On-line analysis of intact olive fruits by vis–NIR spectroscopy: Optimisation of the acquisition parameters

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ABSTRACT

In the last years, the potential of NIRS for quantitative and qualitative analysis of olives fruits and oils has been investigated. However, limited work has been published about the on-line implementation of NIR spectroscopy in this sector. NIRS application at factory level (olive mills) is desirable. However, prior its implementation, many parameters related to the on-line spectrum acquisition, must be studied and optimised. In this paper, the influence of parameters such as focal distance and integration time has been studied. On-line spectral measurements were performed on intact olives in the spectral range of 380–1690 nm using a diode array spectrometer located on the top of a conveyor belt. The statistical criteria used to evaluate the spectral repeatability for each level of parameters considered were the standard deviation (SD) of the log ($1/R$) values and the root mean square statistics (RMS). Results demonstrated that for on-line control of olives in movement, spectra acquisition process was more affected by the focal distance chosen than by the integration time used. A focal distance of 13 mm and an integration time of 5 s have been defined as the optimal operational conditions.

1. Introduction

Near infrared (NIR) spectroscopy is widely accepted as a valuable tool in olive fruits, pastes and oils analysis. Applications have been demonstrated for raw material testing as well as for quality control process (Armenta et al., 2010; Cayuela et al., 2009; Cayuela and Pérez-Camino, 2010; Gracia and León, 2011). This is mainly due to the advantage of this technique over traditional methods. NIRS instruments are characterised by their speed, excellent accuracy and precision, no sample destruction, minimal or no need of sample preparation, ability to provide simultaneous information on several properties and constituents, relatively reduced operational costs and no use of toxic reagents (Garrido, 2006).

Despite these advantages, the use of this technique for on-line process monitoring in industrial plants is still limited. This could mainly be attributed to low scanning speed, high cost and non-portability of some traditional NIRS laboratory instruments (Fernández-Ahumada et al., 2008). Most of these problems happening in the past have been minimised and overcome by the development of new NIR technologies more suitable for on-line analysis. This is the case of instruments based on diode array (DA) detectors,

fibre optic probes (Yi-Tao et al., 2010; Turza et al., 2002) and acousto optical tunable filters (AOTFs) technologies (Kestens et al., 2008).

During the 1990s, several spectrophotometers based on diode array detectors became commercially available and have been used for quality control analysis of different foods such as apple (Alamar et al., 2007), tomato (Flores et al., 2009), milk (Mouazen et al., 2009), nectarine (Pérez-Marín et al., 2011) and beef (Rosenvold et al., 2009; Rødbotten et al., 2001). The main advantage of these instruments is the possibility to acquire the spectral information at several wavelengths simultaneously within few millisecond. The high speed of diode array NIRS instruments allow analysis of a large sample volume in a short time. Furthermore, these DA spectrometer systems are mechanically robust (no moving parts included), which permits to realise measurements under harsh conditions.

Several NIRS applications of DA technology for on movement analysis of different food and products have been published. One of the first studies was carried out by Axrup et al. (2000). A diode array spectrometer (Ocean Optics Europe) was evaluated as a tool for the on-line determination of several parameters (water content, extractive content, Klason lignin, etc.) in wood chips and bark. Spectral data were acquired while the samples were transported on a conveyor belt moving at 1 ms^{-1}. This study showed that NIR reflectance measurements allow obtaining valuable information about the chemical and physical properties of several components

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in motion. Sinnaeve et al. (2004) studied the quality of wheat (moisture, protein and specific gravity) and forage (dry matter, total protein, cellulose, neutral and acid detergent fibre, soluble sugar and organic matter digestibility) using a diode array instrument (CORONA 45) installed on a harvester machine. The results showed that the robustness of the calibrations models obtained in this way should be further improved. More recently, Hahn et al. (2010) assessed the potential of NIRS on a mobile at-line system for the determination of dry matter, crude protein and starch contain of wheat, barley and triticale grains. Spectra were collected with the same instrument used by latter authors over the wavelength range of 960–1690 nm. Results demonstrated a good determination of dry matter and protein content. On contrary, no good calibrations were developed for starch content.

