GRASAS Y ACEITES, 62 (3),
JULIO-SEPTIEMBRE, 268-274, 2011,
ISSN: 0017-3495
DOI: 10.3989/gya.089610

Non-destructive assessment of olive fruit ripening by portable near infrared spectroscopy

By **A. Gracia** and **L. León***

IFAPA Centro Alameda del Obispo, Avda. Menéndez Pidal s/n, E-14004 Córdoba, Spain.
(* Autor para la correspondencia: lorenzo.leon@juntadeandalucia.es)

RESUMEN

Evaluación no destructiva de la maduración de la aceituna mediante espectroscopia en el infrarrojo cercano portátil.

En este trabajo se estudia la posible aplicación de la tecnología en el infrarrojo cercano (NIR) portátil para la determinación de la humedad y el rendimiento graso en aceitunas intactas. Se han utilizado un total de 144 y 112 muestras recolectadas a lo largo del periodo de maduración en dos ensayos de variedades. Los datos espectroscópicos en la región de 1100 a 2300 nm a intervalos de 1 nm se recogieron bajo dos condiciones experimentales: directamente en el árbol en el ensayo 1 y en condiciones de laboratorio en el ensayo 2. Los modelos de calibración se desarrollaron y evaluaron mediante regresión por mínimos cuadrados parciales (PLS) por separado para cada uno de los ensayos y conjuntamente para todas las muestras. Los resultados obtenidos directamente en el árbol en campo fueron suficientemente precisos para determinar la fecha óptima de recolección de cada variedad aunque los resultados obtenidos en laboratorio fueron ligeramente mejores. El modelo conjunto mostró resultados intermedios (r=0.89 y RMSECV= 1.99 para rendimiento graso y r=0.88 y RMSECV=2.06 para humedad), que se pueden considerar aceptables por la mayor robustez de los modelos. Estos resultados estimulan el uso de la tecnología NIR portátil para el seguimiento de la maduración de la aceituna y la determinación del momento óptimo de recolección en base al contenido graso y humedad.

PALABRAS CLAVE: Ensayos comparativos – Humedad – NIR – Olea europaea – Rendimiento graso.

SUMMARY

Non-destructive assessment of olive fruit ripening by portable near infrared spectroscopy.

The feasibility of portable near infrared spectroscopy (NIR) technology for the determination of oil and moisture contents in intact olive fruits was studied. A total of 144 and 112 samples were collected throughout the ripening period in two different olive cultivar trials. Spectral data were recorded in the wavelength region from 1100 to 2300 nm at 1 nm intervals under two different experimental conditions: on-tree in the field in Trial 1 and under laboratory room conditions in Trial 2. Calibration models were developed and evaluated using partial least squares (PLS) regression separately for each trial set and for the combined group of samples. Although slightly better results were obtained under laboratory room conditions, the results obtained on-tree in the field were also accurate enough to determine the optimal harvest date of each cultivar. The combined model showed predictive statistics within the range of the individual models (r=0.89 and RMSECV= 1.99 for oil content and r=0.88 and RMSECV=2.06 for moisture content), which could be considered acceptable as an increase in the model robustness could be expected. These results encourage the use of portable NIR spectroscopy to monitor olive fruit ripening and to decide the optimal harvesting date on the basis of oil and moisture content.

KEY-WORDS: Comparative field trial – Moisture – NIR – Oil content – Olea europaea.

1. INTRODUCTION

Several biochemical processes occurring during olive fruit ripening are responsible for oil accumulation and for the formation of the characteristic compounds of olive oil. The ripening process of the olive fruit changes with the cultivar, fruit load, environmental conditions and cultural practices; and thus is different for each growing area and harvest season (Beltrán *et al*., 2004; Lavee and Wodner, 2004). For these reasons, an optimal harvesting period in terms of oil quantity and quality must be determined in order to obtain the maximum return to the grower.

Indirect measurements are normally used by growers as guidelines to decide the harvest period. Most of them are based on color changes in the olive fruit during the ripening period. Frías *et al*. (1991) developed a method based on the color changes in peel and pulp classified into eight groups or categories from green intense (category 0) to black with 100% purple flesh (category 7). For oil mill purposes an average index of 3.5 has been suggested for harvesting although these color changes are highly influenced by several genetic and environmental factors. Direct measurements of characteristics such as fruit weight, fruit removal force, oil content, fruit moisture, sugars composition, etc. provide the best way to determine the optimal harvesting period (Wodner *et al*., 1988; Mickelbart and James, 2003; Beltrán *et al*., 2004; Mailer *et al*., 2007; Cherubini *et al*., 2009). However, all of them are based on destructive measurements which are time consuming and impractical for processing a large number of samples.

