Chapter 14 Application of NIR in Agriculture



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Abstract NIR has been used for decades as an innovative technique in agriculture. There are many benefits, and today, researchers active in agronomy science are not the only ones using NIR extensively in their daily research but also breeders, farmers and agri-processors, using it as an efficient tool for the assessment of a large number of parameters and criteria including detection of contaminants. Undoubtedly, NIRS has demonstrated clear advantages in the analysis of soil, crops, forages, silages and faeces, but also for the analysis of agro-food products such as feed and dairy products. These analyses are no more conducted only at the laboratory level but go more and more to the sample. The new generation of instruments (portable and handheld devices) allow to perform the analyses at the field, farm, orchard or greenhouse level in order to get information to take the right decision at the right moment. This chapter aims to summarise some of these applications and attempts to give the trends of a selection of recently completed or current projects. Readers aiming to delve further into the potential of NIR in agriculture can refer to dedicated books (Williams and Norris in Amer Assn of Cereal Chemists, 312 p, 2001 [1]) or recent reviews (Baeten et al. in Handbook of food analysis, pp 591–614, 2015 [2], Dale et al. in Appl. Spectrosc. Rev. 48(2):142-159, 2013 [3], García-Sánchez et al. in Agricultural systems, pp 97–127, 2017 [4]).

Keywords Quality \cdot Agriculture \cdot Agro-food \cdot Forage \cdot Feed \cdot Farm \cdot Crop \cdot Faeces

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14.1 Introduction

Different areas will be considered in this short section regarding applications of NIR in agriculture: soil analysis, field analysis, forage and silage analysis, feed analysis, milk analysis, faeces and effluent analysis and orchard analysis. In order to help the readers to assess the potential of NIR agriculture, some practical results in terms of NIR equations are provided. The figures presented are those of the Belgian REQUASUD network. This network has been in place in the Walloon Region of Belgium (south part of the country) since 1989 and has the ambition of providing classical analyses and NIR analyses of premium quality for the agri-food sector. The network currently includes seven quality control laboratories performing routine analyses for the public and private sectors (www.requasud.be). The Walloon Agriculture Research Centre (CRA-W) manages the NIR instrument network consisting of 10 NIR spectrometers. A brochure in French explaining the organisation of the NIR network, the current achievements and future developments can be downloaded from the following address: http://www.requasud.be/wp-content/uploads/ 2017/07/brochure requasud spectrometrie proche infrarouge.pdf. In this chapter, it has been decided to provide R^2 , SEC and RPD_{sec} of the NIR equations in use in the REQUASUD network. These figures could be considered to be a good estimation of the performance that could be achieved by NIR technology. It is important to underline that the performance figures have been obtained with multi-annual spectral databases covering a wide diversity in terms of origin and assembled in a NIR network equipped with one single type of benchtop instrument (Foss XDS). Moreover, reference values have been provided by different laboratories having high quality standards. Indeed, REQUASUD frequently organises interlaboratory studies to assess the quality of implementation of the Reference and NIR techniques. Frequent tailored trainings are provided to members of the NIR network.

14.2 Applications in the Field and Crop Analysis

Applications of NIR to field and soil analysis are well illustrated in the literature. These topics are also widely presented and discussed in NIR-focused conferences (e.g. ICNIRS = www.icnirs.org; IDRC = www.idrc-chambersburg.org; HELIOSPIR = www.heliospir.net). In the following section, the current status of application of the NIR technique to soil and crop analysis is given. Some ongoing research with their challenging objectives is briefly presented to give an idea of some of the forthcoming developments in these fields.

