

Spectroscopic Evaluation of the Surface Quality of Apple

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Different spectroscopic techniques based on infrared and Raman were used to evaluate the natural wax and related surface quality of apple fruit. Transmission near-infrared (NIR) spectroscopy was applied to solutions of single wax components and extracted apple wax. Fourier transform infrared (FTIR) spectroscopy was used for transmission measurements of wax films on NaCl crystals, diffuse reflectance spectroscopy (DRIFTS) was used to analyze wax powders, and FT-Raman spectroscopy was explored to examine intact wax layers on whole fruit. The natural wax layers of apple fruit from a maximum of three different cultivars (Jonagold, Jonagored, and Elshof) from three picking dates (early, commercial, and late), three controlled atmosphere storage durations (0, 4, and 8 months), and three shelf life periods (0, 1, and 2 weeks) within each storage duration were examined. Canonical discriminant analysis was carried out on the first derivative NIR and FTIR spectra to describe the information contained in the spectra. Discrimination between cultivars and between storage duration based on wax layer properties was achieved with reasonable accuracy from both of the techniques. Information contained in the spectra of apples from different picking dates and shelf life periods was not significant. Differences between cultivars and storage periods in this analysis mostly related to differences in the number of aliphatic chains (e.g., alkanes and esters) and the presence of α -farnesene. No satisfactory results were obtained by means of Raman spectroscopy and DRIFTS.

KEYWORDS: Apples; wax; quality; NIR spectroscopy; FTIR spectroscopy

INTRODUCTION

To obtain optimal returns in apple production, it is important to deliver fruit of high internal and external quality with an extended storability. Especially the visual appearance of the fruit is an important factor determining the consumer demand. This visual appearance is determined by size, color, and intactness of the surface but also by the wax layer, which naturally covers the fruit. This natural wax layer is a secondary metabolite of the fruit, which is produced as a main function to protect the fruit from stress factors such as moisture loss, mechanical damage, and microbial infections. When it develops well, it also provides the fruit with an attractive shine and overall appearance. Natural wax layers tend to change their composition and structure when confronted with stress situations. While positive for offering optimal protection against the present stress, these changes do not always contribute positively to the organoleptic or visual appearance of the fruit. A different structure and composition can turn the wax greasy and sticky so that dust particles stick to the surface and give the apple an unpleasant

bloom. Of some apple cultivars, such as Jonagold, it is known that they are more susceptible to the development of greasy surfaces than other cultivars, such as Elshof. To have a better control of the wax layer, apples are sometimes artificially waxed before commercialization. The fruit is first washed and subsequently coated with a wax such as carnauba or shellac (*1*). Not only is this an expensive procedure, in many countries, this artificial waxing is also restricted to rigorous regulations. That is why often the principal aim is to find new cultivars with a low susceptibility to greasiness development of the wax layer combined with optimal storage conditions, which prevent wax changes.

In this context, insight was gained to the specific surface characteristics of different apple cultivars through a thorough investigation of the wax layer structure and composition of three different apple cultivars (Elshof, Jonagold, and Jonagored) by means of confocal microscopy (*2*) and gas chromatography (GC) (*3*). The susceptibility to greasiness development was evaluated, correlated to specific wax components (such as nonacosane-10-ol) and structures, and compared statistically between all cultivars. From this analysis, Elshof appeared to be a cultivar with a different surface structure and lower susceptibility to chemical or structural changes of the wax during storage and

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shelf life than Jonagold and Jonagored. These latter two had similar surface properties since they are mutant cultivars. GC is, however, a very complex and time-consuming technique. Faster analysis methods are aimed for when only a discrimination between the wax compositions of different cultivars is emphasized in contrast to a detailed chemical analysis of the wax layer.

