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Postharvest quality of apple predicted by NIR-spectroscopy: Study of the effect of biological variability on spectra and model performance

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ABSTRACT

The effect of cultivar, season, shelf-life and origin on the accuracy of near infrared (NIR) calibration models for the soluble solids content (SSC) and firmness of apple was studied based on a large spectral data set based on approximately 6000 apple fruit from different cultivars, origins, shelf-life exposure time and seasons. To interpret the variance in the spectra with respect to biological variability, functional analysis of variance (FANOVA) was used. From the FANOVA analysis it was concluded that the effects of cultivar, origin and shelf-life exposure time on the NIR spectra were all significant. The largest differences in the spectra were found around the water absorption peaks (970, 1170 and 1450 nm). External validations using independent data sets showed that the accuracy of the models increased considerably when more variability was included in the calibration data set. In general the RMSEP for predictions of the SSC were in the range 0.6–0.8 °Brix, while for Magness Taylor firmness it was 5.9–8.8 N, depending on the cultivar. It was shown that atypical data can lead to large validation errors. It is, therefore, important to collect a calibration data set which is sufficiently representative for future samples to be analyzed with the developed calibration models and to develop simple procedures for model adaptation during practical use.

1. Introduction

The use of NIR spectroscopy to measure internal quality attributes of horticultural produce has been investigated extensively during the last decade. The variety of studied fruit is large, ranging from apple (Bellon-Maurel, 1992; Lovász et al., 1994; Lammertyn et al., 1998; Clark et al., 2003; McGlone et al., 2005; Alamar et al., 2007) to melon and pineapple (Guthrie et al., 1998), kiwifruit (Osborne and Künnemeyer, 1999; Schaare and Fraser, 2000; McGlone et al., 2002b), citrus (Kim et al., 2004), mango (Saranwong et al., 2003, 2004; Teerachaichayut et al., 2007), mandarin (McGlone et al., 2003; Guthrie et al., 2005; Gomez et al., 2006), peach (Carlomagno et al., 2004) and pear (Han et al., 2006; Nicolaï et al., 2008; Liu et al., 2008). More applications and recent developments have been reviewed by Nicolaï et al. (2007).

Firmness and soluble solids content are key parameters in determining apple quality. Hence, the prediction of apple quality based on NIR-spectroscopy is usually focused on these two quality attributes (Lovász et al., 1994; Lammertyn et al., 1998; Ventura et al., 1998; Peirs et al., 2000; Lu et al., 2000; McGlone et al., 2002a; Temma et al., 2002; Park et al., 2003; Mehinagic et al., 2004; Johnston et al., 2005; Zude et al., 2006; Gomez et al., 2006). Due to biological variability apples can vary greatly in their quality properties. Few articles have addressed the robustness of NIR calibration models for apple SSC and firmness (Nicolaï et al., 2007). Guthrie et al. (1998) found a lack of robustness of calibration models for the SSC of pineapple and melon when fruit were harvested at different times. McGlone and Kawano (1998) found that when variability in origin, size and firmness were not incorporated in the NIR calibration models for firmness, dry matter content and soluble solids content of kiwifruit, significantly higher validation errors were obtained. Similar results were found by Peirs et al. (2003) for apple, Guthrie et al. (2005) for mandarin, Guthrie et al. (2006) for rockmelon, and Golic and Walsh (2006) for peach and nectarine. Peirs et al. (2003) demonstrated that for pre-climacteric apples more than half of the spectral variability was due to orchard, season and cultivar effects. Different growing conditions (soil type, hours of sunlight and amount of precipitation, fruit age and seasons) affect the maturation process and the quality of apples (Peirs et al., 2003). This biological variability will affect the prediction of the quality parameters and also the developed models (Thomas and Ge, 2000; Peirs et al., 2003, 2005). As more biological variability is

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taken into account, the prediction accuracy becomes less sensitive to unknown changes of external factors (Nicolaï et al., 2007). This makes the calibration models more robust against future changes, but may simultaneously reduce the accuracy of the models.

Model robustness can be evaluated by studying the potential sources of variability of the data and by identifying the factors that can significantly affect the accuracy of the model prediction (Sanchez et al., 2003; Zeaiter et al., 2004; Golic and Walsh, 2006). Relating spectral data to quality attributes is usually done by partial least square regression (PLS) which refers to the computation of the optimal least-squares fit to part of a correlation or covariance matrix (Wold, 1982). PLS is quite similar to principal components regression (PCR), but defines the latent variables (principal components) based on the covariance between the independent and dependent variables, rather than on the variance in the independent variables alone. The advantage of PLS regression is the ability to analyze data with many, noisy, collinear, and even incomplete variables in both *X* and *Y* (Naes et al., 2004).

Saeys et al. (2008a) demonstrated the potential of functional data analysis in chemometrics. They found that this technique, which describes the spectrum as a function instead of a set of points, can obtain a predictive power comparable to that of PLS while taking into account the spatial correlation between the variables. Also functional analysis of variance (FANOVA) was shown to be a useful tool to analyze the sources of variance in spectroscopic data.

The objective of this study was to investigate the effects of the different sources of biological variability on the measured spectra and to evaluate their impact on the performance of NIR calibration models for predicting postharvest soluble solids content and firmness. The effects on the spectra and their significance were studied using functional analysis of variance (FANOVA) (Saeys et al., 2008a). Their impact on the prediction performance is evaluated through external validation of the calibration models with apples of different varieties, origins and seasons. The size and variability of the datasets used in this article are considerably larger than in previously published studies (Ventura et al., 1998; Liu and Ying, 2005; Zude et al., 2006; Qing et al., 2007).

2. Materials and methods

2.1. Fruit material

In total, approximately 6000 apple fruit were analyzed during 1 year (2006). Different cultivars and origins were included (Table 1): 'Golden Delicious' (Belgium, South Africa, Italy and France), 'Royal Gala' (Chile, Argentina, New Zealand, France and Belgium), 'Jonagold' (Belgium – 4 different growers), 'Braeburn' (South Africa, Chile, France and New Zealand), 'Pink Lady' (Chile, Argentina, France, South Africa and New Zealand) and 'Fuji' (China, France). The apples were purchased from several local supermarkets and were put in cold storage (1 °C). For each cultivar and origin, the sample size was about 200 apples. Half of the amount was measured the day after purchase and the other half was measured after 1 week of shelf-life (18 °C). This experiment was carried out from June until August, and repeated in November. Both periods will be referred to as season 1 and season 2, respectively, although obviously only the apples originating from the Northern Hemisphere were actually from two different seasons.

2.2. Quality attributes

The apple quality attributes, firmness and soluble solids content (SSC), were measured destructively with traditional standard methods (reference measurements). The firmness was determined with a Magness-Taylor Texture Analyzer (TA-XT2i, Stable Micro Systems, the Netherlands). Fruit firmness was measured as the maximum force to penetrate the fruit with a self-cutting plunger (11 mm diameter) over a distance of 8 mm at a speed of 8 mm/s. The apple juice which was released during penetration was used to measure the soluble solids content with a digital refractometer (PR-101, Palette Series, ATAGO CO. Ltd, Tokyo, Japan). Both reference measurements were taken on 2 equatorial positions (green and blush side) of the apple. In further data analyses the average value was used.