Near infrared (NIR) spectroscopy analysis offers many advantages such as a rapid, simultaneous and non-destructive analysis with low cost per sample. In recent years, NIR spectroscopy has been used for nondestructive measurements of the internal quality attributes of several fruits and vegetables. In a recent review, Nicolaï *et al*. (2007) reported the application of NIR to determine the soluble solid contents of many fruits such as apple, apricot, cherry, citrus, grape, kiwifruit, mango, nectarine, papaya, peach, pear, pineapple and plum. Other applications of NIR in fruits include the determination of firmness, pH, acidity, sugars, starch, dry matter, pectin, etc. In olives, previous works have demonstrated the enormous possibilities offered by NIR spectroscopy for the on site quality control process of olive fruits, pastes, and oils for both a quantitative analysis of the main components or the discrimination of samples according to cultivar, origin, common olive alterations, classification, authentication and detection of olive oils (reviewed by Armenta *et al*. 2010). However, direct analysis of intact fruit has been scarcely reported (León *et al*., 2003, 2004; Cayuela *et al*., 2009; Cayuela and Pérez-Camino, 2010).

In recent years, the availability of low cost miniature spectrophotometers has opened up the possibility of portable devices which can be useful for monitoring fruit ripening in the field to determine the optimal harvesting date. Applications of portable NIR for the determination of fruit quality characteristics have been recently reported in apricot, apple, citrus, tomato, peach and nectarine (Golding *et al*., 2006; Peano *et al*., 2006; Bessho *et al*., 2007; Kusumiyati *et al*., 2008; Zude *et al*., 2008; Camps and Christen, 2009).

The aim of this work was to asses the feasibility of portable NIR technology for the determination of olive oil and moisture contents, the two main components of the olive fruit. Samples both on-tree in the field and under laboratory room conditions were scanned throughout the harvest season in order to track their evolution during the ripening process to determine the optimal harvesting period.

2. MATERIALS AND METHODS

2.1. Plant material and sampling procedure

Two different comparative field trials of olive cultivars were used in this study. Trial 1 located in Cordoba, Spain, includes four different cultivars: 'Arbequina', 'Picual' and two selections from a breeding program (Sel1 and Sel2) coming from crosses between these two cultivars. Three trees per cultivar and two samples of five fruits per tree were collected every two weeks in six consecutive dates from September 29th to December 3rd for a total of 144 samples. Trial 2 located in Huelva, Spain, also includes four different cultivars: 'Arbequina', 'Verdial de Huévar', 'Manzanilla de Sevilla' and 'Picual'. In this trial, four trees per cultivar and one sample of five fruits per tree were collected every two weeks on seven consecutive dates from September 16th to December 12th for a total of 112 samples.

These samples were selected to provide enough heterogeneity in terms of cultivar, environmental conditions and ripening stage.

2.2. Spectral collection and analysis by reference methods

In trial 1, NIR spectra of fruits on-tree were obtained in the open field and fruit samples were then taken to laboratory. In trial 2 fruits were harvested and brought to laboratory where spectral collection was carried out. The average spectra of five fruits per sample date were used for subsequent analyses in both trials. All spectra were acquired in absorbance (log (1/Reflectance)) with a portable acousto-optical tunable filters AOFT-NIR spectrophotometer (Luminar 5030, Brimrose Corp., Maryland). Spectra were collected in the wavelength region of 1100 to 2300 nm at 1 nm intervals. Each olive fruit spectrum represents the average of 50 spectra acquired on the fruit equator with continuous measuring configuration for a total scanning time of around 5 seconds. Spectra collection was controlled with a laptop computer using SNAP32 software.

After spectral collection, fruit samples were processed in the laboratory for analysis by reference methods. Fresh samples were weighed and then dried in a forced-air oven at 105 °C for 42 h to determine moisture content. The oil content of dried samples was recorded by NMR Minispec NMS100 (Bruker Optik GmbH, Ettlingen, Germany).

