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Citrus Internal Quality Predictions by NIR Spectroscopy

José A. Cayuela Sánchez

Department of Physiology and Technology of Plant Products, Instituto de la Grasa – CSIC Avda. Padre García Tejero 4, 41012 Sevilla, Spain

Correspondence: * jacayuela@ig.csic.es

ABSTRACT

Consumer perception and satisfaction regarding fruit quality is an important issue in marketing. However, there is a lack of objective information that will allow consumers to choose fruit of a desired quality. Good correlations, well established in the literature, exist between soluble solid content and consumer acceptance of several fruit. Commercial application of near infrared spectroscopy (NIR) to fruit sorting by soluble solids was first initiated in Japan in 1990, and this technology has been applied to pack-house fruit sorting lines for sweetness of citrus, apples, pears and peaches, since the mid 1990s in Japan, and more recently in other countries. At present, NIR technology applied to nondestructive measurement of fruit quality is in development. Commercially available instruments for these applications are few and scientific evaluation of calibration models for the measurement of the most important fruit internal quality parameters is needed. NIR technology has the potential to become a suitable instrument, not only to sorting citrus fruit by some quality parameters but also to develop 'electronic tasters' based on the correlation among NIR measuring and sensory analysis. As well, the direct use of portable instruments by consumers for purchasing decision, could become of interest. A brief review is done in this work on the state of the art of NIR applied to citrus quality prediction.

Keywords: chemometrics, model, spectroscopy

Abbreviations: ANN, artificial neural networks; **AOTF**, acousto-optic tunable filters; **LCTF**, liquid crystal tunable filters; **MLP**, multilayer perceptron; **MPLS**, modified partial least squares; **MSC**, multiplicative scatter correction; **NIR**, near infrared spectroscopy; **PCR**, principal component regression; **PLS**, partial least squares; **RMSEP**, root mean square error of prediction; **SEP**, standard error of performance; **SSC**, soluble solids content; **TA**, total acidity

CONTENTS

CONSUMER SATISFACTION REGARDING FRUIT QUALITY

Until now, fruit are generally sorted by their appearance using image technology, being the absence of defects, fruit size and colour the main attributes considered. The awareness and rising requirements of food security and food quality of the consumers has led to value fruit sorting systems based on the attributes of internal quality. However, there is a lack of objective information that will allow consumers to choose fruit of desired quality (Poole *et al*. 2006).

There exist good correlations between SSC and the consumer acceptance of several fruits as melon (Lester and Shellie 1992), cherry (Crisosto *et al*. 2003), plum (Crisosto *et al*. 2004, 2005), apple (Hoehn *et al*. 2003), kiwifruit (Crisosto 2001), tomato (Serrano-Megías and López-Nicolás 2006) and citrus fruit (Sala *et al*. 1971; Ino and Osodo 1978; Costell 1992). The ratio of soluble solids to titratable acidity is widely used as a maturity criterion for non-climateric fruits such as citrus and grapes, where the sugars-to-acid balance is the key to acceptability and where the content of these constituents is fairly stable before and after harvest. Maturity indices for climateric fruit are more difficult to devise, as until the fruit has ripened only the potential quality of the fruit can be assessed (Beattie and Wade 1996).

Due to the high correlation between SSC and citrus consumer acceptance, there is an interest in the main producing countries to rapidly and non-destructively determine SSC, assuring that all fruits meet a minimum quality. The classification of the fruits according to their internal quality should influence the structure of markets and price formation, mainly through increased transparency and competitiveness; products of better quality would be rewarded with better prices.

Sorting systems based upon synthetic quality indices, obtained as ratios between the main analytical parameters as the soluble solids content to total acidity ratio in citrus, could be more effective and useful. Moreover, perhaps synthetic quality indices more sophisticated could be developed based upon correlations between different attributes evaluated by sensory analysis and non-destructive analytical techniques.

