QUANTIFICATION OF SUNFLOWER MINOR COMPONENTS BY NEAR INFRARED SPECTROMETRY (NIR)

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ABSTRACT

Both plant breeding programs and food industry need to develop rapid and low cost methods to characterize sunflower prior to allotment. Near infrared spectrometry (NIRS) is widely used to classify sunflower lots according to fatty acid content. This study proposes an extension of NIRS potential use concerning sunflower by tocopherol and phytosterol content quantifications. Approximately 800 samples of grinded sunflower kernels were scanned by NIRS at 2 nm intervals from 400 to 2500 nm. For each sample standard measurements of tocopherol and phytosterol contents were performed. Total tocopherol content was obtained by High Performance Liquid Chromatography (HPLC) coupled with a fluorescence detector while total phytosterol content was assessed by gas chromatography (GC). For tocopherol, the calibration data set ranged from 190 to 1280 mg·kg^{-1} oil (mean value around 615 ± 165 mg·kg^{-1} oil) whereas for phytosterol content, the calibration data set ranged from 125 to 765 mg·100 g^{-1} oil (mean value of 334 ± 75 mg·100 g^{-1} oil). The NIRS calibration showed a relative good correlation (R^2 = 0.62) between predicted by NIRS and real values for total tocopherol content but a poor correlation for total phytosterol content (R^2 = 0.21). These results indicate that NIR spectrometry could be usefull to classify high and low tocopherol containing lots despite possible improvement of calibration by inclusion of a larger number of data in tocopherols NIRS calibration. In contrast, the determination of phytosterol content by NIRS needs more investigations. In this study, only one mathematical calibration was tested and other mathematical methods are under investigations.

INTRODUCTION

Sunflower is an important oilseed crop around the word. The most common sunflower oil use concerns the edible oil industry (e.g. salad oil, margarine…). But recently, the high oleic type of sunflower is considered as a suitable material for non food applications such as lubricants, biocarburants (Ballerini, 2006, De Caro and Cecutti, 2005). In addition, classic or oleic sunflower seeds are rich in micronutrients such as tocopherols and phytosterols exhibiting different health benefits (cardiovasculars diseases, ageing disorders…). Tocopherols (Vitamin E) are fat-soluble compounds exhibiting

antioxidant properties leading to protection against chronic diseases (Beardsell, et al., 2002, Bramley, et al., 2000). The sunflower tocopherol complex is known to contain predominantly α-tocopherol form represents the vitamin for humans. Furthermore tocopherols are the most important compounds having antioxidant activity *in vivo* by quenching active oxygen and peroxide radicals (Hofius and Sonnewald, 2003). On the other hand, phytosterols (plant sterols) have a chemical structure similar to human cholesterol (Dutta and Normen, 1998). According to their particular amphiphyl properties, phytosterols reduce the absorption of dietary cholesterol (Normen, et al., 2000, Trautwein, et al., 2003). Morever phytosterols, especially β-sitosterol, exhibit other beneficial properties such as anti-inflammatory and antitumor activity (Ling and Jones, 1995).

Thus, breeding sunflower programs are not based only on the fatty acid profile of hybrids but also start to include other selection characteristics like minor components (tocopherols and phytosterols). To include such new characteristics breeders need larger analytical input. Nowadays, tocopherol content is mainly determined by high performance liquid chromatography (HPLC) whereas phytosterol content is determined by gas chromatography (GC). These two analytical methods are long, expensive, using hazardous chemicals and requiring high qualification. Therefore these methods are poorly adapted to industrial uses which require a rapid and a low cost method to make easier the screening of vegetal material. Near-infrared reflectance spectrometry (NIRS) is a rapid, low cost and simple analytical tool. Indeed, NIRS is the most common tool used in seed breeding programs, to determine fatty acid composition in sunflower seeds (Moschner and Biskupek-Korel, 2006, Pérez-Vich, et al., 1998). This simple analytical tool allows breeders to accelerate selection programs in order to obtain varieties with a high oleic acid.

Taken into account the increasing interest of breeding program concerning minor components of sunflower oil as breeding characteristics, the aim of this study was to develop a NIRS calibration to predict tocopherol and phytosterol contents in milled sunflower seeds in order to make a rapid selection between high or low minor component contents.