2.3. Data analysis

Spectral data were exported to The Unscrambler 9.5 software (CAMO A/S, Trondheim, Norway) for chemometric analysis. Average spectral data of five fruits per tree were used in all subsequent analyses. Calibration models were developed and evaluated using partial least squares (PLS) regression. Baseline offset transformation was used in all cases and several other mathematical pretreatments were also tested to improve the prediction accuracy of the PLS models. Calibration models were developed separately for each trial set and for the combined group of samples.

Full cross-validation (i.e. leaving-one-out) was used to determine the performance of the models and no outliers were removed in any step of the calibration process. Correlation between actual and predicted constituent values (r) and standard error of cross validation (RMSECV) were used to test the performance of calibrations (Shenk and Westerhaus, 1995). The range error ratio (RER), defined as the ratio between the data set range for any given constituent and the standard error of cross validation for the same constituent was also determined to indicate the relative accuracey of each model (Williams and Sobering, 1996).

3. RESULTS AND DISCUSSION

3.1. Reference data and spectral features

As expected, the selection of different cultivars, environmental conditions and ripening stages have provided wide ranges of variability for the characteristics evaluated (Table 1). For the combined group oil content ranged from 4.80 to 29.84 % and moisture content from 48.12 to 69.57 %. In both trials 1 and 2 oil content increases at the beginning of the experiment and then stabilizes or even slightly decreases at the end of the ripening period (Figure 1). Moisture content showed the opposite trend decreasing during the ripening period. In fact, both characteristics, oil and moisture content, were highly correlated ($r = -0.61$, $p < 0.001$). However, different patterns were observed in trials 1 and 2 probably due to the different climatic conditions of each experimental area.

The average raw spectra and coefficient of variation of olive fruit samples from T1 and T2 are shown in Figure 2. Spectra are characterized by two principal water absorption bands of around 1460 nm and 1950 nm and oil of around 1210 nm and 1730 nm (Shenk *et al.*, 2001). No clear trends were observed among spectra collected in the different trials or different harvesting dates in any of the trials (data not shown).

3.2. Calibration development for each trial set

Calibration models were developed for moisture and oil contents for each trial independently and for the combined data set. The number of PLS factors, correlation coefficient and predictive error (RMSECV and RER) obtained for models are shown in Table 2. R values for oil content and moisture were slightly lower and RMSECV was higher for

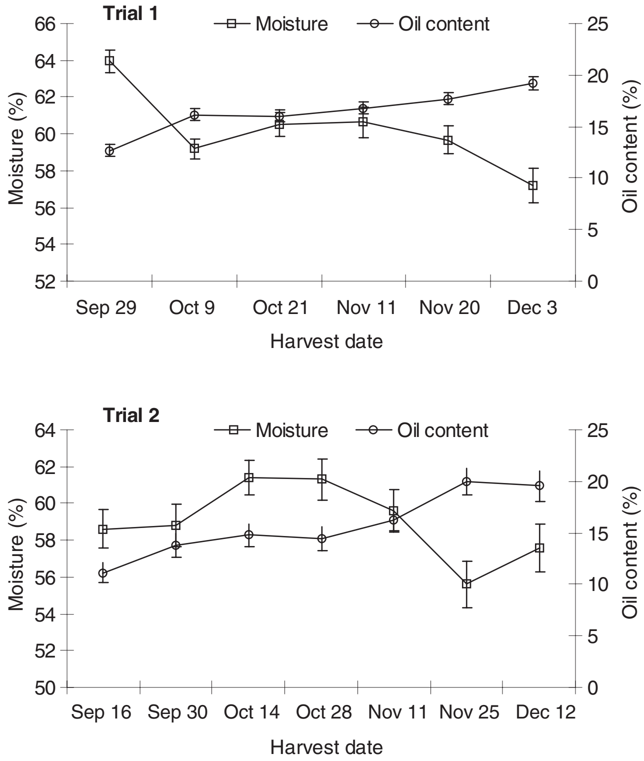

Figure 1
Change in oil content and moisture during the ripening period. Each point represents the mean value of 24 samples (Trial 1) and 16 samples (Trial 2) and error bars indicate the SE.

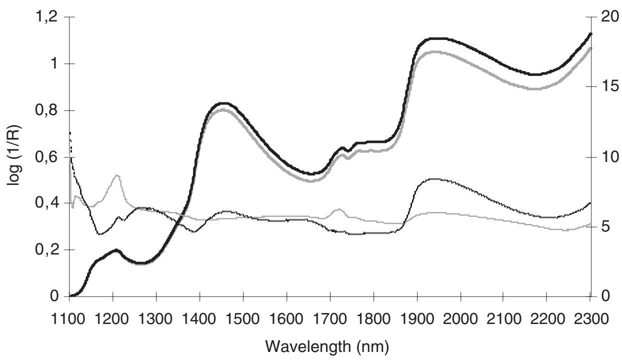

Figure 2
Average spectra and coefficient of variation of olive fruit samples from Trial 1(Black) and Trial 2 (grey) showing the main absorption bands by water and oil.

