

## **Comparison and Application of Near-Infrared (NIR) and Mid-Infrared (MIR) Spectroscopy for Determination of Quality Parameters in Walnut Samples**

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**ABSTRACT:** Walnut composition is directly related to maintenance of quality. Chemical analyses have been determined using traditional and laborious methods, which are time-consuming and generate chemical waste. This justifies the development of fast and accurate alternative methodologies to control the composition. Near-infrared (NIR) and mid-infrared (MIR) spectroscopy techniques associated with chemometric tools have been applied in the development of several analytical methodologies for agricultural products. The aim of this study is to develop and compare these two spectroscopic techniques to determine the parameters of quality, such as moisture, protein, lipid, mineral composition and fatty acid which is grown in Iran, totally 66 samples. Proteins and fats accounted for more than 70% of the walnut kernel weight. Among other healthful properties, consumption of all the studied cultivars would be potentially beneficial to health. It was used near-infrared and mid-infrared spectroscopy associated with multivariate calibration methods based on partial least squares (PLS) algorithm. The determination coefficient ( $R^2$ ) for moisture, protein, lipid content and fatty acid were 0.78, 0.76, 0.85 and 0.87 for NIR and 0.66, 0.91, 0.92 and 0.62 for MIR, respectively, having an RMSECV (root mean square error of cross-validation) < 2.09%. The results show that both infrared (NIR and MIR) techniques have predictive abilities.

**Key words:** Walnut, NIR spectroscopy, MIR spectroscopy, PLS algorithm

### **INTRODUCTION**

Persian walnuts are thought to have originated in present day Iran and Iraq – once part of ancient Persia. The Persian walnut is a medium-sized tree growing to a mature height of approximately 8 m and spread of 6 m. It is also known by several other names including the 'Carpathian' walnut, English walnut, or California walnut. Persian walnut, *Juglans regia* L., is grown as an economically valuable crop in a number of semi-arid and temperate regions worldwide.

Nuts are rich sources of polyphenols, polyunsaturated fatty acids (PUFA) [mainly the essential fatty acids linoleic (n<sub>6</sub>) and α-linolenic (n<sub>3</sub>)], a high n<sub>6</sub> to n<sub>3</sub> ratio, proteins unusually rich in essential amino acids (and thus with a high nutritive value), minerals (magnesium, potassium, calcium, etc.), fiber, etc. (Yang et al., 2009). Trends in analytical chemistry are towards simple and less

time consuming analytical methods. Several advances in new technologies, automation, development of qualitative and screening methodologies, and the increasing use of chemometrics are being achieved. Spectroscopy techniques are well-established techniques with a minimum sample preparation for determining the chemical components of foods (Chen et al. 2013). Infrared spectroscopy is a technique that has been proposed as an excellent alternative to traditional methods due to its multiple characteristics such as rapidity, simplicity, cost effectiveness, potential for routine analysis if proper calibration and validation is developed, and non-requirement of special skilled operator among others. Identification of compounds by infrared spectroscopy is based on the property of molecules to absorb the infrared light and experience a wide variety of vibrational motions

characteristic of their composition. The MIR region (4000–400  $\text{cm}^{-1}$ ) is a very robust and reproducible region of the electromagnetic spectrum in which very small differences in composition of samples can be measured reliably (Subramanian et al. 2011). MIR spectra provide information arising from fundamental molecular vibrational frequencies, while NIR spectra contain information arising from overtones and combinations of such vibrations, making them more difficult to interpret (Reid et al., 2005). MIR and NIR have been used in authentication procedures, in order to assess if a product strictly conforms to description provided by its label and complies with legislation (Sinelli et al. 2010). Chemometric statistical techniques are useful to apply in multidimensional data sets to reduce the original space into a few components keeping the most of the relevant information of the original matrix.

Common factor analysis methods used are partial least squares regression (PLSR) and principal component regression (PCR) that reduce the calibration spectral intensity data at many frequencies to a relatively small number of intensities in a transformed full-spectrum coordinate system (Haaland and Thomas, 1988). PLSR has been particularly successful in developing multivariate calibration models for infrared spectroscopy by reducing the impact of irrelevant spectral-variations in the calibration model (Bjorsvik and Martens, 1992). This capability provides a more information-rich data set of reduced dimensionality and eliminates data noise which results in more accurate and reproducible calibration models.

The aim of this study is to investigate the potential of the use of two infrared spectral regions (MIR and NIR), in association with chemometric techniques, for characterize the Iranian walnut from a nutritive point of view.

