evaluation of fruit authenticity and determination of the fruit content of fruit products using FT-NIR spectroscopy of cell wall components

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ABSTRACT

An analytical procedure using Fourier transform near infrared (FT-NIR) spectroscopy and chemometrics with multivariate techniques for the rapid determination of the fruit authenticity and for the quantification of the fruit content was developed, based on the cell wall constituents (alcohol-insoluble residue, AIR, and hemicellulose, HC). The contents of rhamnose, fucose, arabinose, xylose, mannose, galactose, and glucose in the hemicellulose fraction of apricots, peaches, and pumpkins determined by gas chromatography were used as references. Furthermore, spectral information was correlated with the fruit content and the gravimetric data obtained from sequential fractionation of the alcohol-insoluble residue. Samples of self-made and commercial apricot and peach fruit preparations, jams, and spreads were included in the investigations. Hemicellulose from 109 samples and AIR from 92 samples was recorded, and principal component regression was used to create calibration models relating chemical and gravimetrical reference values to spectral data. The calibration models provided a good predictability in comparison with the results obtained by reference methods. Good agreement was also obtained for the prediction of the neutral sugar composition of the HC and the fruit content from the AIR. FT-NIR spectroscopy allowed a rapid, accurate and non-destructive assignment of specified fruit from spectral data of the HC fraction and the AIR. Thus, FT-NIR could be applied for investigations on quality control complementing, or even replacing, gas chromatography as the most widespread method for the determination of neutral sugars. Furthermore, preliminary investigation on classification of fruit blends was performed. The probability of belonging to a specific fruit was significantly lowered or precluded for samples with fraudulent admixture like peach or pumpkin to apricot, or pumpkin to peach.

1. Introduction

Jams, spreads, and fruit preparations are high-value foodstuffs which are a potential target for adulteration by unscrupulous producers. Determination of the fruit authenticity and fruit content is a prerequisite for quality control and consumer protection. Food inspectors and producers need analytical tools to verify the nature of high-value foods. As a rapid, inexpensive, and non-destructive method, near infrared (NIR) spectroscopy has been widely implemented in the food industry for analysing ingredients in foodstuff (Downey, Kelly, & Petisco Rodriguez, 2006). It can be used without sample preparation and provides the advantage that more than one quality parameter can be monitored at the same time. Furthermore, the method also allows replicate measurement. Infrared spectroscopy has already been applied in a number of studies on the characterisation and structural analysis of macromolecular cell wall compounds, e.g. pectin, hemicellulose (HC), cellulose, and lignin (Coimbra, Barros, Rutledge, & Degadillo, 1999; Séné, McCann, Wilson, & Grinter, 1994; Workman, 2001). Polesello, Giangiacomo, Forni, and Braga (1990) previously applied NIR spectroscopy to estimate pectic substances in fruit and fruit products with good prospects under optimised experimental conditions. Ferreira, Barros, Coimbra, and Delgadillo (2001) used FT-IR spectroscopy to investigate the effects of processing on cell wall polysaccharides of pears. Spectroscopic techniques in the visible, near- and mid-infrared regions, which have been used with some success to detect and quantify food adulteration and even for determination of fruit content, were summarised by Fügel, Carle, and Schieber (2005a). However, investigations on the adulteration of apricot and peach products are lacking and the technique is limited by product composition, especially in complex systems like jams, spreads and fruit preparations (Defernez & Wilson, 1995). Furthermore, only a few IR investigations focused on cell wall components in relation to the evaluation and determination of vegetable or fruit authenticity (Belton et al., 1995; Kemsley et al., 1994). Particularly, NIR spectroscopy of hemicellulose has so far not received much attention with respect to quality and authenticity control.

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In our former investigations on quality and authenticity control of fruit products we have demonstrated that the neutral sugar profile of the HC fraction and the carotenoid profiles represent diagnostically important tools for plant species determination (Kurz, Carle, & Schieber, 2008a, 2008b). Furthermore, approaches described by Kurz, Münz, Schieber, and Carle (2008), Schieber, Fügel, Henke, and Carle (2005), Fügel, Förch, Carle, and Schieber (2005b) and Fügel, Schieber, and Carle (2006) showed that the HC fraction is a suitable parameter for fruit content determination. However, the methods reported require extensive sample preparation and therefore allow the investigation only of a limited number of samples, which represents a major disadvantage for its broader application in the food industry and food inspection board laboratories. Therefore, our earlier approach was combined with FT-NIR spectroscopy. The objective of the present study was to evaluate the potential of NIR as a complementary technique for authenticity control and determination of fruit content via cell wall components.

Although there are numerous potential ingredients that could be used in apricot products for fraudulent purposes, peaches and pumpkins were considered the most likely candidates, since peaches are cheaper than apricots, and pumpkins usually contain relatively large amount of carotenoids and may therefore be used to improve the visual appearance of fruit products and feign higher fruit contents.

Our investigations included the spectra of pure apricots, peaches, and pumpkins, as well as apricot and peach purées and apricot jams which have been blended with pumpkin to mimic fraudulent practices. The majority of samples have been prepared from fresh fruits purchased from the local market, or from IQF fruits. In addition, products supplied by industry collaborators and commercial products were investigated.