Spectroscopic techniques are based on the analysis of the information contained in the light, which is absorbed, reflected, or transmitted by a sample after its incidence on the sample or analyte of interest. For several years, spectroscopy has been employed in the qualitative analysis of food products (4, 5) offering a fast, noninvasive alternative to more common analytical techniques. It also holds potential for at-line or even on-line application. Near-infrared spectroscopy (NIR) has been used in apple quality analysis for predicting firmness and the soluble solid content of apples (6–8), apple sorting (9), and bruise detection (10). Fourier transform infrared (FTIR) spectrometers use a system of moving mirrors and interferometry to generate infrared spectra and have an accuracy of less than 1 cm^{-1} . FTIR analysis has an advantage over NIR analysis since the spectra are based on fundamental vibrational modes rather than overtones. Interpretation of the different bands in the spectrum can thus provide insightful interpretations and findings based on specific component groups of the sample. The penetration depth of IR radiation is also much less than that of NIR radiation. FTIR has been used as a monitoring tool for the detection of acid content and sugars in apple juice (11) and for the presence of adulterants in apple juice (12). FTIR photoacoustic spectroscopy (PAS) has been used for the analysis of surface quality of apple fruit (13, 14). In this work, FTIR/PAS was used to detect the presence of bacteria, yeasts, and fungi on the surface of apple. FTIR was reported to be used for analysis of whole cuticle of cherry tomato fruit, which includes the wax and cutin layer (15). These spectra, therefore, show typical bands for the components of the cutin layer.

The objective of this paper was to evaluate the potential of NIR and FTIR for rapid analysis of the quality of epicuticular apple wax. If sufficient information on wax composition could be obtained from spectra, it would be possible to discriminate different wax samples up to six times faster than with chromatographic techniques. Also, the ability of diffuse reflectance spectroscopy (DRIFTS) and FT-Raman spectroscopy for wax analysis was tested as these systems can be used for solvent free wax powder and wax on intact apple, respectively.

MATERIALS AND METHODS

Fruit. Apples were harvested at the Proeftuin voor Pit-en Steenfruit in Velm (Belgium) on September 2, 1999 (early) and September 11, 1999 (commercial) for Elshof and on September 22, 1999 (early), September 29, 1999 (commercial), and October 7, 1999 (late) for Jonagold and Jonagored. All fruit was stored under controlled atmosphere (CA) conditions (Elshof: 2% O₂, <1% CO₂, 1 °C, 95% relative humidity; Jonagold and Jonagored: 1% O₂, 2.5% CO₂, 1 °C, 95% relative humidity) until wax extraction. After different periods of CA storage (0, 4, and 8 months), some apples were also stored at shelf life conditions at 20 °C for 0, 1, or 2 weeks. The wax of these apples was then also extracted.

Sample Preparation. Except for Raman spectroscopy, the wax of all apples was extracted by immersion of the fruit in hexane for 45 s. These extracts were then used for GC analysis (3) and subsequently stored at -20 °C until spectral analysis. For the spectral analysis, the solvent of each extract solution was first completely evaporated under a stream of nitrogen and the total weight of the dry, extracted wax was then determined by an analytical balance (Mettler AE 200, Mettler-Toledo, Inc., Columbus, OH). The dried wax was used directly for

DRIFTS analysis. For NIR and FTIR analysis, the wax was redissolved in 2 mL of chloroform and 1.5 mL of hexane, respectively.

NIR Analysis. For the NIR experiments a VIS/NIR spectrophotometer was used (OSA 6602, Rees instruments, England) operating in the range from 300 to 1700 nm at a 1 nm resolution. In a first series of experiments, different solvents were tested based on their interference with wax components in the NIR spectra. Therefore, 1 and 5% solutions of octacosane (Sigma-Aldrich, St. Louis, MO) in toluene, hexane, diethyl ether, acetone, and chloroform were examined. Octacosane is a known component of apple wax, and the different solvents are known extraction solvents.

For measurements on extracted wax, 90 samples were selected from the total data set for Jonagold and Jonagored and 72 for Elshof, equally divided over the different picking dates, two storage durations (4 and 8 months), and three shelf life periods (0, 1, and 2 weeks) within each storage duration. The wax was first dissolved in 2 mL of chloroform and then transferred to a cuvette. Three transmission NIR spectra were recorded per sample. The spectra of pure chloroform were used for correction.

