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Olive oil quantification of edible vegetable oil blends using triacylglycerols chromatographic fingerprints and chemometric tools

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ABSTRACT

The present work studies the effectiveness of the use of triacylglycerols (TAGs) for the quantification of olive oil in blends with vegetable oils. The determinations were obtained using high-performance liquid chromatography (HPLC) coupled to a Charged Aerosol Detector (CAD), in combination with Partial Least Squares (PLS) regression and using interval PLS (iPLS) for variable selection.

Results revealed that PLS models can predict olive oil concentrations with reasonable errors. Variable selection through iPLS did not improve predictions significantly, but revealed the chemical information important in the chromatogram to quantify olive oil in vegetable oil blends.

1. Introduction

Olive oil (OO) is defined as the oil obtained solely from the fruit of the olive tree (*Olea europaea* L.). There are different botanical varieties of olive trees, so olive oils can be obtained as monovarietal oils (arbequina, picual, hojiblanca, etc.) or as a blend of two or more of these varieties. Moreover, the International Olive Council (IOC) recognizes four main categories of olive oil according to the final quality product: extra-virgin, virgin, refined and pomace [1]. Refined olive oil is a tasteless product and, in the market, it is always blended with a low proportion of virgin olive oil. OO consumption per person in the European Union (EU) has increased considerably in recent decades and EU is the biggest olive oil producer in the world [2]. Moreover OO is recognized nowadays as oil with beneficial effects for health (skin, cardiovascular system, cancer, etc.) [3]. Due to these facts, companies have been taking advantage of selling OO blends at the same price as pure OO, obtaining important economic benefits. The adulterants used in blends are the ones with similar physical and chemical properties and usually they are cheaper and easy to obtain. In the case of OO this usually implies the dilution with other inferior quality olive oils or cheaper vegetable oils [4].

It is necessary to be able to verify authenticity of blends with edible oils. When the prepared blend deviates from the mixture proportions given on the product label, it is considered that the oil is adulterated [5]. In the EU, requirements has being established in Regulation (EC) no. 1019/2002, concerning commercialization and labeling of products which contain olive oil. For example, foodstuffs containing olive oil have to specify the percentage of olive oil in the labeling. In addition, if blends of olive and other edible vegetable oils are marketed, the presence of olive oil higher than 50% has to be indicated on the label, but if the percentage is lower than 50% the name of olive oil cannot be used in the label [6].

There is a demand for rapid methods to detect adulteration of OO present in edible oil blends. For this, authentication and/or quantification methods have been developed. Different chemical and physical techniques have been studied to quantify olive oil in blends with other vegetable oils in combination with chemometric tools, for example, Headspace and Mass Spectrometry [7], ^{1}H and ^{31}P Nuclear Magnetic Resonance [8,9], and Fourier Transform-IR, Near IR, Mid-IR and FT-Raman [10–14]. Chromatographic techniques, such as Gas Chromatography (GC) and High Performance Liquid Chromatography (HPLC) are the most frequently used for this pur-

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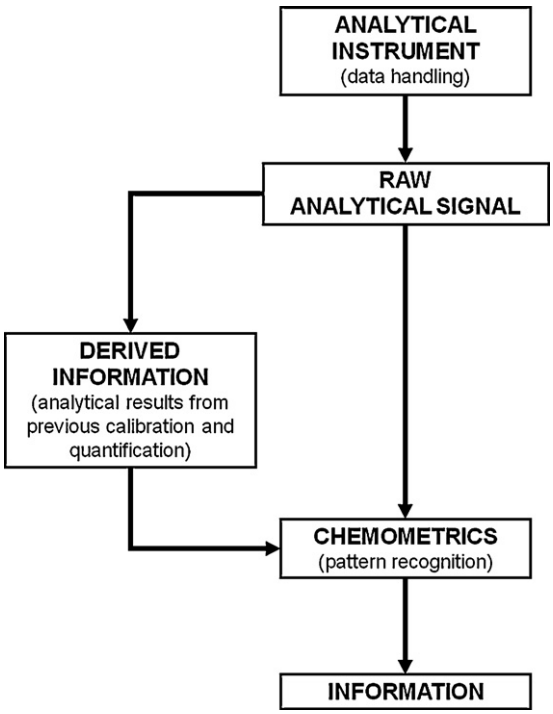