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Invited Mini-Review

NONDESTRUCTIVE TECHNIQUES FOR THE ANALYSIS OF FRUIT QUALITY

There are different reasons why it might be interesting the ability of using non-destructive techniques for fruit quality analysis in various stages of the production chain. The first relates to the pre-harvest stage. The ability to monitor and instantly analyze fruit quality parameters in the field can be a big advantage to develop strategies for harvesting; furthermore, the transport and laboratory analysis of samples are avoided, and time and money are saved. The next stage is handling and packing in the fruit pack-house, where the availability of techniques which allows the automatic fruit sorting according with certain quality parameters is most interesting. Another stage can be the distribution of food; at this phase, non-destructive techniques of fruit quality assessment could be useful both for distributors and consumers, who could verify easily and quickly that the product acquired meets the desired characteristics; this option would bring transparency to the marketing and competitiveness at the price formation process based on the fruit internal quality. By these reasons, there is currently great interest on non-destructive fruit quality analytical techniques. A recognized expert in post-harvest said: 'Future research and development efforts should focus on developing better methods of monitoring quality and safety attributes of fresh produces as part of a quality assurance system.' (Kader 2000).

The most suitable technology depends on which is the main quality parameter required to be measured. Techniques that have shown promise for non-destructive firmness measurement of fruits and vegetables are acoustic impulse transmission (Abbot *et al*. 1995; Miyamoto *et al*. 1996), electrical and mechanical impedance (Steinmetz *et al*. 1996) and chlorophyll fluorescence (DeEll *et al*. 1996). Maturity assessment could be affordable by chlorophyll fluorescence (van Kooten *at al*. 1997; Schreiber and Bilger 1993) and by NIR (Peirs *et al*. 2005; Zude *et al*. 2006). The detection of external and internal defects can be carried out by X-Ray imaging (Schatzki *et al*. 1997) acoustic and compression measurements (Armstrong *et al*. 1997) and nuclear magnetic resonance imaging (Upchurch and Throop 1994). Nevertheless, regarding the non-destructive determination of internal and maturity attributes, near-infrared detectors have great potential, especially for the estimation of sugar content in fruits (Abbot 1999).

NEAR INFRARED SPECTROSCOPY

NIR is a spectroscopic method utilising the near infrared region of the electromagnetic spectrum, from about 800 to 2500 nm. Typical applications include pharmaceutical, medical diagnostics as blood sugar and oximetry, food and agrochemical quality control. NIR is based on molecular overtone and combination vibrations, being the molar absorptivity in the NIR region typically quite small. One advantage of NIR is that it can typically penetrate much farther into a sample than mid infrared radiation. NIR is therefore not a particularly sensitive technique, but it can be very useful in probing bulk material with little or no sample preparation.

The molecular overtone and combination bands seen in the NIR are typically very broad, leading to complex spectra and to assign specific features to specific chemical components can be difficult. Multivariate calibration techniques, e.g., principal components analysis or partial least squares are often employed to extract the desired chemical information. Careful development of a set of calibration samples and application of multivariate calibration techniques is essential for near infrared analytical methods.

The discovery of near-infrared energy is ascribed to F.W. Herschel in the 19^{th} century, but the first industrial application began in the 1950s. In the first applications, NIR was used only as an add-on unit to other optical devices that used other wavelengths such as ultraviolet, visible, or mid-infrared spectrometers. In the 1980s, a single unit, stand-alone NIR system was made available, but the application of NIR was focused more on chemical analysis. With the introduction of light-fiber optics in the mid 80s and the monochromator-detector developments in early nineties, NIR became a more powerful tool for scientific research.

Currently, NIR is increasingly being used to monitor chemical and physical properties in the refining and petrochemical industries. Prediction of nitrogen fertiliser requirement of crops, particularly rice and wheat, via NIR leaf tissue analysis, can represent an important advance in cost-efficient application of fertiliser and has environmental benefits. Soil and fertiliser analysis by NIR, particularly for carbon, nitrogen and anions is becoming more common. Estimation of wool quality was one of the first NIR application ever attempted (Connell 1996) and after much research, measurement of scoured wool properties as residual grease and colour are now routine.