FTIR Analysis. Spectra were collected on a Bio Rad (Cambridge, MA) 3000 Excalibur spectrometer equipped with a deuterated triglycine sulfate (DTGS) detector, operating in the mid infrared (400–4000 cm^{-1}) region at 4 cm^{-1} resolution and 0.32 cm per second mirror velocity. Two hundred fifty-six interferograms were coadded before Fourier transformation. The instrument was allowed to purge for 5 min with nitrogen gas (grade 1) prior to acquisition of the spectra in order to minimize spectral contribution due to atmospheric carbon dioxide and water vapor.

The sampling station was equipped with a special holder for transmission measurements with crystal disks (Pike Technologies, Madison, WI), which positions the crystals perpendicular to the infrared beam. For these experiments, sodium chloride (NaCl, Pike Technologies) crystal disks with a diameter of 25 mm and a thickness of 4 mm were used. NaCl crystal is a hygroscopic crystal, which withstands thermal and some mechanical shock. It is very sensitive to water, lower alcohols, and “wet” solvents but can be used with wax, which is an apolar mixture of very long chain components. The refractive index of NaCl crystal at 1000 cm^{-1} is 1.49, and the transmission range is 40000 to 625 cm^{-1} .

For measurements on extracted apple wax, 130 samples were selected from the total data set for Jonagold and 90 for Elshof, equally divided over the different picking dates, three storage durations (0, 4, and 8 months), and three shelf life periods (1, 2, and 3 weeks) within each storage duration. The dried wax was first dissolved in a 1.5 mL of hexane and then heated in the oven at 50 °C for 15 min. Twenty-five microliters of the warm wax solution was then sprayed over the NaCl crystals by means of a micropipet, and the solvent was allowed to evaporate to obtain a wax film. Heating of the wax solutions was done to obtain a more homogeneous film and to make sure that all chemical components were dissolved. After measurement, the crystals were cleaned by means of hexane. The cleaned crystal was checked spectrally to ensure that no sample residue from the previous sample was retained on the crystal surface.

A background spectrum was recorded using a blank NaCl crystal. Single beam spectra of all of the samples were obtained and ratioed against the background spectrum to present the spectra in transmission units.

DRIFTS and FT-Raman Analysis. DRIFTS spectra were obtained using a Nicolet 870 FTIR spectrometer (Madison, WI) with a DTGS detector and XT-KBr beam splitter. Recordings were done in the midinfrared (400–4000 cm^{-1}) region at 4 cm^{-1} resolution. Two hundred fifty-six interferograms were coadded before Fourier transformation. In the sampling unit, a DRIFTS accessory was provided for placement of the sample in cups about 4 mm in diameter. Background spectra were recorded using an empty cup. Dried wax powders were directly transferred to the cups without dilution. The system was operated using the OMNIC 5.1 software.

FT-Raman measurements were performed on the Nicolet 870 FTIR spectrometer with the Nicolet Raman module 32B. Excitation was provided at 1064 nm with a HeNe laser, and detection was done by an InGaAs (indium–gallium arsenide) detector. The system was also

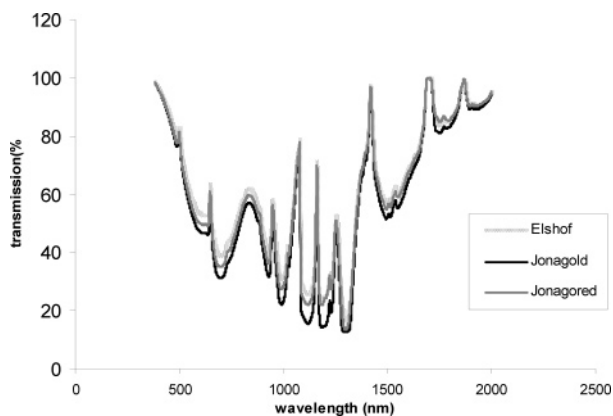


Figure 1. Comparison of NIR transmission spectra of solutions of wax in chloroform of the apple cultivars Elshof, Jonagold, and Jonagored.

equipped with an XT-KBr beam splitter with 180° reflective optics and a fully motorized sample position adjustment feature. Data were recorded in the Raman shift range (200–4000 cm^{-1}) with 8 cm^{-1} resolution and coaddition of 256 interferograms before Fourier transformation. Samples for Raman analysis were intact apples or pieces of intact apple skin. Also, this system was operated using the OMNIC 5.1 software.

