Classical and NIR measurements of the quality and nutritional parameters of apples: a methodological study of intra-fruit variability

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For the past few years, research has focused on the application of Near Infrared Spectroscopy (NIR) techniques to analyze the quality of apple varieties. As all fruit quality and nutritional parameters are affected by many factors, to standardize the sampling methods prior to analysis is very important. In this study, the intra-fruit variability of apple varieties in terms of quality parameters (sugar, total polyphenol and vitamin C content) was examined. The adequacy of the protocols usually applied to analyze apple quality (reference analyses of a representative sample of the apple and NIR measurements collected at four points 45° from each other in the equatorial region of the apple) was then investigated. The reference values for the quality parameters showed important intra-fruit variability for all quality parameters analyzed. The results also showed that there was little difference between the mean value at the four points and the mean value of the entire apple. This suggested that a mean value obtained from a representative sample of an apple is enough to assess the variability within the fruit and to estimate, with precision, the content of the quality parameters. The results of NIR spectroscopy showed that measurement at the four target points is suitable for predicting quality parameters precisely. The study concluded that standardized reference analyses should be done on a representative fruit sample of an apple and spectral measurements made at four points 45° from each other in the equatorial region of the apple.

Keywords. Fruits, apples, varieties, ascorbic acid, polyphenols, sugar, vitamin content, quality, quality controls.

Analyse de la qualité et des paramètres nutritionnels des pommes par les méthodes NIR et classiques : étude méthodologique de la variabilité intra-fruit. Dans le passé, les recherches ont porté sur l'application de la technique de Spectroscopie Proche Infrarouge (NIR) pour l'analyse de la qualité des pommes. Étant donné que les paramètres de qualité et nutritionnels sont influencés par de nombreux facteurs, il est très important de standardiser les méthodes d'échantillonnage avant de réaliser les analyses. Dans cette étude, la variabilité intra-fruit des variétés de pommes en termes de paramètres de qualité (contenu en sucre, polyphénols totaux et vitamine C) a été étudiée. L'adéquation des protocoles généralement utilisés pour analyser la qualité des fruits (mesures de référence réalisées sur un échantillon moyen du fruit et mesures NIR réalisées en quatre points situés à l'équateur du fruit) a ensuite été étudiée. Les mesures de référence ont montré une importante variabilité intra-fruit pour tous les paramètres de qualité étudiés. Les résultats ont aussi montré la faible différence entre la moyenne des valeurs aux quatre points de l'équateur et la valeur moyenne de la pomme entière. Ceci suggère que la valeur moyenne obtenue à partir d'un échantillon représentatif du fruit est suffisante pour estimer la variabilité au sein du fruit et pour estimer avec précision les paramètres de qualité. Les résultats obtenus avec la technique NIR montre que la mesure aux quatre points de l'équateur est adéquate pour prédire précisément les paramètres de qualité. En conclusion, les analyses de référence devraient être réalisées sur un échantillon représentatif du fruit et les mesures spectrales réalisées en quatre points à l'équateur du fruit.

Mots-clés. Fruits, pomme, variété, acide ascorbique, polyphénol, sucre, teneur en vitamines, qualité, contrôle de qualité.

1. INTRODUCTION

Fruit quality has traditionally been evaluated by visual inspection of the external appearance and by using destructive methods to measure the internal quality parameters. Due to the destructive nature of the methods used, only a certain number of batches can be analyzed, even though fruit and vegetable batches are known to be highly variable (Flores et al., 2008). In the past decade, research has focused on developing non-destructive measurement techniques to analyze the quality attributes of agricultural products. Near Infrared Spectroscopy (NIR) is based on the reflection and selective absorption of NIR radiation, depending on the structure and chemical composition of the product. The resulting spectral data are analyzed using multivariate regression techniques in order to establish a relationship with the quality characteristics of interest (measured by reference method or by standardized observation). This is done by developing prediction models. NIR spectroscopy offers decisive advantages over traditional methods, the main ones being that it is rapid (a few seconds per sample) and non-destructive, which is very important for controlling and sorting agricultural products. It is also very simple, direct (no preparation of sample) and chemical-free (limited to the reagents required for reference analyses). Finally, it allows several product parameters to be studied simultaneously (Lammertyn et al., 1998).

NIR spectroscopy has been applied widely to determine the internal quality of numerous agricultural and food products, including fruit, particularly apples. It has been used to predict firmness (Mehinagic et al., 2003; Lu, 2004; Zude et al., 2006), sugar content (Lu et al., 2000; Park et al., 2003; Peirs et al., 2003; Sanchez et al., 2003; Liu et al., 2006; Zude et al., 2006; Liu et al., 2007; Angra et al., 2009; Paz et al., 2009), water content (Qing et al., 2008), acidity (Lammertyn et al., 1998; Liu et al., 2005; Liu et al., 2006) and post-harvest quality (Bobelyn et al., 2010). It has also been used to discriminate varieties (Ciotti et al., 1998; He et al., 2007) and to detect such defects as bitter pit (Nicolaï et al., 2006), bruises on apples (Guillermin et al., 2005) and internal browning (Clark et al., 2003).