14.2.1 Soil Analysis by NIR—A Technique in Development

There is great interest for farmers having a good knowledge of the quality of their soil. Specifically, it is strategic and crucial to be able to adequately determine the physical and chemical characteristics and properties of the soils proposed and used for agricultural purposes. Based on different quality parameters, farmers will decide which crop to plant and which agricultural practices to follow in order to maximise the intrinsic value of their plots. Adequate management of inputs is of prime importance in order to maximise the benefits and to maintain the soil fertility. Soil analysis by classical techniques is tedious, time consuming and requires standardised methods using significant amounts of reagents, making such analyses particularly unfriendly for the environment. Several reviews have been published dealing with the perspectives offered by NIRS for soil analysis [5-7]. Chabrillat et al. reviewed the achievements and perspectives in soil mapping and monitoring based on imaging spectroscopy from air and space-borne sensors [8]. This review underlines that the next generation of hyperspectral satellite sensors could greatly help to meet the demand for global soil mapping and monitoring. Recently, Hutengs and co-authors have compared the performance of portable NIR and mid-infrared (MIR) devices for assessment of organic carbon in soils. Even though a comparison has not been made on the same sample batches, they conclude with the fact that handheld MIR gives significantly more accurate results for on-field analyses. This work clearly demonstrates that the prediction of soil properties (whatever the technique) is improved when the samples are dried and ground (RMSE = 0.155 for NIR) instead of being analysed in situ (RMSE = 0.243 for NIR). Ongoing projects also aim to develop the application of NIR to tackle the current challenges faced by soil analysis. For instance, the INDIGGES project (http://www.cra.wallonie.be/fr/indigges) aims to develop direct and indirect indicators to evaluate greenhouse gas emissions and carbon storage at the farm. In this project, the NIRS technique is tested for characterising both fresh and dried soils. NIR benchtop, portable, handheld and hyperspectral imaging devices are tested (Fig. 14.1). Another challenging issue where NIR approaches are interesting is the detection of foreign material in soil. An example is the detection, identification and quantification of macro- and micro-plastics in cultivated lands. Micro-plastics are emerging as persistent contaminants of increasing concern. They come from mulching film, sludge, wastewater irrigation and atmospheric deposition. It seems that the micro-plastics influence soil physio-chemistry and biota [9].

A key issue regarding soil analysis is the heterogeneity of samples proposed for classical or NIR analyses. It is crucial to take care and devote the required resources to be sure that the sample specimen analysed is representative of the soil for which we want to determine the physical and chemical properties.

Table 14.1 presents the performance of equations used in the REQUASUD network for soil analysis (2018 status). Only the parameters for which a RPD_{sec} is higher than 2 are presented, i.e. organic carbon content, nitrogen, CEC and clay content. The performance for soil from grasslands and lands under cultivation is given [6, 10]. The NIR analysis protocol for soil and the spectral database has been



Fig. 14.1 Illustrations of NIR soil measurements taken in the framework of the INDIGGES project. Portable VIS-NIR measurements taken directly in the field (*Source* CRA-W)

 Table 14.1
 Performance of equations implemented in the REQUASUD network for analysis of soil from grasslands and lands under cultivation

Grasslands									
Properties	Ν	Min	Max	Mean	SD	R^2	SEC	RPD	
Total organic carbon % MS	8849	0.01	14.91	3.64	1.49	0.91	0.49	3.4	
CEC (meq/100 g)	855	0.02	71.2	9.6	7.03	0.85	3.15	2.6	
Nitrogen (g/kg)	1077	0.2	6.92	3.18	1.25	0.82	0.59	2.4	
Clay % MS	210	2.56	57.7	18.52	8.23	0.82	4.12	2.3	
Lands under cultivation									
Total organic carbon % MS	10,139	0.05	7.66	1.54	0.69	0.93	0.21	3.8	
CEC (meq/100 g)	1228	0.48	44.3	12.01	4.3	0.81	2.47	2.3	
Nitrogen (g/kg)	3240	0.17	9.31	1.61	0.75	0.92	0.25	3.6	
Clay % MS	575	1.9	72.65	19.92	8.41	0.84	4.08	2.5	

