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Determination of oleic acid in sunflower seeds by infrared spectroscopy and multivariate calibration method

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ABSTRACT

A method for the determination of oleic acid in sunflower seeds is proposed. One hundred samples of sunflower seeds were analyzed by near-infrared diffuse reflectance spectroscopy (NIRDRS). The direct measures were realized in ground and sifted seeds. The PLS multivariate calibration model was obtained using first derivate absorbance values as response matrix, while the oleic acid concentration matrix was obtained analyzing the seed samples by gas chromatography with a flame ionization detector (GC-FID). The NIRDRS-PLS model was validated externally using unknown samples of sunflower seeds. The accuracy and precision of the method was evaluated using GC as reference method. The following figures of merit (FOM) were obtained: LOD = 3.4% (w/w); LOQ = 11.3% (w/w); SEN = 8×10^{-5}; SEL = 0.15; analytical sensibility (γ) = 1.5 and linear range (LR) = 18.1–89.2% (w/w). This method is useful for the fast determination of oleic acid in sunflower seeds and for quality control of raw materials.

1. Introduction

In Argentina, sunflower crops represent an important agricultural, industrial and commercial activity due to the high production of this grain. In the year 2007, the total world production of sunflower grains was 2.7×10^7 ton; Argentina occupied the 3rd place in the production of sunflower seeds, with 3.5×10^6 ton, after Russia and Ukraine, with a sunflower cultivated area for the first country of 2.44×10^6 ha [1].

Since 1975, when the crop was introduced in Argentina, varieties of sunflower have been cultivated, with high content of oleic acid, higher than 77% of the total content of fatty acids, higher than the traditional sunflower whose range of oleic acid is 15–25%. Due to its high content of oleic acid, the high oleic sunflower varieties are very much requested by edible oil factories to obtain oils with high oleic acid concentration, which have a higher commercial price in comparison with the rest of the common edible oils. For this reason, the oleic acid analysis is crucial to define the final price of the raw material.

On the other hand, the production of biodiesel around the world involves the use of vegetable and animal oils, including traditional and high oleic sunflower varieties [2]. In previous works, the use of sunflower oil in biodiesel production was reported, including the production of biodiesel at room temperature [3], optimization of biodiesel production from vegetable oils [4] and the study of blended vegetable oils for production of biodiesel [5]. Sunflower oil is frequently used for biodiesel production and, as in the case of edible oil factories, the cost of sunflower raw material depends on the content of oleic acid in the seed.

The percentage of oleic acid in seeds is determined by gas chromatography, which is the more classical method for this determination [6]. The technique requires an important time of analysis, including the sample preparation and chromatographic times, which result in a critical aspect in the commercialization of seeds for biodiesel production and edible oil factories. Since the percentage of oleic acid determines the final price of the raw material, it is an important fact to find a single and fast method that may allow analyzing the content of oleic acid by direct measure on seeds, without chemical pretreatment of samples, reducing considerably the analysis time.

Oleic and fatty acids analysis were widely studied in previous works. In this way, a physiological study of the stearic synthesis and oleic acids in developing high-oleic sunflower seeds has been reported by use of TLC and radio-isotope scanning in biological assays [7]. Nevertheless, spectroscopic methods have been the preferred ones for analysis of fatty and organic acids in different kinds of samples. On the other hand, near-infrared diffuse reflectance