MATERIALS AND METHOD

Samples

A collection of approximately 800 sunflower seeds samples recovered from several cultivars (breeding lines and hybrids) over three growing years (2003, 2004 and 2005) and from different planting locations (all over France and Chili) were used to establish the calibration. For each sample used in NIRS procedure at least two replicate of tocopherols and phytosterols determination were performed by classical methods (HPLC, GC respectively). The mean of these two replicates was considered as reference value.

Solvent extraction of lipids

The analysis of the total oil content was performed by hexane extraction using a soxhlet extractor apparatus. Fifty grams of achenes were grounded and introduced in cartridges. The grounded achenes were submitted to hexane extraction for 4 hours. Then, the solvent was removed from the extracts under low pressure evaporation. 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 - 250 cm × 4 mm × 5µm (CIL Cluzeau, France). 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 (Tocopherol Kit; ChromaDex, USA). 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 a saponification and a preparation with trimethylsylil (TMS) esther derivatives. 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 analysis

A FOSS NIR System 6500 (Foss Analytical, Denmark) was used to collect spectra from the milled sunflower seed samples (around 30 g) using 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, the 687 spectra database was used for the calibration set and for phytosterol prediction, the 281 spectra database was used.

Statistical analysis

Prediction equations were calculated with a modified partial least-squares regression (MPLS) model after 4 outliers elimination passes (WINISI 1.02 - Infrasoft International LLC). Previous mathematical treatment was applied on each spectra: a standard normal variate and detrend (SNV/detrend) scatter correction, a first derivative transformation, a gap as well as a smoothing of four data points. The equation with the highest coefficient of determination (R^2) and the lowest standard error (SE) in the calibration was use to predict the tocopherol and the phytosterol values of the validation set.

RESULTS AND DISCUSSION

Determination of reference values

Tocopherol content determination in sunflower oil by HPLC showed that α-tocopherol was the major tocopherol and represented more than 95% of the total tocopherol content which corresponded to the sum of the 4 isomers α, β, γ and traces of δ-tocopherol. The total tocopherol content observed in this study was ranged from 190 g/kg oil to 1187.9 g/kg oil and this range was similar than the values reported by Nolasco *et al* (2004).

The most abundant sterol content observed in all cultivars was β-sitosterol (around 60%), followed by campesterol and stigmasterol. Other phytosterols like Δ7-campesterol, Δ5-avenasterol, Δ7-stigmasterol and Δ7-avenasterol were detected at very low levels. These seven sterols represented the total phytosterol content in sunflower oil tested. The total phytosterol content in this study was ranged between 125 mg/100 g oil and 765 mg/100 g oil. These results were similar than the values reported by Vlahakis and Hazebroek (2000).

Calibration dataset presented large variations for the alpha and total tocopherol content as shown in Table 1 whereas these variations were less important for the total phytosterols content in sunflower oil sample tested. Such discrepancies could be explained by the number of samples (lower numbered than for tocopherols) and by the lower variability due to experiment or genotype concerning phytosterols (Vlahakis and

Hazebroek, 2000) (Ayerdi Gotor, et al., 2006). Moreover, a normal distribution of tocopherol and phytosterol content values was observed (Figure 1).

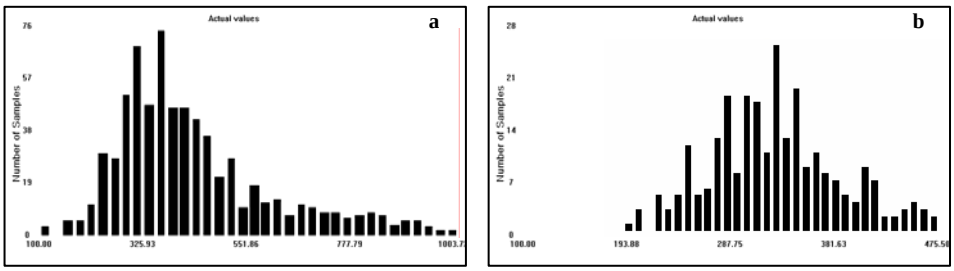

Figure 1. Histograms of (Tasinato, et al.) total tocopherol content (mg/kg oil) and (b) total phytosterol (mg/100 g oil) used for the calibration equation

NIRS calibration

The reference values of all samples were used to make mathematical treatments in order to obtain the prediction equation with a modified PLS. The equations were used to calculate predicted values. The predictive values were given in Table 1 and the calibration graphics were shown in Figure 2.

