Near infrared spectrometry (NIRS) prediction of minor components in sunflower seeds

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ABSTRACT

Minor components such as tocopherol and phytosterol present in sunflower seed are becoming interesting to consumers for their health properties, and to the food industry and sunflower breeders to obtain an added value to their products. The near infrared spectrometry (NIRS) technique could be a rapid and accurate method to determine the content of these useful molecules. The total tocopherol content reference values used to make calibration by NIRS varied from 62.8 to 451.9 mg/kg of dry matter (DM) and total phytosterol content values ranged from 53.9 to 189.0 mg/100g DM. The calibration equations obtained by NIRS showed a relatively good correlation coefficient between reference values and predicted values $R^2 = 0.58$ for the tocopherol content and of 0.61 for the phytosterol content. These encouraging results showed that NIRS could be employed to estimate minor components such as tocopherols and phytosterol rapidly in sunflower seeds. Nevertheless, further investigations are required to improve calibration equations to permit an accurate selection with this method.

Key words: minor components – near-infrared spectrometry – oil – phytosterols – sunflower seeds - tocopherols.

INTRODUCTION

Sunflower contains minor components with interesting properties for human health. Tocopherols and specially α-tocopherol (the main homologue present in sunflower oil) are good antioxidants, and they protect against some cancers and reduce cardiovascular disease problems (Bramley et al., 2000; Niki, 2004). Furthermore, sunflower contains phytosterols which reduce cholesterol levels in blood (Patel and Thompson, 2006). The concept of "food-medicine" or healthy foods is starting to be introduced to consumer philosophy. The food industry is looking for these compounds in current food products and seed producers are becoming interested in this added value for their seeds. Unfortunately, the available methods of analysis of these compounds takes a long time and are expensive, requiring a specialised person to perform it. Therefore, it has become necessary to develop new techniques for the analyses. Near infrared spectrometry (NIRS) is nowadays used by food industry and breeders to determine multiple parameters such as moisture, proteins and oil content or fatty acid composition in a large variety of matrices (Pérez-Vich et al. 1998; Velasco and Becker, 1998; Biskupek-Korel and Moschner, 2007). Few studies have focused their interest on the analysis of minor component by NIRS (González-Martín et al., 2006; Ayerdi Gotor et al., 2007). The objective of this work was to improve the values in our previous work (Ayerdi Gotor et al., 2007) especially in the prediction of total phytosterol content in sunflower seeds.

MATERIALS AND METHODS

Plant material
From a collection of nearly 2000 sunflower mature seeds from 4 growing seasons between 2003 and 2006 in different places all over France and Chile, 600 samples were selected as having the greatest variability for the parameters investigated, and the highest analytical accuracy and repeatability. These samples were used to elaborate the NIRS calibration. Each sample used for NIR spectrometry analysis had at least two replicates of tocopherols and phytosterols determined by classical methods (HPLC and GC respectively). The mean of these two replicates was considered as being the reference value.

NIRS analysis

In this work, 40 g of sunflower seeds per sample were ground in a Knifetec Mill (1975, Foss Tecator, Höganäs, Sweden) three times for 10 s. No sample material adhered to the walls of the mill because it was mixed at certain intervals. A FOSS NIR System 6500 (Foss Analytical, Denmark) was used to collect spectra from the milled sunflower seed samples (around 30 g) using a small round cup with a quartz window. The reflectance values as [log (1/R)] of each sample were measured from 400 to 2500 nm at 2 nm intervals. For each sample, a screening of 32 measures was carried out and compared with the 32 measures of a ceramic reference. For tocopherol prediction, an 860 spectra database was used for the calibration set, and for the phytosterol prediction, a 660 spectra database was used. For the validation set, around 200 samples for the tocopherols analysis and about 260 for the phytosterol content were used

Chemicals

For analysis, hexane, methanol, ethanol, acetone and diethyl ether had an HPLC grade from SDS (France). The trimethyl silyl ether (TMS) derivatives of all sterols were prepared using 1-methyl imidazol and N-methyl-N(trimethylsilyl)-heptafluorobutyramide reagent (Sigma, France). All sterol standards: β-sitosterol, stigmasterol and campesterol and betulin were purchased from Sigma (Paris, France). The four α-, β-, δ- and γ-tocopherol standards (99% minimum purity) were purchased in a Chromadex kit (USA).