Despite these studies, the acquisition of spectral data from a moving sample (for instance, during an on-line process, on a conveyor belt or through a pipe), is quite complex (Andersson et al., 2005). The on-line acquisition of a spectrum from a moving sample is dependent on many different parameters such as physical nature of the material (particle size, shape, orientation and density) as well as the type and the composition of the material (Fernández-Ahumada, 2008). Moreover, the packing of the sample, the surface roughness or the average distance from the sample to the optics are parameters that significantly influence the quality, repeatability and reproducibility of the spectra generated (Berntsson et al., 2001). In order to obtain robust models for NIRS applications with an acceptable level of accuracy and precision, it is essential to set up the optimal operational conditions to assess the adequate on-line measurement.

The performance of a NIR on-line instrument for the monitoring of olive quality parameters in the mill industries is desirable. However, the obtention of spectra of samples in movement is really difficult. Prior its implementation, the operating procedure should be studied and optimised in order to obtain reliable spectra.

The operating procedure usually followed to acquire more accurate spectra involves recording a large number of spectra over a determined time (known as integration time) to procure a unique average spectrum (Blanco et al., 1990). However, the optimal integration time value is not always the same for all kinds of samples. They have to be optimised in each study before starting spectra collection. On the other hand, the focal distance, defined as the distance between NIR sensor head and the surface of the olive sample, is another important parameter that must be optimised in order to guarantee the optimal irradiance into the sample and in order to ensure the maximum detection of the reflected NIR light by the detectors.

The purpose of the present study is to evaluate the effect of some parameters such as focal distance and integration time on the spectral repeatability for the analysis of intact olive fruits on a conveyor belt.

2. Materials and methods

2.1. Olive samples

Olive varieties 'Picual', 'Hojiblanca' and 'Manzanilla' have important morphological and physiological differences, in terms of fruit size and maturity date. For this reason, and in order to provide a high range of variability, these three olive cultivars were selected for this work (Table 1).

A total of 20 kg of olive fruits from each variety were collected sequentially in the period October–December 2009 at the olive culture station IFAPA "Alameda del Obispo" (Córdoba, Spain). As soon as the samples were received in the laboratory of the Postharvest and Food Technology area, olive fruits were stored at 4 °C and 90% of relative humidity until the time of analysis.

Table 1
Characteristics of the three varieties used in this study.

Variety	Mean fruit weight (g)	Pulp/bone relationship	Maturity index
Picual	3.2	5.6	1–2
Hojiblanca	4.8	7.9	6–7
Manzanilla	4.6	8.2	3–4

Before spectroscopy analyses were carried out, the olive fruit samples were stabilised at laboratory temperature (24 °C).

2.2. On-line VISNIR measurements

A single-beam diode array spectrometer, Corona 45 VISNIR (Carl Zeiss, Jena, Germany) was used to acquire reflectance spectra in the wavelength range between 380 and 1690 nm. The instrument interpolates the data to produce one point at every 2 nm, giving a 656 data point spectrum.

The instrument is equipped with a silicon (Hamamatsu S 3904) sensor array of 256 diodes active in the range 380–950 nm and an InGaAs array of 128 diodes active in the range 950–1690 nm. The light source consisted of a 10 V/20 W halogen lamp illuminating perpendicularly to the sample surface. Reflected light was collected by 15 sensors equally spaced around the source and oriented at 45° to the sample plane.

The spectrometer was installed on a structure designed specifically to support it and to perform, in this way, on-line measurements on a conveyor belt (Fig. 1). Spectral readings from intact olives were acquired during the movement of the samples underneath the instrument. Absorbance values were recorded as $\log(1/R)$, where R is the reflectance using the CORA software, version 3.2.2 (Carl Zeiss, Inc.). Before NIRS data acquisition were carried out, spectra of dark and white references were measured using respectively, a black standard with spacer and a white ceramic reflectance standard.

2.3. Experimental parameters

The effects of integration time and focal distance parameters on on-line spectra acquisition were studied. At the same time, the cultivar's influence on spectral response was evaluated.