2. Materials and methods

2.1. Materials

2.1.1. Plant material

Fresh apricots (*Prunus armeniaca* L.) and peaches (*Prunus persica* L.) of different cultivars and provenances and yellow-fleshed pumpkins (*Cucurbita* sp.) were obtained from the local market (Stuttgart, Germany). IQF fruits (individually quick frozen after lye peeling (peaches) and blanching) were provided by Wild (Eppelheim/Heidelberg, Germany). Fresh apricots and peaches were (lye) peeled and manually cored. An aliquot of selected fruit varieties remained unpeeled to determine the influence of (lye) peeling. The pumpkins were manually or lye peeled, cored, blanched at 85 °C for 10 min, and subsequently mashed through a sieve (mesh size: 1.5 mm). In total, 10 different cultivars of apricots, 14 cultivars of peaches and 9 pumpkin cultivars from three pumpkin species (*Cucurbita maxima* Duch., *Cucurbita pepo* L., and *Cucurbita moschata* Duch.) were investigated.

Blended products ("adulterated" samples) were prepared from apricot purées (cv. Bergeron), which were produced from cored fruits treated as described above and mixed with peach (unknown cultivar) and pumpkin purée (cvs. Muscade de Provence and Halloween) in proportions of 95:5, 90:10, 85:15 and 80:20, respectively. Two samples of peach purée were blended with 20% of pumpkin purée cvs. Muscade de Provence and Halloween, respectively. Fruits were harvested from 2003 to 2006. The fruits of the 2003 and 2004 harvests were stored, lyophilised in a Steris Lyovac GT 4 Lyophiliser (Steris, Hürth, Germany) and frozen at −20 °C, respectively, to avoid enzymatic degradation of the cell wall material. Subsequently, the dried product was finely ground in a pre-cooled cutter (UM12, Stephan and Söhne, Hameln, Germany) with liquid nitrogen.

2.1.2. Hydrocolloids

For the production of fruit preparations, modified maize starch NATIONAL 67-0029 from National Starch (Bridgewater, NJ, USA), xanthan "Rhosigel Easy" (Rhodia, Melle, France) and amidated pectin (Pectin Amid AF 010-A) from Herbstreith and Fox (Neuenbürg, Germany) were used. Carrageenan "Gelcarin DG 5264" was purchased from FMC Biopolymer (Brussels, Belgium). For the production of jams, high esterified pectin (Pectin Classic AF 401) provided by Herbstreith and Fox (Neuenbürg, Germany) was used. Pectins were dispersed in distilled water using an Ultra-Turrax blender (IKA, Staufen, Germany) before admixture to the fruits. Starch and xanthan were blended with sucrose (Südzucker, Mannheim, Germany).

2.1.3. Enzymes

For the enzymatic degradation of the hydrocolloid matrices the following preparations were used: Fructamyl HT containing a solution of amylolytic enzymes was kindly provided by Erbslöh (Geisenheim, Germany). Hazyme DCL comprising α-amylase and amyloglucosidase was a gift from DSM Food Specialities (Seclin, France). Galactomannanase granules (0.2 U/mg) from *Aspergillus niger* were obtained from Sigma–Aldrich (Steinheim, Germany).

2.2. Production of fruit products and sample preparation

2.2.1. Fruit preparations

Peach fruit preparations were produced in quantities of 1.0 kg according to industrial recipes. For this purpose, thawed peach cubes, water, sugar, and the hydrocolloids were blended. The mixture was heated at 96 °C for 6 min in a reaction vessel (EL 3, ESCO-Labor, Riehen, Switzerland). After heating, the fruit preparations containing starch were cooled to the digestion temperature (50 °C) and enzymes were added. Digestion by adding 0.75 mL/kg amylolytic enzymes was performed for 1 h. Subsequently, 1.5 mL/kg amyloglucosidase and 6 mg/kg galactomannanase were added and hydrocolloids were digested successively within 5 h. Industrial fruit preparations were heated at 50 °C and enzymes were added as described above. After incubation, the fruit preparations were cooled to room temperature, filled on metal trays and frozen at −20 °C in a deep freezer for 24 h. The frozen samples were lyophilised for 120 h in a Steris Lyovac GT 4 Lyophiliser (Steris, Hürth, Germany). Subsequently, the dried product was homogenised to a fine powder in a cutter (UM12, Stephan and Söhne, Hameln, Germany) pre-cooled with liquid nitrogen.

2.2.2. Jams

The jams (1.0–2.0 kg) were prepared by cooking of a defined amount of pure apricot purée or blended purée with inverted sugar (72.7° Brix, Schließmann, Schwäbisch Hall, Germany) and/or sucrose (Südzucker, Mannheim, Germany) and high esterified pectin (Classic AF 401, Herbstreith and Fox, Neuenbürg, Germany) under reduced pressure in a reaction vessel (EL 3, ESCO-Labor, Riehen, Switzerland) at 75 °C until a dry matter of 60% was obtained. Subsequently, the jams were cooled to room temperature, acidified with citric acid to pH 3, filled on metal trays, frozen at −20 °C for 24 h, freeze-dried and powdered with liquid nitrogen as described above. Randomly selected commercial apricot jams and spreads purchased in the central market in Stuttgart were filled on metal trays, frozen at −20 °C, then freeze-dried and powdered with liquid nitrogen.

