

Discrimination of storage duration of apples stored in a cooled room and shelf-life by visible-near infrared spectroscopy

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Improved non-destructive instrumental approaches for grading fruit during post-harvest could be an efficient way to monitor stock in the apple industry. The objective of this study was to evaluate the ability of visible-near infrared (vis-NIR) spectroscopy in reflectance mode for classifying apples left on the shelf or stored in a cooled room. The ability of NIR spectroscopy to classify the duration of storage of three apple cultivars in two storage modalities was evaluated. A total of 450 fruit, sampled after 7, 14, 28, 60, 90 and 120 days of storage in a cooled room (CR) and 7, 14 and 28 days in shelflife (SL), has been studied. The classification of these modalities was analysed by factorial discriminant analysis (FDA) pooling the spectral data of all cultivars (global models) into a common data set. Then, the cultivar effect on the classification of the same modalities was analysed by processing data from each cultivar in separate factorial discriminant analyses. A preliminary analysis showed the genetic variability of spectral data due to the three apple cultivars. We show that vis-NIR spectroscopy allowed the correct classification of the fruits of each cultivar by more than 95%. The classification relied on both vis and NIR absorption bands : 500, 680, 1400 to 1700, 1850, 1950, 2200 and 2300 nm. We show that storage modalities of *global models* can be classified by more than 75% and 83% for fruits stored in a cooled room and shelf, respectively. Classification of the same storage modalities was improved by *cultivar models* with percentage of individuals correctly classified of 86% (Gala), 89% (Elstar) and 85% (Smoothie) for fruits stored in a cooled room and 95% (Gala), 98% (Elstar) and 95% (Smoothie) for fruits left in shelflife. We conclude that despite the slight increase of efficiency of the models when we considered each apple cultivar separately, global models applicable to a set of different cultivars presents a correct level of classification and could be usefull for some commercial applications.

Keywords: apple, FDA, storage, visible-NIR spectroscopy, non-destructive, shelf-life

Introduction

The duration and the conditions during storage of apples (*Malus domestica* L.) play an important role in the final quality of the fruit^{1–5} and may depreciate their commercial value on the market. In order to carry out a better diagnosis of fruit quality, various studies have reported fast and non-destructive measurements of fruit quality.^{6–12}

Among these measurements, visible-near infrared (vis-NIR) spectroscopy in reflectance mode presents the advantages of being a non-destructive and non-invasive analytical method. Vis-NIR spectroscopy makes it possible to obtain information on inner properties of products through the measurement of vis and NIR light absorption at specific wavelengths.¹³ The light beam is able to penetrate the fruit beyond an apple cuticle, allowing analysis of biochemical properties of the parenchyma tissue.¹⁴ For these reasons, vis-NIR spectroscopy appears to be well adapted to assist the fruit industry and growers.

However, biological information cannot be directly extracted from raw spectral data. Several treatments are necessary to reduce the uncontrolled baseline variations and chemometric techniques are then able to correlate the spectral data to the quality attributes of the fruit.

Treatments used to cope with the uncontrolled variations of absorbance include standard normal variate (SNV)¹⁵ and the first and second derivatives. Furthermore, the first and second derivative methods can cope with the overlapping effect of wide wavelength bands in the NIR region.

Chemometric approaches can be divided into two groups. The first corresponds to the regression methods aiming to predict quantitative values of quality attributes of fruit and a second group that corresponds to discriminant methods aiming to classify different qualitative groups of fruit according to a given variability.

Among regression methods, the most used are partial least square regression (PLSR), principal component

regression (PCR) and multiple linear regression (MLR). These methods have been used to build predictive models of various quality attributes, such as soluble solids content (SSC), titrable acidity (TA) or dry matter (DM), for apples,^{7,10,16} kiwi fruits⁶ or mangos.¹¹ Such quantitative predictions present two limits. First, such models need to be developed for each apple cultivar separately to be efficient enough and second, because the biological changes in fruit which occur during storage are multi-factorial, the prediction of a given quality parameter (SSC, TA, etc.) is not enough to have a complete view of the fruit evolution in post-harvest.

Other chemometric methods could help to access the global changes of fruit by analysing the variability of whole vis-NIR spectra. Among the most used methods are factorial discriminant analysis (FDA), partial least square discrimination (PLSD) and neural networks.^{17,18} However, such models are used considerably less compared to those based on regression methods for following fruit quality during storage. This under-utilisation is probably due to the difficulties for scientists to give a biological interpretation of absorbance at some wavelengths and, consequently, explain the variability measured between fruits.