N—Number of samples in the spectral database; Min—Minimum; Max—Maximum; SD— Standard Deviation; SEC—Standard Error of Calibration; R^2 —Coefficient of determination; RPD—Ratio of Performance to Deviation = SD_{ref}/SEC; DM—Dry Matter Basis; CEC—Cation Exchange Capacity

Source CRA-W, Adapted from [10]

built since 2011 by the University of Liège in collaboration with CRA-W. Different regression algorithms have been tested. The LOCAL approach with the use of PLS is the most appropriate [6]. An important public resource is the NIR spectral database developed in the framework of the European LUCAS initiative (https://esdac.jrc.ec. europa.eu/projects/lucas). In this initiative, about 20,000 topsoil samples have been collected in 25 European Union (EU) Member States with the goal of producing a European physical and chemical topsoil database with the aim to harmonise soil monitoring [11].

14.2.2 Crop Analysis—Direct Analysis in the Field or Laboratory Analysis to Support Farmers and Breeders

For the monitoring the crop before and during harvesting, NIR spectroscopy can be an interesting solution. Indeed, it can be used for optimising the harvest date and for crop management. NIR technology is used, among other things, for measuring moisture, production yield, nitrogen status of the crop and to monitor the occurrence of plant pests and diseases. Determining these chemical compositions and properties can be done directly in the field during farm operations or at the harvesting stage for optimal monitoring of crops throughout their life cycle. The objective is to support breeders or farmers in their management. It can also be done in the field to support breeder observations or farmer choices, as well as at the receipt stages of storage facilities or of industry. Today, there is a common effort by farmers, researchers, instrument manufacturers and farm advisory services to develop operational solutions for assessing optimal crop management, to optimise the use of inputs, to assure the best product quality and to maximise the financial benefits [2]. Classical NIR benchtop instruments are also used by researchers and breeders for routine analysis of the dried and ground aerial parts of the crop in order to determine key parameters such as nitrogen and carbon content [12]. Moreover, near-infrared microscopy (NIRM) has also been proposed as a rapid technique to predict the chemical composition (e.g. nitrogen content) of dried and ground materials when the material quantity is insufficient to perform analysis by classical instrumentation. It has been demonstrated with very small samples ($\ll 1$ g) of tomato (Solanum lycopersicum L.) leaf powder coming from experiments. The calibration model obtained for nitrogen content proved to be excellent, with a calibration coefficient of determination (R_c^2) higher than 0.9 and a ratio of performance to deviation (RPD_c) higher than 3. It appears that NIRM is a promising and suitable tool for a rapid, non-destructive and reliable determination of nitrogen content of tiny samples of leaf powder [13]. The use of the NIR hyperspectral imaging instrument for crop analysis seems to be an increasingly interesting approach as it provides spatial information in addition to chemical information from the spectral data. In that sense, this approach is being investigated to build phenotyping strategies useful in breeding programmes that focus on wheat varieties (e.g. PhenWheat project; http://www.cra.wallonie.be/fr/phenwheat), sugar beet varieties (e.g. BeetPhen project; http://www.cra.wallonie.be/en/beetphen) and potato varieties (e.g. First project; http://www.cra.wallonie.be/fr/first). Figure 14.2 shows the NIR hyperspectral imaging device used at CRA-W. Challenges are presentation of the device to the crop and the development of a robust protocol to calibrate and validate the system using spectral data not collected in the controlled environment of a laboratory.