2.3. Isolation of the alcohol-insoluble residue (AIR)

Quantities of 25 g of lyophilised fruits and 50 g of fruit products, respectively, were homogenised in boiling aqueous ethanol (300 mL, 80%, v/v) using an Ultra-Turrax blender (IKA, Staufen,

Germany). After boiling for 1 h, the insoluble solids were collected on a Büchner funnel. Ethanol extraction was repeated 5 or 6 times until a clear extract was obtained. The AIR was stirred overnight in acetone, passed through a G1 glass sinter filter and air-dried at 50 °C for 24 h in a compartment drier (UT 6120, Heraeus, Hanau, Germany). The dried AIR was ball-milled (Retsch, Haan, Germany), sealed in lever lid glass bottles and kept in a desiccator until further analysis. Alternatively, the enzymatic digestion of the hydrocolloids was performed after AIR isolation. For this purpose, 3.8 g of AIR was suspended in 150 mL McIlvaine buffer (pH 4.18). Digestion was also performed by amylolytic enzymes (80 μL/g AIR) and amyloglucosidase (150 μL/g AIR) under the conditions described above. After enzymatic treatment, the suspension was centrifuged at 15,000*g* for 20 min, and boiling ethanol (96% v/v) was added to the filtrate to precipitate residual macromolecular compounds. Pellets were collected on a Büchner funnel, stirred in acetone overnight, dried, and stored as described above.

2.4. Sequential extraction of the AIR

The AIR (800–1000 mg) of raw materials and products was suspended in 50 mL of hydrochloric acid (0.05 M) and stirred at 60 °C for 1 h. After centrifugation at 15,000*g* for 20 min, the pellet was washed twice with 50 mL of distilled water. For further extraction of pectins from the residue, alkaline EDTA solution (0.05 M NaOH; 0.5 mM EDTA) was added at 30 °C for 1.5 h. The suspension was centrifuged for 20 min at 15,000*g*, and the pellet washed twice with distilled water. The final hemicellulose extraction was carried out using 50 mL of aqueous sodium hydroxide solution (16%, w/w) for 5 h at 30 °C. After centrifugation at 15,000*g* for 20 min, the pellet was rinsed twice with distilled water. The supernatants were pooled and adjusted to pH 6.5–7.0 with HCl, dialysed exhaustively against distilled water for 48 h using dialytic membranes (Visking, type 36/32, pore size 25–50 Å, Roth, Karlsruhe, Germany) and freeze-dried to yield the hemicellulose (HC) fraction. The remaining pellet consisted of insoluble solids such as lignin and cellulose.

The hemicellulose fraction was ball-milled in an oscillating grinder, which was pre-cooled with liquid nitrogen. The milled hemicellulose was sealed in glass vials and stored in a desiccator until FT-NIR analysis.

2.5. Chemical reference analyses

2.5.1. Hydrolysis of the HC fractions

HC fractions were hydrolysed with sulphuric acid. For this purpose, 300 μL of 2-propanol and 300 μL of H_2SO_4 (72%, w/w) were added to 20–30 mg of the HC fraction. 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 in a sealed Pyrex glass test tube. The hydrolysate was neutralised with 750 μL of barium hydroxide saturated aqueous ammonia (25%, w/w). The volume was made up to 10 mL and an aliquot was centrifuged at 14,100*g* for 2 min.