The global objective of the present work was to study the effects of the duration of storage on apples during post-harvest by taking into account the variability of the entire vis-NIR spectra. Our approach, never validated until now, could allow for the integration of the majority of the biochemical changes occurring in fruit during storage. Such analysis could provide an objective and complete view of the fruit as it changes during post-harvest compared to the prediction of a single quality parameter such as SSC, TA, etc. Also, the objective was to assess models able to follow the fruit changes during storage whatever the cultivar and not one model per cultivar which is currently an important limit for the professional application of NIR spectroscopy.

In concrete terms, we performed factorial discriminant analyses on spectral data of three apple cultivars stored in cooled room and in shelf-life in order to classify the fruits according to their duration of storage. The efficiency of the models was discussed and the interpretation of the relevant wavelengths of the models was attempted.

Materials and methods

Sample collection

Fruit from three apple cultivars (*Gala*, *Elstar* and *Smoothie*), harvested at commercial maturity, were collected from the orchards of the National Institute of Agronomic Research (INRA) of Angers (France). *Gala* and *Elstar* were picked on the same day and *Smoothie* one week later. The apples were selected on the basis of size uniformity (~60–65 mm) and lack of physical damage to the surface. They were then stored in a cooled room [2°C,

95% relative humidity, (RH)] until the beginning of the trial.

The fruit was then split up into two groups. The first group (CR) was kept in the cooled room (2°C, 95% RH) and the second (SL) was stored in shelf-life conditions (~20°C, 40% RH). For this study, 15 CR fruits by cultivar were drawn out after 7, 14, 28, 60, 90 and 120 days of storage and 15 SL fruits were collected after 7, 14 and 28 days for further study. A total of 450 fruit (150 fruit of three cultivars) were tested in this way.

Spectra acquisition

Spectra were directly acquired, in reflectance mode, on the whole fruit using a vis-NIR spectrometer (NIRSystems, Model 6500; Foss NIRSystems Inc., Laurel, MD, USA). The fruit was equilibrated at room temperature 24h before analysis. Spectral acquisition was carried out in direct contact analysis mode, without a fibre-optic, by placing the fruit directly on a circular ring located at the top of the spectrometer. Absorbance spectra, using 32 scans, were recorded at a resolution of 2 nm from 400 to 2498 nm. A white reference scan was carried out before each sample. For each fruit, vis-NIR measurements were done on opposite sides (intermediate colour sides) along the equator of the fruit. Then, in order to have a representative spectral signature of the fruit, the two spectra recorded per fruit were averaged for further calculation. A total of 450 spectra were used.

Data analysis

Spectral data pre-treatment

In order to reduce the effects of uncontrolled baseline and intensity variations, spectra were corrected using the standard normal variate (SNV) method.¹⁵ The corrected spectral data are given by Equation (1):

$$x_{ij}(\text{corrected}) = \frac{x_{ij} - \bar{x}_i}{S} \quad (1)$$

With $i = 1, 2, \dots, p$, where x_{ij} is the absorbance of the spectrum i (p data points) measured at wavelength j , \bar{x}_i the average value of the absorbance of the uncorrected spectrum and S is the standard deviation of the spectrum, defined as Equation (2):

$$S = \sqrt{\frac{\sum_{i=1}^p -x_{ij} - \bar{x}_i}{p-1}} \quad (2)$$

Factorial discriminant analysis

Factorial discriminant analyses (FDA) were carried out on the spectral data. A given spectrum curve forms a vector x_i of p wavelengths. The n spectra were gathered into a matrix X dimensioned $n \times p$. Due to the collinear nature of the wavelength absorbances, it was impossible to directly

perform FDA. In order to cope with this collinearity, a modified version of FDA was applied.¹⁹

In FDA, the qualitative groups to be discriminated were the cultivars (*Gala*, *Elstar* and *Smoothie*) and the storage duration for each condition of storage (CR and SL).

There were, for each cultivar, six durations of storage for fruit stored in CR (7, 14, 28, 60, 90 and 120 days) and three durations for fruit stored in SL (7, 14 and 28 days). The starting point (*day 0: harvest time*) was the same for both conditions of storage, giving a single group.

A criterion of efficiency of the FDA is the proportion of correctly classified observations in validation sets. These validation tests were carried out by dividing the data matrices, X , into a training and a validation set. The FDA model was computed on the calibration set. The observations of the validation set were then classified using the established model. The observations correctly classified were then counted and expressed in percentages. Such validation tests were independently carried out ten times, placing two thirds ($2n/3$) of the observations in the calibration set and the remaining ones ($n/3$) in the validation set.