In apples, a major indication of internal quality is sugar content, as this will affect consumer acceptance and therefore influence the product's market value (**Table 1**). Polyphenols and vitamin C are also important criteria for evaluating the nutritional quality of apples. They are antioxidants and have many biological activities in the human body. The health benefits of apple phytochemicals have been widely studied (Boyer et al., 2004), but the chemical analysis of these nutritional characteristics involves destructive, laborious, costly and time-consuming methods. NIR spectroscopy could therefore be an interesting

Table 1. Mean nutritional composition of apples (per 100 g edible portion) (Westwood, 1993). Quantities can change considerably, depending on cultivar, season, maturity stage, mode of production, etc. — *Composition nutritionnelle moyenne des pommes (par portion de 100 g) (Westwood, 1993). Les quantités peuvent changer de façon importante selon le cultivar, la saison, la maturité, le mode de production, etc.*

Components (per 100 g)	**Content**
Water (%)	84.8
Carbohydrates (g)	14.1
Protein (g)	0.2
Fat (g)	0.6
Vitamins	
Vitamin C (ascorbic acid) (mg)	7
Vitamin A (i.u.)	90
Vitamin B1 (thiamine)(mg)	0.03
Vitamin B2 (riboflavin)(mg)	0.02
Minerals	
Phosphorus (mg)	10
Calcium (mg)	7
Sodium (mg)	1
Iron (mg)	0,3
Potassium (mg)	110

alternative for analyzing these nutritional parameters. Unlike the analysis of sugar content, firmness and acidity (see Nicolaï et al., 2007 and Camps et al., 2008 for review), there are no reports on the use of NIR for measuring the polyphenol and vitamin C content in apples in particular or fruit in general.

With NIR analyses, to obtain a robust prediction model, the calibration dataset should be sufficiently rich in variation and should contain samples from diverse varieties, orchards and seasons. The influence of biological variability (variety, orchard, and season) has been demonstrated (Peirs et al., 2003). The dataset should therefore represent the existing variability in the species, but should also take account of the existing variability within the fruit itself. This is especially relevant when fresh and heterogeneous products such as fruits are analyzed. The anisotropic differences of NIR spectra and quality parameters could be observed in apples, as they have been demonstrated for peaches (Fu et al., 2008). In this case, it is essential to know more about the distribution of constituents in the fruit and to determine where and how many points need to be measured with NIR, as well as to assess the sample portion to be analyzed using reference methods, in order to have a representative figure of the product analyzed. In the literature, NIR usually involves

taking measurements at up to four points located in the equatorial region of the fruit. In most studies on apples, four points situated at 45° from each other in the equatorial region are measured with NIR (Lammertyn et al., 1998; Peirs et al., 2003; Liu et al., 2004; Liu et al., 2005; Sinnaeve et al., 2009). However, no study has yet demonstrated the adequacy of this protocol. The mean spectrum at four points might not be enough to provide a representative spectrum of the entire fruit. Similarly, reference analyses are generally done on a representative sample of the fruit, but no study has demonstrated the adequacy of this protocol for apple. The adequacy of the protocol for apple therefore needs to be investigated. This could be done by analyzing and relating the quality parameters to the target points measured by NIR.

Since 1973, an important research area at CRA-W has been the study of a large fruit genetic resources collection (Lateur et al., 1994; Lateur, 2003). The study has several objectives, including the conservation of the fruits' patrimony, as well as the evaluation, conservation and valorization of the fruits. There is a particular focus on the selection and valorization of varieties that have low susceptibility to diseases and are adapted to low-input production modes (Lateur et al., 2009) and of varieties with specific nutritional properties (*e.g.*, with a high content of ascorbic acid and phenolic compounds). The study of the quality and nutritional properties of fruits (sugar, ascorbic acid, malic acid, fibers and phenolic compounds contents) is one of the institution's main areas of expertise (Planchon et al., 1999; Planchon et al., 2004; Romnée et al., 2009; Sinnaeve et al., 2009).

The aims of this study were:
- to investigate the intra-fruit variability of apples for quality parameters, particularly for sugar, polyphenol and vitamin C content;
- depending on this intra-fruit variability, to determine if the protocol usually applied for apple analyses (reference analyses done on a representative sample of the fruit and NIR measurements collected at four points 45° from each other in the apple's equatorial region) is suitable.

2. MATERIALS AND METHODS

2.1. Fruit

Two apple varieties, 'Hidala' (red sort of 'Braeburn') and 'Pilot', were investigated. They were chosen for their distinct sugar content and coloration: 'Pilot' contains more sugar than 'Hidala' and is bi-colored, whereas 'Hidala' is completely red. Five apples per variety were selected. Each apple was divided into eight longitudinal pieces from its central axis (1 to 8) and

three vertical pieces (A-B-C) with A being the upper part between the equator and the stalk; B being the equatorial region; and C being the lower part between the equator and the calyx. The division into longitudinal pieces took account of skin color, with piece 1 being the most highly colored. There was therefore a total of 24 target areas for each apple (**Figure 1**).

2.2. NIR analysis

Part of the electromagnetic spectrum of each target area was measured using an XDS spectrophotometer (FOSS NIRSystems, Inc., USA) covering the visible and NIR range from 408 nm to 2,498 nm and taking data every 2 nm (Sinnaeve et al., 2009). For each apple, 24 spectra corresponding to the 24 target areas were collected. In the literature, only four target areas of apples are usually measured and averaged; they correspond, in this study, to the longitudinal pieces 1, 3, 5, and 7 of the equatorial region of the apple (*i.e.*, areas B1, B3, B5, and B7).

2.3. Reference analysis

The quality parameters (sugar and total polyphenols) were determined using standard protocols for fruits (Vaysse et al., 2004). Sugar content (°Brix), or more precisely the percentage of soluble solid content, was analyzed in apple juice using a digital hand-held refractometer (ATAGO CO., LTD., Japan). The polyphenol content was estimated using the Folin-Ciocalteau method. Vitamin C (ascorbic acid) content was determined using UPLC-MSMS (Waters Corp., USA) (Romnée et al., 2009). The repeatability of these analytical methods as defined by ISO 5725 had been previously estimated for each parameter using six 'Hidala' apples. For each apple, the reference analyses
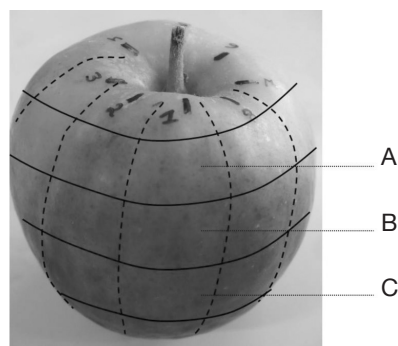

Figure 1. A 'Pilot' apple divided into eight longitudinal pieces (1-8) (indicated by dashed lines) and into three vertical pieces (A-B-C) (indicated by continuous lines) — *Pomme de la variété 'Pilot' divisée en huit portions longitudinales (1-8) (indiquées par des lignes en pointillés) et en trois portions verticales (A-B-C) (indiquées par des lignes continues).*

were repeated four times. The repeatability standard deviation was estimated using the formula:

$$Sr = \sqrt{S^2r}$$

where $S^2r = (S^2i)/k$, with S^2i = variance of the ith essay (apple) and k = number of essays.