2.5.2. Analysis of neutral sugars by gas chromatography

The monosaccharides obtained after hydrolysis of the cell wall fractions were analysed by gas chromatography with flame ionisation detection (GC–FID). For this purpose, the neutral sugars from an aliquot of the hydrolysed cell wall fractions were reduced with sodium borohydride and derivatised to their alditol acetates with acetic acid anhydride, ethanol, and 1-methylimidazole as the catalyst. For derivatisation, 1 mL of the hydrolysed sample, 100 μL of the internal standard (2.0 g/L *myo*-inositol) and 100 μL of ammonia (12 mol/L) were mixed. Subsequently, 100 μL of freshly prepared sodium borohydride solution (750 mg $NaBH_4$, 1.25 mL ammonia (12 mol/L) and 3.75 mL distilled water) was added. After 1 h at 40 °C, 100 μL of glacial acetic acid was pipetted and tempered at 20 °C. Aliquots of 500 μL were transferred in a glass tube and mixed with 500 mL of cold 1-methylimidazole and 5 mL of acetic acid anhydride. After 10 min at room temperature, 750 μL of absolute ethanol was added. After another 10 min, 5 mL of distilled water and 10 mL of potassium hydroxide solution (7.5 mol/L) were added. The upper layer was transferred to vials and stored over anhydrous sodium sulphate overnight at −20 °C until analysis. Aliquots of 1 μL were injected with a split ratio of 1:10. Helium was used as the carrier gas at a column flow of 1.47 mL/min. The GC (Chrompack CP 9001, Chrompack, Middleburg, NL) was fitted with a 30 m × 0.25 mm i.d., 0.15 μm fused silica capillary column (DB-225, J&W Scientific, Folsom, CA, USA) and equipped with a flame ionisation detector operated at 240 °C. The oven temperature was kept at 140 °C for 2.5 min, subsequently increased to 200 °C in 2 min and held isothermally for 4.5 min, followed by an increase to 220 °C in 1 min and finally an isothermal hold at 220 °C for 18 min. Individual sugars were identified by comparison of their retention times with those of authentic standards of D(+)-galactose, D(+)-glucose-monohydrate, D(+)-xylose, L(+)-arabinose, L(+)-rhamnose-monohydrate (Merck, Darmstadt, Germany), D(+)-mannose (Serva, Heidelberg, Germany), and L(−)-fucose (Sigma Chemical, St. Louis, MO, USA), and quantified using *myo*-inositol (Merck, Darmstadt, Germany) as an internal standard. Data analysis was carried out with the Maestro II 2.4 version software (Chrompack, Middleburg, NL).

2.6. FT-NIR measurement

Near-infrared analysis was performed in the reflectance mode using Fourier transform near infrared spectroscopy, Spectrum Identicheck (Perkin Elmer, Norwalk, USA) equipped with an Identicheck Reflectance Accessory (ICRA) and a lead sulphide detector. The reflected NIR light was collected in the integrating sphere. The sampling port was tightly fitted to the dimension of the vials by a black paperboard to prevent the access of ambient light.

Powdered AIR and HC samples in glass vials were scanned from 650 to 2500 nm at 0.2 nm intervals with a resolution of 4.0 cm^{-1}. Each scan represents 9251 points. Individual samples were scanned 16 times to acquire an automatically calculated average NIR spectrum. To eliminate the instrument characteristics, all spectra were collected against a white background on the sampling port during background scanning.

2.7. Data analysis

For the fruits and fruit products, the reflectance of the complete NIR spectra of the AIR and the HC fraction and the neutral sugar contents obtained from GC analysis of the HC fraction were subjected to multivariate calibration techniques using principal component analysis (PCA) by Spectrum Quant+ software (Perkin Elmer). This software allows the statistical analysis of the data sets and the construction of the prediction models. The quality of the calibration model was characterised by the coefficients of determination (R^2) between the predicted and measured parameters, the standard error of estimation (SEE), the standard error of prediction (SEP) and the number of principal components.

Initially, data were collected in the range of 650–2500 nm with an interval of 0.2 nm. The region from 650–1100 nm did not improve calibration and showed large intensity variations (which is probably noise). Transmission data were transformed into absorbance spectra for optimal calibration of the NIR data. Subsequently, auto scaling and final correction of the baseline offset was performed prior to calibration. No smoothing was applied. Normalisation was performed by standard normal variate (SNV) (Candolfi, De Maesschalck, Jouan-Rimbaud, Hailey, & Massart, 1999), which

effectively removes the multiplicative interferences of scatter and particle size that are a particular problem with NIR reflectance spectra. SNV was used without de-trending, which accounts for the variation in baseline shift and curvilinearity also occurring in NIR diffuse reflectance spectra. Calibration performance was not improved by the mathematical transformation of the spectra to first and second derivative. Blank regions and multiple scatter correction did not advance the method. Full cross validation was applied to avoid overfitting, validate the calibration model, and generate the prediction model. The validation procedure started by omitting one sample from the calibration set. From the remaining samples a calibration model was deduced to predict the state of the selected sample. Outliers were eliminated using expert mode from the data set during the calibration process. The algorithm PCR+, a principal component regression algorithm, was used (Perkin Elmer, 1994, chap. 7). PLS 1 and PLS 2 algorithms working with partial least squares were not able to establish a successful calibration.

Classification studies were performed using soft independent modelling class analogy (SIMCA) from spectral data at wavelength ranges from 650 to 2500 nm (Perkin Elmer, 1996). Classification models of each fruit were developed using the spectral data from the HC fraction and the AIR, respectively, of pure fruits and authentic mono fruit products, i.e., products only consisting of the named fruit.

3. Results and discussion

3.1. Methodology

The objective of this study was to simplify the methods for authenticity and quality control of fruit products by isolation and characterisation of cell wall components (Kurz et al., 2008a; Kurz, Münz, et al., 2008). The major problem consisted in the distinction of fruit species belonging to the genus *Prunus* L. (Rosaceae), i.e., peaches (*P. persica* L.) and apricots (*P. armeniaca* L.), and to differentiate between these and pumpkins (*Cucurbita* sp.) using NIR spectral data of their AIR and HC fractions. The neutral sugar contents of the HC fraction determined by gas chromatography and gravimetrical data obtained from sequential fractionation were used as references. Additionally, the prediction of the fruit content from the spectral data of the HC fraction and the AIR was evaluated.