Solvent extraction of lipids

The analysis of the total oil content was performed by hexane (n-hexane, Prolabo/Subra, Toulouse, France) extraction using a soxhlet extractor apparatus for a 4 extraction of 15g of the ground seeds (NF EN ISO 659, 1998) or with an accelerated solvent extractor apparatus (ASE 200, Dionex, France) with an isopropanol/hexane mixture (5:95 v/v) during 20min. Then, the solvent was removed from the extracts under low pressure evaporation (Rotavapor, Bioblock Scientific HS 40 HUBER, Heildorph, Germany). Lipid extracts were weighed and conserved at − 18°C.

Tocopherol determination

The complete separation of all native tocopherols was achieved using a high-performance liquid chromatography (HPLC) (SpectraPhysics, Thermo Separation Products, USA) with a normal phase LiChrorosorb Si60 column - 250cm, 4mm, 5μm (CIL Cluzeau, France) (ISO 9936, 1997, Ayerdi Gotor et al., 2006). The mobile phase was a mixture of hexane/isopropanol (99.7:0.3 v/v) at 1mL/min flow rate. One gram of oil sample was diluted in 25 mL of hexane and injected directly into the HPLC. Detection was performed with a fluorescence detector (excitation wavelength = 298 nm and emission wavelength = 344 nm; Waters 2475 multi λ). Tocopherols were identified by comparison of retention times with respective standards. Total tocopherol content was calculated as the sum of α- , β-, γ- and δ-tocopherol contents.

Sterol determination

The total and individual sterol content was analyzed by gas chromatography (GC) after saponification and a preparation with trimethylsylil (TMS) ether derivatives (NF EN ISO 12228, 1999). 1μl of the TMS solutions were injected into a fused silica capillary (ZB-5) column (Phenomenex, Paris, France) in a Fisons gas chromatograph (GC 8000 series MMFC 800 Multi-function controller, Italy) fitted with a flame ionization detector. Sterols were identified using the ratio obtained between betulin (Internal standard, Sigma-Aldrich, France) and sterol standards.

NIRS calibration and validation procedures

Prediction equations were calculated with a modified partial least-squares regression (MPLS) model after 4 outlier elimination passes (WINISI 1.02 - Infrasoft International LLC). With the MPLS regression method, factors are extracted in decreasing order of reliance measured by covariance with the response variable. To prevent overfitting in calibration, the number of factors is optimized by cross-validation in calibration sample. Previous mathematical treatment was applied on each spectrum as described in Ayerdi Gotor, et al. (2007). The equation with the highest coefficient of determination (R^2) and the lowest standard error (SE) in the calibration was used to predict the tocopherol and the phytosterol values of the validation set.

The validation of this NIRS method, for the estimation of tocopherols and phytosterols, was determined by the following parameters: the standard error of calibration (SEC), the multiple coefficient of determination in calibration (RSQ), the standard error of cross-validation (SECV), the multiple coefficient of determination of cross-validation (1–VR) and the standard error of prediction (SEP).

RESULTS

Reference values were calculated for tocopherol and phytosterol content expressed as a function of oil weight but in order to improve NIRS's calibration, data were converted with the oil yield (g/g of dry matter) into other units referring to dry matter (seeds at maturity stage). Data set used for the calibrations and the statistical parameters employed to make calibrations and cross-calibration are shown in Table 1.

Total tocopherol content represented the sum of the four homologues α, β, γ and δ-tocopherol present in the sunflower oil. Total phytosterol content represented the sum of seven sterols: Campesterol, Stigmasterol, Δ7- campesterol, β-sitosterol, Δ5-avenasterol, Δ7-stigmasterol and Δ7-avenasterol.

