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Title:
APPLICATION OF NEAR INFRARED SPECTROSCOPY TO THE NON-DESTRUCTIVE ASSESSMENT OF THE DEVELOPMENT STAGES OF APPLES.

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Abstract:
The objective of the study was to evaluate the interest of Near Infrared (NIR) Spectroscopy for the rapid characterisation of the development stages of apples. The sample collection included 1066 apples (4 table apples, namely *Elstar (E)*, *Gala (G)*, *Fuji (F)* and *Smoothee (S)* and 2 cider apples: *Douce Moen* and *Kermerrien*). The fruits were collected at 6 different physiological development stages corresponding to end of cell division; 3 different steps of cell enlargement; 2 harvest stages. The NIR spectra of the fruits were non-destructively acquired with a spectrometer fitted with an optic fibre probe. The spectral data were then processed by discriminant analysis (DA) in order to identify the cultivars or the development stages. The quality of the predictions was estimated from series of cross-validation tests.
The cultivars were in general well identified by DA with 77.7, 80.9, 90, 91.5, 98.1, 97.2 percent of correct identifications for *E*, *G*, *S*, *F*, *D* and *K*. On the whole collection, the development stages *1*, *2*, *4*, *5* and *6* where also correctly identified with more than 83 percent of correct classifications in validation tests. The development stage *3* (intermediary growth stage) was more difficult to identify, with only about 73 percent of correct classifications. It was concluded that NIRS is a valuable tool for identifying the cultivars and non-destructively characterising the development stages of apples, in complement with other biochemical and physical methods.

Many elements of the quality of fruits such as apples depend on their evolution during the phases of growth and maturation. The size of the fruits is greatly influenced by the rate and the duration of cell division during the first weeks after full bloom. A poor mitotic activity during this period can be only partly compensated by the formation of cells of bigger size and by the increase in intercellular spaces (Bergh, 1985; Goffinet *et al*, 1995). This early phase, and consequently the final weight, seem to be highly responsive to the temperature during the first 80 days after bloom. The maturation of apples (colour evolution, firmness, starch

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hydrolysis) can also be related to this postbloom temperature (Tromp, 1997; Warrington *et al*, 1999). The accumulation of carbohydrates in fruits is a continuous process depending on the rate of photosynthesis and of crop load, but it can be strongly slowed down at two critical periods, early and late in the season (Lakso *et al*, 1999). Many works have shown that water deficit or mineral fertilisation differently affect the fruit quality (Irving and Drost, 1987) and the texture (Harker *et al*, 1997) according to the period of application. Moreover it is well known that the development of apples includes two stages which follow one another: the cell division that can last 3 to 5 weeks after full bloom and the cell enlargement that includes the maturation step. Nevertheless the date of appearance of these two phases and the exact growth pattern are still debated. Different models have been proposed: sigmoïdal pattern (Pratt, 1988), expolinear pattern (Lakso *et al* 1995), division in three stages (Schechter *et al* 1993). In order to predict and control the final quality of apples, it is therefore necessary to better characterise these stages of development and to identify the critical steps that lead to the variability among the fruits. For a long time, Near Infrared Spectroscopy (NIRS) has been used for the rapid assessment of many food products, in almost all the food industries. Several studies have shown the interest of NIRS for the non-destructive and rapid analysis of apples. Analytical parameters such as Brix degree, pH, firmness, acidity or dry matter can be estimated from Near Infrared (NIR) spectra of intact apples (Robert *et al*, 1989; Bellon-Maurel *et al*, 1996; Kwang-Cho *et al*, 1996; Moons and Dardenne, 2000).

The objective of the present study was to evaluate the interest of NIRS for the rapid characterisation of the development stages of apples. A large collection of apples belonging to 6 different commercial cultivars has been studied. The fruits were collected at 6 representative development stages. The acquired spectral data were then processed by chemometric methods in order to extract the more relevant information related to the nature of the cultivar and the fruit development.