2.3. Spectral acquisition

In order to be able to build calibration models for the prediction of apple quality from NIR spectra, all reference measurements were preceded by the NIR reflectance measurements. Spectra were collected in the 380–1690 nm range with an increment of 2 nm with a Diode Array Spectrophotometer (Corona for Agriculture and Food, Carl Zeiss AG, Germany). The spectrophotometer worked with a single-beam diode array (polychromator) and two sensors: a Si array, Hamamatsu S 3904 (380–950 nm) and an InGaAs array (950–1690 nm). The light source consisted of a 9 W, 5 V stabilized Halogen lamp. A 0°/45° sample setup was used to take the reflectance spectra. The green and blush sides of the apples were scanned. The measured intensity spectra were converted into relative reflectance spectra by subtracting the dark current response of the detector and dividing by a white reference spectrum ($BaSO_4$). For each spectrum, 100 optical scans with an integration time of 30 ms were averaged. Prior to the spectral measurements, all apples were equilibrated at room temperature.

2.4. Statistical data analysis

The spectral data were analyzed with the statistical software program for multivariate calibration 'The Unscrambler' (version 9.6, Camo Process SA, Trondheim, Norway). Prior to the multivariate analysis, the reflectance spectra (R) were converted to absorbance units using the $\log(1/R)$ transformation. Raw absorbance spectra as well as pre-processed data were studied. Two spectral transformation techniques, standard normal variate (SNV) and second derivative (Savitzky-Golay, second order polynomial) were compared in order to verify their usefulness in removing the additive and multiplicative effects and other useless information from the spectra (Naes et al., 2004).

Table 1
Overview of measured apple cultivars, origins and seasons (1: June–August, 2: November).

Cultivar	Origin							
	Belgium	France	Chile	Argentina	New Zealand	South Africa	Italy	China
Golden Delicious	1–2	1–2				1	1	
Royal Gala	2	2	1	1	1			
Jonagold	1–2							
Braeburn	2	1–2	1		1	1		
Pink Lady		1–2	1	1	1	1		
Fuji		2						1

Partial least squares (PLS) regression models were built for the prediction of SSC and firmness using the destructive measurements as a reference. For the cultivar another model was constructed. The calibration models were based on the spectral range 800–1690 nm. Cross validation was carried out with 40 segments and approximately 25 samples per segment.

To test the effect of the biological variability on the model performance different external validations were performed. A random validation test, with one third of the original data as validation set was used. Also test sets with data of apples from different origins, seasons and storage conditions (fresh from storage vs. 1 week shelf-life) were analyzed for validation of the model robustness. The calibration model accuracy was described by the coefficient of determination (R^2) and the root mean square errors for cross validation (RMSECV) or prediction (RMSEP):

$$\text{RMSECV or RMSEP} = \sqrt{\sum_{i=1}^{n_p} (\hat{y}_i - y_i)^2 \frac{1}{n_p}} \quad (1)$$

where $\hat{y}_i$ and y_i are the predicted and measured values of the ith observation in the validation/test set, and n_p the number of validated objects. Also the RPD value (ratio of prediction to deviation) was calculated. This is the ratio of the SD (standard deviation) to the RMSECV or the RMSEP, explaining by which factor the prediction accuracy has been increased compared to using the mean composition for all samples (Saeys et al., 2005). RPD values below 1.5 indicate that the calibration is not useful. When the value for the RPD is higher than 2, quantitative predictions are possible.

To analyze and quantify the variance in the spectra with respect to the biological variability, functional analysis of variance (FANOVA) was used. This adapted traditional analysis of variance uses a functional representation of spectra. Saeys et al. (2008a) have shown that a functional approach in chemometrics has some advantages. A functional description based on B-splines is taking into account the physical background of the spectrum, being a function of the wavelength. More specifically, the spectrum of a sample is the combined result of the absorption peaks for different chemical components, such that the absorbance at neighbouring wavelengths is highly correlated. This functional approach is beneficial because of the combination of dimensionality reduction and smoothing while preserving the information in the derivatives. With this technique the spectral information is decomposed in functional effects comprising the overall mean and the main effects (Saeys et al., 2008a).

In this study the considered main effects are shelf-life, cultivar and the combined effect of geographical and seasonal origin. The effects of geographical and seasonal origin could not be separated efficiently, because the experimental data did not follow a full factorial design, due to the higher mentioned reasons of availability. For the same reason different FANOVA models had to be constructed for the NIR spectra using subsets of the experimental data.

To eliminate the influence of the physiological age on the origin effect the analysis was done separately for the samples from the Northern Hemisphere and the Southern Hemisphere. To explore the origin (geographical and seasonal) and the shelf-life effect the following FANOVA model was constructed per cultivar:

$$A_{ijk}(\lambda) = \mu(\lambda) + O_i(\lambda) + S_j(\lambda) + OS_{ij}(\lambda) + \varepsilon_{ijk}(\lambda) \quad (2)$$

where $A_{ijk}(\lambda)$ is the measured absorbance at wavelength λ, $\mu(\lambda)$ is the overall mean (average spectrum), $O_i(\lambda)$ is the main effect of the ith origin, $S_j(\lambda)$ is the main effect of the jth shelf-life condition, $OS_{ij}(\lambda)$ is the interaction effect between the ith origin and the jth shelf-life condition and $\varepsilon_{ijk}(\lambda)$ the residual. The origin effect also partly includes the season effect. A separate season effect could not be defined because not for each origin apples from 2 seasons were available. The number of replicates is k. In order to obtain a unique solution, the following constraints were applied:

$$\sum_i O_i(\lambda) = 0 \quad \sum_j S_j(\lambda) = 0 \quad \sum_j O_i S_j(\lambda) = 0 \quad (3)$$

To analyze the cultivar effect only the apples of the origin France and season 2 were taken into account. All cultivars were included, except 'Jonagold'. Consequently the FANOVA model covers the overall mean, the cultivar and the shelf-life effect.

$$A_{ijk}(\lambda) = \mu(\lambda) + C_i(\lambda) + S_j(\lambda) + CS_{ij}(\lambda) + \varepsilon_{ijk}(\lambda) \quad (4)$$

where all symbols are as described before, $C_i(\lambda)$ is the main effect of the ith cultivar, and $CS_{ij}(\lambda)$ is the interaction effect between the ith cultivar and the jth shelf-life condition. In order to obtain a unique solution, similar constraints as those for the first model were necessary. The overall mean effect $\mu(\lambda)$ in this model differs from the mean effect in Eq. (2) because the model is based on a different subset of the acquired spectra. In both FANOVA analyses a basis of 100 B-splines was used for the functional description of the spectra.