## **MATERIALS and METHODS**

### **Sample**

Walnut samples come from an orchards located in a west of Iran (Toyserkan, Hamadan). This cultivar was harvested in 2013 from October to November. The nuts were manually cracked and shelled, packaged in plastic bags and transported to the laboratory and stored at 4-8C in the dark until

analysis. The walnuts were finely chopped and ground in an appliance.

### **Reference method**

Analyses of moisture, total fat, protein contents were carried out according to AOAC Official Methods (AOAC, 1981). The carbohydrate content was estimated by difference of the other compounds using the following formula: carbohydrate content =  $100\% - (\% \text{ moisture} + \% \text{ fat} + \% \text{ protein} + \% \text{ ash})$ . The analysis was performed in quadruplicate and results are expressed as percentage.

Water content was determined by freeze-drying, until a constant weight was reached. Protein content was estimated according to the Kjeldahl method (Bligh and Dyer, 1959). For the estimation of crude fat Bligh and Dyer extraction was used (Wirth et al. 1997). The fatty acid was determined by gas-liquid chromatography (Folch et al. 1957). The samples were homogenised in trichloromethane by means of Ultra-Turrax and the total lipid was extracted as described by Folch. The Fatty Acid Methyl Esters (FAMES) were extracted with 2mL  $\times$  2mL of hexane and 1 $\mu$ L was injected to the gas-chromatograph, in split mode. Fatty acids analysis was carried out on an Agilent gas- fitted to an automatic sampler and FID detector. The relative proportion of each fatty acid in the fatty acids patterns was expressed as percentage of the sum of fatty acids resolved. Two replicate analyses per sample were performed.

### **Near-infrared and Mid-infrared spectroscopy**

The 66 samples of walnut were analyzed by spectroscopy in the mid-infrared and near-infrared region. The equipment used were FT-NIR Spectrum 100N from Perkin Elmer and FT-IR MB-100 from Bomem. The NIR and MIR spectra were collected in the range of 10,000–4000  $\text{cm}^{-1}$ , and 4000 to 700  $\text{cm}^{-1}$  respectively. Spectra were displayed in terms of absorbance and the measure of diffuse reflectance was accomplished using the software Spectrum of NIRA e v. 6.3.1.0132 (Perkin Elmer) or Win Bomem Easy (ABB Bomem). Before scanning each sample, the background spectrum was taken with a clean ATR crystal. Infrared spectra of reference blanks and samples were observed on a personal computer using Win-IR Pro Software 3.0 (Varian Inc., Palo Alto,

CA). The samples were scanned in triplicated and the mean value was used.

### Chemometric analyses

Prior to multivariate analysis, MIR spectra were mathematically transformed by multiplicative scatter correction (MSC) to remove the multiplicative interference of scatter and particle size (Helland et al. 1995). NIR spectra were treated with Normalization and the Savitzky and Golay's second derivative to reduce peak overlap and eliminate baseline shift (Savitzky and Golay, 1964). All spectra were mean-center before the analysis. The transformed spectra were analyzed by Partial Least Square Regression (PLSR) that was cross-validated (leave-one-out) to generate calibration models. It has been reported that the number of samples to develop the calibration model should cover the desired quantitation range for the specific analyst, with a minimum of 30–50 samples depending on the complexity of the problem (Chen et al. 2007). In this study, 46 samples were used to cover the wide range of moisture, ash, carbohydrate, fat, protein contents and fatty acids that have been reported in walnuts, providing robustness to the model.

The quality of the models was checked by calculation of the root mean square error of calibration (RMSEC), root mean square error of cross-validation (RMSECV) and determination coefficient ( $R^2$ ) (Williams and Norris, 2001). Values closer to one for  $R^2$  and low values for RMSE indicate the good performance of the model for the prediction of the quality parameters of walnuts.

## RESULTS and DISCUSSIONS

### Chemical composition

Table 1 and Table 2 show the statistical summary of levels of protein, ash, fat, carbohydrate and fatty acids of walnut samples. Fat was the predominant component, followed by protein and carbohydrates. Proteins and fats accounted for more than 70% of the walnut kernel weight, confirming the high energy value of walnuts. Ash content ranged from 0.98 to 1.21%. Walnut kernels, which represent from 40 to 60% of the walnut weight, contained about 60% oil (although this can vary from 50 to 72%), up to 24%

of proteins (usually 13–17%), 1.5–2% of fiber and 1.7–2% of minerals (Pereira et al., 2008; Rabrenovic et al., 2008).