Table 1. Data set samples used to make calibration equations and statistical results. The standard error of calibration (SEC), the multiple coefficient of determination in calibration (RSQ), the standard error of cross-validation (SECV), the multiple coefficient of determination of cross-validation (1–VR) and the standard error of prediction (SEP).

	Calibration sets				Calibration		Cross-validation	
	SN[1]	Range	Mean	SD[1]	SEC	RSQ	SECV	1- VR
Oil (g/gDM[1])	513	16.4 – 54.4	36.38	8.57	2.41	0.92	2,57	0.91
Total tocopherol (mg/Kg DM)	511	62.8 – 451.9	191.90	70.14	41,93	0.64	45.73	0.58
Total phytosterol (mg/100gDM)	489	53.9 – 189.0	117.81	26.89	15.72	0.66	17.19	0.61

[1]DM: Dry matter ; SN: Sample number ; SD: Standard deviation.

The high correlation level of oil content (g/g DM) between the reference and predicted values confirmed that the change in units would not affect the estimation of minor components by NIRS, because it was higher than 0.9 (Fig. 1 A). The calibration for the total phytosterol content (mg/100g DM) (Fig. 1 B) showed that samples were uniformly distributed. The correlation between reference and predicted values for the calibration set of phytosterol was 1–VR = 0.61, better results than those obtained in the previous work (1–VR = 0.27) (Ayerdi Gotor et al., 2007). For the total tocopherol content (mg/kg DM) the results found were not better than those in the previous work.
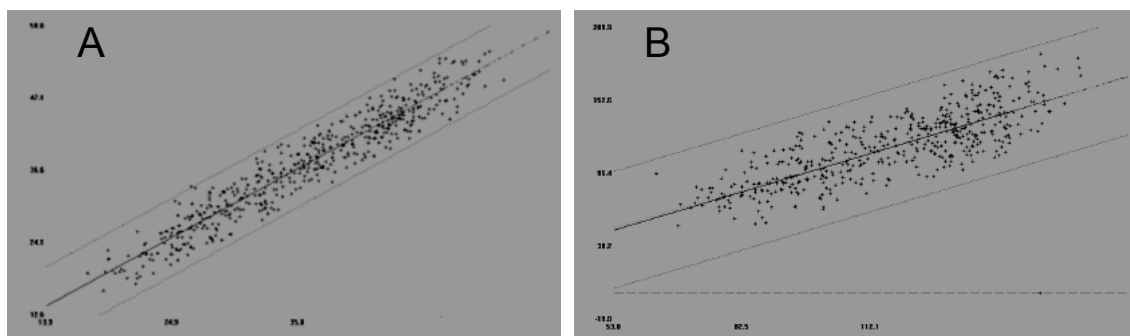

Fig. 1. Reference values *vs*. predicted values of: A- Oil (g/gDM); B- Total phytosterol content (mg/100 g DM) for the calibration group of samples.

The comparison of the two total phytosterol populations of reference values and predicted values (Fig. 2) showed that the reference values were a normal population, contrary to the predicted values that showed clearly two different populations, a fact that could explain the problems experienced for obtaining an accurate equation for this parameter with the modified partial least-square mathematical treatment.
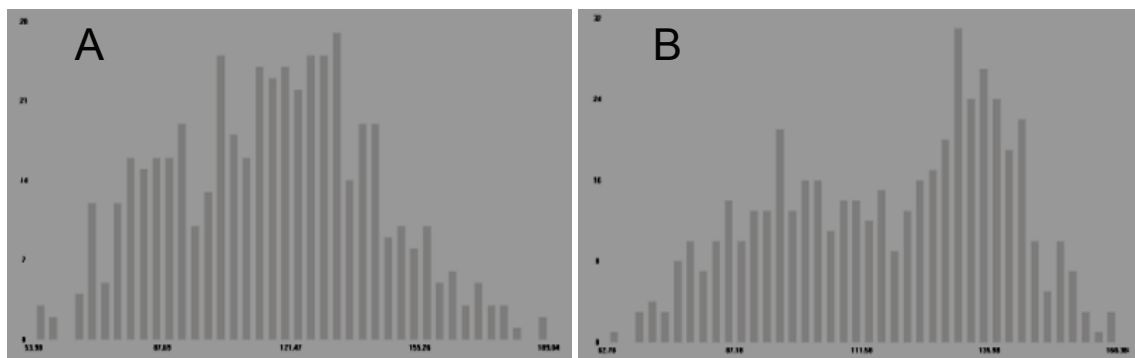
Fig. 2. Distribution of Total phytosterol population (mg/100g DM) and the sample number from each group of: A– Reference values used as calibration set; B– Predicted values for the calibration set.