The potential of NIR hyperspectral imaging spectroscopy and chemometrics for the discrimination of roots and crop residues extracted from soil samples has also been demonstrated. The study of these materials in different field conditions is important to identify suitable soil management practices for sustainable crop production. In



Fig. 14.2 Use of Hyperspectral NIR imaging for crop status monitoring (Source CRA-W)

order to eliminate the cumbersome hand-sorting step, avoid confusion between these elements and reduce the time needed to quantify roots, a protocol based on nearinfrared hyperspectral imaging spectroscopy has been established. The best results have been achieved using a support vector machine to first discriminate the materials and then to quantify them in the soil samples [14]. The methodology has been used, for instance, to better understand the effect of tillage or fertilisation on root system development. Another interesting application of NIR to crops is the use of NIR hyperspectral imaging in the study of legume root systems. This technology has been used in the framework of several studies conducted on pea root systems. First, the suitability of this approach to quantify the mass of pea roots in root samples collected under pea–wheat intercropping has been demonstrated. Secondly, this analytical method was used to quantify leghaemoglobin in individual pea nodules. Fixation activity of the nodules is related to the concentration of this molecule in the pea nodules (Fig. 14.3; [15]).

14.3 Applications on Farm Products or Effluents

Different studies have proposed NIR technology to assess at the farm the control of feed, forages (fresh, dried and silages), milk and effluents [16]. The interest is in optimising costs and reducing the impact on the environment.

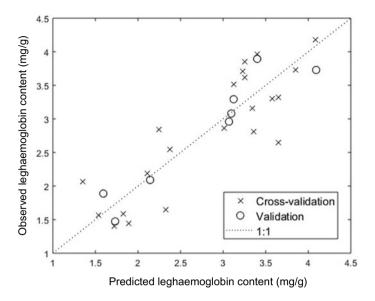


Fig. 14.3 Cross-validation (open circles) and validation (crosses) results of the PLS regression model. Leghaemoglobin content was measured with the cyanmethaemoglobin method and predicted on the basis of nodule NIR imaging spectra. Results are expressed in mg leghaemoglobin g^{-1} of fresh nodules. Leghaemoglobin was predicted with a RMSECV of 0.45 and a determination coefficient (R^2) of 0.74 (*Source* CRA-W)

14.3.1 An Efficient Tool to Assess Forage and Silage Quality for Precision Feeding

In the current economic (e.g. price volatility of main inputs and agricultural productions) and environmental (e.g. reduction of inputs, optimal valuation of farm production by maximising the use of productions and reducing the impact of effluents) context, the appropriate control of forage quality is of prime importance. In some region (e.g. Walloon Region of Belgium), feed produced at the farm contributes significantly (around 50%) to the feeding autonomy of the farm. Different types of forage are generally identified: green forages (i.e. grazed grass, whole plant maize, immature cereals and protein mixed crop); silage forages of grass, whole plant maize or beet pulp obtained by the application of a process to preserve wet forages through anaerobic lactic fermentation; dry fodder; artificially dehydrated and pelleted fodder and cereal/pea straws (Minet et al., to be published). One of the most important issues of forages is their high heterogeneity in terms of physical appearance and nutritional value. This heterogeneity is observed between different types, but also inside each class of forages making determination of forage quality essential in farm management. Sampling is a critical step for forage quality assessment whether analysed by classical techniques or NIR techniques. Samples must be as representative of the whole forage batch as possible regardless of its conditioning. When sampling has to

be performed, several portions of the batch must be taken. It is advisable to collect at least 10 sub-samples (and even better 20) of 50 g each. Then, the sub-samples, also called primary samples, are mixed and transferred to a hermetic plastic bag, stored and transported at low temperature to the laboratory in order to avoid deterioration.

For the farmer, knowing the composition and nutrition value of their forage precisely and throughout the year enables feeding of the animals to be optimised for meeting the animals' requirements [10]. Economic losses can be avoided by ensuring that animals receive the diet that allows them to reach optimal milk production (for instance) without a risk of underfeeding or overfeeding. On the basis of forage quality, a farmer may either overestimate the nutritive value of feed and not cover his animals' needs, or underestimate it, with the risk of producing manure that is too rich and could potentially pollute the environment (Minet et al., to be published). Determining the chemical composition and nutritive value of feed ingredients produced at the farm is crucial. Even though several initiatives are being taken to perform it at the farm on fresh samples, this determination is usually done on previously dried and ground samples in an external laboratory using classical chemical methods or NIR methods. Several studies and reviews on the potential of NIR for assessing feeding values of forages exist. Generally, the LOCAL approach gives better results for the analysis of forages [17].