1. Material and methods

The sample collection included 1066 apples collected in orchards from Angers region (France), during the year 2000. The studied cultivars corresponded to 4 table apples, namely *Elstar* (code *E*), *Gala* (*G*), *Fuji* (*F*) and *Smoothee* (*S*), and 2 cider apples: *Douce Moen* (*D*) and *Kermerrien* (*K*). The fruits were collected at different physiological development stages (coded from *1* to *6*): ***1*** 35-45 days after full bloom (DAFB), end of cell division; ***2*** 66-72 DAFB beginning of cell enlargement; ***3*** 70-95 DAFB, cell enlargement; ***4*** 112-134 DAFB, cell enlargement and maturing process , precocious harvest for early-maturing cultivars (*E* and *G*); ***5*** 125-137 DAFB, late harvest stage for early-maturing cultivars; ***6*** 135-164 DAFB, harvest stage for late-maturing cultivars (*S*, *F*, *D* and *K)*. At stage *6*, some cider apples were collected on the ground, as that is usually practised in the profession. Due to technical conditions, it was not possible to have the same number of apples in each group of cultivar or development stage. The number of apples in each cultivar group was (cultivar code, number): *E*: 253; *G*: 265; *S*: 47; *F*: 97; *D*: 220; *K*: 184. The number of fruits in each group of development stage was (code of development stage, number): *1*:199; *2*:126; *3*: 228; *4*: 126; *5*:101; *6*: 286. The NIR spectra were acquired directly on the intact fruits using a Visible-NIR spectrometer (NIRSystems 6500, Perstorp Analytical) fitted with an optic probe device (OptiProbe systems, Perstorp Analytical). The NIR spectra of two opposite faces of each fruit were acquired from wavelengths of light ranging from 1100 to 2198 nanometers (nm), at intervals of 2 nm. The two spectra of a same fruit were averaged and mathematically corrected by the standard normal variate method (SNV, Barnes *et al*, 1989) for reducing the effect of uncontrolled baseline and intensity variations. In order to identify the development stages or the cultivars of the apples, the whole corrected spectral data were processed by two methods of discriminant analysis (DA): canonical discriminant analysis (CDA ; Bertrand *et al*, 1990) and stepwise discriminant analysis (SDA; Romeder 1973). The CDA procedure does not require select wavelengths, and takes account of the whole of the spectrum. On the contrary, the SDA method selects one after the others the most significant variables.

2. Results and discussion

2.1 Discrimination of cultivars

In order to assess the source of variability which is related to cultivars, CDA was applied for predicting the nature of the cultivars from the NIR spectra of apples at every development stages (1066 apples). The CDA model was tested in 10 independent cross-validation tests. In each of these tests, the validation group included 100 spectra randomly selected from the whole spectral collection. More than 90 percent of the samples in the validation sets were correctly identified. The accuracy of the classifications differed from one cultivar to another with 77.7, 80.9, 90, 91.5, 98.1, 97.2 percent of correct identifications for cultivars *E*, *G*, *S*, *F*, *D*, *K*, respectively. On the same data set, SDA was applied by introducing up to 10 variables (wavelengths). With only two absorbances (at 1426 and 1726 nm) as much as 623 apples among 1066 were correctly identified (Table 1). Using a model with 10 wavelengths resulted in 910 correct classifications (85.4 expressed as a percentage). SDA therefore appeared as useful method for selecting a few number of relevant discriminating variables. However, it was difficult to interpret this selection on the spectroscopic level.

2.2 Discrimination of the development stages

The average weights of the fresh fruits at each development stages (figure 1) exhibited different growth pattern according to the cultivars. For the early-maturing table apples (*E* and *G*), the evolution of the weight of the fruits according to time was almost linear from the beginning of June to harvest, whereas it was sigmoïdal for the late-maturing cultivars (*F* and *S*). The cider apples showed intermediate growth curves: slightly sigmoïdal for *K* and expolinear for *D*. For the four late-maturing cultivars (*F*, *S*, *D* and *K*), the maximum growth occurred between the third and the fourth development stage. Variations in respective intensity and duration of mitotic activity and cell enlargement could explain these various shapes of growth curves (Austin *et al*, 1999).