Fig. 1. Application of chemometric techniques on analytical signals.

pose [15–20]. Nevertheless, the official method of the International Olive Council (IOC) is based on the use of the reverse phase-liquid chromatography with a refractive index detector (HPLC-RID), to establish the difference between actual and theoretical content of TAGs with Equivalent Carbon Number 42 (ECN42) [21].

There are a limited number of published studies that take into account all the variability (different categories and varieties of olive oil) that can be found in products containing mixtures of olive oil with edible vegetable oils, since the mixtures present in the products do not always specify the category/variety of olive oil.Analytical signals can typically be analyzed in two different ways: (a) using raw analytical signals, which come directly from the analytical instrument (i.e., an elution profile) as input to a multivariate model; or (b) use derived information such as peak areas (∼concentrations). Such concentrations may be used in a univariate manner one at a time or in a multivariate chemometric modeling using all available information (Fig. 1).

In chromatography, chemometric tools are usually applied to the information derived from the signal profile such as peak heights, areas or parameters as ECN for TAGs analysis. Peak areas or similar measures are used partly for historical reasons and partly because several problems typically prevent straightforward use of raw chromatograms. These problems are mainly caused by baseline drift and peak shifting.

The present work studies the effectiveness of the use of TAGs for the quantification of olive oil in vegetable oil blends, including many combinations of vegetable oils and considering the different categories and varieties of olive oils at several percentages. The analyses were obtained using an HPLC–Charged Aerosol Detector (CAD) method, applying chemometrics tools to full TAG chromatogram profiles. To our knowledge, there is only one study about olive and vegetable oils using CAD [22]. This detector has some advantages, among others, it presents sensitive, wide dynamic range and little variation in response between analytes [23]. A broader discussion of the advantages of this detector can be found in the paper Quantification of Triacylglycerols in Olive Oils using HPLC–CAD [24]

2. Experimental

2.1. Instrumentation

The samples were analyzed by a chromatographic system HP Agilent HPLC 1100 Series system composed of quaternary pump, degasser, automated sampler and 1100 ChemStation software (Santa Clara, CA, USA). A thermostatic column compartment from Eppendorf TC-50 was used. Detection was carried out with a Corona CAD (ESA Biosciences Inc., Chemlsford, MA, USA). Data analysis was performed using multivariate statistical methods by MATLAB® 7.8.0 R2009a (The Maths Inc., Natick, MA, USA) and PLS_Toolbox 5.5 (Eigenvector Research Inc., West Eaglerock Drive, Wenatchee, WA).

2.2. Chemicals, reagents

Acetonitrile HPLC grade were purchased from PANREAC (Barcelona, Spain). Hexane and isopropanol HPLC grade were obtained from PROLABO (Barcelona, Spain). The nitrogen (99%) was acquired from AirLiquid (Madrid, Spain).

2.3. Oil samples

Ten olive oils samples, including four categories: extra virgin, virgin, olive oil (blend of virgin and refined) and pomace, and two varieties: arbequina and picual. Ten vegetable oils, representative of the most used edible oils, were used in the study. These vegetable oils were 2 sunflower, 1 high-oleic sunflower, 1 rapeseed, 1 soybean, 1 canola, 1 corn, 1 grape seed and 2 commercial vegetable seed, these are seed mixtures and in the label it is not specified what type of seed are used. All the oils were purchased in retail stores and were maintained in dark at −2 °C until analysis.