Table 14.2 presents the performance of equations used in the REQUASUD network (2018 status). A selection of parameters for which a RPD_{sec} higher than 2 is presented, i.e. dry matter, proteins, cellulose, ash, digestibility of dry matter, digestibility of the organic matter and total soluble sugar. It is commonly accepted that most of the parameters that allow the farmer to estimate nutritional value of

Grass forages								
Properties	N	Min	Max	Mean	SD	R^2	SEC	RPD
Dry matter	1877	88.84	97.49	93.16	1.44	0.78	0.68	2.1
Protein % MS	1877	4.45	31.26	15.49	5.26	0.98	0.76	6.9
Cellulose % MS	1465	11.27	41.10	26.18	4.97	0.95	1.11	4.5
ASH % MS	1989	3.44	16.66	10.05	2.20	0.85	0.86	2.6
Digestibility of dry matter (De Boever) % MS	1156	50.19	108.28	79.23	9.68	0.96	1.89	5.1
Digestibility of the organic dry matter (De Boever) % MS	1291	46.02	108.06	77.04	10.34	0.96	1.97	5.3
Total soluble sugar % MS	629	0.12	36.12	11.47	8.22	0.97	1.35	6.1

 Table 14.2 Performance of equations used in the REQUASUD network for analysis of grass forages

N—Number of samples in the spectral database; Min—Minimum; Max—Maximum; SD— Standard Deviation; SEC—Standard Error of Calibration; R_2 —Coefficient of determination; RPD—Ratio of Performance to Deviation = SD_{ref}/SEC; DM—Dry Matter Basis *Source* CRA-W, Adapted from [10] forages can be determined by NIRS with relevant precision. The cost of this NIR determination is about one-tenth of the cost of determination by classical methods, and it is obtained in less time, which is more compatible with farm management requirements. Table 14.2 presents the performance for grass forages only [10, 17].

Today, with the evolution of technology, forage analysis can be performed at the farm with handheld NIR instruments and applied directly on wet samples. Several private companies have dedicated instruments for testing of forages and silages at farm (e.g. AURORA = http://www.grainit.it/en/portfolio-items/aurora-nir-analisi-dei-foraggi-in-stalla/) and some offer a full service to the farmer (e.g. NIR4FARM = https://www.abvista.com/Products/GB/NIR-4-Farm.aspx). Another new perspective is the use of NIR hyperspectral imaging to detect and discriminate grassland species in forage [3].

14.3.2 Determination of Key Parameters and Detection of Contaminants/Impurities in Feed

Today, for compound feed specialists, NIR spectroscopy is considered an essential analytical tool that can contribute greatly to quality and safety control and enhancement of their products. The technology has been implemented with success at different stages of feed production chains. This provides not only gains in speed of analysis but also larger analytical throughputs. For instance, NIR spectroscopy is used to characterise raw materials entering the factory and allows the production process to be optimised to assess the nutritive features of the different processed feeds. Networks of tens (even hundreds in some cases) of spectrometers are implemented in major feed companies that daily and routinely perform numerous determinations to assess the quality of feed ingredients, feed additives and compound feeds. Several reviews have addressed the application of NIR to feed analysis [18, 19].

Different parameters can be adequately predicted by NIR [20, 21]. Table 14.3 presents the performance of the equation used in the REQUASUD network to assess the quality of feed (2018 status). Only the parameters for which a RPD_{sec} higher than 2 are presented, i.e. moisture, nitrogen, fat, cellulose, ash and starch [10].