The significance of the functional effects can be evaluated globally and locally. For the latter case the estimated pointwise standard error for each effect is computed and a 95% confidence interval is constructed around the effect. When the confidence interval does not comprise zero (no effect) the effect can be considered as significant. In the former case, a permutation based overall significance test is performed (Saeys et al., 2008a). In this test the group labels (origin, shelf-life exposure and cultivar) are assigned randomly to the data, keeping the number of elements in each group the same. For each permutation the group effect is determined and a corresponding p-value is derived. Here 1000 permutations were performed. A p-value ≤ 0.05 corresponds to a significant group effect (significance level of 5%). All FANOVA computations were performed in Matlab 2007b, using the FDA toolbox and the procedure described by Saeys et al. (2008a), which are freely available.

3. Results and discussion

3.1. Reflectance spectra

Fig. 1 shows the measured apple reflectance spectra (R) converted to absorbance (log (1/R) of all measured apples, in the region between 800 and 1690 nm. The spectra are dominated by the water spectrum with overtone bands of the OH-bonds at 970, 1170 and 1450 nm (Polesello and Giangiacomo, 1981). Also strong carbohydrate absorbance bands exist around 980 nm and they are convoluted with the strong water band at 970 nm to give the observed broad peak. Similar spectra were also obtained by Temma et al. (2002), Liu and Ying (2005), Peirs et al. (2005), Nicolaï et al. (2006), Alamar et al. (2007) and Camps et al. (2007). From this figure it should be noted that although all spectra have a very similar shape, there is a large variability in absorbance. The effect of the cultivar, season, shelf-life and origin on the measured spectra and the model performance will be discussed below.

3.2. Calibration model robustness

3.2.1. Calibration model performance

Prior to the PLS modelling, the spectra were pre-processed. For SSC, SNV was generally preferred to second derivative preprocessing and raw data, because it resulted in more accurate models. All further SSC models were, therefore, constructed from SNV pre-processed spectra. Firmness, a texture property, is related to scattering effects and with SNV and second derivative preprocessing a lot of scattering information was removed from the spectra. Consequently, for firmness analysis, raw data were used. The opti-

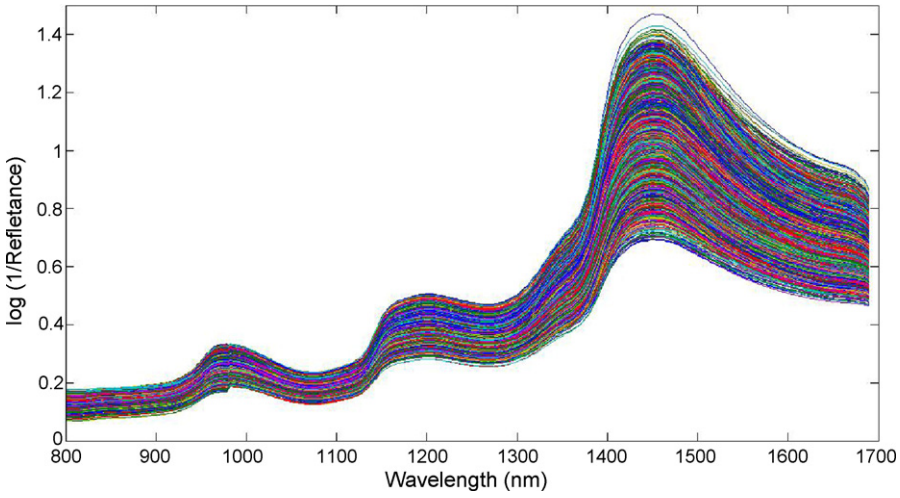

Fig. 1. Typical absorbance spectra of all measured apple samples between 800 and 1690 nm.

mal number of latent variables used in the PLS models (for both firmness and SSC) was between 6 and 8 (Tables 2 and 3).

Tables 2 and 3 summarize the prediction performance of the different calibration models in terms of R^2, RMSEP and RPD for the different cultivars for both firmness and SSC. The best calibration model for SSC was obtained for 'Golden Delicious'. A high R^2 (0.88) was combined with a low RMSECV (0.60) and a high RPD of 2.89 for a model with seven latent variables. For the test set validation with one third of the data (randomly selected) an R^2 of 0.86, RMSEP of 0.59 and RPD of 2.77 were obtained. The latter performance parameters are comparable with the cross validation results. Similar prediction errors were obtained for other cultivars. The correlations were smaller but still acceptable with the exception of 'Pink Lady' where the correlation was poor (R^2 = 0.46, RMSECV = 0.63 and RPD = 1.36). Although the model for 'Pink Lady' was built with a large number of samples (1160), the model clearly failed. This was mainly due to apparently atypical spectra coming from Argentina and France (autumn-winter period) fruit for which the SSC predictions were very poor. No valuable explanation for these atypical spectra could be found. When these origins were excluded from the calibration model data the validation results improved considerably (R^2 = 0.67, RMSECV = 0.50 and RPD = 1.5). The SSC models for 'Fuji' apples performed well with R^2 = 0.74, RMSECV = 0.63 and an RPD around 2. However, to make the model more robust for origin effects more data should be included in the model.

The models for firmness resulted in a lower performance with lower R^2 values and higher validation errors than for SSC. The best results were achieved for 'Royal Gala' and 'Braeburn' apples where the RPD value was higher than 1.5. For 'Golden Delicious', 'Jonagold' and 'Pink Lady' apples the observed validation R^2 values were inadequate (R^2 = 0.41, 0.42 and 0.37, respectively) and also had RPD values <1.5. Lu et al. (2000) also found lower correlations for firmness prediction of apples when compared to SSC prediction. Typically, better results are obtained with models for apples in the pre-climacteric stage. Also including the visible range could improve the prediction results (Peirs et al., 2000; Zude et al., 2006).

Table 2
Model performance of NIR calibration models for SSC for different apple cultivars. CV: cross validation; TSV: test set validation (for further details see Section 3.2); LV: number of latent variables.

SSC		Braeburn	Golden	Jonagold	Royal gala	Pink Lady	Fuji
Number of samples		1086	1295	776	1172	1160	335
CV	R^2	0.66	0.88	0.76	0.66	0.46	0.74
	RMSECV	0.87	0.6	0.63	0.62	0.63	0.76
	RPD	1.71	2.89	2.06	1.71	1.36	1.99
	LV	7	7	7	8	8	6
TSV 1/3 data	R^2	0.65	0.86	0.75	0.6	0.5	0.85
	RMSEP	0.77	0.59	0.63	0.65	0.6	0.6
	RPD	1.53	2.77	1.90	1.57	1.46	2.44
	LV	7	7	7	8	8	6
TSV season 2	R^2	0.77	0.83	0.56	0.62	0.03	0.77
	RMSEP	0.74	0.71	1	0.6	1.2	0.7
	RPD	2.13	2.22	0.95	1.94	0.75	1.93
	LV	7	7	6	8	1	3
TSV Shelf-life	R^2	0.76	0.85	0.86	0.61	0.03	0.8
	RMSEP	0.96	0.7	0.5	0.64	0.85	0.85
	RPD	1.40	2.46	2.55	1.65	1.02	1.73
	LV	8	5	7	4	1	5
TSV Fresh	R^2	0.37	0.81	0.55	0.47	0.4	0.7
	RMSEP	1.31	0.77	0.92	0.68	0.68	0.78
	RPD	1.23	2.27	1.42	1.50	1.20	1.95
	LV	7	7	8	6	9	8

Table 3
Model performance of NIR calibration models for firmness for different apple cultivars. CV: cross validation; TSV: test set validation (for further details see Section 3.2); LV: number of latent variables.