Statistical Analysis. Spectral data were analyzed by means of canonical discriminant analysis (CDA) with SAS/STAT software version 8.2 (SAS Institute, Cary, NC) (16). In CDA, canonical variables (cv) are calculated, which are linear combinations of the original variables. Canonical variables maximize the ratio of between-groups variance over within-groups variance. Applying discriminant analysis on these canonical variables results in a discriminant function, which enables classification of any future measurement depending on Mahalanobis distance to group means. Multidimensional data, in which the number of variables is larger than the number of observations, cannot be used in this statistical method. Regions of wavelengths in the spectra were, therefore, selected for the analysis. For NIR, this was the region from 995 to 1745 nm and for FTIR the region from 2780 to 3100 cm^{-1} . Before statistical analysis, the transmission spectra were converted to absorption spectra and the first derivative was calculated for further analysis.

RESULTS AND DISCUSSION

NIR Analysis. Comparison of the spectra of octacosane in different solvents was carried out to select the optimal solvent for transmission measurements with NIR. The spectra of octacosane in chloroform resulted in the best signal-to-noise ratio, and also, concentration differences were presented the best with this solvent. All further measurements were, therefore, carried out in chloroform.

Comparison of the pure NIR transmission spectra of Jonagold, Jonagored, and Elshof wax solutions revealed many similarities in the general pattern but a difference in total transmission (Figure 1).

More detailed dissimilarities in the total set of spectra, which provided insight to the differences in wax composition of different cultivars, picking dates, storage periods, and shelf life durations were not visually detectable in the original spectra. CDA was, therefore, performed on the total data set of spectra. CDA was chosen over other linear discriminant analysis to avoid multicollinearity.

A first good discrimination was found between the wax compositions of the different cultivars. This is visualized in the score plot of canonical variables cv 1 and cv 2 (Figure 2). Elshof is discriminated from Jonagold and Jonagored by cv 2. Jonagold and Jonagored are discriminated by cv 1. This compares well with the data obtained from GC analysis where

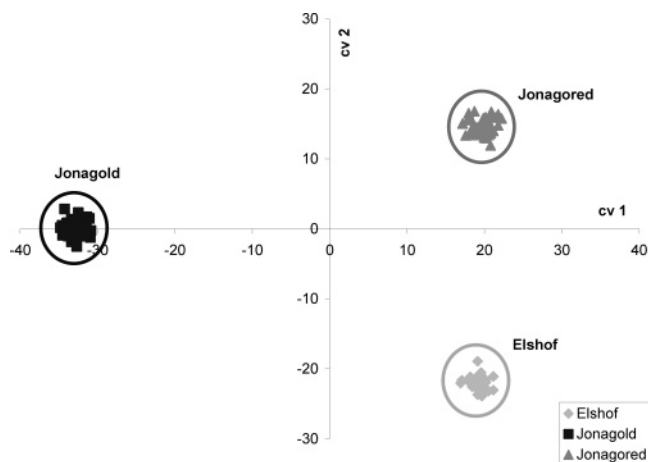


Figure 2. CDA of the cultivars Jonagold, Jonagored, and Elshof based on transmission NIR data of the wax layer.