FDA computes a set of discriminant scores, which are linear combinations of the original variables. The discriminant scores are new “synthetic variables” calculated so they can discriminate the observations. It is interesting to examine the correlation between the discriminant scores and the predictive variables. For this purpose, the correlation coefficients between the discriminant scores and the original variables (1050 wavelength absorbencies) were computed. As it is impossible to show 1050 correlation coefficients, the correlations values were graphically represented as curves giving the correlation coefficient of the absorbance at each wavelength with a given discriminant score.

All the statistical procedures were carried out using the Matlab 6.0 environment (The MathWorks, Inc., Natick, MA USA).

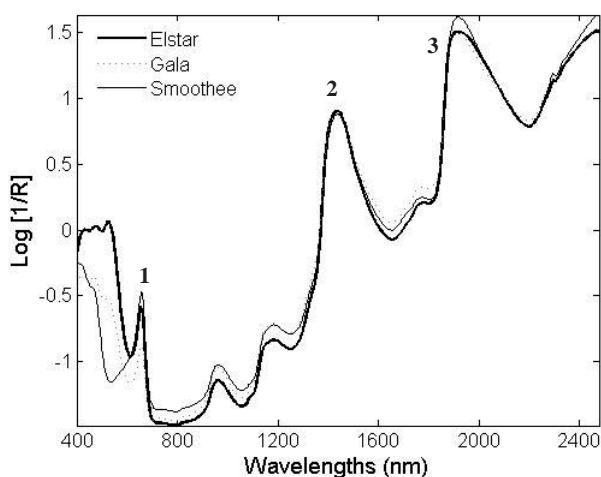


Figure 1. Mean spectrum of each apple cultivar at harvest time (*day 0*). 1: chlorophyll peak, 2 and 3: water absorption peak.

Results and discussion

Preliminary study on genetic variability of apples

Natural variability of apple cultivars can influence the fruit quality at harvest and during long term storage.²⁰ In order to evaluate the cultivar variability, we processed the spectral data of fruit at harvest (*day 0*) in a FDA.

The average spectrum of each apple cultivar at the time of harvest is presented in the Figure 1. These spectra show quite different patterns in the vis region and very close patterns in the NIR region. Variation in vis absorption (400–800 nm) is related to chlorophyll content with a weak absorption band in the vicinity of 680 nm (peak 1) and more globally related to fruit colour. Previous studies have shown that absorbance in the vicinity of 1450 nm and 1900 nm are mainly related to water content of apple fruit.^{12,14,21}

The efficiency and robustness of the discrimination mainly depend on the number of introduced principal component (PC) scores in the discriminant model of calibration. An important number of PCs may lead to an apparently efficient but non-robust model. In order to identify the most robust and efficient model, a compromise must be found. The determination of the optimal number of PCs was carried out by examining the number of fruits correctly classified as a function of the number of PCs introduced in the mathematical model, where a large number of PC has been voluntarily introduced (20 PC). Figure 2 shows the results obtained in each cross-validation. The first PC correctly classified between 58% and 82% of the fruit. The introduction of a second PC led to about a 90% of correct classification. This percentage reached a plateau at 97% when the third PC was introduced. Thus, the following FDA on apple cultivars was built with three PCs.

FDA applied on validation set correctly classified 97%, 95% and 100% of *Gala*, *Elstar* and *Smoothie* fruit, respectively. Figure 3 shows the factorial map according the first two factorial scores from FDA.

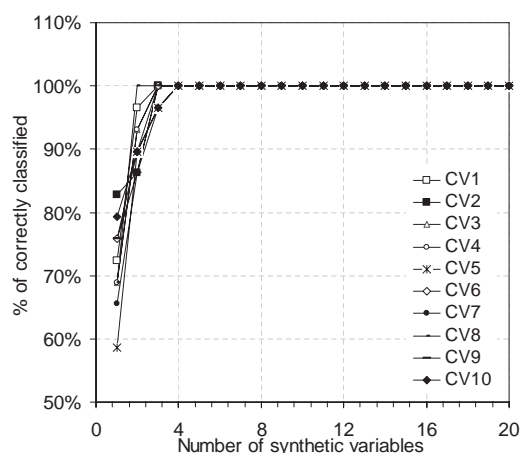


Figure 2. Percent of fruits in the validation set correctly classified expressed as a function of the number of dimensions for each cross-validation (CV). FDA applied on apple cultivars.

