Use of NIR Spectroscopy in an Apple Breeding Program for Quality and Nutritional Parameters

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Abstract

Antioxidants are of great interest because they are known to have many health benefits. Our current apple breeding program aims to create and select new apple cultivars presenting a differentiated quality (high antioxidant content combined with scab tolerance and high fruit quality). This work investigated the use of NIR spectroscopy as a rapid and non-destructive tool which could be applied in breeding programs to determine, in addition to the traditional quality characteristics, the vitamin C and polyphenol content. After NIR measurements and reference analyses of quality parameters, the PLS regression was used to create calibration models. The best results were obtained for the determination of sugar, acidity and total polyphenol content. The calibration models presented good precision of prediction and the Ratio of Prediction to Deviation (RPD values) obtained showed that almost three groups of values (low, medium and high) could be created when apples are analyzed for breeding purposes. The performance of the calibration models were less for the determination of maturity, firmness and vitamin C content. These results suggest that NIR is a potential powerful tool for breeding programs. It allows the prediction with good precision of quality parameters of apples and allows sorting of fruits according to their contents. This is of great importance for breeders, who are more interested in classifying cultivars to a range of concentrations rather than determining precisely the concentration. Further work will be necessary to improve the precision of prediction of the vitamin C content.

INTRODUCTION

Most actual apple breeding programs aim to provide more suitable cultivars adapted to low input, which also helps to decrease the fruit production costs. Since the early 1990s, CRA-W started a breeding program based on the use of local old cultivars as parents with the objective to enlarge the genetic diversity of the common breeding work. It especially focused on scab and powdery mildew tolerance, long storage capacity, flesh firmness and good flavor (Lateur et al., 2009; Warlop et al., 2010). In addition to these properties, the current breeding program aims to create new cultivars with improved specific quality and nutritional characteristics. Indeed, it does not only consider the traditional characteristics involved in the general taste and flavor of fruits (i.e., acidity, firmness and sugar content) but it also takes into account nutritional properties particularly the antioxidant content (vitamin C and phenolic compounds). These nutrients are of great interest for consumers' health. Their health benefits have been reviewed by Boyer and Liu (2004). As apples are commonly eaten in Europe and North America under diverse forms (fresh fruit, juice, cider, sauce) and all the year round, they are an important source of these antioxidants in the human diet. Increasing their content in apple cultivars should therefore contribute to increasing their intake in the diet, which might be associated with health benefits. Creating new cultivars specifically enriched with these nutrients could be particularly interesting since fruit and vegetables (sources of antioxidants) are generally lacking in our modern diet.

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Quality parameters are generally measured by breeders and producers with classical methods. The maturity, firmness, acidity and sugar content are usually determined using standard protocols. The vitamin C content is generally measured using HPLC (Planchon et al., 2003; Odriozola-Serrano et al., 2007) and polyphenol content using the Folin-Ciocalteau method (total polyphenol content) (Khanizadeh et al., 2008) or using HPLC (individual polyphenol) (Verberic et al., 2005; Khanizadeh et al., 2008). However, these methods are destructive, laborious, time and reagent consuming. In contrast, near infrared spectroscopy (NIR) possesses the advantages of being rapid, non-destructive, direct (no preparation of sample) and chemical-free (except the reagents required for the reference analyses). Therefore, it presents relevant advantages for the analysis of quality and nutritional parameters in breeding programs. Most research on the use of NIR spectroscopy on apples was focused on the determination of the soluble solids content, firmness, and acidity (Lammertyn et al., 1998; McGlone et al., 2002; Paz et al., 2009; Bobelyn et al., 2010). To date, the use of NIR spectroscopy to measure vitamin C and polyphenol content of apples has not been investigated.

In addition to its use as an alternative analysis method, NIR spectroscopy could also be used as a tool to monitor the maturation of fruits in orchards. Indeed, as rapid and non-destructive analyses could be realized with NIR, the maturity stage of apples could be followed (especially with portable devices) during the entire maturation period to precisely determine the optimal picking date. This is particularly interesting because apples harvested at the optimal maturity stage present advantages compared with apples picked at an inadequate stage (before or after optimal maturity stage) (Kviklienė et al., 2006). Saranwong et al. (2003) already demonstrated that NIR spectroscopy could be used as a tool to monitor the internal composition changes during maturation and to determine the maturity stages of mango fruit.

This research was developed within the framework of the internal POMINNO project. It investigated the use of NIR spectroscopy as a rapid and non-destructive tool which could be applied in the apple breeding program for differentiated quality and nutritional parameters. The aim of this preliminary study was to assess the potential of NIR for the determination of six important parameters for apple breeding, namely, the maturity, firmness, sugar content, acidity, and the vitamin C and total polyphenol content.