NIR research is underway to assess quality, physical and mechanical properties and even the aging process of wood. On-line NIR determination of ash and dry matter content of recovered paper is also being investigated. Many more applications of NIR spectroscopy can be cited in a wide variety of technological areas.

FOOD QUALITY MEASURING BY NIR

The implementation of NIR to food products, due to the variability of its composition, presents characteristics differential regarding its use on homogeneous industrial products (Garrido *et al*. 2000). Food products in which NIR has been used for the analysis of different quality parameters are very numerous. How near infrared affects daily life has been reported in a very enjoyable form in an article whose reading we recommend by Flinn (2005), an extract of whose description follows in the next lines. Measurement of protein, fat and fibre in processed cereal foods, is a routine NIR application. Protein and moisture measuring in wheat and other grains are probably the most widespread and successful NIR application in the world. Other applications of NIR are on quality, adulteration and botanical origin of honey, caffeine content of coffee and discrimination between the two major cultivars Arabica and Robusta, estimating moisture, fibre and sugar content in sugarcane. Also, assessment of energy, fibre, protein, sugars and other indicators of animal performance in forage, grain and mixed feed using NIR is now frequently used across extensive and intensive livestock industries in many countries. NIR is also used to estimate fermentation characteristics of silage, such as pH, ammonia nitrogen and volatile fatty acids. NIR has as well an important role in monitoring fat, protein and water content in meat, and authenticity. Colour and cooking quality of fresh pasta can be estimated by NIR. In the dairy farms, milk composition can be tested on line during milking for fat and protein, and dairy products such as powdered milk, butter and cheese are routinely analysed on-line in dairy factories for gross composition, texture and process control. NIR has been reported as a reliable method to assess the quality of olives and detect adulteration of olive oil (Bertran *et al*. 2000; Galtier *et al*. 2007).

Measurement of sugars, acidity, colour and pH of grapes are now frequently performed by NIR. Estimation of alcohol, glycerol and discrimination of various white and red wines has been conducted. Also, some studies have shown it is possible to use NIR to assess wine composition through the bottle (Flinn 2005).

A major application of NIR is the quality assessment of intact fruit; in this area, NIR has been used to determine nondestructively the soluble solids content (SSC) in fruit as apples (Iyo and Kawano 2001; Hernández-Sánchez *et al*. 2003; Zude *et al*. 2006), peaches (Slaughter 1992; Peiris *et al*. 1997), cherries (Lu 2001), melons (Dull *et al*. 1989; Dull *et al*. 1992; Ito *et al*. 2002; Guthrie *et al*. 2006), and citrus between others. Notwithstanding, most research regarding fresh plant products has been carried out on apples and only a few reports are on vegetables; the feasibility of NIR to measure SSC in a wide variety of fruit is clear. The first commercial application of NIR to fruit sorting was held in Japan in 1990 (Kawano 1994). This technology has been applied to pack-house fruit sorting lines on sweetness of citrus, apples, pears, and peaches, since the mid 1990s in Japan and since 2000s in the Western countries (Guthrie *et al*. 2005). Other applications of NIR spectroscopy on fruit and vegetables are acidity, dry matter, firmness, hardness, colour, stiffness and sensory attributes (Nicolaï *et al*. 2007).

Hardness accurate predictions are very important in stone fruits and different destructive and nondestructive mechanical instruments are widely used. The possibility of nondestructive determination of hardness of plum fruit by NIR was reported (Onda *et al*. 1994), and a combined method for hardness peach measuring using nondestructive impact and NIR have also been proposed (Ortiz *et al*. 2001), but little research on NIR monitoring of the hardness of stone fruit at the tree have been reported.

The fruit juiciness is in general an important attribute; citrus sometimes shows a juiciness reduction induced by several factors, as freezing. Some results of NIR calibrations for citrus juiciness prediction were informed (Guthrie *et al*. 2005); the possibility of carrying out on-line routinely measures of the juiciness of the fruit can be useful and must be encouraged.