Table 1
Descriptive statistics for oil and moisture contents of olive fruit samples

		Oil content (%)				Moisture (%)			
Group	N	Min	Max	Mean	SD	Min	Max	Mean	SD
Trial 1	144	7.71	26.37	16.42	3.52	48.12	66.96	60.18	4.08
Trial 2	112	4.80	29.84	15.62	5.32	48.63	69.57	59.01	4.78
Combined	256	4.80	29.84	16.08	4.40	48.12	69.57	59.67	4.43

Table 2
Cross-validation results for calibration models developed separately for each trial set and for the combined group of samples

	Oil content (%)				Moisture (%)			
Group	**nPLS**	**r**	**RMSECV**	**RER**	**nPLS**	**r**	**RMSECV**	**RER**
Trial 1	8	0.85	1.89	9.87	8	0.83	2.26	8.34
Trial 2	11	0.96	1.52	16.47	11	0.93	1.65	12.69
Combined	9	0.89	1.99	12.58	11	0.88	2.06	10.41

NPLS, number of factors in partial least squares regression; r, Correlation between actual and predicted constituent values; RMSECV, standard error of cross validation; RER, range error ratio.

model 1 probably due to different conditions during spectral data acquisition. On-tree fruit spectral data were used in model 1 while the spectral data of model 2 were obtained under constant laboratory room conditions. Similar results have been reported for the on-tree and after harvest evaluation of firmness, color and lycopene content of tomato fruit (Kusumiyati *et al*., 2008). Pérez-Marín *et al*. (2009) also reported better results with laboratory instruments for the prediction of nectarine quality parameters, although a handheld instrument also proved to be a promising instrument for the in-field evaluation as well as providing the additional advantages of portability and low cost.

The NIR prediction of fruit moisture and oil content in intact olives has been previously reported. León *et al*. (2003, 2004) obtained calibration models accurate enough to predict oil content and moisture with r values of 0.94 and 0.93 respectively using a laboratory instrument (not portable). Cayuela *et al*. (2009), working with the same instrument used in this work under laboratory conditions, obtained variable predictive ability depending on the sample presentation procedure and the reference laboratory method. The best results in cross-validation provided r values of 0.83 and 0.88 and RER values of 7.8 and 11.8 for oil content and moisture respectively, although samples from a single batch from a single cultivar were used. Using a different portable instrument, Cayuela and Pérez-Camino (2010) obtained r values of 0.78 and 0.76 and RER values of 10.6 and 10.3 for oil content and moisture respectively.

The different cultivars evaluated in this work showed differences in lipid synthesis both in total amount of oil formed and the period of time, as well as the evolution of fruit moisture during ripening (Figures 3 and 4). The prediction values for oil content and moisture by cultivar and sampling date were closely correlated with reference