Reference data on the composition of apricots, peaches, pumpkins, and mono fruit products obtained from chemical and gravimetrical analysis are shown in Table 1. Neutral sugar amounts from a part of the HC fractions from apricots, peaches, and pumpkins investigated in the present study have been previously published by Kurz et al. (2008a). The data from the former study was complemented with data obtained in the present investigation on fruits and fruit containing products. For some HC samples the corresponding AIR sample failed as it was completely used up for the fractionation procedure in the former studies.

Table 2
NIR calibration statistics for individual parameter of all HC fraction samples.

Property	No. of principal components	Coefficient of determination (R^2)	SEE	SEP	Mean value[a] (%)
Rhamnose	11	0.63	0.38	0.42	0.83
Fucose	26	0.95	0.36	0.44	3.16
Arabinose	32	0.96	1.11	1.39	7.29
Xylose	23	0.97	1.53	1.92	22.77
Mannose	27	0.96	1.00	1.24	7.76
Galactose	32	0.96	1.18	1.65	14.95
Glucose	14	0.98	3.04	3.58	42.15
AIR (g/100 g DM)	13	0.87	5.32	5.69	15.31
HC (g/100 g AIR)	7	0.85	4.55	4.91	14.57
Fruit content	23	0.88	9.94	12.99	80.92

DM = dry matter.
[a] Mean value of the respective property from all investigated samples.

The NIR region ranges from 750 to 2500 nm. This region consists only of overtone and combination bands of fundamental molecular vibrations. The effective range for calibration in this study was 1100–2500 nm. No spectral information could be obtained from wavelengths below 1100 nm, probably because of high absorbance of the powdered sample caused by oxidised polyphenols, which could be visually detected in the ground powder. Mathematical pre-treatment processes such as auto scaling and correction of baseline offset needed to be applied to the raw spectra data before calibration.

3.2. Calibration method of the HC fraction and the AIR

Some preliminary conclusions could be drawn from visual inspection of the spectra. As expected, the spectra of the cell wall material from fruits of the genus *Prunus* L. were very similar. The spectra obtained from the HC fraction isolated from pumpkins were more heterogenous (data not shown). This is attributed to the variability of the neutral sugar composition of their HC fraction, which was previously reported by Kurz et al. (2008a) and confirmed in the present investigation (Table 1). The quality of the calibration models and the high correlation of the NIR spectra of the samples with the neutral sugar contents determined by gas chromatography was verified by a high coefficient of determination (R^2), and a low standard error of estimation (SEE) and prediction (SEP).

3.2.1. Calibration model for HC composition

Due to the low contents of rhamnose in the hydrolysate of the HC fraction, only a moderate R^2 value of 0.63 was found from the spectral data of the HC fraction (Table 2). The best calibration results for neutral sugars were obtained by correlation of HC fraction spectral data with specified contents of fucose, arabinose, xylose,

Table 1
Composition of apricots, peaches, pumpkins, and mono fruit products obtained from chemical and gravimetrical reference analysis.

Samples (numbers)	Rhamnose	Fucose	Arabinose	Xylose	Mannose	Galactose	Glucose	AIR (g/100 g DM)	HC (g/100 g AIS)
	Range (%) of neutral sugar content in the HC fraction								
Apricots (17)	0.5–1.8	2.9–3.6	4.0–7.2	23.2–27.8	9.8–12.4	16.6–19.3	31.5–38.3	9.4–21.9	9.7–15.3
Apricot jams, spreads (22)	0.0–1.3	2.8–4.1	3.5–6.0	23.0–33.2	10.5–24.0	16.2–19.9	26.7–38.9	1.3–9.3	4.1–13.1
Apricot fruit preparations (4)	0.0–0.5	1.4–3.3	2.6–4.8	12.99–24.79	6.10–12.10	9.05–17.65	37.47–67.91	1.95–11.31	8.91–14.47
Peaches (27)	0.0–1.5	4.2–5.1	10.2–16.8	21.7–27.7	3.9–7.4	14.8–17.5	32.7–39.2	12.9–18.9	11.9–17.5
Peach jam (1)	1.1	4.5	14.4	26.4	5.2	15.5	32.8	1.3	13.5
Peach fruit preparation (4)	0.0–0.6	4.1–4.6	13.7–14.6	21.9–23.2	4.3–5.4	15.6–16.5	36.6–39.9	4.0–13.6	11.5–124.3
Pumpkins (18)	0.0–5.0	0.0–2.8	0.0–6.1	0.0–55.2	0.0–1.0	0.0–27.3	29.5–98.5	18.3–64.5	4.4–64.1

mannose, and galactose. R^2 ranged from 0.95 to 0.97, with corresponding low SEP of 0.4–1.2. Glucose showed also high R^2 but also a relatively high SEP of 3.6. Prediction of arabinose, xylose, and mannose can serve as a tool for authenticity evaluation, whereas glucose and galactose are less suitable, due to their similar contents and the highly variable amounts of glucose originating from residual starch in the HC fraction of pumpkins. These findings are in accordance with the results reported by Kurz et al. (2008a).