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spectroscopy (NIRDRS) has been proved to be a convenient and fast quantitative method for complex samples and its properties have been improved by means of the multivariate calibration methods [8]. Near-infrared reflectance hyperspectral imaging and multivariate calibration methods were used to determine the concentration of oleic acid in corn kernel [9]. An automated method for the determination of total fatty acids and peroxide value by FTIR with spectral reconstitution was reported [10]. In a previous work, the determination of total fatty acids in poultry feed by FTIR and attenuated total reflectance was reported [11]. Also, fatty acids composition in intact seed of perilla was studied using NIRS and PLS [12]. The profile of fatty acids was determined in cotyledon soybean seeds by reflectance NIR and PLS [13]. A study of acidity and peroxide index in edible oil was carried out by near-infrared spectrometry and PLS [14]. The determination of trans isomers of fatty acids was carried out for edible oil by FTIR and spectral reconstitution procedure [15]. The monitoring of oleic, linoleic, un-saturated, mono-unsaturated and poly-unsaturated fatty acids in virgin olive oil and FTIR and PLS was proposed [16]. The determination of oleic, linoleic, total saturated and unsaturated fatty acids was carried out in edible oils samples by IR spectroscopy, second derivate data and multivariate calibration analysis [17]. Also, a work has been reported that uses the second derivate spectra of NIRS and PLS to estimate the content of total free fatty acids in high oleic sunflower seeds, using a titrimetric method as reference [18] and there is a preliminary technical report that uses NIRS and PLS for the estimation of oleic and linoleic acids in sunflower seed using a special device for seeds analysis and absorbance measures without preprocessing [19]. On the other hand, previous works have been reported related to the classification of edible oils by use of multivariate calibration methods to evaluate quality/authenticity [6,20], designation of origin [21] and a study of oxidative index for determination of aging of edible oils by NIRS and chemometric methods [22].

This paper discusses a fast and complete method to quantify a wide range of oleic acid concentrations in all varieties of sunflower seeds, by near-infrared reflectance diffuse spectroscopy (NIRDRS) and data evaluation using the partial least square regression (PLS) multivariate calibration method. In comparison with previous works, the proposed method was of easy application, because it requires a very simple treatment of sunflower seed samples and simple modeling, and also because it uses only 100 variables (first derivate absorbance spectra) and 2 PLS components. Another important contribution of this method is the report of the complete study of analytical properties: accuracy, precision and figures of merit (FOM) of the NIRDRS-PLS method. FOM have not been reported previously for the determination of oleic acid in sunflower seeds by infrared spectroscopy. The reported FOM include limit of detection (LOD), limit of quantification (LOQ), sensibility (SEN), selectivity (SEL) and analytical sensibility (γ) and linear range (LR) [23–24].

2. Experimental

2.1. Instrumental

The chromatographic analysis was carried out with a Varian Gas Chromatograph model GC 3900 (CA, USA) with a Varian flame ionization detector (FID). A capillary column Varian factor FOUR VF-23 (cyanopropyl stationary phase, 30 m, 0.25 mm ID) was used.

Diffuse reflectance infrared measures were taken by a Brimrose NIRS model Luminar (MA, USA) with acoustic–optic tuning filter (AOTF) and a rotator cup as sample cell.

The Unscrambler 6.11 by CAMO-AS (Trondheim, Norway) software was used for the PLS-1 modeling and the figures of merit were calculated using the MVC1 software [25].

2.2. Reagents

A 1000 $\mu g\,mL^{-1}$ of fatty acid Supelco 31 mix FAME (MO, USA) standard solution was used for the chromatographic analysis. Heptane and methanol were provided by Merck (Darmstad, Germany) and potassium hydroxide was provided by Sigma (MO, USA). The N_2 gas carrier and H_2 and air (chromatographic quality) were provided by Air Liquid (Buenos Aires, Argentina).

2.3. Sampling and sample treatment

The varieties of sunflower seeds (from low to high oleic acid content) were obtained from different places of Argentina and harvested from 2007 to 2008. Validation was carried out with 20 samples, different from those used to obtain the model. The criteria for selection of samples for the calibration step covered a wide range of oleic acid concentrations, from low to high oleic sunflower varieties, to improve the predictive ability of the model. For the validation step all samples were selected randomly.