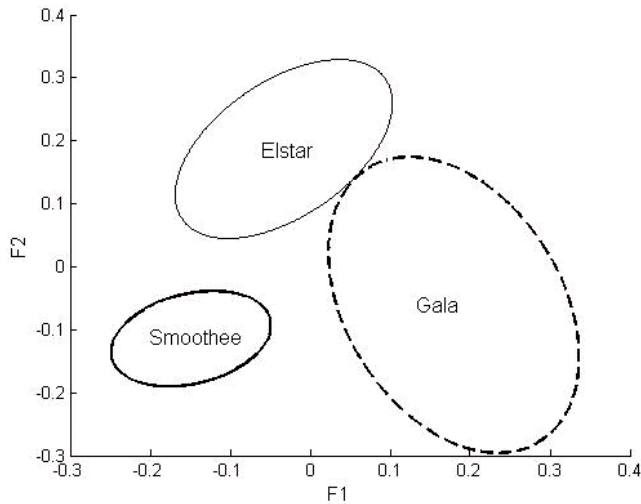


Figure 3. Factorial map according the first two factorial scores (FDA of cultivars). The ellipses present the confidence intervals of each group of cultivar at a threshold of 0.05.

The first factorial score allows the discrimination of the three apple cultivars whereas the second one only separates *Elstar* from a second group consisting of *Smoothee* and *Gala*.

Figure 4 shows the correlation between the first two factorial scores and the absorbance at each wavelength. In the vis range, the first factorial score was negatively correlated to absorbance at 680 nm, whereas the second factorial score was positively correlated to absorbance in the vicinity of 500 nm. These correlations can be explained by the difference in the background colour of the three cultivars, *Smoothee* being a yellow apple and *Elstar* a red one. Absorbance at 680 nm is related to chlorophyll pigment and absorbance in the vicinity of 500 nm is related to anthocyanins responsible for the red colour of exocarp (skin). Wavelength absorbance in the vis-NIR transient region, between 800 nm and 1300 nm, was negatively correlated with the second factorial score, exclusively. In the NIR region, the first factorial score was positively correlated to absorbance in the vicinity of 1400 nm, 1850 nm and 2200 nm and negatively correlated to absorbance at 1950 nm. The second factorial score was negatively correlated to absorbance at 1700 nm and 2300 nm. The interpretation of some of these absorbances is given later on.

A previous study¹⁴ showed that the depth of penetration of vis-NIR energy is wavelength dependent with maximum penetration for wavelengths of the vis-NIR transient region between 700 nm and 900 nm. In this range, light can penetrate up to 4 mm in apple flesh. In the NIR range, it penetrates to about 2 mm. Our results show that some of the most important wavelengths for discriminating the apple cultivars are located in the vis range between 600 and 800 nm. Thus, we can think that more useful information could be collected in the vis range because of the greater distance covered by the visible light in fruit flesh. Also, the importance of the vis

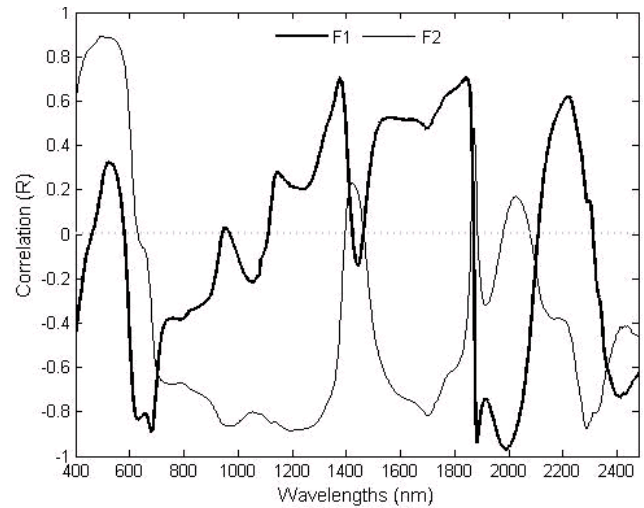


Figure 4. Correlation between the first two factorial scores and the wavelength absorbencies (discrimination of cultivars).

range can be classically related to colour change of apple skin with the duration of storage.^{22,23} The importance of absorbance in the NIR region could be related more to biological differences between cultivars.

Storage effect

The following FDA models analysed the storage effect on spectral data of fruit stored in CR and SL. The preceding analysis showed the importance of genetic variability existing between the three studied apple cultivars. Thus, we analysed spectra variability during storage by building two kinds of FDA models:

- First, spectra of the three apple cultivars were pooled in a single data set. From this set, two FDA models were built to predict the duration of storage in CR and SL separately: “*global models*”.
- Second, FDA models were built for each apple cultivar independently. Thus, three models for fruit stored in CR (*Gala*, *Elstar* and *Smoothee*) and three models for fruit stored in SL were tested: “*cultivar models*”.