The reference analyses were performed in the same 24 target areas (about 1 cm³) as those measured with NIR; the portion of flesh illuminated by NIR was taken and analyzed. Each quality parameter was measured on only one apple per variety. After the NIR measurement, apple no. 1 was analyzed for the sugar content, apple no. 2 for the polyphenol content and apple no. 3 for the vitamin C content. All the reference analyses were performed immediately after the NIR measurements.

2.4. Data analysis

For each quality parameter, the reference values were analyzed using ANOVA (Minitab 15 Inc., USA) in order to highlight the differentiation according to "longitudinal piece" or "vertical piece". The reference values were then used to calibrate spectroscopic models using Winisi software (Infrasoft International LLC, USA). Multiple Linear Regression (MLR) and Partial Least Squares (PLS) regression techniques using different spectral ranges (408-2,498 nm, 950-1,774 nm and 1,600-2,390 nm) had been previously applied. In MLR, the forward stepwise selection of wavelengths has been used in order to avoid over-fitting as there are many more wavelengths than samples. For each quality parameter, the different models were compared on the basis of the calibration characteristics. The MLR regression method gave the most accurate models (lower SEC and higher RSQ and RPD) and was therefore used in the present study.

For each quality parameter, a spectroscopic model was calculated for each variety separately. The performances of the models were estimated according to the following statistics: Standard Deviation (SD), Standard Error of Calibration (SEC), Standard Error of Cross-Validation (SECV), coefficient of determination of calibration (RSQ) and the ratio of prediction to deviation (RPD = SD/ SECV). The SECV was calculated independently by leaving out one longitudinal/vertical piece. Precisely, each longitudinal (or vertical) piece was left out and the SECV was calculated using a calibration set of n = 21 (or 16) and a test set of n = 3 (or 8). Finally, the SECV values obtained after having leaved each longitudinal (or vertical) piece were averaged to provide a mean SECV reported in the tables. Only results for the leaving out one vertical piece are presented in the paper.

The calibration models were then used to predict the quality parameters of the five apples used in the study (including the one used for the calibration step).

For the apples used for the calibration models, the first step was to analyze the reference values. In order to study the influence of the sampling on the calibration, the mean of the values for the four target areas usually analyzed (longitudinal pieces 1, 3, 5, and 7 of the equatorial region of the apple, hereafter referred to simply as "the four target areas usually measured") was compared with the mean value of the entire apple. The NIR measurements were then analyzed. In order to evaluate the adequacy of the four NIR measurements for the target areas usually analyzed, the predicted values at these target areas were averaged and the mean was compared with the mean content of the entire apple (reference value). In addition, the four spectra were averaged and the predicted value of the mean spectrum was compared with the mean of the predicted values of the entire apple and with the mean content of the entire apple.

For the apples with no reference values, the quality parameters were predicted using the calibration models. In order to evaluate the adequacy of the NIR measurements for the four target areas usually measured, the predicted values at these target areas were averaged and the mean was compared with the mean of the predicted values of the entire apple.

3. RESULTS AND DISCUSSION

3.1. Sugar content

Reference analyses. The sugar content of apple no. 1 varied from 10.6 to 13.4 °Brix for 'Hidala' and from 14 to 15.8 °Brix for 'Pilot', depending on the target area (**Table 2**). With the repeatability standard deviation of the method being 0.10, the sugar content data revealed intra-fruit variability.

For both varieties, the highest values were observed for longitudinal pieces 1 and 8, which were the most colored regions of the apples. However, 'Hidala' showed greater variability and a greater range of sugar content than 'Pilot'. Variance analysis showed very highly significant differentiation between the longitudinal pieces of 'Hidala' (p-value < 0.001), but only significant differentiation for 'Pilot' (p-value = 0.037). From a vertical point of view, sugar content did not vary much and was similar for all the vertical pieces. No differentiation was revealed by ANOVA (p-value = 0.22 and 0.89 for 'Hidala' and 'Pilot', respectively).

For 'Hidala', the mean sugar content was 11.8 °Brix. If only the four target areas usually measured were considered, the mean sugar content was 12.02 °Brix [(13.4 + 11.7 + 11.1 + 11.9)/4].

Table 2. Sugar content (°Brix) for apple no. 1 of 'Hidala' and 'Pilot' varieties, according to target area: longitudinal piece (1-8) and vertical piece (A, B, C) — *Contenu en sucre (°Brix) pour la pomme n°1 des variétés 'Hidala' et 'Pilot' en fonction de la zone : portion longitudinale (1-8) et portion verticale (A, B, C).*

'Hidala'									
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**Mean**
A	13.4	13.0	12.2	11.1	11.0	11.0	12.2	12.8	12.1
B	13.4	12.8	11.7	11.1	11.1	11.2	11.9	12.4	12.0
C	13.1	12.1	10.6	10.8	11.4	10.9	11.4	11.0	11.4
Mean	13.3	12.6	11.5	11.0	11.2	11.0	11.8	12.1	11.8
'Pilot'									
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**Mean**
A	15.0	14.9	15.6	14.6	14.2	14.5	15.2	15.2	14.9
B	14.5	14.0	15.7	14.7	14.0	15.1	14.4	15.8	14.8
C	15.6	15.5	14.9	14.1	14.4	15.0	14.4	15.5	14.9
Mean	15.0	14.8	15.4	14.5	14.2	14.9	14.7	15.5	14.9

There was less than a 2% difference (Δ = 0.22 °Brix) between the mean sugar content of the entire apple and the mean sugar content of the four target areas at the equatorial region. For 'Pilot', the mean sugar content was 14.9 °Brix. The mean sugar content in the four target areas usually measured was 14.65 °Brix [(14.5 + 15.7 + 14 + 14.4)/4], which was very close to the mean sugar content of the entire apple (24 target areas) (Δ = 2%).