The ratio SD/SECV for oil content was 3:3, for the total tocopherol content 4:1 and for the total phytosterol content 1:6. Values over 3 are considered as being good for a NIRS calibration for agricultural raw materials in the literature (Moschner and Biskupek-Korell, 2006). But, taking into account that tocopherols and sterols in sunflower represented less than 1% of the total dry matter, these values are promising.
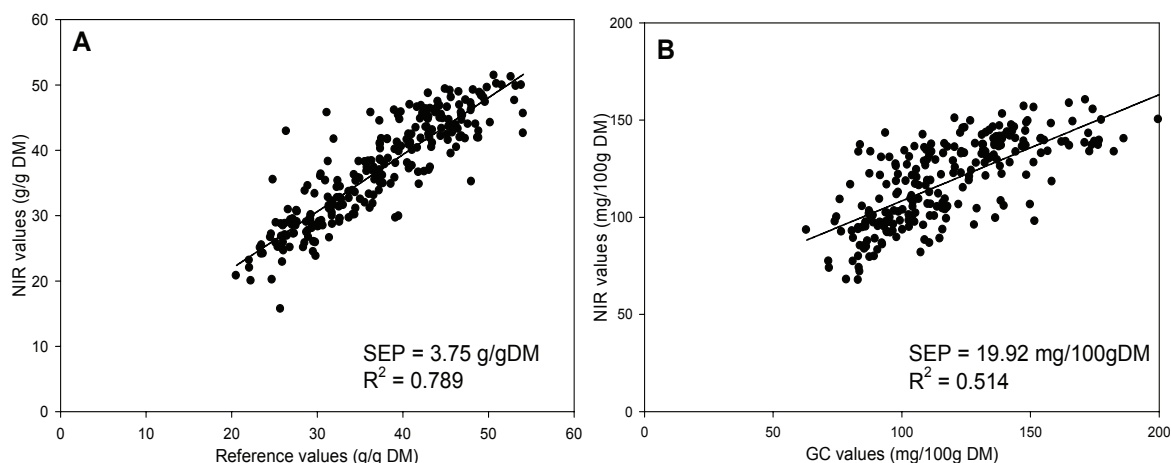

Fig. 3. Scatter plots of reference values vs NIRS predicted values for: A– Oil (g/g DM); B– Total phytosterol content (mg/100g DM).

The validation of the prediction equations was made with 260 independent samples. The comparison of the reference values with the predicted values was performed with these equations (Fig. 3). The SEP values are similar to the SECV values obtained in the cross-validation (Table 1), R^2 values were lower than the 1–VR values of cross-validation. The lower R^2 values for the oil content could be explained by the fact that inside the selected samples there were some parental lines with poor oil extraction results that could affect the validation, but that they were necessary to the calibration of tocopherols and phytosterol.

DISCUSSION

The analysis of minor components by near infrared spectrometry is starting to be used because of its potential: multi-parameter analysis, rapidity and low cost. But the establishment of this methodology requires accurate measurements to obtain reference values and powerful mathematical treatments to calculate prediction equations.

The selection of total tocopherol and phytosterol content based on NIR spectra for plant breeding or food industry allotment could be possible by this method. But better results could be obtained with a selection of a better mathematical treatment to generate equations.

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