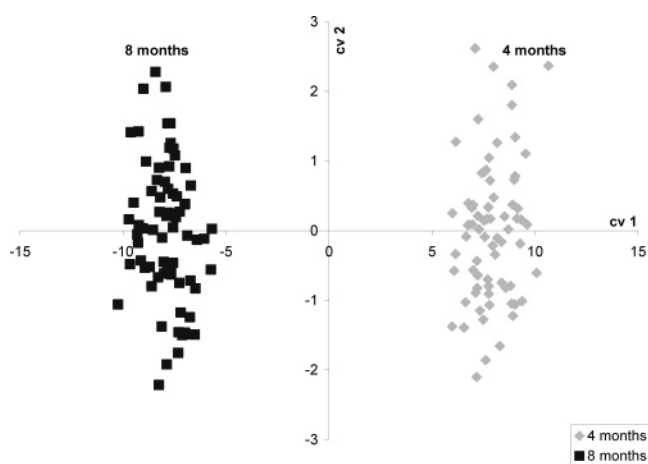


Figure 3. Effect of CA storage on the wax layer composition of Jonagold, Jonagored, and Elshof based on NIR transmission spectra of solutions of isolated wax.

the wax from the cultivar Elshof was shown to have a different composition than the wax from Jonagored and Jonagold. The cultivar-related difference in wax composition from the cultivars Jonagold and Jonagored was only situated in the concentration of certain wax components (e.g., nonacosane and nonacosan-10-ol) (3).

Good discrimination was obtained based on differences in wax composition for different CA storage periods. This was again visualized in the score plot of cv 1 and cv 2 (Figure 3) where 4 and 8 months of storage were discriminated along the axis of cv 1. From the chemical analysis, the changes in wax composition during storage could mostly be attributed to the ester fraction (3).

A first validation of the two canonical discriminant models performed by means of a cross-validation on the entire data set resulted in a successful classification of 92 and 98%, respectively, of the data based on variety and storage period.

A successful discrimination for picking date and shelf life was not obtained based on the transmission NIR spectra of wax solutions. This could be due to the fact that the changes in wax composition that occur over different picking periods and during shelf life are too small to be detected by means of this technique. It could also be due to the selection of the wavelength region. Selection of an appropriate region from the spectra is an important factor in spectra analysis.

FTIR Analysis. FTIR analysis was performed with wax films on crystals instead of wax solutions, which prevented domination

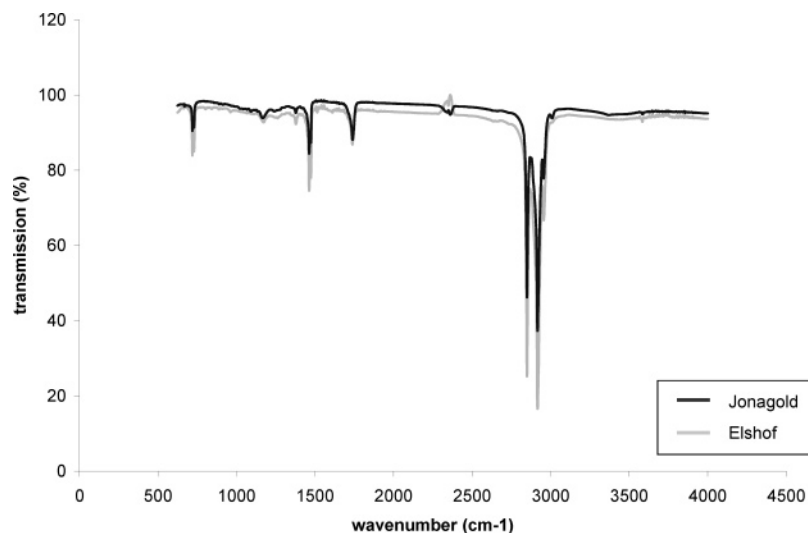


Figure 4. Original FTIR transmission spectra of wax films of two samples of the apple cultivars Elshof (light) and Jonagold (dark).