For both varieties, therefore, the mean sugar content in the four target areas usually measured was very close to the mean sugar content of the entire apple, which demonstrates that analyzing four points is enough to assess the sugar variability within the fruits and to estimate, with precision, the sugar content of the apples.

Calibration and prediction of sugar content. Various regression methods and wavelength ranges were tested to calibrate the data of both varieties. The best results (lowest SEC and highest RSQ and RPD) were obtained using MLR on the spectral range of 408-2,498 nm. The calibration models were realized separately for

both the varieties (n = 24) (**Table 3**). Sugar content predictions for all five apples were made using these calibration models.

'Hidala'. The predicted values for the five apples (data not shown) showed that there was both intra-fruit and inter-fruit variability. In addition to the variation of the predicted mean sugar content among the apples, the sugar content varied within apples, depending on the target area (longitudinal piece and vertical piece) of the apple. This sugar distribution was not constant and varied greatly among apples (data not shown).

For apple no. 1 used in the calibration model, the predicted values were very close to those measured in the laboratory (reference values) (**Table 4**). The predicted mean sugar content was identical to the mean sugar content measured in the laboratory (11.8 °Brix). In addition, the mean of the predicted sugar content in the four target areas usually measured was 11.8 °Brix [(13.2 + 11.6 + 10.7 + 11.7)/4], which was identical to the mean sugar content (reference value). Finally, the predicted value of the mean spectrum for the four target areas was 11.7 °Brix, which was very close to

Table 3. Calibration results for the sugar content (°Brix) obtained using MLR on the spectral range of 408-2,498 nm for the 'Hidala' and 'Pilot' varieties — *Résultats du modèle de calibration pour le contenu en sucre (°Brix) obtenu avec MLR sur la gamme spectrale allant de 408 à 2 498 nm pour les variétés 'Hidala' et 'Pilot'.*

	n	**Mean**	**SD**	**SEC**	**RSQ**	**SECV**	**RPD**	**Nb terms**
'Hidala'	24	11.80	0.89	0.25	0.92	0.35	0.71	3
'Pilot'	24	14.90	0.56	0.32	0.67	1.02	0.55	3

SD: Standard Deviation — *écart-type*; SEC: Standard Error of Calibration — *erreur standard de calibration*; RSQ: coefficient of determination of calibration — *coefficient de détermination de la calibration*; SECV: Standard Error of Cross-Validation — *erreur standard de validation croisée*; RPD = SD/SECV: the ratio of prediction to deviation — *rapport performance/déviation*.

Table 4. Predicted sugar content (°Brix) for apple no. 1 of 'Hidala' and 'Pilot' varieties, according to target area: longitudinal piece (1-8) and vertical piece (A, B, C) — *Contenu en sucre calculé (°Brix) pour la pomme n°1 des variétés 'Hidala' et 'Pilot' en fonction de la zone : portion longitudinale (1-8) et portion verticale (A, B, C).*

	'Hidala'								
	1	2	3	4	5	6	7	8	Mean
A	13.6	12.7	12.2	11.5	11.1	11.3	12.5	12.5	12.2
B	13.2	12.8	11.6	11.1	10.7	10.9	11.7	12.3	11.7
C	12.9	12.3	10.9	10.7	11.2	10.9	11.2	11.7	11.5
Mean	13.2	12.6	11.6	11.1	11.0	11.0	11.8	12.2	11.8
	'Pilot'								
	1	2	3	4	5	6	7	8	Mean
A	15.5	14.9	15.1	15.0	14.2	14.6	14.8	14.7	14.9
B	14.3	14.2	15.5	14.6	14.4	14.9	14.9	15.5	14.8
C	15.8	15.3	14.7	14.2	14.5	14.8	14.7	15.5	14.9
Mean	15.2	14.8	15.1	14.6	14.4	14.8	14.8	15.3	14.9

the mean of the predicted sugar content in the four target areas and to the mean sugar content.

These results proved the robustness of the calibration model and suggested that the measurements in the four target areas usually measured were enough to predict, with precision, the mean sugar content in apples.

For the other apples (with no reference values), the predicted values showed sugar content to be in the same range as that of apple no. 1. The adequacy of the NIR measurements for the four target areas usually measured was also evaluated. The results showed that the means of the predicted values in these target areas were always very close to the means of the predicted values of the entire apple. The difference between the mean of the predicted values in these target areas and the mean of the predicted values of the entire apple was always equal to or less than 1%.

'Pilot'. The same observations can be made for the predicted values of the 'Pilot' apples. There was both intra- and inter-fruit variability, as well as great variability in sugar distribution among the apples (data not shown).

For apple no. 1 used in the calibration model, as observed for 'Hidala', the predicted values were very close to the reference ones (**Table 4**). The predicted mean sugar content (14.9 °Brix) was identical to the mean sugar content measured in the laboratory (14.9 °Brix). The mean of the predicted sugar content for the four target areas usually measured was 14.8 °Brix [(14.3 + 15.5 + 14.4 + 14.9)/4], which was very close to the mean sugar content. Finally, as observed for 'Hidala', the predicted value of the mean spectrum of the four target areas was 14.7 °Brix, which

was very close to the mean of the predicted sugar content in the four target areas and to the mean sugar content.