The different values studied of focal distance were 13, 15, 20 and 25 mm and of integration time were 0.1, 0.5, 1, 2 and 5 s. Due to the different sample sizes analysed, the focal distance has to be adjusted for each variety of olive studied, by the vertical movement of the support structure, in order to maintain always
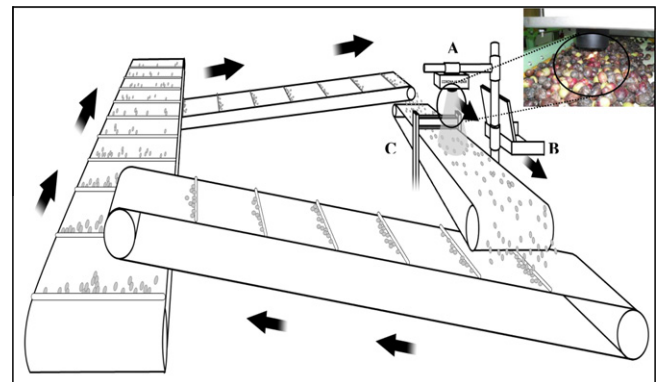

Fig. 1. Scheme showing on-line analysis developed on the conveyor belt and the localisation of the NIRS sensor used. (A) CORONA 45 VISNIR, (B) computer and (C) edge lipping planer.

the same distance between the NIR sensor head and the surface of the sample, independently on the size of the sample analysed.

Distances lower than 13 mm were not studied because they put olives in direct contact with lens, leaving stains and greasy residues on the sensor head. Parameters, such sample layer thickness and conveyor belt speed were also taken into account. A screed guide was fixed on the conveyor belt at a suitable height to obtain a constant bilayer of olives, ensuring an adequate coverage in order to avoid collect spectra from belt surface. The conveyor belt speed was fixed to 0.1 m/s.

In this study, 60 on-line NIR responses were obtained as a result of the combination of the 3 different cultivars, the 4 focal distances and the 5 different integration times.

Each NIR response was the average of 40 spectra acquired during the movement of the olives on the conveyor belt. The time interval measurement was established in 100 ms. Therefore, for each of the three olive varieties, a total of 200 spectra were collected for each focal distance and 160 spectra were collected for each integration time.

2.4. Spectral data processing and statistical analysis

Initial data handling and treatment were performed using Win-ISI III v.1.50 (Infrasoft International, Port Matilda, PA, USA) and Matlab v.7.0. (The Mathworks, Inc., Natick, MA, USA) softwares.

The statistical criteria used to evaluate the spectral repeatability for NIR response were the standard deviation (SD) and the root mean square statistic (RMS). RMS calculates the similarities in absorbance values between the different spectra from the same response (Gaitán-Jurado et al., 2008). As already mentioned, in the present study, 40 spectra per response were obtained.

This statistic is defined as the square root of the mean squared value of the difference between $\log(1/R)$ value of a specific spectrum and $\log(1/R)$ value of the mean spectrum of various spectra from a same sample (in this study, response) for the whole range of wavelength values. RMS value of each spectrum of the 40 spectra from a same sample was calculated according to the following formula (1):

$$RMSj = \sqrt{\frac{\sum_{i=1}^{n}(Y_{ij} - \overline{Y_i})^2}{n}} \tag{1}$$

of which Y_{ij} is the $\log(1/R)$ value for spectrum j at wavelength $i(\lambda_i)$, $\overline{Y_i}$ is the $\log(1/R)$ value for the mean spectrum at wavelength i (λ_i) and n is the number of datapoints (656 in our case).

Finally, the 40 RMS values from each NIR response were averaged in order to obtain a unique RMS Mean value per response. Consequently, a total of 60 RMS Mean values were obtained.

After obtaining the average values of RMS a *Multifactorial* ANOVA (analysis of variance) procedure was designed in order to build a statistical model that describe the impact of cultivar type, integration time and focal distance parameters (defined as categorical factors Xj) in the RMS Mean values (dependent variable Y). After ANOVA, comparisons among individual means were made by Fisher's least significant difference (LSD) and finally, interaction effects between pairs of factors over RMS Mean values were studied.

ANOVA and LSD analysis were performed using Statgraphics Centurion XVI software v.16.1.15 (StatPoint Technologies, Warrenton-Virginia, USA).

3. Results and discussion

The effect of the focal distance and the integration time on the acquired spectra was studied. All olive spectra (from the three varieties) acquired with the same focal distance (as mentioned above, 600 spectra) and with the same integration time (480 spectra)

were averaged in order to obtain a mean spectrum. In this way, 4 mean spectra were obtained for the study of focal distance and 5 mean spectra for the study of integration time.