**Table 1. Proximate composition of walnut cultivar**

	Fat	Moisture	Protein	Ash	Carbohydrate
Mean	61.8	4.12	16.9	1.08	16.1
SD	0.6	0.15	0.23	0.02	0.7

**Table 2. Fatty acids composition of walnuts**

Fatty acids	Mean	SD
C16:0	7.06	0.00
C18:0	2.51	0.00
C18:1cis	16.40	0.01
C18:1trans	0.76	0.00
C18:2(n-6)	60.01	0.11
C18:3(n-3)	13.50	0.01
C20:0	0.09	0.00
C20:1	0.22	0.00
C21:0	0.00	0.00
(n-6)/(n-3)	4.40	0.01
SFA	9.83	0.02
MUFA	16.97	0.12
PUFA	72.88	0.09

### Spectra Analysis

The characteristics of the MIR and NIR spectra for the walnut samples represent functional groups associated mainly to the content of moisture, proteins, fat, carbohydrates and fatty acids. NIR can be highlight the spectral range comprised between 6200 and 5100  $\text{cm}^{-1}$  related to the O-H functional group (1st overtone of the combination mode), (McClure et al. 1996), 5000-5600  $\text{cm}^{-1}$  related to the C-H functional group (1st overtone of  $\text{CH}_3$  and  $\text{CH}=\text{CH}$ , of fatty acids), 4400-4033  $\text{cm}^{-1}$  related to the C-H group and also 5000-4500  $\text{cm}^{-1}$  related to the N-H and C=O stretching, corresponding to proteins (Liu et al. 1994). MIR spectral range from 1200 to 900  $\text{cm}^{-1}$  is known as fingerprint, where most of the fundamental vibrational frequencies are found and containing complex information related to C–O, C–C stretching and C–O–H and C–O–C deformation of carbohydrates. In the MIR region, it was possible to identify the C-O, C-C and C-O-H stretching; moreover, it can be highlight the region between 3040 and 2850  $\text{cm}^{-1}$  related to the C-H groups for asymmetrical and

symmetrical stretching's of CH<sub>2</sub> and CH<sub>3</sub>, characterizing fatty acid chains. The small band at 3005 cm<sup>-1</sup> is characteristic of the cis double-bond stretching of unsaturated fats. The strongest absorption of the spectra was at 1745 cm<sup>-1</sup> related to the ester carbonyl (C-O) stretching mode of fatty acids. Some important differences were found such a small band near 1654 cm<sup>-1</sup> that corresponds to the amide I (N-H) stretching vibration of proteins (Lambert et al., 1998) and 1654 cm<sup>-1</sup> related to N-H vibrational stretching, for proteins.

### Regression Analysis

The MIR and NIR techniques allowed the development of calibration models for the quantification of the content of moisture, protein, fat, carbohydrate and ash. Cross-validation (leave one-out) was applied to all models to obtain the number of principal components for each model. PLSR models were developed to determine the different content of samples and to enter relevant chemical information from their spectra by using the loading vectors. Multiplicative scatter correction (MSC) transformation of the MIR spectra removed both additive and multiplicative noise effects in reflectance spectroscopy and provided the best prediction models, while the first and second derivatives accentuated the spectral information for the NIR techniques. The generated models from MSC-corrected MIR and derivative NIR spectral data (Table 3), provided similar performance statistics for contents with standard error of cross validation (SECV) and correlation coefficients (r). According to Table 3, the cross-validation errors were low and the determination coefficient was similar for NIR and MIR. However, the results suggest the use of MIR for prediction of protein and fat and the use of NIR for SFA, MUFA and PUFA.