For the other apples (with no reference values), the predicted values showed sugar content to be in the same range as that of apple no. 1. The adequacy of the NIR measurements for the four target areas usually measured was also evaluated. The results showed that the means of the predicted values for these target areas were always very close to the means of the predicted values of the entire apple. The difference between the mean of the predicted values for these target areas and the mean of the predicted values of the entire apple was always equal to or less than 1%.

The results did not highlight a particular sugar distribution pattern in the 'Hidala' or 'Pilot' apples. However, they revealed that using NIR to measure the four target areas in the equatorial region of the fruit was enough to predict, with precision, the mean sugar content of apples.

3.2. Polyphenol content

Reference analyses. The polyphenol content of apple no. 2 varied from 554 to 876 $\mu g \cdot g^{-1}$ FW for 'Hidala' and from 688 to 1,484 $\mu g \cdot g^{-1}$ FW for 'Pilot', depending on the target area of the apple (**Table 5**). These values accord with the findings of Vrohvsek et al. (2004) and Vanzani et al. (2005), who found an average concentration of 75.4 $mg \cdot 100g^{-1}$ FW and 2.6 $mmol \cdot kg^{-1}$ FW, respectively, in 'Braeburn' apples. 'Pilot' apples showed greater variability in the polyphenol content than 'Braeburn'. With the repeatability standard deviation of the method being 74.5, the data revealed intra-fruit variability in terms of polyphenol content.

Table 5. Polyphenol content (μg·g^{-1} FW) for apple no. 2 of 'Hidala' and 'Pilot' varieties, according to target area: longitudinal piece (1-8) and vertical piece (A, B, C) — *Contenu en polyphénols totaux (μg·g^{-1} MF) pour la pomme n°2 des variétés 'Hidala' et 'Pilot' en fonction de la zone : portion longitudinale (1-8) et portion verticale (A, B, C).*

	'Hidala'								
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**Mean**
A	864	795	770	769	766	756	727	684	766
B	731	706	797	568	744	554	785	798	710
C	686	876	732	771	752	771	749	656	749
Mean	760	793	766	703	754	694	754	713	742
	'Pilot'								
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**Mean**
A	819	1,258	988	869	992	1,012	873	1,353	1,021
B	997	1,117	974	688	1,013	1,293	1,217	942	1,030
C	1,276	1,484	1,228	1,264	1,104	1,249	1,247	1,244	1,262
Mean	1,031	1,286	1,063	940	1,036	1,185	1,112	1,180	1,104

For both varieties the data showed that there was no particular distribution of polyphenol content among the longitudinal pieces. No differentiation for this parameter was highlighted (p-value = 0.77 and 0.51 for 'Hidala' and 'Pilot', respectively). With regard to the vertical pieces, for 'Hidala' the polyphenol content was highly variable and there was no particular distribution. There was no differentiation among vertical pieces (p-value = 0.96). For 'Pilot', a higher polyphenol content was observed for vertical piece C than for vertical pieces A and B. The variance analysis showed significant differentiation among vertical pieces (p-value = 0.012).

For 'Hidala', the mean polyphenol content was 742 μg·g^{-1} FW. If only the four target areas usually measured were considered, the mean content was 764 μg·g^{-1} FW [(731 + 797 + 744 + 785)/4]. The difference in the results obtained using the two protocols was only 3% (Δ = 22) between the mean polyphenol content of the entire apple and the mean content in the four target areas usually measured. For 'Pilot', the mean polyphenol content was 1,104 μg·g^{-1} FW. The mean content in the four target areas usually measured was 1,050 μg·g^{-1} FW [(997 + 974 + 1,013 + 1,217)/4], which revealed a 5% difference (Δ = 54) from the results obtained for the entire fruit.

For both varieties, therefore, the mean polyphenol content in the four target areas usually measured was very close to the mean content of the entire apple, which again demonstrates that analyzing the four points is enough to assess polyphenol variability within fruits and to estimate, with precision, the polyphenol content.

Calibration and prediction of polyphenol content. Various regression methods and wavelength ranges were tested and compared. The best results (lowest SEC and highest RSQ and RPD) were obtained using MLR on the full spectrum of the XDS spectrophotometer. The calibration models are shown in **table 6**. **Figure 2** shows the correlation between the predicted and reference polyphenol values for both varieties. Polyphenol content predictions for all five apples were made using these calibration models.

'Hidala'. As in the case of sugar content, there was both intra-fruit and inter-fruit variability in polyphenol content. The mean content varied slightly among the apples. However, the distribution of polyphenols within apples varied among the fruits, depending mainly on the quarter (data not shown).

Table 6. Calibration results for the polyphenol content (μg·g^{-1} FW) obtained using MLR on the spectral range of 408-2,498 nm for the 'Hidala' and 'Pilot' varieties — *Résultats du modèle de calibration pour le contenu en polyphénols totaux (μg·g^{-1} MF) obtenu avec MLR sur la gamme spectrale allant de 408 à 2 498 nm pour les variétés 'Hidala' et 'Pilot'.*

	n	**Mean**	**SD**	**SEC**	**RSQ**	**SECV**	**RPD**	**Nb terms**
'Hidala'	24	742.00	74.78	43.47	0.66	197.25	0.38	3
'Pilot'	24	1,104.00	194.58	142.64	0.46	238.79	0.81	3

abbreviations — *abréviations* : see **table 3** — *voir **tableau 3**.*
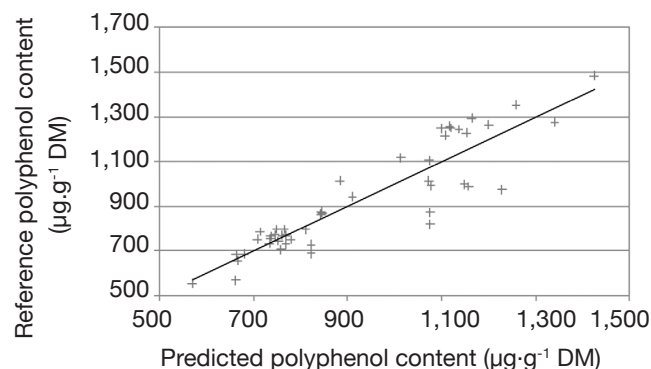
Figure 2. Correlation between the predicted and reference polyphenol values expressed in $\mu g \cdot g^{-1}$ FW for both varieties — *Corrélation entre les valeurs prédites et de référence des polyphénols totaux exprimés en $\mu g \cdot g^{-1}$ MF pour les deux variétés.*