Table 1. Main Spectral Frequencies of Apple Wax and Their Assignment

spectral frequency/ region (cm ⁻¹)	assignment
720–730	δ (CH ₂) _n plane rotation of linear long carbon chains (fatty acids, <i>n</i> -alkanes, esters)
1175	ν (C–O) vibration of esters
1377	hydrocarbon chains
1464	hydrocarbon chains
1473	hydrocarbon chains
1711	ν (C=O) stretch of fatty acids
1737	ν (C=O) stretch of esters
2850	hydrocarbon chains
2918	hydrocarbon chains
2950	hydrocarbon chains
3012	=C–H stretch of olefinic groups (fatty acids or triene systems)

of the spectra by solvent bands. Original FTIR transmission spectra for the apple cultivars Elshof and Jonagold are shown in **Figure 4**. Main spectral frequencies and their assignments are listed in **Table 1** (17, 18). The spectra of apple wax corresponded well to the spectrum of a more general wax system such as beeswax (17). The obtained spectra were very similar between both cultivars; yet, some differences were found. The band around wavenumber 3012 cm⁻¹ was only found in the spectra of Jonagold wax. This frequency is typical for =C–H stretching of olefinic groups. This can be found in fatty acids but also in conjugated triene systems (19) such as the oxidation products of α -farnesene (20). From the chemical gas chromatographic analysis, α -farnesene was the only component that was found to be present in Jonagold and not in Elshof as is the band at 3012 cm⁻¹. Therefore, this band is possibly related to α -farnesene and its oxidation products, which cause the storage disorder scald. The bands between 3000 and 2800 cm⁻¹, which are typical for hydrocarbons, were in general larger for the wax of Elshof as compared to the wax of Jonagold. The band around 2850 cm⁻¹ is, for example, associated with the symmetric stretching vibrations of –CH₂– moieties along alkyl chains (18) and thus with the aliphatic chains in the wax. This corresponded to the findings of the chemical analysis where it was also concluded that Elshof wax had longer aliphatic chains with less substitution than the wax of Jonagold contributing to the increased crystallinity of Elshof wax through better alignment

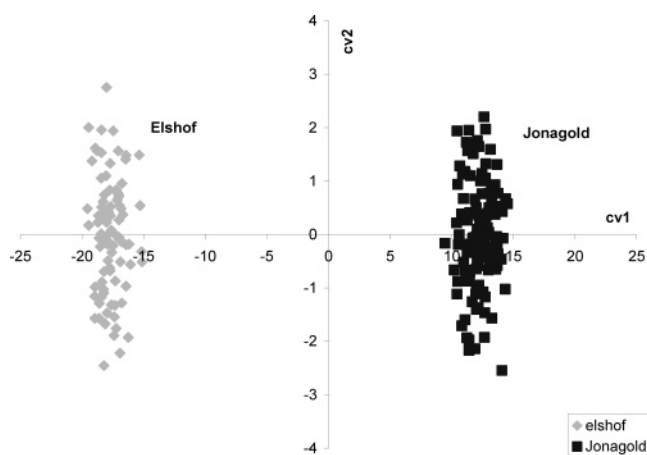


Figure 5. CDA of the cultivars Jonagold and Elshof based on FTIR data of the wax film.

of these chains. Finally, in the region between 1800 and 700 cm⁻¹, the bands were in general larger for Elshof wax than for Jonagold wax.

CDA was also performed on the total data set of FTIR spectra to classify the different cultivars, picking dates, storage periods, and shelf life durations into different groups based on wax composition. Only the region between 2780 and 3100 cm⁻¹ was used for this analysis. This is the region mainly containing information on the aliphatic chains in the wax and possibly on α -farnesene.

As with NIR, also with FTIR spectra, a good discrimination based on the wax compositions of the different cultivars was possible. This was visualized in the score plot of canonical variables cv 1 and cv 2 (**Figure 5**). Elshof was discriminated from Jonagold along the axis of cv 1. Differences were possibly due to the higher concentrations of nonsubstituted aliphatic chains in Elshof wax and the absence of α -farnesene, which was only present in Jonagold wax (3).