In the feed area, NIR technology can be also relevant to detect contamination by plant, animal, mineral, chemical contaminants or any undesirable substances [22]. It has to be admitted that the use of NIR for detecting contaminants and undesirable substances in feed products is not widely practised. However, several studies have demonstrated the unique advantages of using this fingerprinting technique in the continuing effort to give stakeholders the means to check the safety of the feed chains [23]. Examples include the potential of NIR (NIR microscope and NIR hyperspectral imaging devices) for detection of animal protein in feed ingredients and compound feeds [24–26], detection of plant contaminants [21, 27], the detection of chemical contaminants such as melamine [28, 21], paper and plastic residues coming from packaging, assessment of the origin of feed ingredients [28–30] and the presence of

Compound feeds									
Properties	N	Min	Max	Mean	SD	R^2	SEC	RPD	
Moisture	24,962	2.60	16.65	11.27	1.99	0.88	0.68	2.9	
Proteins	23,734	7.10	62.10	20.91	8.66	0.97	1.39	6.2	
Fat	8391	0.70	31.40	5.61	4.49	0.97	0.73	6.2	
Fibre	5792	0.20	17.90	5.45	2.99	0.91	0.91	3.3	
Ash	21,678	1.30	33.00	7.54	3.49	0.79	1.59	2.2	
Starch	961	3.30	59.20	30.77	10.86	0.96	2.10	5.2	

 Table 14.3
 Performance of equations used in the REQUASUD network for analysis of compound feeds

N—Number of samples in the spectral database; Min—Minimum; Max—Maximum; SD— Standard Deviation; SEC—Standard Error of Calibration; R^2 —Coefficient of determination; RPD—Ratio of Performance to Deviation = SD_{ref}/SEC; DM—Dry Matter Basis *Source* CRA-W, Adapted from [10]

insects [5]. A study conducted in a feed factory has also demonstrated the interest to use NIR technique coupled to a fibre optic probe to detect at the early stage non-conformity of feed ingredients [21]. In this study, issued from a EC project (Q-saffe output project = https://cordis.europa.eu/project/rcn/97821/factsheet/en), online spectrometer allows automatically and sequentially acquiring NIR spectra of sub-samples from incoming batch and detect if it differs to the spectra of the rest of the batch and to the spectra obtained from similar feed ingredient.

14.3.3 A Tool to Assess the Quality of Dairy Products and to Track Milk Quality in the Milking Parlour

Whereas NIR analysis of derived dairy products is common in the industry (for instance, determination of composition parameters and properties in cheese and butter), NIR analysis of milk is more anecdotal [31]. The main reason seems to be the fact that milk should be ideally measured in the transmission mode, and also that control of the temperature and homogenisation of the milk have to be properly addressed [22]. As far we know, only a few dedicated and appropriate instruments for milk analysis have been developed in the framework of research project and industrial initiatives [32], and only one including a temperature control system and homogenisation system has been commercialised (www.bruker.com). Milk is a complex matrix and contains many components such as lipids, proteins, carbohydrates and minerals in variable concentrations. Several authors have reviewed the potential of NIR in the analysis of milk and dairy products to assess the quality, discriminate the origin and detect adulteration [33, 34]. Quality analysis of dairy products relies mainly on manual sampling followed by chemical or physical measurements. This procedure uses laboratory methods characterised by a significant time lag between sample

collection and generation of a result. One of the characteristics of this procedure is the fact that it does not permit interaction with the industrial process in order to instantly correct for deviations from target parameters of the process. In the framework of the Walloon Milkinir research project [32], a near-infrared (NIR) spectrometer-based system is used for online monitoring of milk quality during the process, allowing the milk quality of an individual cow to be monitored. Daily measurement of milk components individual cows could be a decisive tool for farm management and development of animal breeding or feeding programmes to produce milk with a specific milk composition.