The chromatographic analysis was carried out following the ISO 5590 method for the determination of fatty acids methyl esters by gas chromatography. A portion of 20.00 g of whole seeds was weighted in an OHaus analytical balance (NJ, USA) and pressed in a hydraulic press (Jack, Argentina). A weight of 0.50 g of obtained oil was transferred to a 10 mL volumetric flask, dissolved with 5 mL of n-heptane, added with 0.2 mL of 2 mol L^{-1} potassium hydroxide in methanol, stirred for 20 s and diluted with n-heptane to mark. After phase separation, the supernatant was collected for gas chromatography analysis [26]. This esterification method is recommended due to its low reagent toxicity and cost [27].

For the NIRDRS analysis, whole sunflower seeds, free from dust, samples were ground in a Dalvo grinder, model MCI. The fraction $\leq$2 mm was obtained by passing through a 2 mm sieve and placed in the sample rotator cup.

3. Results and discussion

3.1. Chromatographic analysis

Calibration solutions of oleic acid were prepared by adequate dilutions of 1000 $\mu g\,mL^{-1}$ standard of fatty acids. Five triplicates concentration levels were analyzed, obtaining a calibration fit with an r^2 coefficient of 0.995. Then, the analysis of 100 sunflower samples was carried out to obtain the necessary concentration matrix to build the PLS model for the calibration step. Chromatographic parameters were the following: injection volume: 1 μL; temperature program: 150 °C during 4 min, 3 °C/min to 190 °C, 1 °C/min to 200 °C; final temperature 200 °C during 3 min; detector temperature: 225 °C; injector temperature: 275 °C; gas carrier flow: N_2, 1 mL min^{-1}; split: 1:20; total run time: 30 min and oleic acid time: 12 min.

3.2. Near-infrared diffuse reflectance spectroscopy (NIRDRS)

A portion of the same samples used for chromatographic analysis was analyzed by infrared spectroscopy. A weight of 20 g of whole ground and sifted sample seeds were placed in the rotator cup and the NIRDRS spectra were obtained. The total spectral range went from 1100 to 2200 nm every 2 nm. The sample rotator cup with the samples was irradiated with NIR radiation, and detected by acousto-optic tunable filter (AOTF). The instrumental system is based on the measurement of the relative beam intensities produced when a 45° plane polarized beam of radiation passes through a sample cell and is directed to the entrance window of the AOTF. The AOTF filter uses an anisotropic crystal of TeO_2, producing two planar and orthogonally polarized beams. The presence of an optically active sample

causes the polarization plane to rotate and a consequent difference in the intensities of the AOTF diffracted beams is registered as a function of the optical activity of the sample. Thirty scans were obtained for every standard and sample seeds and the total number of scans collected from every sample was averaged by Brimrose software into a single spectrum, which was used in the response matrix. Measurements were carried out at room temperature.

3.3. PLS model: calibration step

The concentration of oleic acid obtained by the GC method was used as the concentration matrix for the NIRDRS-PLS model. The response matrix was obtained using the first derivate absorbance values from the NIRDRS spectrum (a single averaged spectrum for every sample was used). The first derivate absorbance signal was preferred due to the fact it had yielded better results in the previous multivariate models in comparison with the second derivate and conventional absorbance signal. The range from 1596 to 1794 nm was selected by analysis of the correlation coefficient and important variables as function of wavelength [28]. This selected range produced a response matrix with 100 variables (wavelength) and 100 objects (calibration samples). Fig. 1 shows the first derivate spectral data of 100 calibration samples. With the selected range of wavelength, the final model was built using internal validation (cross-validation method), where the model leaves out one standard of the calibration set every time. Then, the calibration and validation model error was calculated through root mean square of calibration (RMSEC) and root mean square of prediction (RMSEP), finding a minimum value for both cases in the second PLS component: for this component, RMSEC and RMSEP showed a value of 4.65 and 4.72 respectively. The explained variance, obtained in the calibration and validation processes for the NIRDRS-PLS model, were 99.46 and 99.45% respectively, using 2 PLS components, which are in agreement with the minimum value found for the RMSEC and RMSEP. With this 2 PLS components for the NIRDRS-PLS model, the observed-predicted concentrations plot for oleic acid was obtained, showing an r^2 coefficient = 0.995, abscise and slope (mean ± standard deviation): 0.63 ± 1.10 and 0.99 ± 0.02 respectively, which suggest a good fit of the model (ideal values of $r^2 = 1$, abscise = 0 and slope = 1).