Global models

Table 1 shows the percentage of individuals correctly classified using the global models (CR and SL). Average percentages reached 75% for fruit stored in CR and 83% for fruit stored in SL. Two reasons can explain the higher efficiency of the SL model compared to the CR model: (1) the most important changes of fruit quality in SL compared to CR and (2) the most important number of qualitative groups to be discriminated for fruits stored in CR compared to SL, leading to confusion about the different modalities of storage.

An FDA map of global models is presented in Figures 5(a) and 5(b). The first two factorial scores allowed the ranking of all qualitative groups of fruit stored in CR according

Table 1. Percentage of fruits correctly classified in each FDA. PC: number of synthetic variables, CR: cooled room storage, SL: shelflife storage.

Qualitative groups (storage in days)	Global models		Gala		Elstar		Smoother	
	CR (PC:12)	SL (PC:12)	CR (PC:12)	SL (PC:6)	CR (PC:12)	SL (PC:6)	CR (PC:10)	SL (PC:6)
Day 0	77	91	93	100	100	98	80	100
7	82	93	100	87	100	94	100	87
14	78	67	80	100	93	98	80	100
28	71	83	80	93	80	100	87	93
60	64	—	80	—	87	—	67	—
90	82	—	87	—	93	—	80	—
120	74	—	79	—	71	—	100	—

to the duration of storage [Figure 5(a)]. The first factorial score allowed a separation in the duration of storage between the 7th and the 120th day. The second factorial score allowed the separation of the fruit stored between 0 and 14 days on

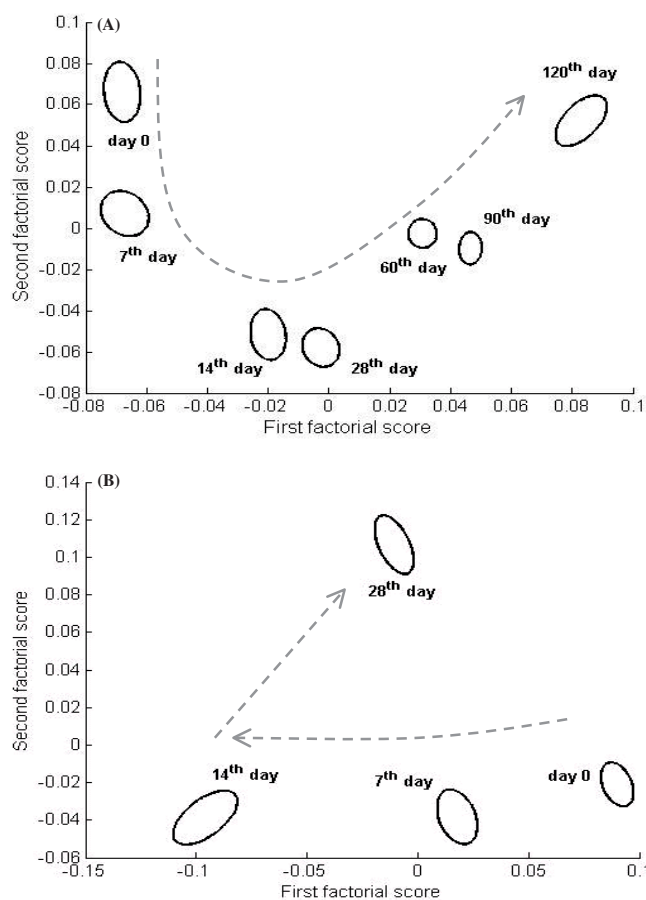


Figure 5. Factorial map of (A) CR and (B) SL storage according the first two factorial scores (global models). The ellipses present the confidence intervals of barycentre. The number in each ellipse represents the duration of storage in days.

the one hand, or the fruit stored between 28 and 120 days on the other hand.

Fruit stored in SL were separated and ranked according to the duration of storage by the first factorial score until the 14th day. Fruits stored for 28 days were discriminated from the other by the second factorial score [Figure 5(b)].

Thus, global models show relative efficiency, but confusion exists between the apple cultivars, particularly in CR condition where 75% of fruit are correctly classified meaning that 25% remain incorrectly classified (Table 1).

Cultivar models of the duration of storage

The collection of 450 spectra was split into six sub-sets corresponding to cultivar (three modalities) stored at a given temperature (CR and SL). FDA was applied on each of these sub-sets, considering the storage duration as qualitative groups. The ability of each FDA to discriminate the storage conditions was tested by ten cross-validations. The optimal number of PCs was determined independently for each discriminant model.