Table 1. Formation of the calibration sets for the total tocopherol, α-tocopherol and total phytosterol content and calibration and cross calibration results for tocopherols and phytosterols prediction. (RSQ : Coefficient of linear regression ; SECV : Standard error of cross validation ; 1-VR : Coefficient of determination in cross validation (R^2) ; SEC : Standard error of calibration).

	Tocopherol (mg·kg^{-1} oil)		**Total Phytosterol (mg·100g^{-1} oil)**
	alpha–T	**Total–T**	
Number of samples	687	687	282
Range	178.3 – 957.7	197.1 – 1003.7	179.8 – 475.5
Mean	489.70	462.10	318.46
SD	151.84	143.56	80.85
RSQ	0.735	0.727	0.356
SECV	91.150	94.182	54.823
1- VR (R^2)	0.620	0.619	0.207
SEC	74.658	80.093	48.964

The correlations between the predicted values and the reference values show that alpha and total tocopherol were better correlated (R^2 = 0.62) than phytosterols (R^2 =0.21). For the tocopherol the repartition of samples was homogeneous, on the contrary for the total phytosterol content all samples were localized around the mean value.

The ratio (SD/SECV) for all minor components was ranged from 1.5 to 1.7. However these values are quite different from 3, considered as the minimal value for this ratio in good NIRS calibration for agricultural raw materials in the literature (Moschner and Biskupek-Korell, 2006). Nevertheless, considering that the total tocopherol content or sterol content in sunflower seed is inferior to 1% of the composition. These results are promising for possible improvement of the procedure, these results encourage further studies.

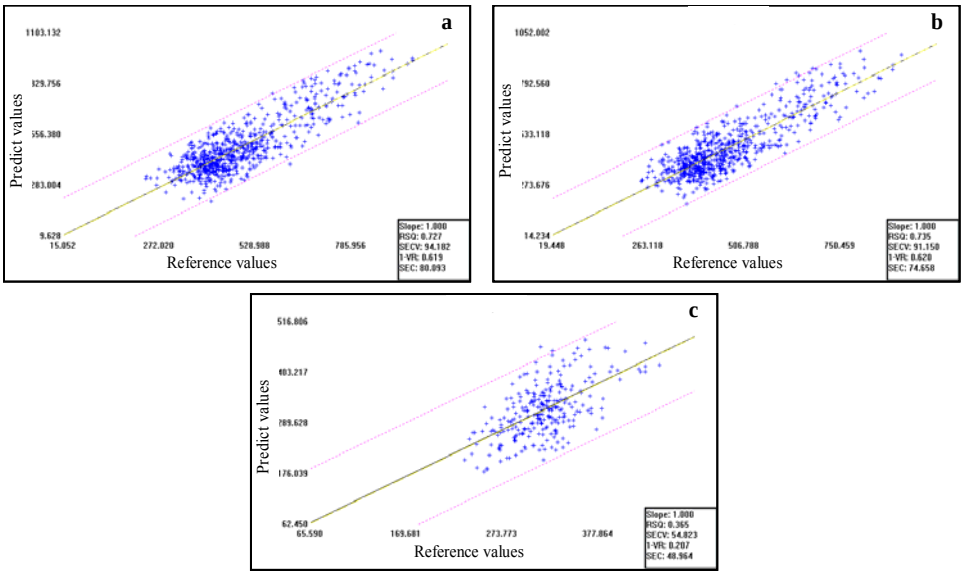

Figure 2. Reference vs. predicted (**a**) Total tocopherol (mg/kg oil) (sum of the four isomers α, β, γ and δ) ; (**b**) α-tocopherol (mg/kg oil) and (**c**) Total phytosterols (mg/100g oil) values for the calibration set.

Indeed the introduction of selected samples prior to enlarge variability of the content in tocopherol we propose to establish a larger calibration dataset and to use appropriate mathematical treatment in order to improve these results.

In conclusion, a selection for the tocopherol content based on NIRS information could be possible for plant breeding or allotment whereas it seems more difficult to determinate by this method the phytosterol content. Further investigations will be undertaken to improve the two prediction models, based on the above propositions.

ACKNOWLEGMENTS

The authors thank Caussade semences, Maïsadour semences, Monsanto Dekalb, RAGT-R2N, Soltis, Syngenta Seeds for field experiments and sunflower plant material providing. Financial support was provided by ASEDIS-SO, ONIDOL (French Oil seeds organization) and Midi-Pyrénées Region. We also thank Cognis-France for their technical support.

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