For apple no. 2 used in the calibration model, predicted values were generally quite close to the reference values (**Table 7**). The predicted mean polyphenol content (742 $\mu g \cdot g^{-1}$ FW) was identical to the reference value (742 $\mu g \cdot g^{-1}$ FW). The mean of predicted values for the four target areas usually measured was 750 $\mu g \cdot g^{-1}$ FW [(769 + 765 + 753 + 713)/4], which was close to the mean polyphenol content (742 $\mu g \cdot g^{-1}$ FW). Finally, the predicted value of the mean spectrum for the four target areas was 751 $\mu g \cdot g^{-1}$ FW, which was very close to the mean of the predicted polyphenol content in the four target areas (750 $\mu g \cdot g^{-1}$ FW) and also close to the mean polyphenol content.

For the other apples (with no reference values), the predicted values showed the polyphenol content to be in the same range as that for apple no. 2. The adequacy of the NIR measurements for the four target areas usually measured was also evaluated. The results showed that

the means of the predicted values at these target areas were always very close to the means of the predicted values of the entire apple. The difference between the mean of the predicted values at these target areas and the mean of the predicted values of the entire apple was always less than 5%.

'Pilot'. As observed for 'Hidala', the mean polyphenol content varied slightly among the apples. Its distribution however varied among the apples, depending mainly on the quarter. The variation among the vertical pieces was less important (data not shown).

For apple no. 2, the predicted values were fairly close to those measured in the laboratory (**Table 7**). Despite some differences between values, the predicted mean content (1,104 $\mu g \cdot g^{-1}$ FW) was identical to the reference value (1,104 $\mu g \cdot g^{-1}$ FW). The mean of the predicted values for the four target areas usually measured was 1,091 $\mu g \cdot g^{-1}$ FW [(1,147 + 1,228 + 883 + 1,107)/4], which was close to the mean content. Finally, the predicted value of the mean spectrum for the four target areas was 1,098 $\mu g \cdot g^{-1}$ FW, which was close to the mean of the predicted polyphenol contents in the four target areas (1,091 $\mu g \cdot g^{-1}$ FW) and close to the mean polyphenol content.

For the other apples (with no reference values), the predicted values showed polyphenol content to be in the same range as that for apple no. 2. For 'Hidala', the adequacy of the NIR measurements for the four target areas usually measured was also evaluated. The results showed that the means of the predicted values in these target areas were always close to the means of the predicted values of the entire apple. The difference between the mean of the predicted values

Table 7. Predicted polyphenol content ($\mu g \cdot g^{-1}$ FW) for apple no. 2 of 'Hidala' and 'Pilot' varieties, according to target area: longitudinal piece (1-8) and vertical piece (A, B, C) — *Contenu en polyphénols totaux calculé ($\mu g \cdot g^{-1}$ MF) pour la pomme n°2 des variétés 'Hidala' et 'Pilot' en fonction de la zone : portion longitudinale (1-8) et portion verticale (A, B, C).*

	'Hidala'								
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**Mean**
A	842	811	761	768	736	735	822	662	767
B	769	758	765	661	753	569	713	749	717
C	681	844	734	769	708	746	779	665	741
Mean	764	804	753	733	732	683	771	692	742
	'Pilot'								
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**Mean**
A	1,075	1,116	1,157	846	1,077	1,072	1,075	1,258	1,084
B	1,147	1,012	1,228	822	883	1,163	1,107	910	1,034
C	1,340	1,424	1,152	1,199	1,073	1,118	1,099	1,136	1,193
Mean	1,187	1,184	1,179	956	1,011	1,118	1,094	1,101	1,104

in these target areas and the mean of the predicted values of the entire apple was always less than 5%.

As concluded for the sugar analysis, the results for both the 'Hidala' and 'Pilot' varieties showed that using NIR to measure the four target areas in the equatorial region of the fruit was enough to predict, with precision, the mean total polyphenol content of apples.

3.3. Vitamin C

Reference analyses. The vitamin C content of apple no. 3 varied from 12.6 to 27.3 mg·100g^{-1} FW for 'Hidala' and from 12.9 to 28.1 mg·100g^{-1} FW for 'Pilot', depending on the target area of the apple (**Table 8**). These values accord with previous vitamin C analyses carried out in our laboratory and are close to those reported by Vrohvsek et al. (2004), who found an average concentration of 8.1 mg·100g^{-1} FW in 'Braeburn'. The data showed that there was even higher intra-fruit variability than observed for sugar and total polyphenol content. The repeatability standard deviation of the method was 0.9.

For both varieties, the highest values were observed for longitudinal pieces 1 and 2. Both varieties showed a great variation in vitamin C content according to longitudinal pieces. 'Hidala' showed a greater range of vitamin C content and greater variability than 'Pilot'. Variance analysis showed a very highly significant differentiation among longitudinal pieces for 'Hidala' (p-value < 0.001) and a highly significant differentiation for 'Pilot' (p-value = 0.002). From a vertical point of view, for both varieties the vitamin C content did not vary much. No differentiation according to the vertical

pieces was revealed using ANOVA (p-value = 0.38 and 0.12 for 'Hidala' and 'Pilot', respectively).