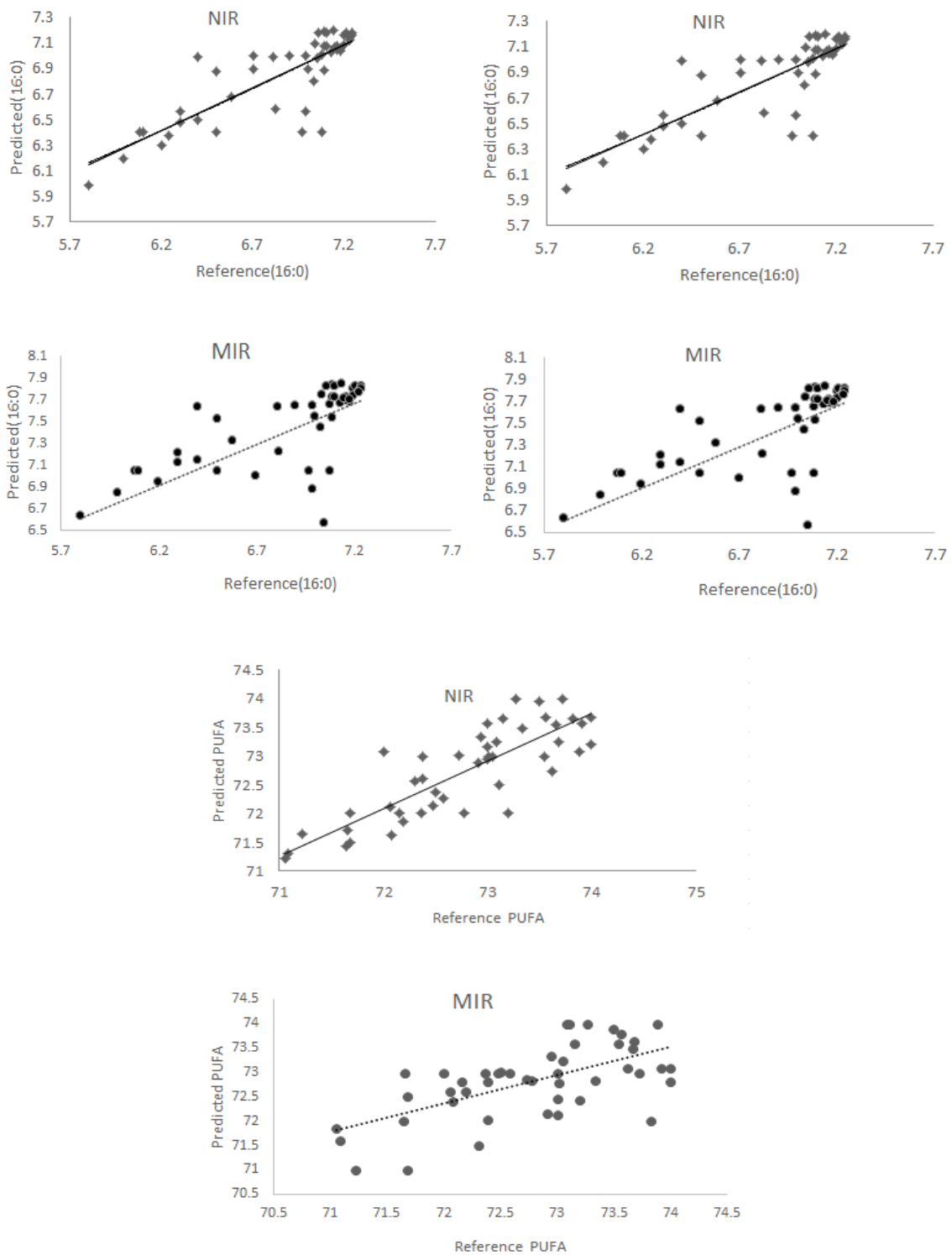
The PLSR regression graphs (Fig. 1) showed good correlation between the infrared estimated concentrations and the reference analysis for both NIR and MIR reflectance spectroscopy.

For the representation of a good relation between the experimentally determined values and the predicted values for the set of 20 validation samples, it was calculated the relative error, which is the ratio between the measured value minus the predicted value divided by the measured value.

**Table 3. Fatty acids composition of walnuts**

Components	NIR		MIR	
	R <sup>2</sup> <sub>cv</sub>	SECV	R <sup>2</sup> <sub>cv</sub>	SECV
Fat	0.85	1.51	0.92	1.85
Moisture	0.78	0.21	0.66	0.27
Protein	0.75	1.81	0.91	1.25
Ash	0.94	1.84	0.92	2.07
Carbohydrates	0.93	1.81	0.91	1.09
C16:0	0.88	2.08	0.91	1.07
C18:0	0.85	2.01	0.90	0.82
C18:1cis	0.83	2.05	0.89	0.64
C18:1trans	0.82	1.95	0.90	0.72
C18:2(n-6)	0.87	2.09	0.85	1.22
C18:3(n-3)	0.86	2.02	0.82	1.51
SFA	0.91	0.98	0.86	0.28
MUFA	0.87	1.50	0.62	0.72
PUFA	0.91	1.68	0.76	1.90

From the results presented here, it was found that both the NIR and MIR techniques can be applied to quality parameters determination in walnuts also concluded that both technique is rapid, accurate and cost effective for analyzing the composition of protein, lipids, moisture, carbohydrates and ash in samples also showed that the two techniques are fast, robust and cost effective.



**Figure 1. Partial least squares regression (PLSR) plots for some contents in walnut analyzed with NIR and MIR spectroscopy techniques.**

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