A visual comparison of the different mean spectra obtained in the studies of focal distance and integration time was realised. In both cases, the spectral variations were also examined by the statistical standard deviation (SD).

Fig. 2 illustrates the average spectral curves obtained in the study of the focal distance parameter, as well as, the mean and SD spectra. Mean and SD spectra are obtained on the basis of 4 mean spectra.

The analysis of the visible region, between 380 and 580 nm, shows that high SD values were observed due to noise affecting this region (Malley et al., 2007; Kemps et al., 2010). Spectra show a particular profile in the range located in the transition area from the visible to near infrared zone (approximately between 950 and 990 nm). In fact, this is due to the change from silicon (380–950 nm) sensor array to the InGaAs array (950–1690 nm) detector.

As can be observed on Fig. 2, the mean spectrum of the higher distance (25 mm) is located on the top of the graph, while the spectrum of the lower distance (13 mm) is located on the bottom. A shift in absorbance values can be observed in spectra acquired with different focal distances, possibly due to the difference in the amount of light reflected from the sample back to the spectrometer. These spectral positions were also observed after reduce the multiplicative effects by MSC (Multiplicative Scatter Correction) pretreatment and adjust spectral baseline using a second derivative (data not shown).

The effect of the integration time parameter on the absorbances was also studied. Mean and SD spectra were obtained on the basis of 5 mean spectra. Fig. 3 represents the mean, SD and average spectral curves for the different integration times studied.

Contrary to the focal distance parameter, no significant visual differences of absorbance values were observed between mean spectra obtained over the complete wavelength range. However, as in the previous case, an important variation in the SD spectra was observed in the visible region between 380 and 480 nm, due probably to the instrument noise.

Results obtained seem to suggest that the choice of an appropriate focal distance will have a major influence on the spectral quality and repeatability than the choice of an adequate integration time (mean $SD_{Focal\ distance}$ = 0.12 vs. mean $SD_{Integration\ time}$ = 0.02).

In order to understand how the selection of a determined focal distance and integration time affect the repeatability of results, RMS Mean values were obtained and evaluated using *Multifactorial*
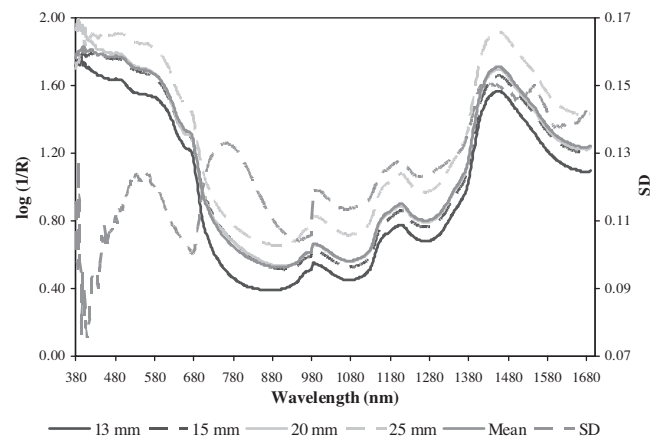

Fig. 2. Mean, SD and average spectral curves collected for the different focal distances (13, 15, 20 and 25 mm).
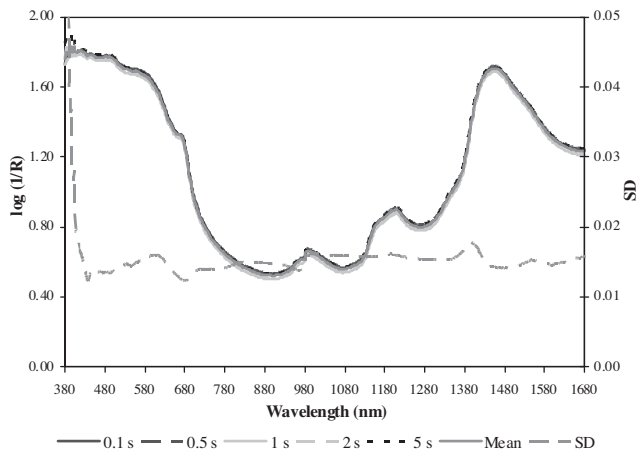
Fig. 3. Mean, SD and average spectral curves collected for the different integration times (0.1, 0.5, 1, 2 and 5 s).

Table 3
Multiple Range Test. Method: 95.0 percent LSD.