At present, NIR technology applied to non destructive measurement of fruit quality is in development. Obtaining calibrations on-line and scientific evaluation of the calibration models for on-line predictions is needed. Thus, is particularly useful the collaboration between food technologists and enterprises specialized in the development of NIR instruments.

Also, the direct use of portable instruments by consumers for purchasing decisions, could become of interest.

NIR INSTRUMENTATION

The development of new techniques for selecting the measurement wavelength has been the driving force in the increasing diversity of NIR instrumentation (Stark and Luchter 2003). The main wavelength selection methods are based on fixed and variable filters, diffraction gratings, scanning monochromators, diode-array and interferometer technology. Within the filters, the acousto-optic tunable filters (AOTF) and the liquid crystal tunable filters (LCTF) must be emphasized, that allows rapid wavelength switching and the sequential scanning (Stark and Luchter 2005). A shift towards diode-array systems because their high acquisition speed and the absence of moving parts, that enables them to be mounted on fruit grading lines, has been reported (Nicolaï *et al*. 2007). However, also in AOTF moving parts are absent and as with diode-array, measuring is extremely fast.

MEASUREMENT CONFIGURATIONS

There is considerable literature on different NIR and Visible/NIR measurement modes and configurations for intact fruit SSC and TA prediction. Reflectance, transmittance and interactance modes are used. Transmittance mode is the optical configuration which showed the best calibrations for different fruit (e.g. Dull *et al*. 1989; Miyamoto and Kitano 1995; McGlone *et al*. 2003). Nevertheless, good results for the reflectance mode have also been reported (Guthrie *et al*. 2006; Hernández *et al*. 2006). In the reflectance mode, the field of view of the light detector is constituted by a part of the fruit surface directly illuminated by the source (Mowat and Poole 1997), being the easiest mode to obtain measurements because they require no contact with the fruit and light levels are relatively high. Even so, calibrations are susceptible to variations in superficial or surface properties of the fruit (Schaare and Fraser 2000). In the transmission mode, the fruit surface viewed by the detector is diametrically opposite to the illuminated surface (Kawano *et al*. 1993), the measurements may be less sensitive to surface properties but expected to be more influenced by fruit size; the amount of light penetrating the fruit is often very small, thus is difficult to obtain accurate transmission measurements at grading line speeds. In the interactance mode a fiber optic probe is connected to a digital analyzer that indirectly measures the sample composition.

CITRUS INTERNAL QUALITY ANALYSIS BY NIR SPECTROSCOPY

The rind of citrus has been considered a specific handicap for NIR quality measurement in these fruits (Nicolaï *et al*. 2007), what may explain relatively poor predictions of SSC when compared with thin-skinned fruits. Citrus fruit includes the exocarp or skin, with numerous oil glands, the white mesocarp and the space comprised within the carpels where the juice sacs are that is the the edible part, the endocarp. The application of NIR to a given kind of fruit needs to know how the attribute to be measured is distributed within the fruit. Miyamoto and Kitano (1995) reported that total soluble solids of Satsuma mandarin were highest in the distal apex of the fruit, decreasing towards the pedicel. Peiris *et al.* (1999) described the coefficients of variation of the same attribute in orange as 10.2, 1.8 and 5.6% in the proximal to distal, around the fruit circumference and radial orientations, respectively. NIR assessment of citrus fruit at an equatorial position is therefore logical (Guthrie *et al*. 2005).

The performance of the models for SSC prediction by NIR has been reported from several works, as **Table 1** shows; research on the robustness of multivariate calibretion models in citrus are currently less numerous, although at least one can be cited (Guthrie *et al*. 2005).