3.4. External validation set: accuracy and precision

In order to corroborate the predictive ability of the model, 20 triplicates of the unknown samples were predicted by the NIRDRS-PLS method and the obtained concentrations were compared with gas chromatography analysis. Table 1 shows the mean values with standard deviation of 20 validation samples. The accuracy of the method was evaluated by the elliptical joint confidence region (EJCR) test, which is frequently used to evaluate accuracy of new analytical methods [29,30]. This ellipse must contain values of abscise = 0 and slope = 1, which indicate the absence of systematic errors [31,32]. The ellipse for oleic acid includes these theoretical values (1, 0) at a level of confidence of 95%, which indicates absence of systematic errors of the IR-PLS method in comparison with gas chromatography.

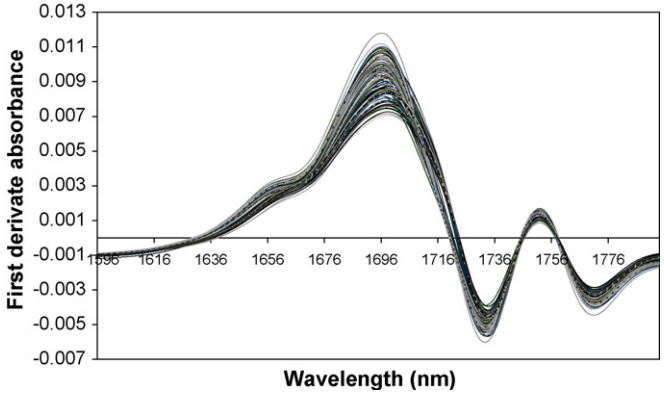

Fig. 1. Spectra data plot. First derivate absorbance – from 1596 to 1794 nm – for 100 calibration samples of sunflower seeds.

Table 1
Results of validation test set.

Sample	GC[a]	NIRDRS-PLS[a]	Sample	GC[a]	NIRDRS-PLS[a]
1	20.5 ± 0.1	21.2 ± 0.3	11	69.7 ± 0.2	69.0 ± 0.7
2	27.2 ± 0.1	27.0 ± 0.4	12	27.5 ± 0.1	28.4 ± 0.3
3	30.2 ± 0.2	29.0 ± 0.5	13	42.3 ± 0.1	44.1 ± 0.4
4	26.9 ± 0.1	25.4 ± 0.4	14	81.9 ± 0.3	82.2 ± 0.9
5	59.8 ± 0.2	55.7 ± 0.6	15	51.3 ± 0.2	49.2 ± 0.5
6	85.5 ± 0.3	86.0 ± 1.0	16	89.2 ± 0.3	90.1 ± 1.1
7	82.7 ± 0.2	85.3 ± 0.6	17	25.5 ± 0.1	24.2 ± 0.3
8	65.8 ± 0.2	63.5 ± 0.7	18	79.6 ± 0.2	75.9 ± 0.7
9	27.5 ± 0.1	28.4 ± 0.3	19	75.7 ± 0.2	78.4 ± 0.6
10	72.9 ± 0.3	76.2 ± 1.0	20	48.5 ± 0.1	46.4 ± 0.4

[a] Concentration of oleic acid (%, w/w) ± standard deviation ($n = 3$).

Precision of the NIRDRS-PLS model was evaluated using the F test, through the analysis of 10 replicates of the same sample, analyzed by both, gas chromatography and the NIRDRS-PLS methods. The critical value is $F = 4.026$ ($n_1 - 1 = 9$ and $n_2 - 1 = 9$). The calculated F value was 3.810, lower than the F critical value, which indicates that the proposed method was precise in comparison to the classical chromatographic method. The percentage of precision obtained for a same day (intra-day precision) was 1.2%, while the precision found for a week (inter-day precision) was 2.0%.