Firmness		Braeburn	Golden	Jonagold	Royal gala	Pink Lady	Fuji
Number of samples		1086	1295	776	1172	1160	335
CV	R^2	0.67	0.41	0.42	0.69	0.37	0.55
	RMSEP	8.83	6.38	7.85	7.36	8.53	7.85
	RPD	1.71	1.31	1.30	1.81	1.28	1.51
	LV	8	8	8	8	8	8
TSV 1/3 data	R^2	0.62	0.4	0.42	0.72	0.39	0.47
	RMSEP	8.93	6.18	7.85	6.77	8.34	8.83
	RPD	1.38	1.29	1.26	1.82	1.35	1.33
	LV	8	8	8	8	8	5
TSV season 2	R^2	0.4	0.2	0.1	0.24	0.03	0.03
	RMSEP	17.66	10.79	8.14	7.85	6.87	23.54
	RPD	0.60	0.63	1.22	1.97	1.68	0.30
	LV	8	6	4	1	7	7
TSV Shelf-life	R^2	0.4	0.31	0.21	0.62	0.25	0.41
	RMSEP	12.75	6.57	12.75	9.03	9.81	9.81
	RPD	1.32	1.34	0.68	1.31	1.14	1.13
	LV	9	4	4	8	8	5
TSV Fresh	R^2	0.58	0.14	0.06	0.41	0.21	0.47
	RMSEP	10.79	8.34	13.73	9.12	10.20	8.24
	RPD	1.15	0.94	0.52	1.57	1.38	1.40
	LV	5	7	6	4	7	6

3.2.2. *Effect of season*

In order to evaluate the effect of the season on the prediction performance, the different models per cultivar were validated with apples from a different season. The apples from season 2 were used for the validation of the models based on the apples from season 1. The model was not based on a full factorial design due to reasons of availability, and therefore there are some confounding effects with season and origin (Table 1).

Generally, good results were obtained for the external validation of the SSC calibration models with data of another season, although for some cultivars the external validation resulted in slightly higher RMSEP and lower RPD values compared to the cross validation results. For 'Golden Delicious' the RMSEP increased with 0.11 °Brix compared to the cross validation results for the same number of LVs. This is mainly due to the lower SSC of the validation samples compared to the calibration samples (Fig. 2). For 'Jonagold' a higher RPD value was observed which could be attributed to the smaller SSC range of the validation samples. Oddly, for 'Braeburn' a lower RMSEP was found for the external validation. In other words, the calibration model gave more accurate predictions of the SSC values for the new season apples. This can probably be explained by the fact that their SSC values fitted very well into the calibration range (Fig. 2).

Similar conclusions can be drawn for the calibration models for firmness. For certain cultivars the RMSEP increased while for others the prediction error decreased when apples from season 2 were used for validation. However, the correlations were remarkably lower, which is probably due to the smaller firmness range of the validation samples compared to the calibration range. For 'Braeburn' a large increase in RMSEP was observed (from 8.8 to 17.7 N) which may be related to the fact that the Belgian apples in the validation set were recently harvested and still much firmer than the apples in the calibration set which had already been stored for a considerable period of time. Hence, the seasonal effect also comprises the effect of physiological age because we are dealing with the begin and the end of a storage season.

From these results, it can be concluded that by including more relevant variability in the calibration data the model robustness can be improved considerably.

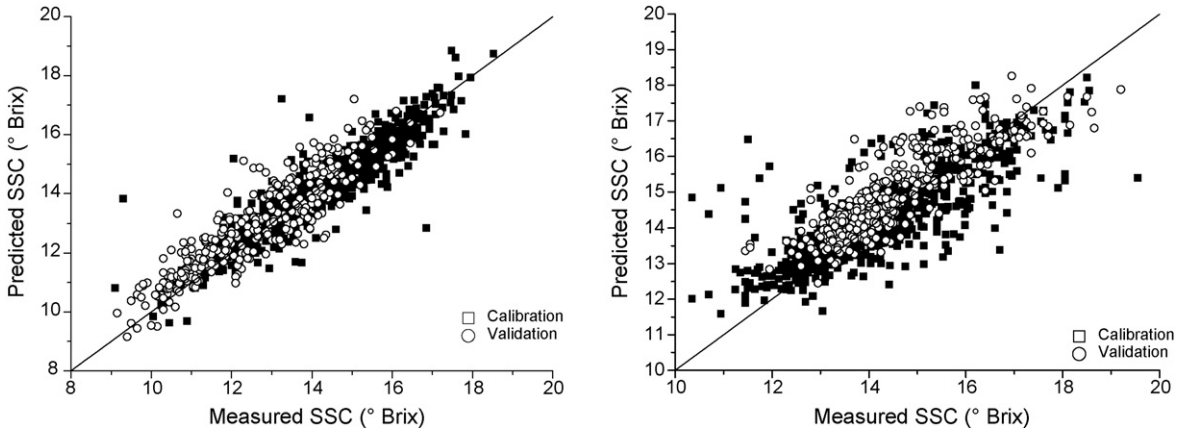

Fig. 2. Predicted vs. measured SSC for 'Golden Delicious' (left) and 'Braeburn' (right). Apples of season 1 were used for the model calibration; apples of seasons 2 for validation purposes.

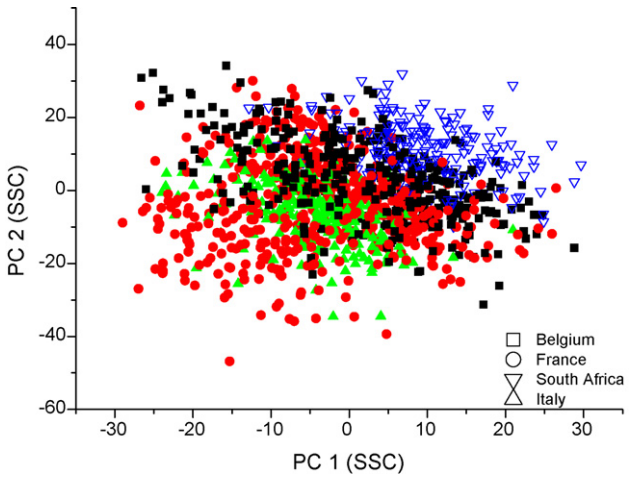

Fig. 3. PCA score plot (PCA) of 'Golden Delicious' NIR spectra of different origins.

3.2.3. *Effect of origin*

Because of the large variation in origins there was a considerable biological variability due to climate, growing and storage conditions, etc. Hence, the measured range of SSC and firmness was quite large. From the PCA score plots and the predicted vs. measured scatter plots it can be clearly observed that the different origins in the model cause large variations in the spectra (Fig. 3) as well as variation in SSC and firmness. In addition the mean values for SSC and firmness per cultivar/origin/shelf-life exposure are listed in Table 4. In this experimental design, studying the variation caused by the different origins also includes the effect of physiological age of the fruit because we are dealing with different hemispheres. Moreover the fruit was bought in the supermarkets so there are no details known about the physiological age of the measured fruit. To interpret the origin effect as clear as possible, this effect was studied separately for the apples originating from the 2 hemispheres.