The commercial application of NIR to pack-house fruit sorting lines for the sorting of citrus by its SSC was initiated in Japan in the mid 1990s. Grading lines equipped with NIR sensors potentially applicable to citrus are now commercially available from, Aweta (IQA, http://www.aweta.nl), Color Vision Systems (Insight, http://www.cvs.com.au), Greefa (iFA, http://www.greefa.nl), Mitsui-Kinzoku (http://www.mitsui-kinzoku.co.jp), Sacmi (http://www.sacmi.it), TasteMark (http://www.taste-technologies.com), and others, although no scientific evidence is available about the accuracy of these systems (Nicolaï *et al*. 2007).

Since the ratio of soluble solids to titratable acidity is linked to the consumer acceptability and it's very important

Table 1 Soluble solids prediction by NIR spectroscopy in citrus.

Species	Cultivar	Authors	Technology	Configuration	Wavelengths	RMSEP
C. reticulata (mandarin)	Satsuma	Kawano *et al*. 1993	Scanning	Transmittance	680-1235	0.3
	Satsuma	Miyamoto *et al*. 1995	Scanning	Transmittance	680-1235	0.1-0.3
	Satsuma	Miyamoto 1998	Scanning	Transmittance	700-1000	0.1 (SEP)
	Imperial	Greensill and Walsh 2002	Diode Array	Interactance	730-930	0.2-0.4
	Miyagawa	McGlone *et al*. 2003	Diode Array	Various	700-930	0.3
	-	Walsh *et al*. 2004	Diode Array	Interactance	300-1100	0.5
	Imperial	Guthrie *et al*. 2005	Diode Array	Interactance	720-950	0.3-0.7
	Satsuma	Hernández *et al*. 2006	Scanning	Reflectance	300-2500	0.3
C. sinensis (orange)	Valencia late	Cayuela 2008	Scanning	Reflectance	800-2500	0.5
Citrus x limon (citrus fruit)	Jeju	Lee *et al*. 2004	Diode Array	Transmittance	636-1236	0.4 (SEP)

as maturity criterion, the achievement of good NIR calibration models for total acidity prediction is most valuable. In general, NIR total acidity predictions has been considered difficult to achieve in fruits, due to the relatively low levels of organic acids in these commodities (McGlone *et al*. 2003; Guthrie *et al*. 2005). Organic acids form a contribution to the soluble solids content that is in the ten percent range in citrus fruit.

However, Shiina *et al*. (1993), Onda *et al*. (1994), Sohn *et al*. (2000), Schmilovitch *et al*. (2002), Guthrie *et al*. (2005), Hernández *et al*. (2006) and Cayuela (2008), among others, have reported various levels of success in measuring total acidity (TA) of intact pineapple, plum, apple, mango, Imperial mandarin, Satsuma mandarin and orange.

Fruit sorting by its acidity can be also done with a discrete classes approach; when the objective is to classify a batch of fruit in e.g. three different groups, a high accuracy measure of total acidity is not required, and therefore de results of NIR TA prediction could be most useful (Miyamoto 1998), and NIR obtained relations between classes of SSC and TA could become perhaps even more interesting.

CHEMOMETRICS AND REGRESSION TECHNIQUES

The near infrared spectrum is composed of a large set of overtones and combination bands. In the case of fruit and vegetables, the NIR spectrum is dominated by water, as water highly absorbs near infrared radiation. Moreover, fruit and vegetable composition is complex, each chemical component affecting the shape of the spectrum in proportion to their concentration. The heterogeneity of the commodity, instrumental noise, differences between room and sample temperatures and scattering effects wavelength dependent may affect also the spectrum. For the foregoing reasons, it is difficult to assign specific absorption bands to specific chemical components. However, the spectrum content information related with chemical components and the multivariate statistical analysis can extract it, through the process known as 'model calibration'; it essentially involves multivariate regression techniques coupled with spectral preprocessing. An overview of linear and nonlinear techniques for classification of fruit based on Vis/NIR spectra was reported by Kim *et al*. (2000). Many calibrations use hundreds of wavelengths, being necessary to use methods more complex than multilinear regression as principal component regression (PCR), partial least squares regression (PLS) or modified partial least squares regression (MPLS). However, PLS has been reported as the more suitable method for NIR predictive calibrations by many authors.