CDA was again applied for discriminating the development stages of the fruits. On the whole collection, the development stages *1*, *2*, *4*, *5* and *6* where correctly identified with more than

83 percent of correct classifications in validation tests (table 2). Moreover, the confusion logically appeared between consecutive development stages. The development stage *3* (cell enlargement) was more difficult to identify, with only 72.9 percent of correct classifications. By considering the physiology of the fruit development, it appeared that this stage was indeed less clearly defined than the others because several biological processes (cell enlargement and beginning of maturation) occur together. The factorial map (figure 2) issued from CDA of NIR spectra gave a rather satisfying representation of the successive stages of apple development and of the growth pattern differences among the cultivars. The first stage (end of cell division) appeared to be very particular and formed a well isolated group. On the contrary, all the stages ranging from cell enlargement to harvest (*2* to *6*) showed a continuous evolution. The classical division of fruit growth into two phases therefore clearly appeared through the NIR measurements. In order to characterise the evolution of spectroscopic properties of fruits during their development, we have traced the mean of the two first discriminant scores, number 1 and 2, according to time and cultivars (figures 3a and 3b). According to the first factor, the evolution was regular from stage *2* to stages *5* or *6*, except during few days before harvest when this evolution might be inverted (cultivars *E*, *G* and *D*). It was supposed that the first factor was related to the soluble sugar concentration or the starch regression. It is indeed known (Schechter *et al.* 1993) that at the beginning of July (stage *2*), the starch content of fruits is high while their soluble sugar content is low. Moreover, the stages of ripening maturity at harvest were quite different between cultivars. Such differences were able to explain the inverted evolution observed at stage *5* or *6*: cultivars *E*, *G* and *D* were a little over the commercial maturity, and could thus consume a lot of carbohydrate just after harvest during climacteric phase. Some measures of the refractometric index and the starch regression (not presented here) on the fruits collected at stages *4*, *5* and *6* agreed with this hypothesis. On the second factor (figure 3b), we could observe a similitude between the evolutions of the discriminant scores associated with the second factor and the growth curves for the different cultivars. This evolution was rather continuous and linear for *E* and *G* (which presented linear growth curves), whereas it was

inverted during the phase of maximal growth rate for the other cultivars with sigmoïdal or expolinear growth curves. The second factor could thus be partly related with the size, or the number, of fruit cells and intercellular spaces.

Table 3 gives the results of SDA of development stages, introducing up to 10 absorbances. The more complex model (with 10 introduced variables) made it possible to rightly classify about 79% of the samples according to their development stage. A few number of wavelengths was sufficient for correctly classifying the fruits. It seems therefore possible to control the maturity of the fruits even with simple spectrometers equipped with photosensitive diodes or optic filters.

More detailed studies (in progress) are required to propose a biological interpretation of the discriminant scores and to confirm our first hypotheses. From this preliminary work, it is concluded that NIRS may appear as a relevant tool for predicting and controlling the development stages of apples.

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Table 1: Stepwise discriminant analysis of the cultivars from the NIR spectra.

Introduced variables: wavelength expressed as nanometers

Step	Introduced variables	Percentage of correct classification
1	1426	53.4
2	1726	58.4
3	1998	61.9
4	1696	70.7
5	1730	72.6
6	1686	76.5
7	1658	80.3
8	1662	82.3
9	1408	84.0
10	1456	85.4

Table 2: Discriminant analysis of the development stages of apples from their NIR spectra. Percentage of classifications (validation sets)

Code of the groups: 1 end of cell division; 2, 3 and 4 different steps of cell enlargement; 5 harvest stage for early-maturing cultivars; 6 harvest stage for late-maturing cultivars).

		Predicted group					
		1	**2**	**3**	**4**	**5**	**6**
Actual group	**1**	**97.5**	1.5	1.0			
	2		**99.2**	0.8			
	3		11.0	**72.9**	7.6	8.1	0.5
	4		0.9	0.0	**96.5**	2.7	0.0
	5		3.0	0.0	1.0	**93.0**	3.0
	6		0.4	0.8	2.0	12.9	**83.9**

Table 3: Stepwise discriminant analysis of the development stages from the NIR spectra.