For the Northern Hemisphere the origin effect is further illustrated with the 'Golden Delicious' data. SSC models were built using the data of season 1 per origin (France, Belgium and Italy) and using a combination of the different origins. Seven latent variables were used for all models. Each model was externally validated with the data for the apples from the origin Italy. The models calibrated with the apples from Belgium and France were able to predict the SSC of the apples from Italy with an RMSEP = 0.52–0.66 and RPD = 1.4–0.9, respectively. The combined model resulted in a model with a higher performance (RMSEP = 0.66 and RPD = 1.6).

'Pink Lady' was chosen to test the origin effect for the Southern Hemisphere. SSC models calibrated using either all apples from Chile, Argentina or New Zealand were validated on samples from South Africa. The model error varied from 0.64 to 1. The RPD value was in the range 0.75–0.90, which is not sufficiently accurate to do powerful predictions. The combined model (Argentina, New Zealand and Chile together) resulted in an RMSEP = 0.6 and RPD = 1.2, which was better, but still not accurate enough. As earlier mentioned, the spectra from Argentina were atypical enlarging the model error. Leaving out Argentina in the combined model gave a much better model resulting in an RMSEP = 0.45 and RPD = 1.5 when applied to the samples from South Africa.

Both these examples illustrate the importance of having a calibration model which is sufficiently representative for the future samples to be predicted with it. This can be obtained by including the relevant variability in the calibration data or by incorporating prior information about this variability in the modelling process (Saeys et al., 2008b).

Table 4
Mean and standard deviation for SSC and firmness per cultivar/origin/storage condition.

SSC (°Brix)	Braeburn		Golden		Jonagold		Royal gala		Pink Lady		Fuji	
Origin/storage	Fresh	Shelf-life	Fresh	Shelf-life	Fresh	Shelf-life	Fresh	Shelf-life	Fresh	Shelf-life	Fresh	Shelf-life
Belgium	14.9 ± 1.2	15.8 ± 1.3	13.8 ± 1.1	13.6 ± 1.1	14.4 ± 1.3	14.2 ± 1.3	13.1 ± 07	12.7 ± 0.8				
France	13.2 ± 0.8	13.0 ± 0.8	13.7 ± 0.7	13.5 ± 0.6			13.3 ± 0.7	12.7 ± 0.8	13.4 ± 0.9	13.3 ± 0.9	15.1 ± 1.2	14.9 ± 1.5
Chile	13.8 ± 1.4	13.8 ± 1.4					14 ± 0.8	13.7 ± 0.9	14.1 ± 0.5	13.8 ± 0.6		
Argentina							13.9 ± 0.9	13.5 ± 0.8	14.5 ± 0.6	14.3 ± 1		
New Zealand	13.8 ± 0.8	13.8 ± 1.0					12.0 ± 0.8	11.8 ± 0.7	14 ± 0.7	13.8 ± 0.8		
South Africa	15.7 ± 1.2	16.3 ± 1.0	15.3 ± 0.9	15.4 ± 1.1					15 ± 0.8	15.2 ± 0.7		
Italy			12.3 ± 0.9	12.2 ± 0.9							13.9 ± 1.4	13.6 ± 1.3
China												
Firmness (N)	Braeburn		Golden		Jonagold		Royal gala		Pink Lady		Fuji	
Origin/storage	Fresh	Shelf-life	Fresh	Shelf-life	Fresh	Shelf-life	Fresh	Shelf-life	Fresh	Shelf-life	Fresh	Shelf-life
Belgium	97.0 ± 6.7	80.9 ± 8.0	54.9 ± 5.7	51.7 ± 4.7	67.7 ± 8.6	54.9 ± 7.2	65.8 ± 4.7	58.9 ± 4.3				
France	58.5 ± 10.0	55.5 ± 8.5	60.4 ± 4.3	59.3 ± 5.9			57 ± 5.3	51.3 ± 5.9	61.1 ± 8.8	57.1 ± 9.3	79.1 ± 7.1	78.1 ± 8.7
Chile	62.5 ± 7.0	57.8 ± 7.9					63.7 ± 6.0	60.0 ± 6.4	87.4 ± 7.3	79.1 ± 6.8		
Argentina							54.4 ± 6.1	49.8 ± 7.2	81.9 ± 7.1	75.7 ± 7.2		
New Zealand	78 ± 6.9	69.4 ± 7.4					81.2 ± 7.2	81.8 ± 6.0	76.7 ± 5.2	74.8 ± 6.4		
South Africa	60.8 ± 6.8	59.4 ± 7.0	49.7 ± 5.1	52.1 ± 4.9					68.1 ± 8.4	61.2 ± 5.9		
Italy			51.9 ± 6.2	47.1 ± 4.7								
China											61.5 ± 6.7	58.2 ± 6.9

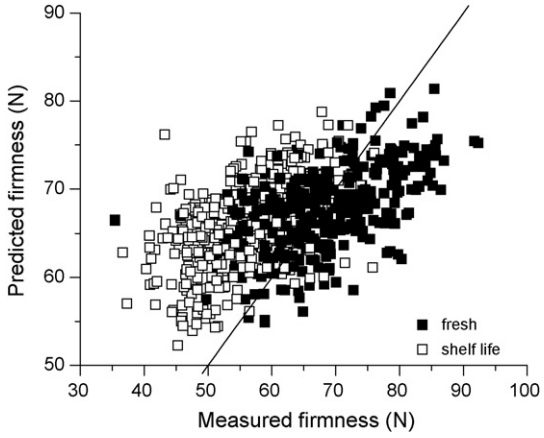

Fig. 4. Predicted vs. measured firmness for 'Jonagold' using a model trained on fresh apples (closed symbols) applied to test sets of apples exposed to shelf-life conditions (open symbols).

3.2.4. Effect of shelf-life conditions

Exposing the apples to shelf-life conditions is another factor which creates variability in the data. As expected, during 1 week at shelf-life conditions the apple firmness decreased considerably while the SSC remained rather stable. In parallel, a decrease in the NIR absorbance was observed. To study the effect of shelf-life exposure on the model performance, both fresh apples as well as apples exposed to shelf-life conditions were used for external validation. Zude et al. (2006) showed that shelf-life exposure affects the accuracy of NIR calibration models for SSC and firmness.

From Table 2 it is clear that better results were obtained when the calibration model for SSC was based on fresh apples and the validation on apples exposed to shelf-life exposure than the other way around. In the former case, the RMSEPs were slightly higher than the RMSECVs, but still acceptable for most cultivars. In the latter case, the RMSEP was as high as 1.3 °Brix ('Braeburn'). Only for 'Golden Delicious' and 'Fuji' the RPD values were larger than 1.5. Similar results were obtained by Zude et al. (2006). For firmness neither ways provided predictions with acceptable RMSEPs (Table 3). The effect of applying a calibration model for firmness prediction based on fresh apples to apples which had been exposed to 1 week of shelf-life is illustrated in Fig. 4 for 'Jonagold'. The firmness values for the stored apples are considerably overestimated, leading to a high RMSEP. This can be explained by the fact that the ranges of firmness values for fresh and shelf-life exposed apples is considerably different.