Even more sophisticated methods try to deal non-linear regressions between concentration and absorbance (Fearn 2005). Artificial Neural Networks (ANN) have been used to construct NIR calibration models (Næs *et al*. 1993; Geladi *et al*. 1996). The most used ANN is the so-called multilayer perceptron (MLP) which typically consist on three layers of so-called neurons, the input, hidden, and output layer. A neuron is a computational device that calculated the weighted sum of its inputs and calculates the output signal from this using an appropiate algorithm based on a calibration set using cross validation (Kim *et al*. 2000). Most applications of ANNs in postharvest technology have been for classification purposes (Guyer and Yang 2000; Hahn *et al*. 2004). Næs *et al*. (1993) concluded that even though ANNs may in certain cases be better than linear techniques, they are more difficult to understand and the results are more difficult to visualize and interpret. Kernel-based techniques (Nicolaï *et al*. 2006) are other non-linear alternative which have as advantage that allow interpretation of the calibration model.

Spectral preprocessing techniques are used to remove any irrelevant information which cannot be handled properly by the regression techniques (Nicolaï *et al*. 2007).

One of the most important data pre-treatment is spectra averaging, what is usually done during the acquisition of the spectrum to reduce the thermal noise of the detector. The number of spectral measures needed to obtain an averaged spectrum depends on the technology used and on the application, the signal to noise ratio of the instrument being a very important parameter. A major factor to be considered in the on-line NIR fruit classification is the speed of the measure and therefore the limited time in acquiring spectrum. Usually, NIR diode-array spectrophotometers operate at an acquisition time around 50 ms, therefore it is difficult to obtain more than one spectrum by fruit unit at the conveyor speed. Most frequently, however, the main interest is focussed on the quality attributes of the batch rather than in those of the individual fruits.

Smoothing can be used to remove random noise, but can also remove useful information and should be applied carefully. Mean normalisation and multiplicative scatter correction (MSC) are frequently used for compensate for additive and multiplicative effects in the spectral data which are induced by physical causes (Næs *et al*. 2004).

First and second derivatives are both commonly used in NIR spectroscopy, although should be taken into account that it complicated spectra and sometimes can be difficult to distinguish true spectral features from the artifacts created by the calculation (Mark and Workman 2003). They are usually calculated according Savitzky-Golay algorithm or gap-segment procedures. The choice between MSC and derivative depend on the procedures which will enable us to obtain better results. As well, standard normal variate transformation and de-trending are routines frequently used to remove the multiplicative interferences of scatter and particle size and the variation in base-line shift found in the reflectance spectra (Barnes *et al*. 1989).

MODEL ROBUSTNESS

Model robustness can be defined as the validity of the calibration models for future predictions on samples completely independent that those used for obtaining the model, from different harvest year and geographical origin. The validity of the calibration model for future predictions depends on how well the calibration set represents the composition of the new samples which will be assessed (Peirs *et al*. 2003).

There exist three closed related causes which may be responsible for deviations towards samples, inducing errors: 1) the use of the calibration model in an instrument different of that used to develop it; 2) temperature fluctuations in the measurement room, changes in the wavelength or detector intensity stability or in other electronic components, can induce drift into the measures; 3) the concentration of the attribute of interest as well as the whole composition of samples belonging to different batches might deviate from the calibration set. Considering the above factors, is important that the specimens used for calibration are selected for both the attribute of interest as other physical and chemical components comprise an appropriate range of variation (Thomas and Ge 2000).

In any case and whatever the predictive model used if are desired guaranteed results, the introduction of validation exercises within the model use protocol is required by the variability in the composition of the fruit. In fact, a proper maintenance of any NIR calibration does not only rely on regular instrument checking, but also requires procedures ensuring model validity (Wiedeman *et al*. 1998).

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