In **Figure 6**, discrimination based on differences in wax composition over different CA storage periods indicates that FTIR could be a potential tool for such analysis. This was again visualized in the score plot of cv 1 and cv 2 (**Figure 6**) where 0 months of storage (or fresh produce) was discriminated along the axis of cv 1 from 4 and 8 months of storage and the 4 and 8 month stored samples were in turn discriminated by cv 2 (along the axis of the second variate).

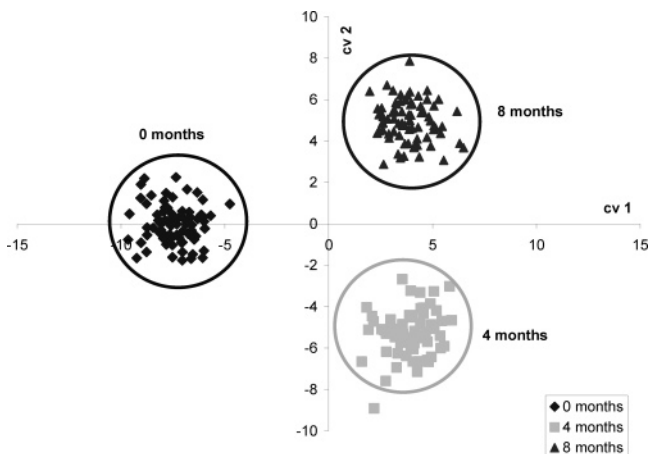


Figure 6. Effect of CA storage on wax layer composition of Jonagold and Elshof based on FTIR spectra of wax films with two scores using CDA.

A first validation of both canonical discriminant models for FTIR data of wax was performed by means of cross-validation on the entire data set to obtain a 99 and 76% classification based on variety and storage, respectively. The model for storage clearly had a lower representation than the one based on variety. This could be due to the fact that the changes, which occur during storage, are not fully represented by the specific region chosen for this analysis.

A successful discrimination for picking date and shelf life was not obtained based on the FTIR spectra of wax films. This could be due to the fact that the changes in wax composition, which occur over different picking periods and during shelf life, were too small to be detected by means of this technique or perhaps the selected region in the spectra did not contain sufficient information. Selecting a different region for each model would then offer a possible solution.

DRIFTS and FT-Raman Analysis. DRIFTS and FT-Raman analysis did not result in a good analysis of wax layer composition. DRIFTS was performed on dried wax powders but was very sensitive to compaction and particle size of the powders. Especially for the wax of the cultivar Jonagold, which had a very high plasticity and much lower crystallinity than Elshof wax, the DRIFT analysis resulted in very broad bands and a very high noise-to-signal ratio. Mixing the wax powders with KBr could be a possible solution, but this will increase the sample preparation time and, hence, the analysis cost. Raman spectroscopy was tested for its use on intact apple skin. Because of the high fluorescence of apple tissue, no stable interferogram was obtained.

In conclusion, for NIR transmission measurements on wax solutions, chloroform was the best solvent. Differences in total transmission were observed between the pure spectra of wax solutions of the cultivars Jonagold, Jonagored, and Elshof. A CDA on the NIR data resulted in a good discrimination between the wax compositions of different apple cultivars and between the wax compositions of each individual cultivar at different CA storage times (4 or 8 months). FTIR transmission spectroscopy was carried out on wax films. The main spectral frequencies of the transmission spectra could be assigned and compared to the information in gas chromatographic spectra of the same wax samples. The wax of the cultivar Elshof contained longer aliphatic chains with less substitution than the wax of the cultivar Jonagold but lacked the component α -farnesene. CDA performed on the data of the spectral region between 2780 and

3100 cm^{-1} resulted in a good discrimination between the wax composition of the cultivars Elshof and Jonagold and also for each cultivar between the wax composition at different CA storage times (0, 4, and 8 months). All NIR and FTIR models were successfully validated by means of cross-validation. No successful discrimination was obtained for picking date and shelf life. DRIFTS on wax powder and FT-Raman on intact apple fruit did not result in a good analysis of the wax composition.

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