Introduced variables: wavelength expressed as nanometers

Step	Introduced variables	Percentage of correct classification
1	1360	42.5
2	1886	45.6
3	1460	47.2
4	1444	58.9
5	1670	63.9
6	1746	66.9
7	1684	67.1
8	1702	72.1
9	1844	78.7
10	1708	79.5

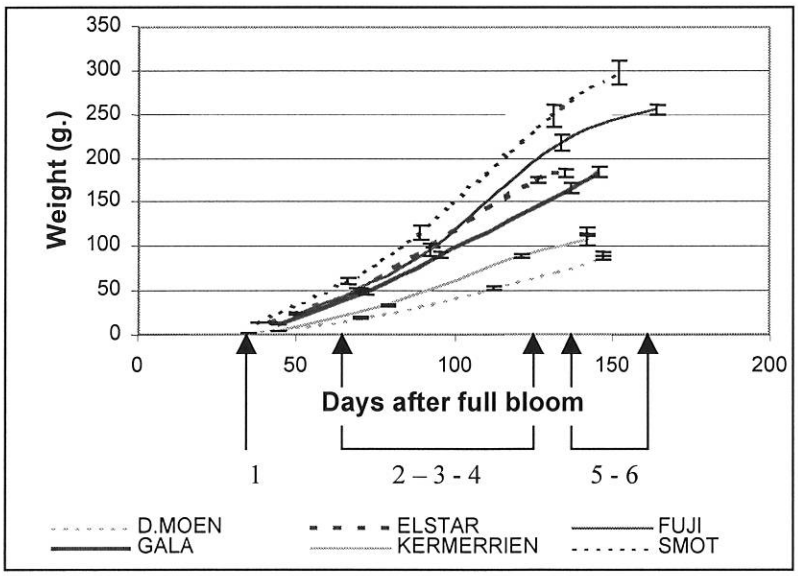


Figure 1: Evolution of fresh weight according to time and cultivars.

Arrows indicate the main development stages: 1 end of cell division; 2, 3 and 4 different steps of cell enlargement; 5 harvest stage for early-maturing cultivars; 6 harvest stage for late-maturing cultivars .