For 'Hidala', the mean vitamin C content was 20.5 mg·100 g^{-1} FW. If only the four target areas usually measured were considered, the mean content was 18.9 mg·100 g^{-1} FW [(27.3 + 19.9 + 12.6 + 15.8)/4]. For vitamin C, the difference between the mean vitamin C content of the entire apple and the mean content of the four target areas in the equatorial region of the apple was higher than that observed for the other parameters, and reached 8% ($\Delta = 1.6$). For 'Pilot', the mean content was 21 mg·100 g^{-1} FW. The mean content for the four target areas usually measured was 18.1 mg·100 g^{-1} FW [(25.4 + 16.4 + 12.9 + 17.8)/4], which is close to the mean polyphenol content of the entire apple ($\Delta = 2.9$) and represents a difference of 14%. For both varieties, therefore, the mean vitamin C content in the four target areas could be considered as representative of the mean content for the entire apple.

Calibration and prediction of vitamin C content. As for sugar and polyphenol content, the calibration models for vitamin C were realized using MLR on the full spectrum of the XDS spectrophotometer (**Table 9**). **Figure 3** shows the correlation between the predicted and reference vitamin C values for both varieties. Vitamin C content predictions for all five apples were made using these calibration models.

'Hidala'. As observed for sugar and polyphenol content, both intra-fruit and inter-fruit variability were observed. Predicted mean vitamin C content varied slightly among the apples. The distribution of vitamin C within apples varied slightly among fruits, according to both the longitudinal and vertical pieces (data not shown).

Table 8. Vitamin C content (mg·100 g^{-1} FW) for apple no. 3 of 'Hidala' and 'Pilot' varieties, according to target area: longitudinal piece (1-8) and vertical piece (A, B, C) — *Contenu en vitamine C (mg·100 g^{-1} MF) pour la pomme n°3 des variétés 'Hidala' et 'Pilot' en fonction de la zone : portion longitudinale (1-8) et portion verticale (A, B, C).*

	'Hidala'								
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**Mean**
A	26.6	29.0	22.8	23.5	13.5	20.3	21.2	19.2	22.0
B	27.3	24.8	19.9	14.6	12.6	13.4	15.8	19.7	18.5
C	26.7	24.4	24.3	19.8	13.8	13.5	22.8	21.6	20.9
Mean	26.9	26.1	22.4	19.3	13.3	15.7	19.9	20.1	20.5
	'Pilot'								
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**Mean**
A	24.3	28.1	25.0	19.2	16.6	20.3	21.0	26.3	22.6
B	25.4	23.4	16.4	14.3	12.9	18.3	17.8	19.0	18.4
C	25.7	26.0	22.4	17.8	15.9	18.8	21.2	27.5	21.9
Mean	25.1	25.9	21.2	17.1	15.1	19.1	20.0	24.3	21.0

Table 9. Calibration results for the vitamin C content (mg·100 g⁻¹ FW) obtained using MLR on the spectral range of 408-2,498 nm for the varieties 'Hidala' and 'Pilot' — *Résultats du modèle de calibration pour le contenu en vitamine C (mg·100 g⁻¹ MF) obtenu avec MLR sur la gamme spectrale allant de 408 à 2 498 nm pour les variétés 'Hidala' et 'Pilot'.*

	n	Mean	SD	SEC	RSQ	SECV	RPD	Nb terms
'Hidala'	24	20.50	5.02	2.70	0.71	4.53	1.11	3
'Pilot'	24	21.00	4.37	1.90	0.81	2.79	1.57	3

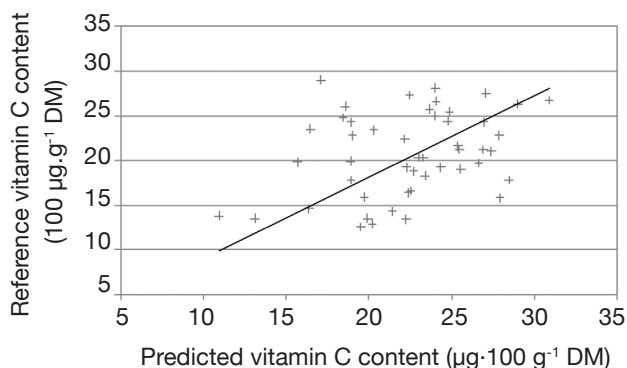Abbreviations — *abréviations* : see **table 3** — *voir **tableau 3**.*

Figure 3. Correlation between the predicted and reference vitamin C values expressed in mg·100 g⁻¹ FW for both varieties — *Corrélation entre les valeurs prédites et de référence de la vitamine C exprimée en mg·100 g⁻¹ MF pour les deux variétés.*

For apple no. 3 used in the calibration model, the predicted values showed some differences from the reference values (**Table 10**). The predicted mean vitamin C content (21.2 mg·100g⁻¹ FW) however was close to the reference value (20.5 mg·100 g⁻¹ FW).

The mean of the predicted values for the four target areas usually measured was 22.2 mg·100 g⁻¹ FW [(22.5 + 18.9 + 19.5 + 27.9)/4], which was quite close to the mean vitamin C content. Finally, the predicted value of the mean spectrum for the four target areas was 20.4, which was quite close to the mean of the predicted vitamin C content in the four target areas and very close to the mean vitamin C content.

For the other apples (with no reference values), the predicted values showed vitamin C content to be in the same range as that for apple no. 3. Again, the results showed that the means of the predicted values for the four target areas usually measured were always close to the means of the predicted values of the entire apple. The differences between the mean of the predicted values in these target areas and the mean of the predicted values of the entire apple ranged from 0.7 to 5%.