Table 1 shows the number of introduced PCs in each model. All FDAs correctly classify at least 90% of individuals of the training set. It can be noticed that the optimal number of PCs was higher when applying FDA on fruit stored in CR compared to fruit stored in SL. As the evolution of the fruit in CR is logically less marked than in SL, it is logical that a more complex model is required for predicting the storage duration in CR than in SL.

The efficiency of the FDA models is presented in Table 1. About 86% (*Gala*), 89% (*Elstar*) and 85% (*Smoother*) of the fruit stored in CR was correctly classified in the validation sets against only 75% for the global model. For fruit stored in SL, these percentages reached 95% (*Gala*), 98% (*Elstar*) and 95% (*Smoother*) against 83% for the global model. The higher efficiency of the discrimination of fruit stored in SL, compared to those stored in CR, was probably due to the most important changes of fruit quality attributes in SL. According to our results, we

also conclude that FDA models built by cultivar offered improved fruit classifications compared to the global models, showing the importance of cultivar effect in such discriminant approach. Nevertheless, the percentage of fruit correctly classified by the global models was reasonable and, while these could be useful for the apple indus-

try, it will be necessary to confirm this result with a larger number of apple cultivars.

Identification of relevant wavelengths

In order to identify the most important wavelengths of each FDA model, the correlation between the first two factorial