Factor	Count	*LS mean	LS sigma	Homogeneous groups
Cultivar				
Picual	20	37336.3	3865.71	X
Hojiblanca	20	55083.2	3865.71	X
Manzanilla	20	72510.9	3865.71	X
Focal distance				
13 mm	15	44824.8	4463.73	X
15 mm	15	49570.3	4463.73	X
20 mm	15	57504.7	4463.73	XX
25 mm	15	68007.3	4463.73	X
Integration time				
5 s	12	43371.3	4990.61	X
2 s	12	47416.2	4990.61	X
1 s	12	49972.9	4990.61	XX
0.1 s	12	62543.6	4990.61	XX
0.5 s	12	71580.0	4990.61	X

* LS, leasts squares.

ANOVA. Before RMS calculation, spectra from 380 to 580 nm were removed to eliminate high frequency noise region.

As can be observed in Table 2, ANOVA procedure decomposes the variability of RMS Mean into contributions due to various factors. Since Type III sums of squares (the default) have been chosen, the contribution of each factor is measured having removed the effects of all other factors. The *P*-values test the statistical significance of each of the factors. Since three *P*-values are less than 0.05, these factors have a statistically significant effect on RMS at the 95.0% confidence level.

In order to determine which means are significantly different from which others it was necessary to apply the Multiple Range Tests. The method used to discriminate among the means was Fisher's least significant difference (LSD) procedure. With this method, there is a 5.0% risk of calling each pair of means significantly different when the actual difference equals 0.

Table 3 shows for the cultivar factor that three different homogenous groups were identified using columns of X's. Within each column, the levels containing X's form a group of means within which there are no statistically significant differences. In this particularly case, the X's form denotes a statistically significant difference among the three cultivars. The following Fig. 4A displays the intervals around each mean graphically. It is clearly seen from this figure that the RMS values of Picual cultivar are lower than those observed for the Hojiblanca variety and about all, for the case of Manzanilla.

In Table 3 can also be observed that two homogeneous groups have been identified according to the alignment of the X's in columns for focal distance factor. As can be noticed, two pairs (13–25 mm and 15–25 mm) show statistically significant differences.

Fig. 4B suggests that at 95 percent LSD, increasing the distance between the NIR sensor and the surface of the olive, increases RMS mean value.

For the particularly case of integration time factor, as seen in Table 3, three different homogenous groups were identified using columns of X's and there are five pairs statistically significant at the 95.0% confidence. In Fig. 4C is observed that for integration time higher than 1 s the RMS values decreases reaching minimum values for 5 s.

Finally, interaction effects were calculated in order to represent the combined effects of factors on the dependent measure. When an interaction effect is present, the impact of one factor depends on the level of the other factor. In the following figure (Fig. 5A) the interaction effects between the cultivar type and integration time factors are shown. As can be observed, there is no interaction between both because the slope of lines follow the same tendency in the graph. The plot of RMS Mean values obtained shows a clear tendency of increase of these values with decreasing the integration time. Smaller RMS Mean values for the three varieties were observed at the integration time 5 s. On the contrary, the highest values were showed for 0.5 and 0.1 s integration times. Also, it is possible notice that RMS Mean values for Picual variety did not show significant differences according to the integration time used.