3.5. Figures of merit (FOM)

The method's calculated figures of merit (FOM) were: limit of detection (LOD), limit of quantification (LOQ), sensibility (SEN_k), selectivity (SEL_k) and analytical sensibility (γ_k). SEN_k is described as:

$$SEN_k = \frac{1}{\|\mathbf{b}_k\|}$$

where $\mathbf{b}_k$ is the vector of regression coefficient for the k analytes. LOD is defined as:

$$LOD = 3.3 \|\delta_r\| \|\mathbf{b}_k\|$$

where $\|\delta_r\|$ is a measure of instrumental noise. LOQ is defined as:

$$LOQ = 3LOD$$

SEL_k is defined as:

$$SEL_k = \frac{\|\mathbf{s}_k^*\|}{\|\mathbf{s}_k\|}$$

Table 2
Figures of merit (FOM) calculated for the NIRDRS-PLS method.

Figures of merit	Value
Limit of detection (LOD)	3.4% (w/w)
Limit of quantification (LOQ)	11.3% (w/w)
Sensibility (SEN)	0.00008
Selectivity (SEL)	0.15
Analytical sensibility (γ)	1.5
Linear range	18.1–89.2% (w/w)

Table 3
Comparative table for the determination of oleic acid and other analytes by infrared spectroscopy reported in previous works.

IR method	Sample	Analyte	Reported FOM
NIR-reflact–multiv. cal [9]	Corn kernel	Oleic acid	Non-reported
FTIR–spect. reconst. [10]	Edible oils	Total fatty acids and peroxide value	Non-reported
FTIR-ATR [11]	Poultry feed	Total fatty acids	Non-reported
NIR and mult. cal. [12]	Perilla seed	Palmitic, stearic, oleic, linoleic, linolenic acids	Non-reported
NIR reflectance–multiv cal. [13]	Soybean cotyledons	Palmitic, stearic, oleic, linoleic, linolenic acids	Non-reported
NIR-PLS [14]	Edible oil	Peroxide and acidity index	LOD
FTIR-ATR [15]	Edible oil	Trans fatty acids	Non-reported
FTIR-PLS [16]	Virgin olive oil	Oleic, linoleic, unsaturated, mono-unsaturated, poly-unsaturated fatty acids	LOD, LOQ, SEN, γ and SEL
FTIR-PLS [17]	Edible oil	Oleic, linoleic, unsaturated and saturated fatty acids	Non-reported
NIRS-PLS [18]	Sunflower seed	Total free fatty acids	Non-reported
NIRS-PLS [19]	Sunflower seed	Oleic and linoleic acids	Non-reported

where $\mathbf{s}_k$ is the vector of spectral sensitivities of k components in the pure form, while $\mathbf{s}_k^*$ is the projection onto the net analyte signal space [24]. Finally, analytical sensibility is defined as:

$$\gamma_k = \frac{\mathrm{SEN}_k}{\left\|\delta_r\right\|}$$

The figures of merit for determination of oleic acid in sunflower seeds by the proposed NIDRS-PLS method are shown in Table 2. Table 3 shows a summary of the determination of oleic acid and other analytes using infrared spectroscopy, reported in previous works for edible oil and seed samples.

4. Conclusions

This paper shows the result of a complete research for the determination of oleic acid by direct analysis in sunflower seeds, using near-infrared diffuse reflectance spectroscopy and data evaluation by partial least square multivariate calibration. The proposed method is fast, accurate and precise in comparison to the gas chromatography classical method and figures of merit were presented. Due to the fact that pretreatment of solid samples was minimal, this method can be useful for the fast analysis of oleic acid in sunflower seeds, and also for quality control of raw material in edible and biodiesel oil factories.

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