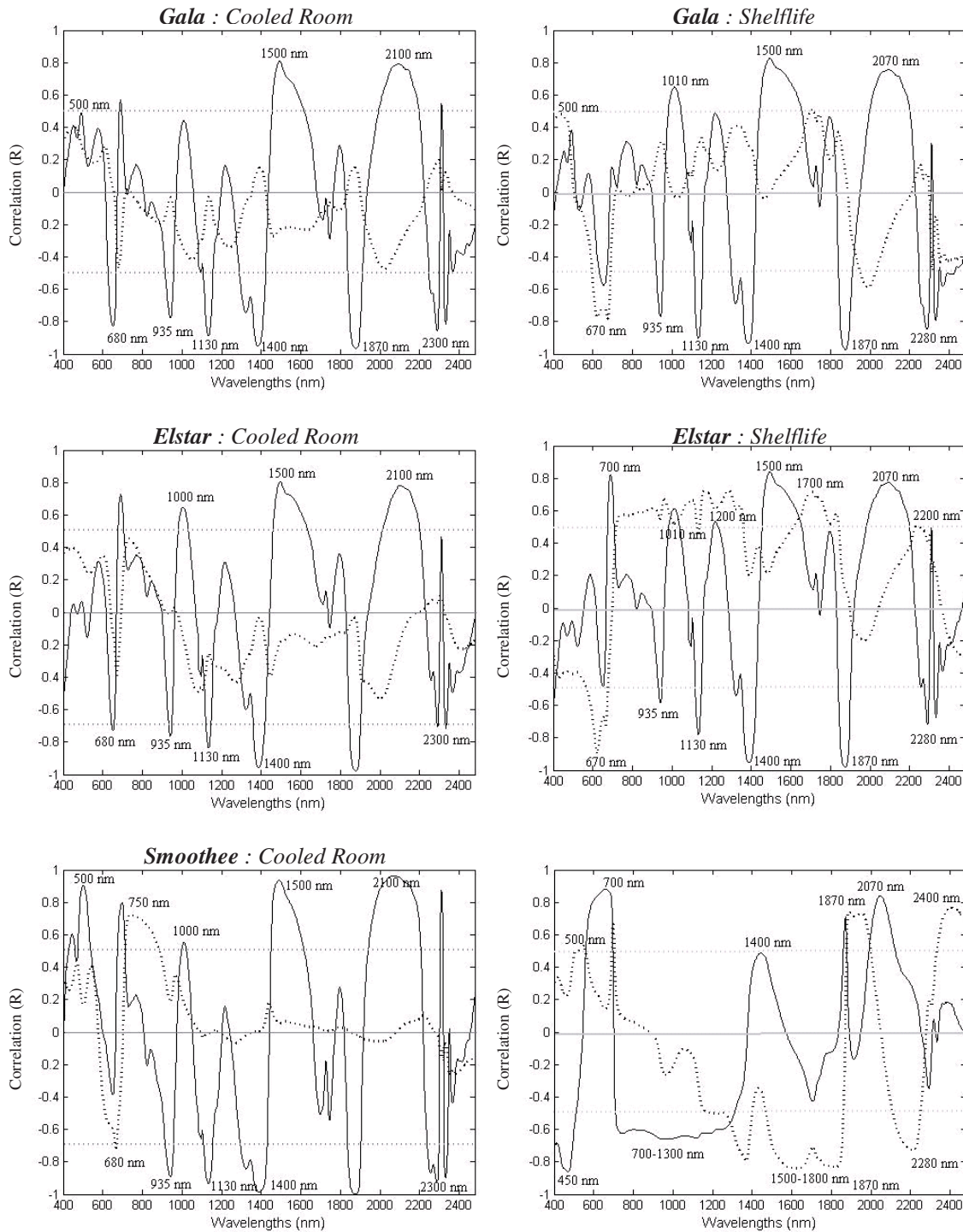


Figure 6. Correlation (R) between the first two factorial scores of FDAs (F1: full line and F2 : dotted line) and the absorbance intensity at each wavelength. FDA models on fruits stored in *Cooled Room* and in *Shelflife*, for each apple cultivar (Gala, Elstar and Smoothie). The numbers expressed in nm on graphs represent the location of the most relevant wavelengths in the established models.

discriminant scores and the absorbance at each wavelength was computed (Figure 6). High correlations were found both in the vis and the NIR regions, showing the importance of the whole spectral region for the discrimination. Most of the relevant wavelengths identified were similar for CR and SL models of each apple cultivar, except for the SL model of *Smoothie*.

In the vis range, two absorption bands can be considered as relevant in the vicinity of 500 nm and 680 nm. Variation of absorbance at 680 nm is related to the decrease in chlorophyll content of fruit skin which occurs during storage. Variation of absorbance in the vicinity of 500 nm is commonly related to the blushed colour of the skin that appears during the fruit senescence.^{14,22} It can be noticed for the *Elstar* models, that absorbance at 500 nm was less important compared to the other cultivar models. This difference can be explained by the natural red background colour of this cultivar. Thus, relevance of this wavelength region, in the vicinity of 500 nm, could be dependant on apple skin colour and be less efficient to grade the fruits with a natural and pronounced red skin. Previous study showed that firmness decrease during storage, an important quality parameter of apple fruit, was closely related to chlorophyll decrease in fruit.⁷ Thus, they showed that an efficient predictive model of this parameter strongly relied on absorbance at 680 nm. Through our results, we confirm the decrease of such absorbance at 680 nm and, consequently, firmness during storage (Figure 7).

Concerning the transient vis-NIR region, two important absorption bands were identified in the vicinity of 935 nm and 1000 nm. Previous studies have shown that absorbance in this region could be correlated to water losses and sugar content.^{24–28} In an earlier study,⁷ it was shown that the increase of SSC during storage is mainly related to absorbance between 800 nm and 1000 nm. Average absorbance in this spectral region for *Gala* and *Elstar* cultivars illustrates the possibility to rank the fruits stored in CR according to their duration of storage (Figure 7). Thus, classification of fruits using this spectral region is certainly related to SSC increase during storage.

In the NIR region, four major absorption bands were identified in the vicinity of 1400 nm, 1870 nm, 2070 nm and 2400 nm. It is generally difficult to explain the absorbance at a given wavelength due to the overlapping effect of several molecular bonds constituting different quality attributes of fruit. Absorbances in the NIR region are related to the first and second overtones or combinations of fundamental absorption bands, these being located in the mid-infrared region (between 3000 cm^{-1} and 1700 cm^{-1} , i.e. between 3333 and 5882 nm). Most molecular bonds involved are C–H, O–H and N–H. So, absorbance in the vicinity of 1400 nm correspond to the first overtone of O–H, absorbance in 1870–2070 nm could be related to O–H and NH combinations and absorbance at 2400 nm could be related to C–H