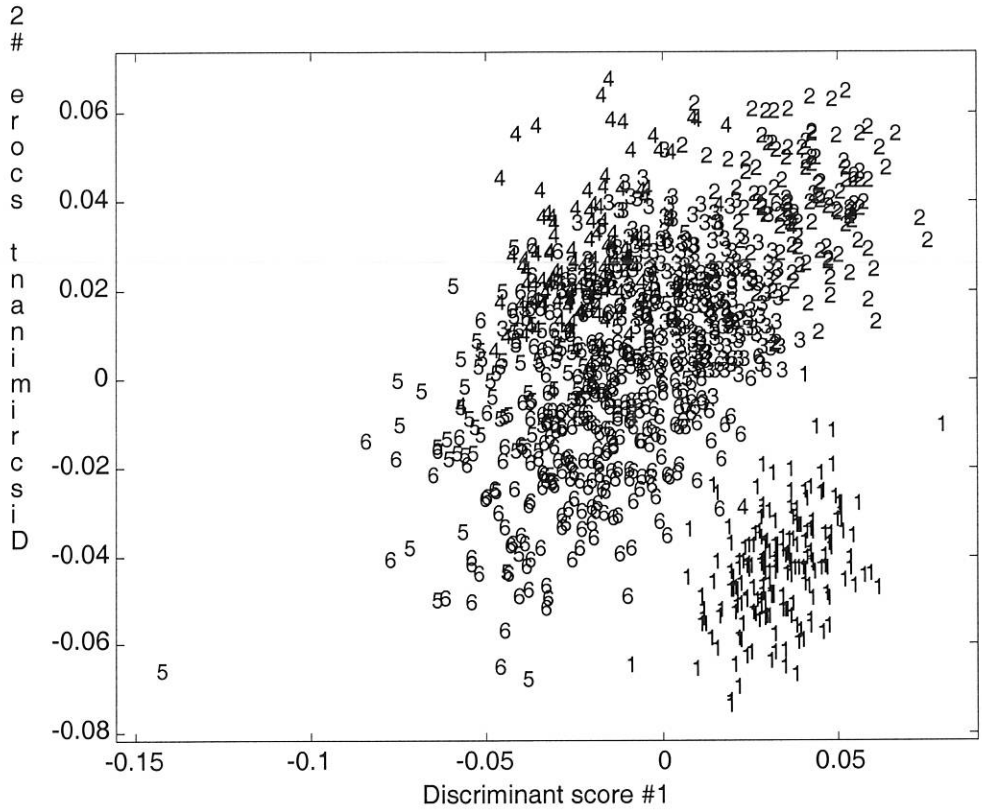
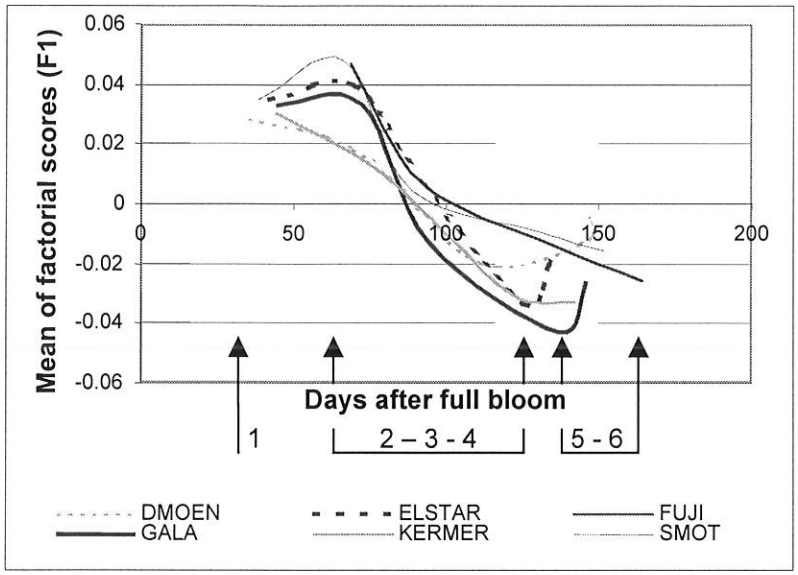


Figure 2: Discriminant analysis of the development stages of apples from their NIR spectra (whole set of apples).Biplot of the two first discriminant scores.

Code of the groups (1 to 6) as indicated on figure 1.



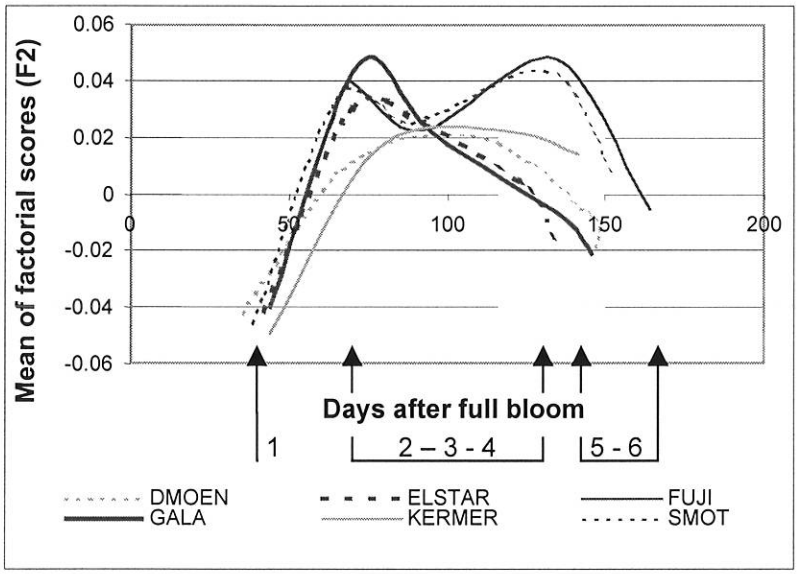


Figure 3: Evolution of factorial coordinates means observed on the first (3a) and second (3b) discriminant scores according to time and cultivars.

Arrows as in figure 1.