In the same way, interaction effects between cultivar and focal distance were studied (Fig. 5B). The comparison of the repeatability measurements for different focal distances shows that, in general, RMS values were much higher at 25 mm than those obtained at 13 mm for the particular case of Manzanilla and Hojiblanca. However, Picual variety showed similar RMS Mean Values for both distances.
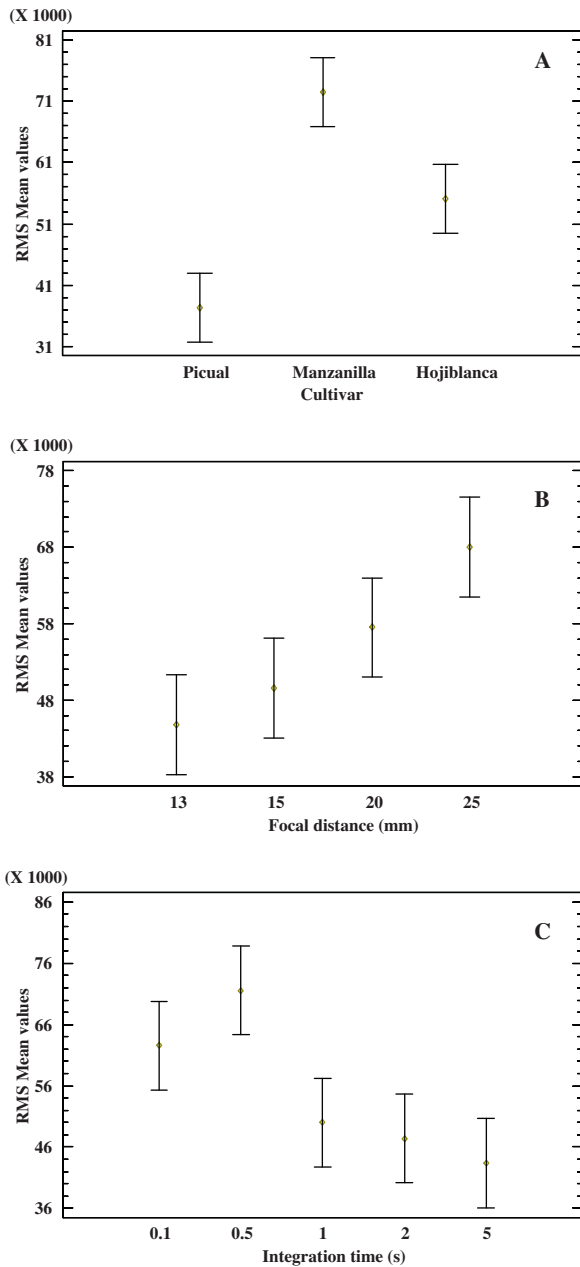
Table 2
Results from the *Multifactorial* ANOVA of the three factors (cultivar type, integration time and focal distance) in the RMS mean values.

Source	Sum of squares	Df*	Mean square	F-ratio**	P-value
Main effects					
A: Cultivar	1.23729E10	2	6.18643E9	20.70	0.0000
B: Integration time	6.59776E9	4	1.64944E9	5.52	0.0027
C: Focal distance	4.62717E9	3	1.54239E9	5.16	0.0068
Interactions					
AB	1.1628E9	8	1.4535E8	0.49	0.8538
AC	4.19238E9	6	6.98729E8	2.34	0.0641
BC	4.08205E9	12	3.40171E8	1.14	0.3772
Residual	7.17297E9	24	2.98874E8		
Total (corrected)	4.0208E10	59			

* Df, degrees of freedom.
** All *F*-ratios are based on the residual mean square error.

Fig. 4. Means and 95.0 Percent LSD Interval for: (A) cultivar; (B) focal distance (mm) and (C) integration time (s).

Fig. 5. Interaction effects between pairs of factors in ANOVA. (A) Cultivar–integration time (s); (B) cultivar-focal distance (mm) and (C) focal distance (mm)-Integration time (s).

Finally, interaction effects between focal distance and integration time factors were studied (Fig. 5C). The combination of a focal distance of 13 mm and an integration time of 5 s gave the smallest RMS value, and consequently the best spectral repeatability. A similar result was showed by the combination of 13 mm and 2 s. In contrast, higher values of RMS were observed when combining the focal distance of 20 mm and 0.5 s of integration time or 25 mm focal distance and 0.5 s of integration time.

The comparison of the mean RMS values obtained with the different focal distances studied shows that no significant differences could be observed using 0.1 s as integration time for all focal distances. The study carried out with an integration time of 5 s gave notable differences between the RMS results according to the focal distance used, showing a minimum value of 28,753 and a maximum of 66,028.