14.3.4 Analysis of Faeces and Farm Effluent, A Way to Optimise Their Valuation

At the farm, NIR technology can be also used for the analysis of effluents and faeces. Farm activities produce organic residues, i.e. farm effluents and manure. These residues are of great interest to improve the fields as they are rich in phosphorus, potassium and nitrogen. Rational use of farm effluent and manure based on their intrinsic quality is interesting from the economic point of view. The challenge is the appropriate strategy in the preparation of the sample submit for analysis in order to take into account the high heterogenic nature of this product. Misuse can lead to reduction of soil fertility, environmental pollution and reduction of the farm's profitability.

NIR technique has been also proposed to analyse faeces in order to correlate spectral information to chemical composition or biological status of the diet. Development of models to determine quantitative and qualitative characteristics of grass and feed on the basis NIR spectra has been proposed [35]. It has been demonstrated, among other things, that this approach is relevant for estimating in vivo digestibility and voluntary intake of animals. Moreover, ruminants' diet composition in terms of plant species can be ranked using NIR data. The current work relates to the development of decision support tools for improving grazing management schemes based on NIR determination.

14.4 Applications in the Orchard and in the Fruit Sector

In the fruit sector and since beginning of the twentieth century, VIS and NIR techniques are becoming more and more widely adopted as a non-destructive technique to rapidly and cost effectively assess the quality of fruit. In production, harvesting, storage, processing and consumption of fruit, it is crucial to determine several quality parameters and criteria. A key issue in the analysis of fruit by NIR is appropriate sample presentation. Different studies have concluded that measurement in the reflection and interaction modes is more appropriate for fruit analysis. It is essential to report that the penetration depth for apples has been measured in the reflection mode and is about 4 mm for the 700–900 nm range and 2–3 mm for the 900–1900 nm range. In the transmission mode and in the 1400–1600 nm range, less than 1% of the initial intensity of the radiation goes through a 1 mm slice. The skin definitely poses a major barrier for the light entering the flesh of the apple, requiring a strict protocol for presentation of the sample to the instrument. This protocol will be adapted to the fruit analysed, the architecture of the instrument (mainly the relative position of the source/sources and detector/detectors). Several reviews summarise the potential of NIR for determining different parameters and criteria of fruit [36, 37]. A specific review has been dedicated to challenges and solutions for quality inspection for robotic fruit instrumentation [38].

Several parameters can be determined with enough precision to be used routinely. A number of authors have reported on the use of NIR spectroscopy to determine apple quality parameters, such as soluble solids, acidity, pulp firmness, maturity indexes, polyphenols and vitamin C [36]. Pissard et al. has shown that NIR technique can be used to determine sugar, vitamin C and total polyphenols contents [39]. This study, based on large spectral databases (between 1274 and 2646 depending on the parameter studied) built in the framework of breeding programmes and European projects, has demonstrated the high precision of models that can be achieved. Low standard error of prediction values, in addition to relatively high ratio to prediction (RPD) values, has been obtained especially for total polyphenol and sugar content (RPD values of 5.1 and 4.3 for polyphenol and sugar, respectively). These same authors have also studied the intra-fruit variability in apples using classical and NIR techniques [40]. This paper proposes and validates a protocol to analyse fruit based on 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 fruit (i.e. apple). It has been demonstrated that there was little difference between the mean value at the four points and the mean value of the entire apple. The potential of NIR spectroscopy on fresh apples to determine the phenolic compounds and dry matter content in peel and flesh has been also studied [41]. In general, one of the challenges is the online analysis of intact fruit.

More and more handheld NIR devices are commercially available and proposed to analyse fruit. NIR uses under field conditions (i.e. orchard) have been limited for many years due to restrictions imposed by the size and low robustness of the instruments available. Recently, the development of new technologies used in the construction of NIR spectrometers and data acquisition strategies has enabled a significant reduction in size and cost of these instruments but often a decreased of the robustness of the methods developed [42, 43]. The challenge is to set up the right procedure to use the historical databases and calibration models, previously developed using benchtop spectrometers.

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