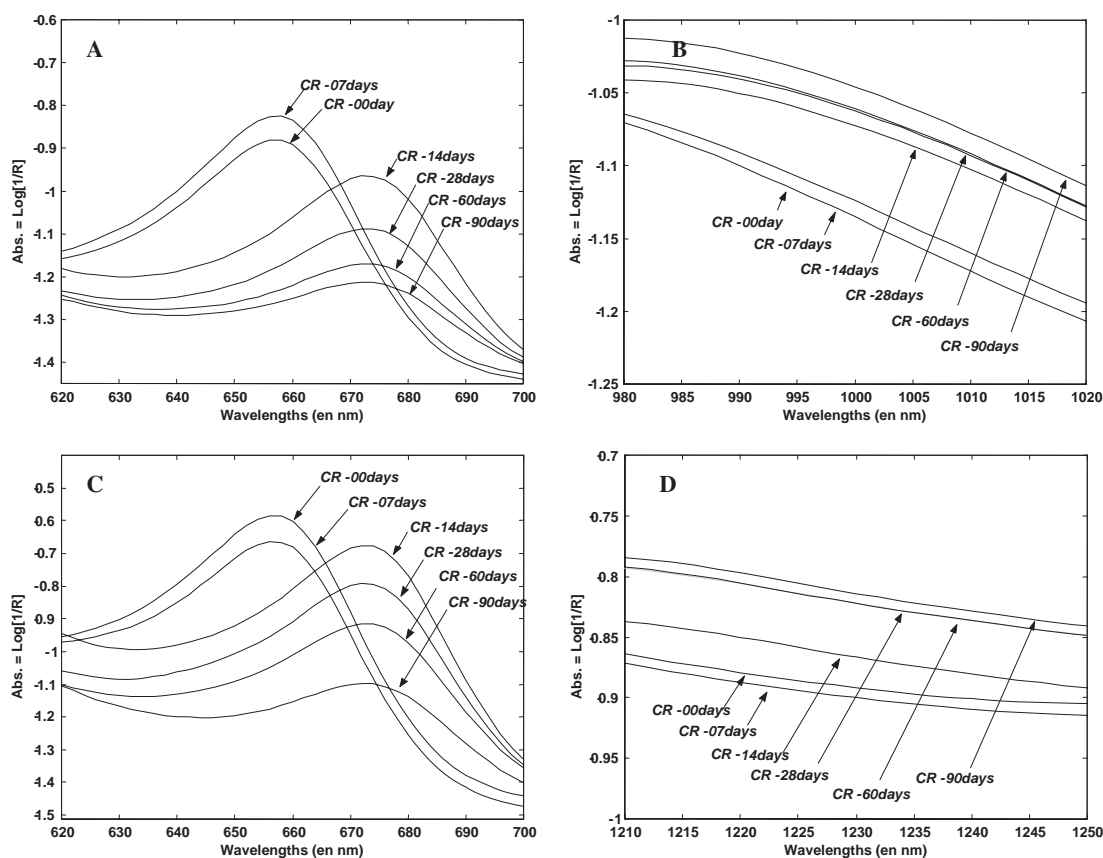


Figure 7. Mean absorbances, A, B, Gala and C, D, Elstar. CR: cooled room storage.

combinations. Previous studies have reported the correlation of these absorption bands to certain quality attributes of apple fruit. Absorbance in the vicinity of 1400nm has been correlated to water content⁶ (also the band in the vicinity of 1900nm) and with less importance to soluble solids content.²⁹ Absorption in the vicinity of 2400nm has been correlated to protein content and pectic substances of apple.³⁰ The relevance of absorption in this last spectral region could be due to depolymerisation of cell-wall pectins that is a classical phenomenon of fruit senescence during post-harvest.³¹

So, the ability of vis-NIR reflectance spectroscopy to classify the fruit according to their evolution during storage could be an alternative to the classical approach of NIR spectroscopy that establishes a quantitative prediction of some indicators of quality. Furthermore, fruit quality and its post-harvest evolution can be defined as a multifactorial process.²⁵ So, grading fruits from whole vis-NIR spectra could allow taking into account the maximum available information to characterise the fruit quality at a given post-harvest moment. Nevertheless, this discriminant approach needs complementary analyses to explain certain relevant absorption bands identified in the NIR region.

Conclusion

In this study, we show that vis-NIR spectroscopy in reflectance mode could be a useful tool to efficiently classify apple fruit according to the duration of storage in two storage conditions. Analysis of cultivar variability showed that notable differences exist in the spectral data of some varieties. This natural variability can be efficiently identified by more than 95%. The most relevant wavelength bands playing a role in the discrimination of the three apple cultivars we studied were identified both in the vis and NIR regions in the vicinity of 500, 680, 1400 to 1700, 1850, 1950, 2200 and 2300 nm.

Also, the evolution of fruit during storage can be characterised with high efficiency using vis-NIR spectroscopy. Each discriminant model was built with particular attention to robustness. We show that classifications of fruit according to the duration of storage with a “global model” was 75 to 83% correct but this was logically less efficient than models which took into account the nature of cultivars. We conclude that despite the slight increase of efficiency of the models when we considered each apple cultivar separately, global models applicable to a set of different cultivars presents an acceptable level of classification. Such models could be complementary to the development of predictive models of quality parameters that need to be developed independently for each cultivar.

Finally, we show that most of the vis and NIR wavelengths of the spectrum play an important role in the grading of the fruit (680, 935, 1000, 1400, 1870, 2070 and 2400nm), showing the multifactorial nature of biological changes in apple fruit during storage.

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