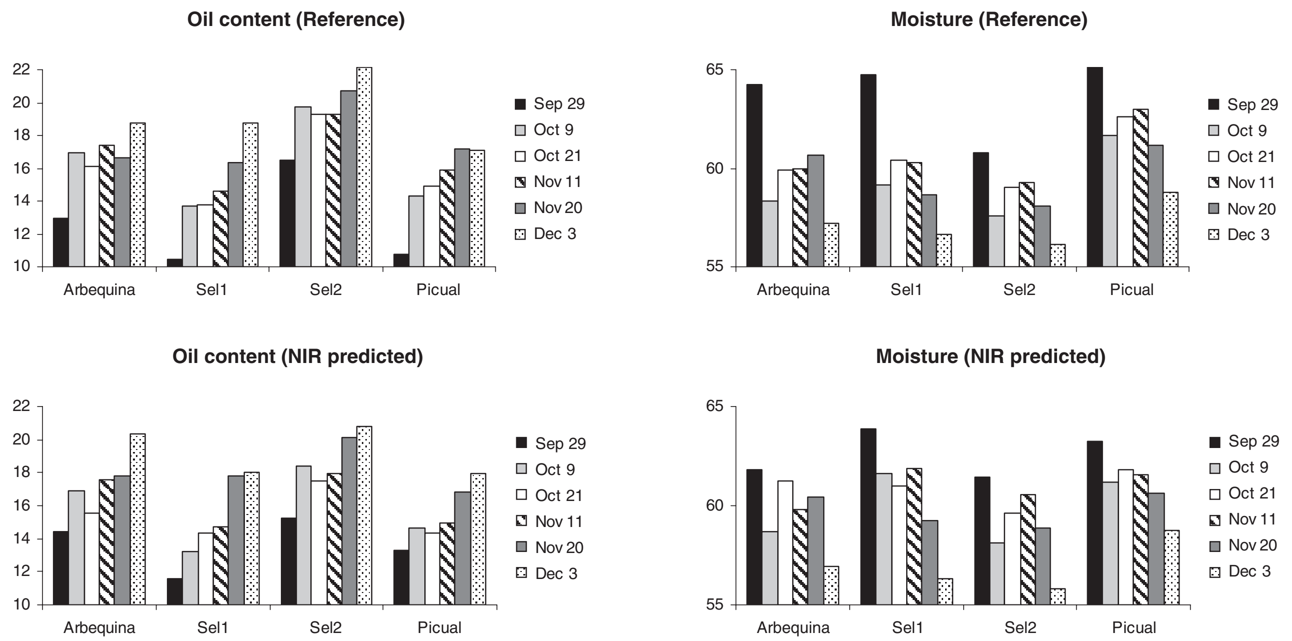

Figure 3
Average reference and NIR predicted (on-tree) oil and moisture contents by cultivar and sampling date in trial 1.

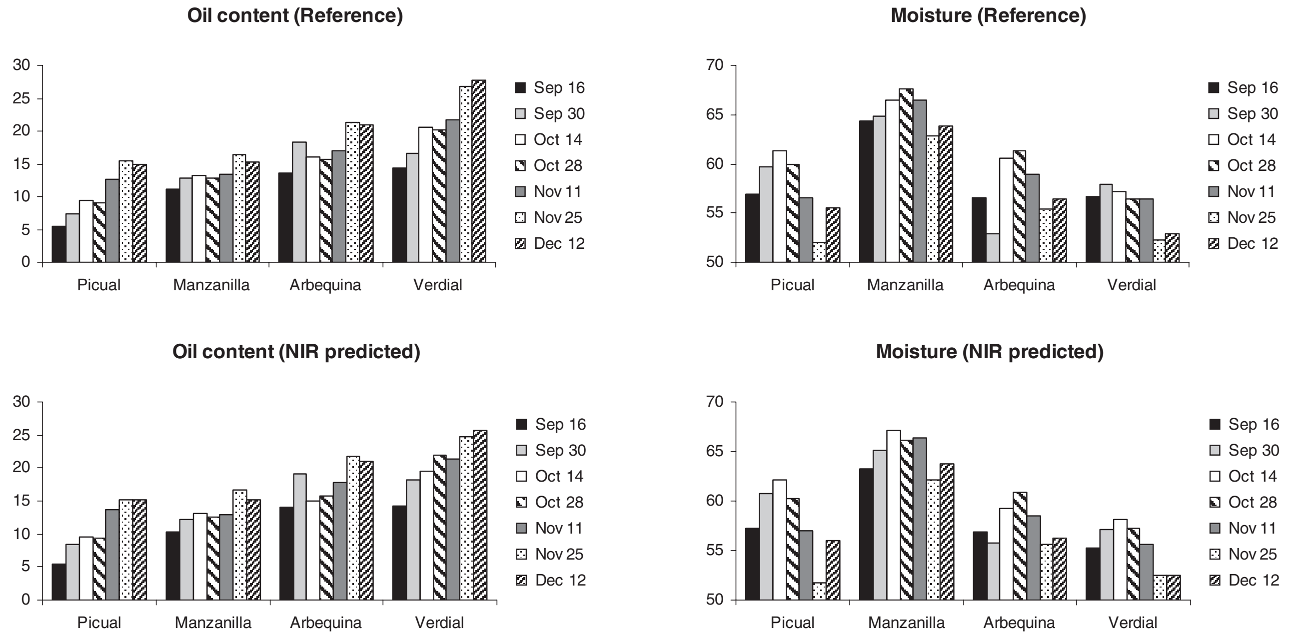

Figure 4
Average reference and NIR predicted (laboratory) oil and moisture contents by cultivar and sampling date in trial 2.