'Pilot'. As observed for 'Hidala', the predicted mean vitamin C content varied slightly among fruits. The distribution varied among apples. The highest values were generally observed for longitudinal pieces 1 and 8, which are the most colored regions of the apples, and for vertical pieces A and B (data not shown).

Table 10. Predicted vitamin C content (mg·100 g⁻¹ FW) for apple no. 3 of 'Hidala' and 'Pilot', according to target area: longitudinal piece (1-8) and vertical piece (A, B, C) — *Contenu en vitamine C (mg·100 g⁻¹ MF) pour la pomme n°3 des variétés 'Hidala' et 'Pilot' en fonction de la zone : portion longitudinale (1-8) et portion verticale (A, B, C).*

	'Hidala'								
	1	2	3	4	5	6	7	8	Mean
A	24.1	17.1	19.0	16.5	13.1	23.0	25.5	24.3	20.3
B	22.5	18.4	18.9	16.4	19.5	22.3	27.9	26.6	21.6
C	30.9	24.8	18.9	15.7	11.0	19.9	27.9	25.4	21.8
Mean	25.8	20.1	19.0	16.2	14.5	21.7	27.1	25.5	21.2
	'Pilot'								
	1	2	3	4	5	6	7	8	Mean
A	26.9	24.0	23.9	22.2	22.5	23.2	27.3	28.9	24.9
B	24.8	20.2	22.3	21.4	20.2	23.4	28.4	25.5	23.3
C	23.6	18.5	22.1	18.9	19.7	22.7	26.9	27.0	22.5
Mean	25.1	20.9	22.7	20.8	20.8	23.1	27.5	27.1	23.9

For apple no. 3, the predicted values also showed some difference from the reference values (**Table 10**). However, the predicted mean content (23.9 mg·100 g^{-1} FW) was close to the mean content (21.0 mg·100 g^{-1} FW). The mean of the predicted values for the four target areas usually measured was 23.9 mg·100 g^{-1} FW [(24.8 + 22.3 + 20.2 + 28.4)/4], which was quite close to the mean content. Finally, the predicted value of the mean spectrum for the four target areas usually measured was 20.1 mg·100 g^{-1} FW, which was quite close to the mean of the predicted vitamin C content for the four target areas and close to the mean vitamin C content.

For the other apples (with no reference values), the predicted values showed vitamin C content to be in the same range as that for apple no. 3. As observed for 'Hidala', the results showed that the means of the predicted values for the target areas usually measured were always close to the means of the predicted values of the entire apple. The differences between the mean of the predicted values for these target areas and the mean of the predicted values of the entire apple ranged from 2 to 7%.

As concluded for the previous quality parameters analyzed, the results for both 'Hidala' and 'Pilot' varieties showed that using NIR to measure the four target areas in the equatorial region of the fruit was enough to predict the mean total vitamin C content of apples. It is however relevant to note that concentrations of vitamin C in apples (around 20 mg·100 g^{-1} for the varieties analyzed here) are very low to be detected by NIR. Indeed, NIR spectroscopy is unlikely to measure such low concentrations. It is generally accepted that only components at least at a mass concentration of 0.1% have a chance to be quantified by NIR (Lin et al., 2009). Consequently, it is very likely that the calibration models used here are related to another component, covariate of vitamin C. In this case, NIR spectroscopy can be used to measure indirectly its concentration.

4. CONCLUSION

The analyses of reference values for the quality parameters show that there is intra-fruit variability in sugar and total polyphenol content and even more variability in vitamin C content. They also show that the difference between the mean value for the four target areas usually measured (longitudinal pieces 1, 3, 5 and 7 of the equatorial region of the fruit) and the mean value of the entire apple is small. Specific attention needs to be paid to the sampling of fruits used for vitamin C content analysis because the intra- and inter-fruit variability can be particularly high. This was discussed by Planchon et al. (2004). A mean value

obtained with a representative sample of an apple is therefore enough to assess the variability within the fruit and to estimate, with precision, the content of the quality parameters (sugar, total polyphenols and vitamin C). It should be noted that the intra-fruit variability may be related to the position of the fruit within the tree. Indeed, the exposition of the fruit to the sun, its height in the tree (in the canopy or in the low part of the tree) and its relative distance to the trunk constitute factors susceptible to influence the distribution of quality parameters within the fruit. This reinforces the attention that should be paid when sampling fruits for analyses. In addition, only two varieties were investigated in this study. It is likely that the 'variety' factor also influences the distribution of quality parameters within the fruits. The intra-fruit variability may be more or less important according to the variety and the environmental conditions under which it is cultivated.

The results on the intra-fruit variability highlighted in this study constitute elements to take into account to develop standardized sampling protocols when analyzing quality parameters of apples. However, the intra-fruit variability may be influenced by other factors, including environmental conditions, which has not been investigated in this study. Further research on this topic should be carried out in order to consider this source of variability in sampling protocols. From a spectroscopic point of view, it is particularly important to consider the intra-fruit variability when analyzing the quality parameters of apples with NIR, to avoid bias in the calibration steps.

Analyses of the different regression methods reveal that MLR is generally better suited than PLS regression. For both the 'Hidala' and 'Pilot' varieties and for all quality parameters, the predicted value of the mean spectrum for the four target areas usually measured and the mean of the predicted values for the four target areas were always close, or very close, to the mean reference value. This clearly shows that the measurement of four target areas in the equatorial region is enough to predict, with precision, the quality parameters. The predicted value of the mean spectra in the four target areas usually measured was also always very close, or identical, to the mean of the predicted values for the four target areas. The two approaches are therefore similar and appropriate.

In conclusion, NIR spectroscopy coupled with MLR method regression constitutes a good tool for predicting, directly or indirectly in the case of vitamin C, the quality parameters analyzed in this study. In further research, the reference analyses could be conducted on a representative sample of the apple and spectral measurements made at four points 45° from each other in the equatorial region of the apple.

Acknowledgements

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