3.3. Functional ANOVA

To study the effect of biological variability on the spectra in more detail, functional ANOVA (FANOVA) was applied based on the functional representation of spectra.

For most studied cultivars the global origin, cultivar and shelf-life effects were found to be significant with a p-value ≤ 0.05 based on the permutation significance test (Table 5). The origin effect was studied separately for the 2 hemispheres to avoid confounding with the physiological age.

Fig. 5 shows the absorbance functions for the different effects as obtained from the functional analysis of variance (FANOVA) using Eq. (2) applied to the apple spectra of 'Pink Lady' only including Southern Hemisphere origins. The upper left plot shows the overall mean function, indicating that it is an apple spectrum. The upper right plot shows the absorbance functions which should be added to the overall mean function to obtain the average spectrum for each of the origin groups. The functional effect of 1 week of shelf-life, is illustrated in the lower left plot. This absorbance function indicates how the apple spectrum on average has changed after 1 week of shelf-life (18 °C). The lower right plot illustrates the difference in shelf-life effect on the absorbance spectra for the 'Pink Lady' apples belonging to different origin groups. In all the different effects, the absorbance peaks around 970, 1170 and 1450 nm can be seen, which indicates variation in the water content of the measured apples due to the different effects investigated. From the shelf-life effect it is clear that the absorbance is decreasing and the largest decrease was found around 1450 nm, mainly due to the water loss during shelf-life. Hertog et al. (2004) showed that during storage a decrease in apple stiffness can be related with both water loss and enzymatic cell wall breakdown. Both phenomena will influence the propagation of light through the apple tissue. The enzymatic cell wall break down will reduce the tissue structure and thus the light scattering at the cell interfaces, leading to less scattering events in the same tissue volume. Due to this reduced light scattering less light will be scattered backward (reflectance) resulting in lower reflectance and thus higher absorbance (Peirs et al., 2000; Peirs, 2002). On the other hand, water loss from the apple tissue will result in smaller cells (denser packing of the scatterers) and more air-filled pores (higher refractive index mismatch) leading to more and stronger scattering events in the same tissue volume (Schotmans et al., 2004; Nguyen et al., 2006). This increased light scattering might explain the higher overall reflectance and thus lower overall absorbance giving rise to the negative shelf-life effect. Depending on the type of fruit and its physiological status, the relative contributions of turgor and the mechanical strength of the cell wall to stiffness might vary. This interplay of increased

Table 5
Results of global significance test for the cultivar, origin and shelf-life effect. Corresponding p-values are shown.

Cultivar	Origin effect: corresponding p-values for Southern hemisphere			
	Chile	Argentina	New Zealand	South Africa
Pink Lady	0.001	0.001	0.001	0.001

Origin	Cultivar effect: corresponding p-values				
	Braeburn	Golden Delicious	Royal Gala	Pink Lady	Fuji
France season 2	0.001	0.5	0.001	0.001	0.001

Cultivar	Shelf-life effect: corresponding p-values
Jonagold	0.001
Braeburn	0.001
Golden Delicious	0.001
Royal Gala	0.001
Pink Lady	0.001
Fuji	0.001

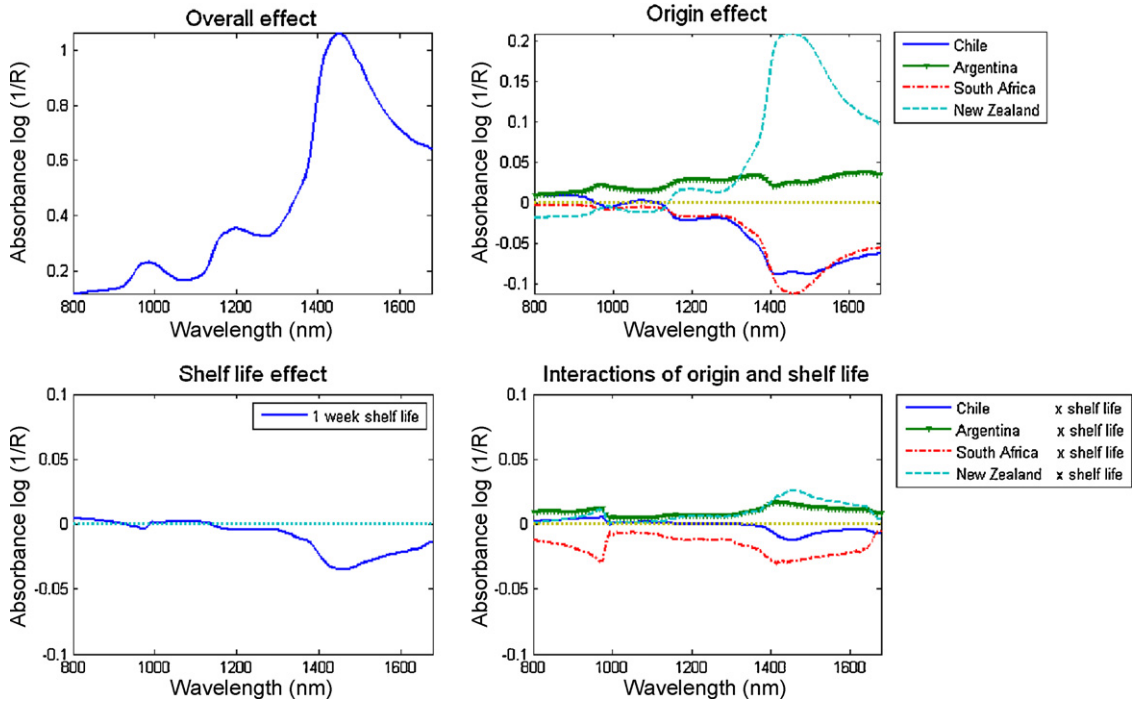

Fig. 5. Plot of the contribution of the overall mean function (upper left), the functional effect of shelf-life (lower left), origin (upper right) and the interaction of origin and shelf-life (lower right) to the NIR absorbance spectrum of 'Pink Lad'y according to the functional analysis of variance (FANOVA).

scattering due to water loss and decreased scattering due to enzymatic cell wall breakdown complicates the relation between the measured spectra and firmness values for postharvest apples, leading to a positive or negative correlation between absorbance and firmness depending on which effect dominates the spectrum.