values both for trial 1 (on-tree prediction) and 2 (laboratory prediction). According to the evolution of oil content and moisture for Trial 1 the optimal harvest date would be in October for 'Arbequina' and Sel2, the end of November for 'Picual' and the beginning of December for Sel1. Similarly, the optimal harvest date for Trial 2 would be in October for 'Arbequina' and 'Manzanilla de Sevilla' and the end of November/beginning of December for 'Picual' and 'Verdial de Huévar'. Therefore, portable NIR spectroscopy may be used for determining the optimal harvest date of each cultivar on the basis of oil content and moisture. It should be noted that the evolution of other oil components which are important for quality should also be taken into account to determine the optimal harvesting period. For instance, the contents of antioxidant compounds and related parameters such as oxidative stability as well as sensory quality decreased as olive fruit ripened (García *et al*., 1996; Beltrán *et al*., 2005; Gómez-Rico *et al*., 2006).

3.3. Combined calibration model

The combined model showed predictive statistics in the range of the individual models (Table 2, Figure 5), which could be considered acceptable as an increase in the model robustness could be expected. As pointed out by Nicolaï *et al*. (2007), model robustness is the single most important concern in NIR spectroscopy for horticultural produce. Taking into account the ranges of variation, RER values of 12.58 and 10.41 for oil and moisture contents respectively were obtained in this combined model. Values of r higher than 0.7 indicate a good fit of the predictive model and an RER value of at least 10 has been suggested as acceptable for use in certain applications (Shenk and Westerhaus, 1995; Williams and Sobering, 1996). None of the mathematical pretreatments tested (multiplicative scatter correction, standard normal variate and Savitzky-Golay derivatives) improved the prediction accuracy of the PLS models. Similar results have been recently reported in models developed for the prediction of moisture, dry matter, oil content, free acidity and fruit maturity index in intact olive fruits using a portable spectrometer, in which none of the tests performed using the same spectral data pre-calibration treatments improved the results (Cayuela and Pérez-Camino, 2010). Moreover, as previously reported by the same authors, the selection of spectral variables did not provide any significant improvement in calibrations compared with the full spectrum between 1100-2300 nm.

These results were similar to those obtained for the prediction of the optimal picking date for different apple cultivars by means of VIS/NIR spectroscopy, in which correlation values of 0.80-0.90 were found for characteristics such as soluble solids, acidity or firmness (Peirs *et al*., 2001). Zude *et al*. (2008) applied NIR for non-destructively monitoring the soluble solids content of citrus fruit on trees concluding that this method has potential for use as a tool in site-specific harvest management. Pérez-Marín *et al*. (2009) also reported the feasibility of NIR technology for monitoring important quality attributes such as soluble solid contents, flesh firmness, fruit weight and diameter in nectarines.