Although the content of AIR is not directly related to the amount of HC, NIR reflectance showed significant correlation (R^2 = 0.87) and the R^2 for the HC content in the AIR was 0.85. However, the SEE and the SEP were not satisfactory (Table 2). The correlation of the specified fruit content of the products with the spectral data of the HC fraction was in an acceptable range. The R^2 of the fruit content from the HC spectra was 0.88. Standard SEE (9.94) and SEP (12.99) were rather high. Thus, the prediction of the hemicellulose and fruit contents from the spectral data of the HC fraction is more limited than the estimation of individual neutral sugar contents. Consequently, the fruit content can be determined more accurately by gravimetric quantification of HC (Kurz et al., 2008b) than by NIR spectroscopy. Since the work of isolation and fractionation of cell wall material to obtain the HC fraction for NIR spectral investigations on the fruit content is the same, NIR does not provide any advantage.

3.2.2. Calibration model for composition of the AIR

Table 3 shows the results obtained from the calibration method correlating spectral data of the AIR with the neutral sugar composition of the HC fraction hydrolysate, gravimetrical data obtained by isolation and fractionation of the AIR and the specified fruit content. In this approach it should be evaluated whether authenticity control and determination of fruit content can be accomplished by characterisation of the AIR, the isolation of which is simple and rapid, as opposed to the isolation of the HC fraction. From comparison of calibration results of the HC fraction (Table 2) with those of the AIR (Table 3) it becomes obvious that the accuracy of the calibration method decreased for the amounts of monosaccharides. Correlating the neutral sugar contents obtained from gas-chromatographic determination of the HC fraction with the AIR spectral data decreased R^2, whereas SEP increased by a factor of two. Only galactose provided an identically high coefficient of determination. These findings were expected since the AIR contains a multitude of macromolecular components like hydrocolloids and proteins. Thus, correlation of AIR spectral data with neutral sugar contents of the HC fraction is influenced by the more complex nature of matrix components decreasing the determination coefficients.

Table 3
NIR calibration statistics for individual parameter of all AIR samples.

Property	No. of principal components	Coefficient of determination (R^2)	SEE	SEP	Mean value[a] (%)
Rhamnose	18	0.64	0.40	0.54	0.79
Fucose	25	0.89	0.53	0.72	3.15
Arabinose	22	0.85	2.09	2.73	7.29
Xylose	11	0.78	4.41	6.21	23.00
Mannose	13	0.84	1.91	2.30	7.70
Galactose	16	0.96	1.10	1.42	14.91
Glucose	14	0.90	6.78	8.66	42.82
AIR (g/100 g DM)	28	0.98	2.57	3.37	15.38
HC (g/100 g AIR)	26	0.98	1.96	2.53	15.14
Fruit content	28	0.94	7.00	9.93	82.39

DM = dry matter.
[a] Mean value of the respective property from all investigated samples.

Spectral data of the AIR correlated very well with the gravimetrical data from fractionation procedure and the fruit content (Fig. 1; Table 3). Although for the fruit content the R^2 value of 0.94 was good, the corresponding SEP of 9.93 was poor. Standard deviation and deviation from specified fruit content of the determined fruit content by gravimetric quantification of the HC fraction did not exceed 6.0% and 6.5%, respectively (Kurz et al., 2008b). According to these results, the gravimetric method has a better accuracy than the NIR method. On the other hand, the NIR method provides a number of advantages, since no fractionation is required and the measurements can be carried out even by untrained personnel. A MIR (mid-IR) method for the determination of the fruit content of strawberry, apricot, raspberry, plum, and bramble jams reported previously provided a correlation of 0.94 between fruit weight and the area of a specific band at 1725 cm^{-1}, thus allowing the quantification of fruit content. However, the potassium bromide (KBr) technique required tedious sample preparation restricting the determination to only few samples per day and still needed some improvement (Wilson, Slack, Appleton, Sun, & Belton, 1993).

3.3. Classification of spectral data of HC faction and AIR from fruits and fruit products

Classification was performed by class-dividing of the fruits and products to apricot, peach, pumpkin, and apricot and peach products and the correlation of their spectral data of the HC fraction and the AIR. Classification results are summarised in Table 4. The