The overall decrease in the absorbance spectrum due to shelf-life (negative shelf-life effect function) suggests that during the shelf-life experiment the effect of the water loss was more pronounced than the changes in microstructure. The 95% confidence interval (Fig. 6) shows that from 1150 to 1680 nm the main shelf-life effect is significant (zero is not included). With respect to the origin, the apples from New Zealand have a higher absorbance in the NIR region compared to the other origins. The apples from South Africa have a lower absorbance. Based on the reference measurements it was found that the apples from South Africa were riper than the others (higher SSC and lower firmness). It is clear that the higher water loss in these apples is playing an important role in the measured absorbance spectrum. Other chemical and textural changes cannot be well related to this result. Peirs et al. (2000) observed that pre-harvest ripening of apples was related with in a decrease in reflectance (≈increase in absorbance) in the NIR region due to different physicochemical changes such as enzymatic cell wall breakdown. In our study, however, postharvest ripeness was examined where water loss from the harvested apple also has an important impact on the spectra.

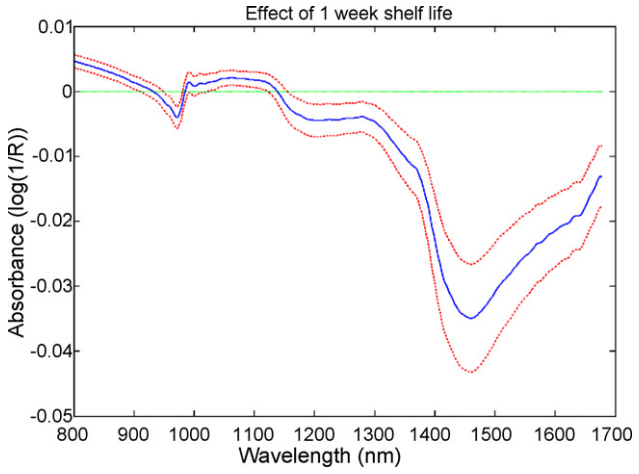

Fig. 6. Shelf-life effect on the NIR reflectance spectrum of 'Pink Lady' with 95% confidence interval (dotted line).

From the 95% confidence intervals around these origin effects (Fig. 7) it can be concluded that for most origins the whole wavelength range is significant (zero is not included). From the interaction (Fig. 5) between origin and shelf-life it is clear that the average apple spectra for each origin are differently affected by shelf-life exposure.

Three different apple cultivars ('Golden Delicious', 'Braeburn' and 'Pink Lady') from the origin France were investigated over two seasons (only the results for 'Braeburn' are shown in Fig. 8). The absorbance functions for season 2 (France s2) show a clear decrease in the absorbance especially around 1450 nm when compared with those for season 1 (France s1). Comparison of the functional effects for 'France s1′ and 'France s2′ shows that the seasonal effects are significantly different around the water peaks, suggesting that the water content has been affected by the season. The overall lower absorbance for season 2 comprises different physicochemical changes. Apples from season 2 were freshly harvested and still at the beginning of the storage season. It seems that the higher water content of these apples is not predominating in the measured spectrum because we are dealing with a lower absorbance. The fresher apples will have a better tissue structure with less cell wall break down resulting in a higher reflectance and thus lower absorbance. These results are in accordance with the findings of Peirs et al. (2000).

Moreover, Bergh (1985) reported that the sugar and acid content as well as cell size, number of cells and amount of

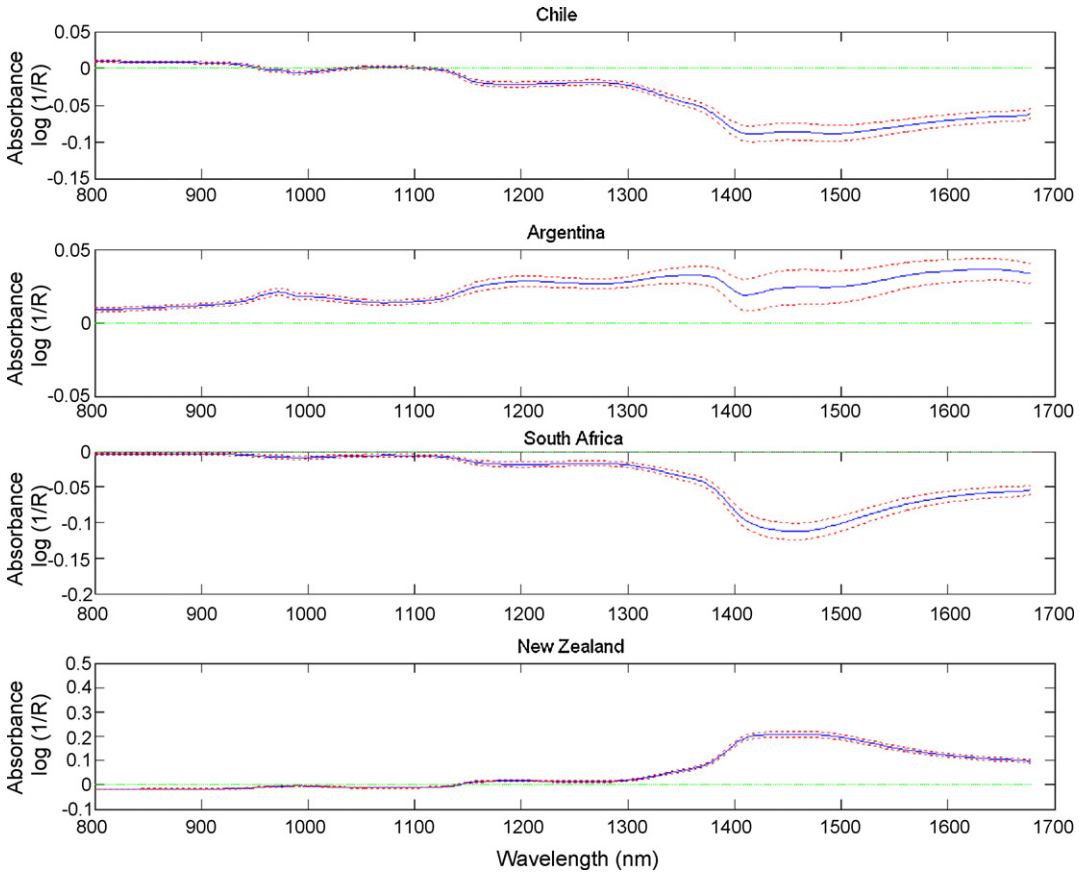

Fig. 7. Origin effect on the NIR absorbance spectrum of 'Pink Lady' with 95% confidence intervals (dotted line).

intercellular spaces differ between seasons due to different weather conditions, such as precipitation, hours of sunshine and temperature.

Apples from the origin France and season 2 (November) were selected to investigate the cultivar effect, as for this origin and season all cultivars (except 'Jonagold') were measured. This subset of apple spectra was then analyzed using FANOVA with the model of Eq. (4) to determine the contributions of the different cultivars to the absorbance spectra. As we could expect from the PLS models there are large differences between the average absorbance spectra for the different cultivars. The highest differences in absorbance between the cultivars were also found around 970, 1170 and 1450 nm (Fig. 9). Since these are OH-absorption bands which dominate the absorption coefficient of apple tissue (Saeys et al., 2008c), this can be related to differences in the water absorption, and thus water content, of the different cultivars. For most cultivars the whole wavelength range was found to be significant in explaining the cultivar effect. However, for 'Golden Delicious' only the three water absorption bands were significant which leads to a cultivar effect which is not globally significant (Table 5).