 GRASAS Y ACEITES, 62 (3), JULIO-SEPTIEMBRE, 268-274, 2011, ISSN: 0017-3495, DOI: 10.3989/gya.089610

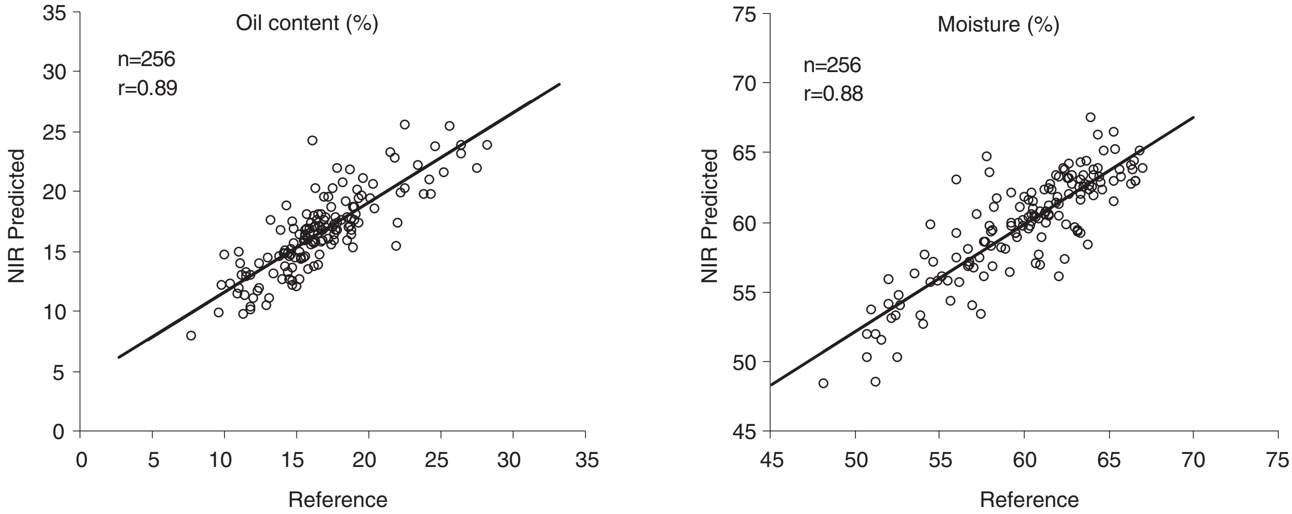

Figure 5
Reference vs. NIR predicted oil and moisture content for the calibration model using the combined group of samples.

4. CONCLUSIONS

The results obtained indicate that portable NIR spectroscopy could be used to estimate oil content and moisture in intact olive fruits both on-tree or under laboratory conditions. This technology offers the possibility of a rapid and non-destructive analysis which is useful to determine several characteristics in one single measurement. Moreover, the use of portable devices allows for the possibility of repeated measurements in the same fruit on-tree without interfering in fruit development, which would be useful to monitor olive fruit ripening and to decide the optimal harvesting date on the basis of oil and moisture contents. Future works which allow for the simultaneous determination of oil quality components could be used for achieving not only the highest oil content but also the best oil quality. The results obtained in this work also indicate the usefulness of this technique as a selection tool in olive breeding programs where many samples must be analyzed to select the most outstanding genotypes where there is a need for fast and cheap analytical procedures to determine the agronomical traits of interest.

ACKNOWLEDGMENTS

This work has been partly supported by project RTA2006-00039-C02-01, National Institute of Agricultural Research (INIA), Ministry of Education and Culture, Spain, partially funded by European Regional Development Fund (ERDF). A. Gracia is grateful to INIA and IFAPA-Junta de Andalucía for his research fellowship. Samples of trial 1 come from selections obtained in the cooperative breeding program carried out by the University of Cordoba and the Institute of Agricultural and Fishery Research and Training (IFAPA) and thanks are due to O. Arquero, N. Serrano and F. Rodríguez for providing samples of trial 2. The authors also thank Raul De la Rosa for critical review of the manuscript.

REFERENCES

Armenta S, Moros J, Garrigues S, De La Guardia, M. 2010. The Use of Near-Infrared Spectrometry in the Olive Oil Industry. *Crit. Rev. Food Sci. Nutr.* **50**, 567-582.

Beltrán G, Aguilera MP, Del Río C, Sánchez S, Martínez L. 2005. Influence of fruit ripening process on the natural antioxidant content of Hojiblanca virgin olive oils. *Food Chem.* **89**, 207-215.

Beltrán G, Del Río C, Sánchez S, Martínez L. 2004. Seasonal changes in olive fruit characteristics and oil accumulation during ripening process. *J. Sci. Food Agric.* **84**, 1783-1790.

Bessho H, Kudo K, Masuda T, Nakamoto Y, Fujisawa H. 2007. A portable non-destructive quality meter for undestanding fruit soluble solids in apple canopies. *Acta Hort.* **732**, 593-597.

Camps C, Christen D. 2009. Non-destructive assessment of apricot fruit quality by portable visible-near infrared spectroscopy. *LWT-Food Sci. Technol.* **42**, 1125-1131.

Cayuela JA, García JM, Caliani N. 2009. NIR prediction of fruit moisture, free acidity and oil content in intact olives. *Grasas y Aceites* **60**, 194-202.

Cayuela JA, Pérez-Camino MC. 2010. Prediction of quality of intact olives by near infrared spectroscopy. *Eur. J. Lipid Sci. Technol.* **112**, 1209-1217.

Cherubini C, Migliorini M, Mugelli M, Viti P, Berti A, Cini E, Zanoni B. 2009. Towards a technological ripening index for olive oil fruits. *J. Sci. Food Agr.* **89**, 671-682.

Frías L, García-Ortiz A, Hermoso M, Jiménez A, Llavero Del Pozo MP, Morales J, Ruano T, Uceda M. 1991. *Analistas de laboratorio de almazara*. Junta de Andalucía, Sevilla, Spain.