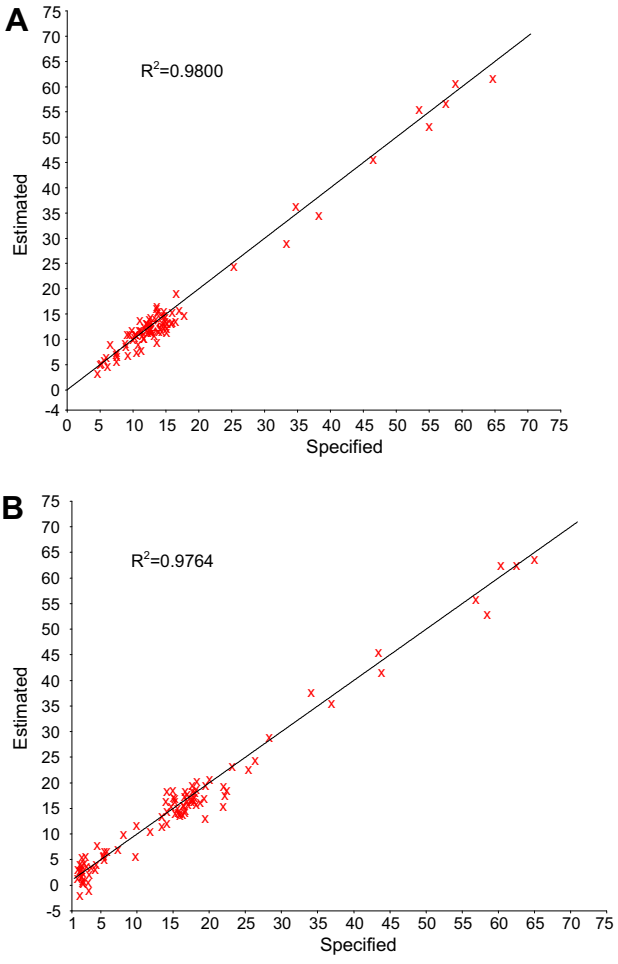

Fig. 1. Calibration plot of estimated versus specified (A) HC content (g/100 g AIR) and (B) AIR content (g/100 g dry matter) from AIR spectral data.

Table 4
Results of the classification of the HC and AIR spectra based on authentic fruit samples.

Class	% Recognition rate	% Rejection rate	Inter class distances	
			Peach	Pumpkin
Apricots HC	94 (33/35)	96 (43/45)	5.7	26.93
Pumpkin HC	100 (18/18)	100 (62/62)	28.79	–
Peaches HC	93 (25/27)	100 (53/53)	–	28.79
Apricots AIR	100 (31/31)	98 (40/41)	17.72	28.39
Pumpkin AIR	100 (17/17)	100 (55/55)	82.78	–
Peaches AIR	100(24/24)	98 (47/48)	–	82.78

critical probability level was 0.01, corresponding to a probability of 99%. The accuracy of classification models was assessed on the basis of the number of false positive and false negative results. False positive means that a sample is erroneously identified as belonging to a specific class. This was observed for two peach HC samples, which were classified as apricots with a 5.3% and 2.0% probability, respectively. With respect to the relatively low inter class distance between HC from apricots and peaches (Table 4) and minor percentage of misclassification probability, a satisfactory differentiation between the two fruits of the genus *Prunus* L. can be accomplished. Furthermore, one apricot HC fraction and one HC sample isolated from apricot jam were misclassified but not assigned to a specific class. The probability of belonging to the apricot class was only 12.1% for one peach HC spectra. The spectrum of HC material from one apricot jam was not unique and had only a 2.4% probability of belonging to the apricot class. Accordingly, two peach samples had a low probability of belonging to the peach class (32.8% and 42.4%) and could therefore not be assigned.

Due to the higher inter class distances, the accuracy of the classification model was better for the AIR. The recognition rate was 100% for all classes, and a false positive classification was observed only for two fruit samples. One apricot sample was misclassified with a 13.7% probability of belonging to the peach class. Accordingly, one peach AIR was erroneously classified to the apricot class with a probability of 2.4%. However, the probability of belonging to the correct class was 99.9% for the peach and 88.6% for the apricot sample. Defernez and Wilson (1995) also obtained a differentiation rate of almost 100% investigating diffuse reflectance IR spectra of strawberry and non-strawberry jams, however, their preparation method was lengthy and still needed some refinement.

Failure to detect adulterations results only by the false positive results, since false negative can be verified by alternative analytical methods, for example neutral sugar composition and carotenoid profiles (Kurz et al., 2008a, 2008b). HC fraction and AIR spectra from adulterated samples were recognised by missing classification or untypical spectral data. Based on these results, the method developed does possess the necessary classification accuracy to be applied in practice. However, the method showed limitations when apricot products have been treated enzymatically for hydrocolloid degradation. Enzymatic digestion before and after AIR isolation resulted in absent classification of the respective AIR and HC fractions. Although the enzymes used for hydrocolloid decomposition in the fruit preparations were shown to be devoid of hemicellulolytic side activities (Schieber et al., 2005), enzymatic digestion affected the neutral sugar composition of their HC fraction and consequently the spectral data. From Table 1 it can be seen that apricot fruit preparations which have been treated enzymatically to remove hydrocolloids display deviating neutral sugar proportions compared to apricot fruits; in particular xylose, galactose, and glucose amounts differ significantly. Similar effects of enzymatic maceration on polysaccharide spectral data were also reported by Coimbra, Gonçalves, Barros, and Delgadillo (2002). In contrast, the neutral sugar composition of peach fruit preparations did not differ from that of the fruit although the same enzyme preparations have been used for hydrocolloid digestion. However, the investigated commercial apricot fruit preparations contained Chinese fruits of unknown cultivar and pre-treatment (Kurz et al., 2008b), which may result in a different composition of the HC fraction.