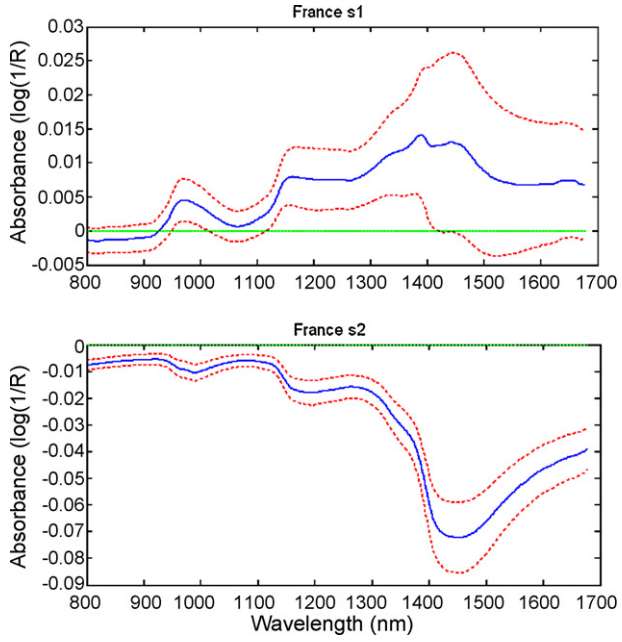

Fig. 8. Seasonal effect on the NIR absorbance spectrum of 'Braeburn' with 95% confidence intervals (dotted line).

The cultivar 'Royal Gala' had the highest absorbance, and thus lowest reflectance. This cultivar is known as a cultivar with a smaller storage potential compared to the other cultivars, so these apples are likely to be in a more advanced ripeness stage compared to the others. Also, 'Royal Gala' has an earlier commercial picking date than the other cultivars. This result is in accordance with the results of Peirs et al. (2000), showing that riper apples have a higher absorbance. When the SSC content and the firmness results for the 'Royal Gala' apples are investigated it can be seen that the apples were rather soft but also the SSC content was quite low. The higher absorbance by the 'Royal Gala' apples can thus most likely be explained by a combination of chemical and textural (less scattering) differences due to the more advanced ripeness stage. McGlone and Kawano (1998) also concluded that a firmness decrease in kiwifruit resulted in an increase of absorbance in the NIR region.

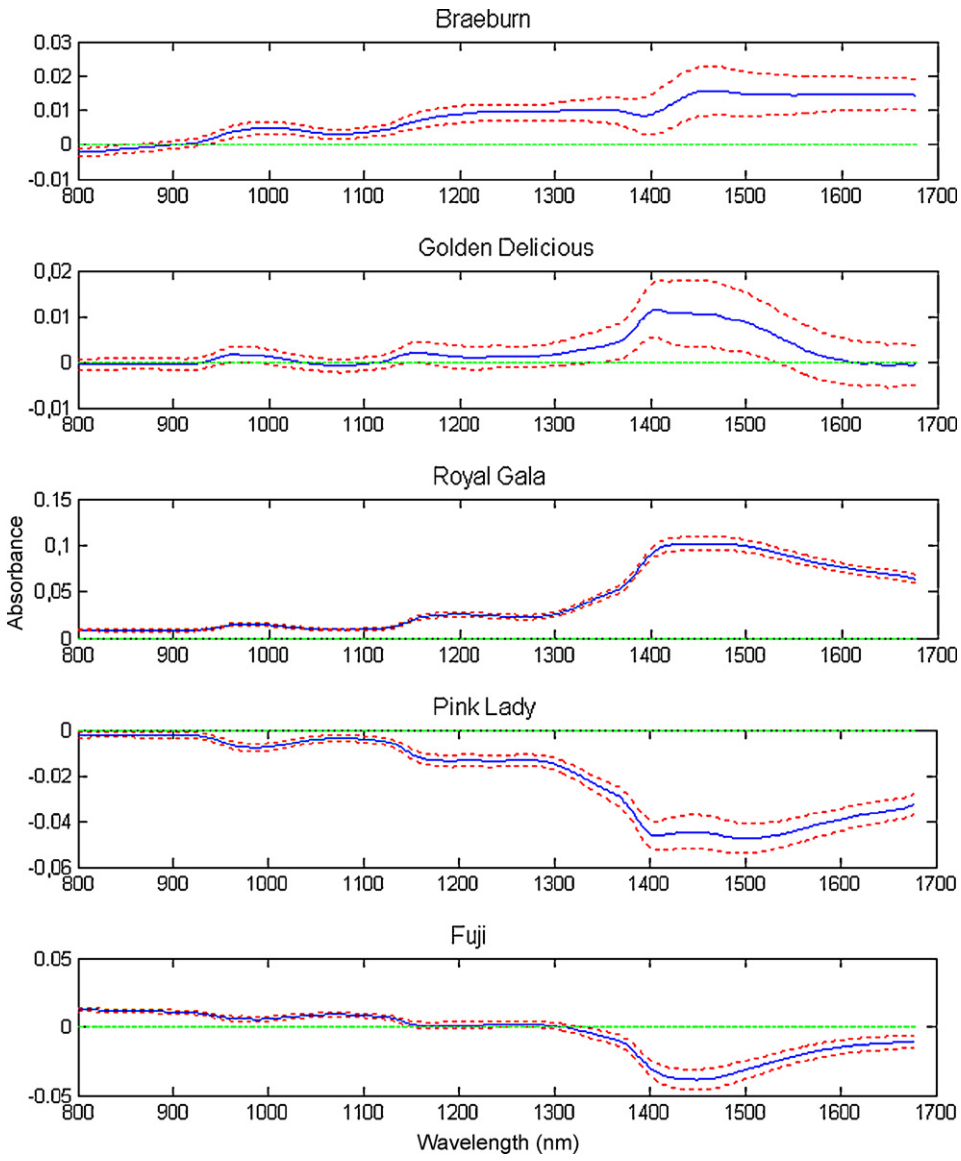

Fig. 9. Cultivar effect on the NIR absorbance spectra from origin France and season 2, with 95% confidence intervals (dotted line).

4. Conclusions

NIR prediction models were constructed for SSC and firmness based on a large dataset of apples from different seasons, origins, cultivars and storage conditions. In general the RMSEP values for predictions of the SSC were in the range 0.6–0.8 °Brix, while for Magness Taylor firmness it was 5.9–8.8 N, depending on the cultivar. Through external validation it was shown that a robust calibration model requires large data sets with sufficient relevant variability. In this study variability was introduced in the calibration data set by including data of different seasons, origins, cultivars and storage conditions. Using FANOVA the effect of biological variability on the measured spectra was further explored. It was found that the origin (geographical and seasonal), cultivar and shelf-life effects significantly affect the spectra at 970, 1170 and 1450 nm, which can be related to differences in the water absorption, and thus the water content in the apples. One week of shelf-life was found to decrease the absorbance at the water peak, which can be attributed to water loss during shelf-life. The FANOVA analysis also revealed large differences between the different apple cultivars.

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