García JM, Seller S, Pérez-Camino MC. 1996. Influence of fruit ripening on olive oil quality. *J. Agric. Food Chem.* **44**, 3516-3520.

Golding JB, Satyan S, Liebenberg C, Walsh K, McGlasson WB. 2006. Application of Portable NIR for Measuring Soluble Solids Concentrations in Peaches. *Acta Hort.* **713**, 461-464.

Gómez-Rico A, Salvador MD, La Greca M, Fregapane G. 2006. Phenolic and volatile compounds of extra

virgin olive oil (*Olea europaea* L. cv. Cornicabra) with regard to fruit ripening and irrigation management. *J. Agr. Food Chem.* **54**, 7130-7136.
Kusumiyati A, Akinaga T, Tanaka M, Kawasaki S. 2008. On-tree and after-harvesting evaluation of firmness, color and lycopene content of tomato fruit using portable NIR spectroscopy. *J. Food. Agr. Environ.* **6**, 327-332.
Lavee S, Wodner M. 2004. The effect of yield, harvest time and fruit size on the oil content in fruits of irrigated olive trees (*Olea europaea*), cvs. Barnea and Manzanillo. *Scientia Hort.* **99**, 267-277.
León L, Rallo L, Garrido A. 2003. Análisis de aceituna intacta mediante espectroscopia en el infrarrojo cercano (NIRS): una herramienta de utilidad en programas de mejora de olivo. *Grasas y Aceites* **54**, 41-47.
León L, Garrido-Varo A, Downey G. 2004. Parent and harvest year effects on near-infrared reflectance spectroscopic analysis of olive (*Olea europaea* L.) fruit traits. *J. Agr. Food Chem.* **52**, 4957-4962.
Mailer RJ, Ayton J, Conlan D. 2007. Influence of harvest timing on olive (*Olea europaea*) oil accumulation and fruit characteristics under Australian conditions. *J. Food. Agr. Environ.* **5**, 58-63.
Mickelbart MV, James D. 2003. Development of a dry matter maturity index for olive (*Olea europaea*). *N. Z. J. Crop Hort. Sci.* **31**, 269-276.
Nicolaï BM, Beullens K, Bobelyn E, Peirs A, Saeys W, Theron KI, Lammertyn J. 2007. Nondestructive measurement of fruit and vegetable quality by means of NIR spectroscopy: A review. *Postharvest Biol. Technol.* **46**, 99-118.
Peano C, Reita G, Chiabrando V. 2006. Firmness and Soluble Solids Assessment of Nectarines by NIRs Spectroscopy. *Acta Hort.* **713**: 465-470.
Peirs A, Lammertyn J, Ooms K, Nicolai BM. 2001. Prediction of the optimal picking date of different apple cultivars by means of VIS/NIR-spectroscopy. *Postharvest Biol. Technol.* **21**, 189-199.
Perez-Marin D, Sanchez MT, Paz P, Soriano MA, Guerrero JE, Garrido-Varo A. 2009. Non-destructive determination of quality parameters in nectarines during on-tree ripening and postharvest storage. *Postharvest Biol. Technol.* **52**, 180-188.
Shenk JS, Westerhaus MO. 1995. *Analysis of Agricultural and Food Products by Near Infrared Reflectance Spectroscopy*. Monograph. NIRSystems, Inc.: Silver Spring, Md.
Shenk JS, Workman JJ, Westerhaus M O. 2001. Application of NIR spectroscopy to agricultural products: In: Burns DA, Ciurczak EW (Eds.) *Handbook of Near-Infrared Analysis*. Marcel Dekker, New York, 419-474.
Williams PC, Sobering D. 1996. How do we do it: a brief summary of the methods we use in developing near infrared calibrations en Near Infrared Spectroscopy, in Davies AMC, Williams PC (Eds.) *Near Infrared Spectroscopy, The Future Waves: Proceedings of the 7th International Conference*. NIR Publications, Chichester, UK, 185-188.
Wodner M, Lavee S, Epstein E. 1988. Identification and Seasonal Changes of Glucose, Fructose and Mannitol in Relation to Oil Accumulation during Fruit Development in *Olea europaea* (L.). *Scientia Hort.* **36**, 47-54.
Zude M, Pflanz M, Kaprielian C, Aivazian BL. 2008. NIRS as a tool for precision horticulture in the citrus industry. *Biosystems Engineering* **99**, 455-459.

Recibido: 20/08/10
Aceptado: 09/11/10