The best RMS values obtained at higher values of integration time in the repeatability study could be explained by the size of
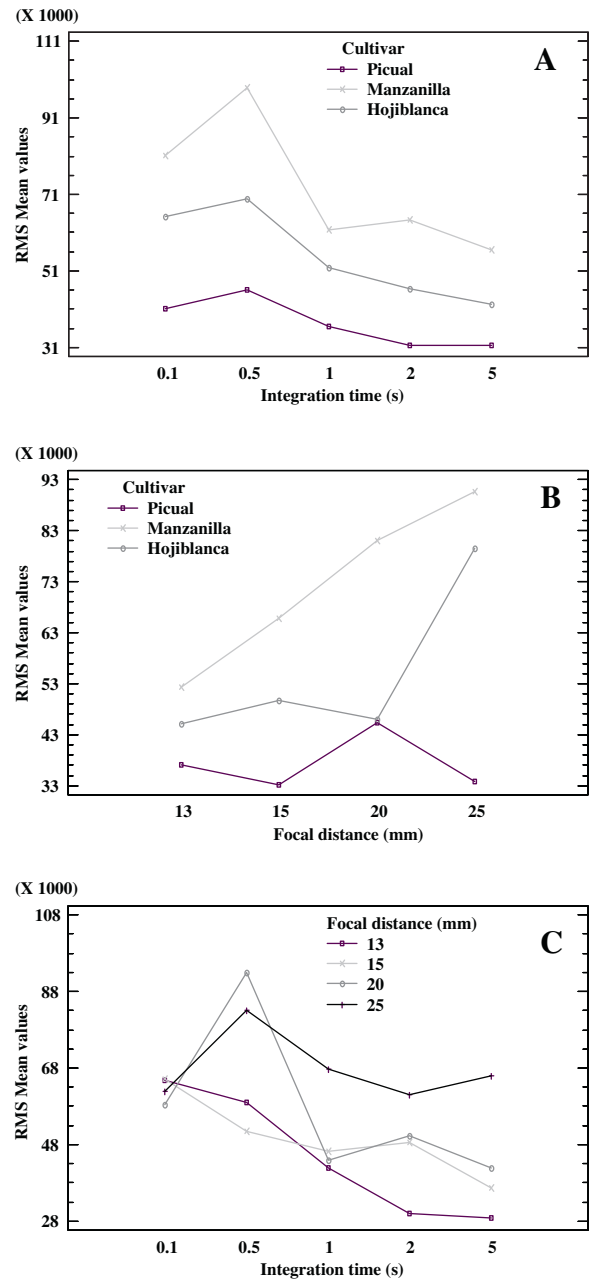
sample area analysed and by the size of the sample. The increase of the integration time permits to analyse more area of one sample and then to include more heterogeneity during one measurement (Fig. 6). In the same way, the size of the sample can also exercises influence due to small olive fruits (as in the case of Picual variety) means a lot of olives analysed by sample area and therefore, more heterogeneity is collected.

In view of the results obtained, it is possible conclude that the best focal distance and integration time is 13 mm and 5 s because the RMS Mean value was the lowest. Furthermore, the type of cultivar have influenced significantly in these values. Picual variety has shown the minimum values regardless of integration time or focal distance used. On the contrary, RMS values obtained for Manzanilla and Hojiblanca have been higher in both cases. Therefore, these results could indicate that the ripeness state of the fruit
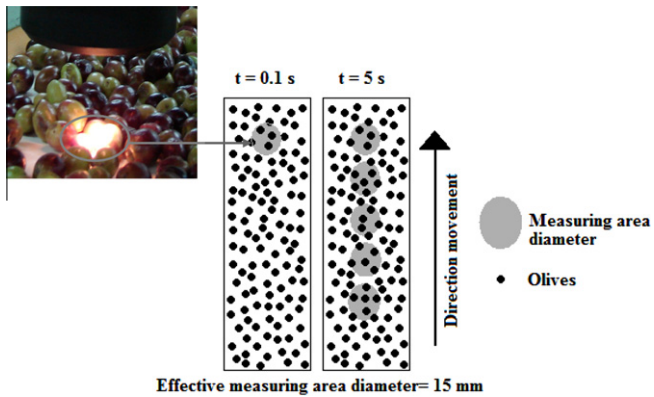
Fig. 6. Illustration of sample area analysed (grey) with 0.1 and 5 s integration times.

has had a significant effect on the RMS Mean values obtained since the Picual cultivar was the least mature and conversely, the olives of the Manzanilla and Hojiblanca varieties analysed had all a high state of maturity.

4. Conclusions

Optimal operational conditions should be defined prior to start a NIRS on-line analysis of samples in movement in order to obtain accurate spectral data. This paper reports the impact of the focal distance and the time integration acquisition parameters on the spectral repeatability for the analysis of intact olive fruits on a conveyor belt. A focal distance of 13 mm and an integration time of 5 s give the best RMS Mean value, showing a higher repeatability than those obtained with the other distances and integration times studied.

Acknowledgements

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