Owing to practical limitations by the preliminary fractionation method, only cell wall material of apricot purée (cv. Bergeron) blended with mash from two pumpkin cultivars (Halloween and Muscade de Provence, respectively) and one unknown peach cultivar were selected. Additionally, selected blends containing 5%, 10%, 15%, and 20% of the admixed fruit were included in the study. Differentiation of intentionally "adulterated" samples from the authentic ones could be accomplished even at admixtures of 5%. Blended samples were not assigned to a specific class, or classification was characterised by a significantly lowered probability, as can be seen in Table 5 for HC fraction of blended apricot puree and jam. Class identification was better from the AIR spectral data than from the HC fraction (Table 5). Apparently, other macromolecular compounds, e.g. proteins which were still included in the AIR but removed during isolation of the HC, enhanced fruit differentiation. No correlation between the admixed proportion of non-authentic fruit product and probability of class identification was observed. Accuracy of classification of the present study is similar or even better compared to former investigations on FT-IR spectroscopy of adulterated fruit purées (Contal, León, & Downey,

Table 5
Classification of HC and AIR from blended apricot and peach samples.

Product	Cell wall component	Class identification (% probability)											
		Peach proportion (%)				Pumpkin (Halloween) proportion (%)				Pumpkin (Muscade de Provence) proportion (%)			
		5	10	15	20	5	10	15	20	5	10	15	20
Apricot purée	HC fraction	n.i	Apricot* (11.18)	–	–	n.i	n.i	n.i	–	n.i	n.i	n.i	–
Apricot jam	HC fraction	Apricot (13.98)	–	–	–	Apricot* (7.69)	–	Apricot (46.64)	Apricot (41.42)	Apricot* (12.45)	–	Apricot (14.35)	Apricot (5.96)
Apricot purée	AIR	n.i	n.i	n.i	–	n.i	n.i	n.i	–	n.i.	n.i	n.i	–
Apricot jam	AIR	–	–	n.i	–	–	n.i	n.i	–	–	–	n.i	–
Peach purée	AIR					n.i	n.i	n.i	Peach (46.06)	n.i	n.i	n.i	–

n.i, Not investigated.
*, Not unique – second closest match is not significantly different from closest match.
–, None.

2002; Defernez, Kemsley, & Wilson, 1995; Downey & Kelly, 2004; Holland, Kemsley, & Wilson, 1998; Kemsley, Holland, Defernez, & Wilson, 1996). However, the detection of adulteration of closely related apricots and peaches and derived products has not yet been investigated using FT-IR spectroscopy. Furthermore, since sugars and consumable acids are eliminated from the AIR during the isolation process, these abundant food ingredients do not affect the spectral data, as reported in former studies (Defernez & Wilson, 1995).

An important factor limiting the applicability of methods for routine authenticity control is their stability over time, as instrument drift, seasonal variations and peeling of the fruit may influence the results. To consider fruit matrix related problems, the study included the investigation of peeled and unpeeled fruit samples harvested 2003–2007. The results showed that the method is not limited by the harvest year and (lye) peeling treatment, respectively (data not shown) and could be applied in subsequent seasons. Thus, the model does not require calibration for each year's fruit, which makes the technique practical for routine screening of AIR and HC obtained for the determination of fruit content over extended periods of time. Nevertheless, further work, also on other fruits, is still required to establish fruit libraries and complement, optimise and implement this technique in daily routine.

4. Conclusion

Compared to former methods on authenticity control and determination of fruit content developed by us, FT-NIR spectroscopy is an easy and rapid technique. This method should contribute to the practicability of the quality and authenticity control of fruit products by isolation of the HC fraction. In a preliminary investigation the potential of NIR spectroscopy for direct differentiation of AIR and HC fraction from apricots, peaches and pumpkins and derived products is described. The multivariate calibration required the preliminary extraction and fractionation of the cell wall components, as well as gas-chromatographic investigation of the neutral sugar profiles of the HC fraction. However, establishing of comprehensive databases of the spectral data of economically important apricot, peach, and pumpkin cultivars and related products leads to easy sampling and reliable authenticity measurement of AIR and HC isolated from fruits and fruit products.

The suitability of the HC fraction for the determination of the fruit content could be clearly demonstrated (Fügel et al., 2005a, 2005b, 2006; Kurz et al., 2008b). The use of FT-NIR spectroscopy of the AIR represents an important improvement of the practicability in comparison with the determination of the fruit content via gravimetric quantification of the HC fraction. Far from being a rapid determination method, the fractionation procedure of the AIR is required for exact fruit content and neutral sugar determination. Varying processing parameters, particularly with regard to enzymatic treatments and complex recipes recommend the reported fractionation of the cell wall constituents and isolation of the HC fraction for determination of the fruit content with the utmost meticulousness.

However, the NIR method proved to be suitable for fruits, jams, and spreads and is more practicable since it does not require gravimetric determination of AIR fractions. Therefore, the AIR isolation and fractionation procedure and the measurements can be carried out even by less qualified and experienced personnel.

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