MATERIALS AND METHODS

Thirty-two cultivars were collected in 2009 and 2010 to cover the broad variability of the CRA-W's collections: old, commercial, cider cultivars, as well as cultivars coming from our breeding program were sampled. Six apples per cultivar, collected at physiological maturity were analyzed.

The spectra were acquired in reflectance mode using the XDS spectrophotometer (FOSS, NIRSystems, Inc.) (408-2,498 nm). In order to integrate the variability of the fruit, 4 measurements 45° from each other were performed at the equator of the apple. Immediately after NIR measurements, reference values were determined. The maturity, firmness, and acidity were determined according to standard protocols (Vaysse and Landry, 2004). The maturity was assessed through the starch-iodine test by visually matching iodine staining patterns on one cut half of the apples to standard starch patterns. The firmness was determined using a hand-held penetrometer. Two measurements on opposite peeled sides of the apples were performed and averaged to give a mean value. The acidity of the apple juice was determined by titration to an endpoint at pH 8.1 using 0.1 N NaOH. The content of vitamin C was determined using the UPLC-MSMS (Romnee et al., 2009), the total polyphenol content using the Folin-Ciocalteau method and the sugar content, more precisely the percentage of soluble solid content, using a digital refractometer (Brix determination).

Finally, calibration models based on PLS regression were developed using the Winisi III package. The performance of the models was estimated using the following statistics: Standard Deviation (SD), Standard Error of Calibration (SEC), Standard Error of Cross-Validation (SECV), and the Ratio of Prediction to Deviation (RPD=SD/SECV).

RESULTS AND DISCUSSION

Results of the calibration models are shown in Table 1. Best results were obtained for the determination of sugar (SEC=0.54, R^2cal=0.91), acidity (SEC=1.60, R^2cal=0.89), and total polyphenol content (SEC=84.89, R^2cal=0.89). Figure 1 shows the scatter plot obtained for the determination of sugar content. RPD values for these quality parameters ranged from 2.48 to 2.89. According to Nicolaï et al. (2007), a RPD value between 2 and 2.5 indicates that coarse quantitative predictions are possible and a value between 2.5 and 3 or above corresponds to good or excellent prediction accuracy. Therefore, our values correspond to models with good precision of prediction. Moreover, it means that almost three groups of values (low, medium and high) could be created when apples are analyzed for breeding purposes. This is of great importance for breeders, who are more interested in classifying cultivars according to a range of concentrations rather than determining precise concentration.

Compared with other studies, accuracy of the model for sugar is of a similar level of performance. Indeed, it is in the same range as the accuracy reported in the literature (accuracy typically around 0.5% SSC) (Lammertyn et al., 1998; McGlone et al., 2002; Paz et al., 2009; Bobelyn et al., 2010). A good calibration model was also obtained for acidity. Acidity seems in general more difficult to predict than sugar content because the concentration of acids in most fruits is typically considerably smaller than that of sugars, and probably too small to affect the NIR spectrum significantly (Nicolaï et al., 2007). As discussed by these authors, when the concentration of such a minor constituent is correlated to, e.g., sugar content, the calibration results could seem reasonable but the model is indirect and robustness issues are to be expected when applied to a different batch.

To our knowledge, the determination of polyphenol content of apple has never been investigated by NIR to date. Therefore, no comparison can be made with the literature. The good precision of prediction obtained in this preliminary study suggests that NIR spectroscopy constitutes a potentially powerful tool to precisely measure this quality parameter.

Performance of the calibration models was less for the determination of maturity and vitamin C content. Despite the relatively good coefficients of determination for the calibration (R^2cal=0.81 and R^2cal=0.82 for the maturity and vitamin C respectively), RPD values for these parameters were less than 2 (RPD of 1.89 and 1.87 for the determination of the maturity and vitamin C respectively). The use of NIR to determine vitamin C content of apple has never been studied. However, it has been used for other species (Fu et al., 2005, 2008; Xia et al., 2007). In particular, Fu et al. (2008) have shown that NIR coupled with LS-SVM regression technique is a feasible and promising method to predict the vitamin C in kiwifruit. Results obtained here were average and should be improved by further analyses, including chemometric analyses.

The least performing results were obtained for the determination of firmness (SEC=1.17; R^2cal=0.68 and RPD=1.54). The difficulty to precisely calibrate this parameter has already been observed and the precision of prediction of this parameter is generally low (Lammertyn et al., 1998; McGlone and Kawano, 1998; Paz et al., 2009). The difficulty to precisely calibrate this texture property with NIR could be explained by its physico-chemical feature. NIR provides an approximate measure of the absorption of light in fruit which is related to chemical constituents such as sugar and acid (Lu, 2004). It could also be explained by the fact that hand-held penetrometers (usually used to measure the firmness) are imprecise analysis devices providing random results (McGlone et al., 2002; Camps et al., 2008) since the detected firmness can vary greatly with the skill and care taken by the operator (Fu et al., 2008).

CONCLUSIONS

These results indicate that NIR is a potentially powerful tool for breeding programs. The calibration models developed for sugar, acidity and polyphenol content presented a good precision of prediction and enabled three groups of values to be distinguished. This is particularly interesting in breeding programs to select cultivars according to a specific parameter. This is the first study focusing on the determination of polyphenol content using NIR spectroscopy and these preliminary results are very promising.

Further work is necessary to improve the precision of prediction of quality parameters, in particular, the vitamin C content. In addition, further work should focus on the development of precise calibration models on portable devices directly useful in orchards.

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Tables

Table 1. Performance of the calibration models for the quality parameters.

Quality parameter	N	Mean	SD[1]	SEC[2]	R^2cal[3]	SECV[4]	R^2val[5]	RPD[6]	Nb factors
Maturity	231	7.89	1.70	0.74	0.81	0.90	0.72	1.89	11
Firmness (kg/cm²)	230	7.00	2.09	1.17	0.68	1.36	0.58	1.54	11
Sugar (°Brix)	224	14.01	1.82	0.54	0.91	0.63	0.88	2.89	11
Acidity (eq.g. malic acid/L)	224	7.91	4.89	1.60	0.89	1.85	0.85	2.64	12
Total polyphenols (µg/g FW)	220	312.31	267.20	84.93	0.89	107.5	0.84	2.48	12
Vitamin C (mg/100 g FW)	205	2.41	1.40	0.59	0.82	0.75	0.71	1.87	12

[1] SD: standard deviation.
[2] SEC: standard error of calibration.
[3] R^2cal: coefficient of determination for the calibration.
[4] SECV: standard error of cross-validation.
[5] R^2val: coefficient of determination for the validation.
[6] RPD: ratio of prediction to deviation (=SD/SECV).

Figures

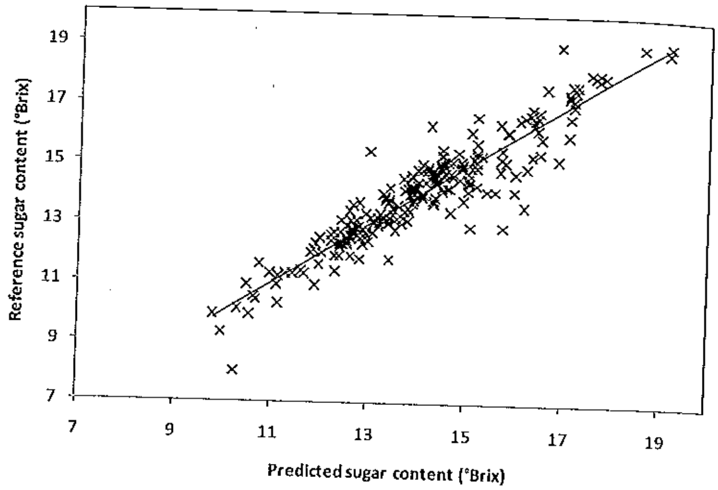

Fig. 1. Results of the calibration model for sugar content.

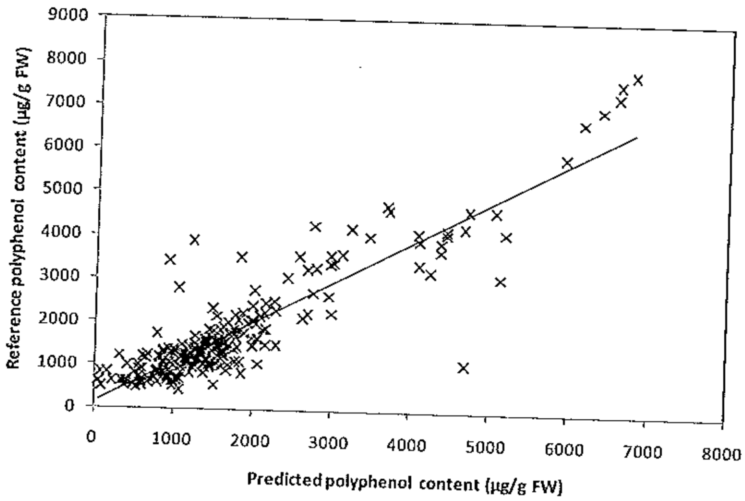

Fig. 2. Results of the calibration model for polyphenol content.