2.4. Chromatographic conditions

Chromatographic analysis was carried out using a LiChrospher 100 RP-18 (250 mm × 4 mm 5 μm) purchased from Agilent Technologies (Waldbronn, Germany). The column temperature was kept at 30 °C. The injection volume was 4 μL. A binary mobile phase composed of acetonitrile and hexane–isopropanol (1:1), was used for gradient analysis (60:40 to 42.5:57.5 in 40 min with a post time of 5 min); the flow rate was 1.0 mL/min. CAD conditions were: nitrogen gas pressure was adjusted to 35 psi, none filter was used for detector signal and a 100 pA output range was used for CAD monitoring.

2.5. Samples preparation

The working samples were prepared mixing one olive oil with one vegetable oil in different percentages. Four batches were prepared: (i) one olive oil and one sunflower oil; (ii) one olive oil and nine different vegetable oils; (iii) one sunflower oil with nine different olive oils (iv) a validation batch. Composition of the samples of the different batches is presented in Table 1. The selection of the sunflower oil for preparing the batches 1 and 3 was due to the importance of this oil as ingredient in foodstuffs, like in chips, bakery products or preserves foods. The concentrations were chosen to cover all the concentration ranges of the blends.

Table 1
Samples composition.

Batch 1		Batch 2		Batch 3		Batch 4	
Sunflower (%)	Olive (VE) (%)	Vegetable (%)	Olive (VE) (%)	Sunflower (%)	Olive (%)	Vegetable (%)	Olive (%)
100	0						
90	10	Soy 90	10	90	Pommace 10	Soy 90	Virgin 10
80	20	Canola 80	20	80	Virgin 20	Rapeseed 90	OO 10
70	30	Seed 70	30	70	OO 30	Sunflower 70	Virgin 30
60	40	HO Sunflower 60	40	60	VE (arbequina) 40	Corn 70	OO 30
50	50	Corn 50	50	50	OO 50	Soy 50	Virgin 50
40	60	Rapeseed 40	60	40	VE (picual) 60	Rapeseed 50	OO 50
30	70	Grape 30	70	30	VE 70	Sunflower 30	Virgin 70
20	80	Sunflower 20	80	20	Virgin 80	Corn 30	OO 70
10	90	Seed 10	90	10	OO 90	Soy 10	Virgin 90
0	100					Rapeseed 10	OO 90

HO: High oleic; VE: virgin extra; OO: olive oil (mixture refined olive oil + virgin). Batches 1–3 calibration sets and batch 4 validation set.

Stock solutions were prepared by dissolving 100 mg of each oil sample into 1.0 g of hexane. Working sample solutions were diluted by hexane to obtain a final concentration of 250 μg/g. The samples were filtrated prior to injection through a 0.22 μm PTFE membrane.

2.6. Statistical analysis

Pre-processing was used to make the data suitable for statistical analysis. Peak shifting was corrected with interval Correlation Optimized shifting, *i*coshift [25], and initial baseline correction was performed by a weighted least squares approach using a second-order polynomial basis. The chromatograms were subsequently mean centred. The prediction capability of the regression models was assessed by cross-validation by removing one sample at a time.

3. Results and discussion

The quantification of olive oil in samples mixed with different vegetable oils was carried out using PLS and iPLS. The reference (dependent variable) was the measured TAG values (%) and therefore, the results, predicted values, were obtained in percentage units of olive oil.

In this study, 29 samples, batch one to three, were used for the calibration model. Firstly, each of the three calibration batches were analyzed separately; and then, the three batches were combined to obtain a more comprehensive model. Finally batch four, with ten samples, was used as external validation.

3.1. Data preprocessing

To avoid bias from chromatographic sources of variation that are unrelated to the chemistry of the samples, preprocessing of the data was used. The aim of this preprocessing is to reduce peak misalignment and drift in the baseline [26].

The first 8 min and the ten last minutes of the chromatograms were eliminated due to lack of information. Fig. 2 shows the chromatograms after baseline correction and alignment. Initial baseline correction was performed on the chromatographic data by a weighted least squares correction based on a second-order polynomial basis [26,27]. The misaligned peaks were corrected with *i*coshift [25]. Six intervals were used for aligning the chromatograms. These intervals were chosen based on the TAGs peaks, and the maximum peak in each interval was used as target (Fig. 2).

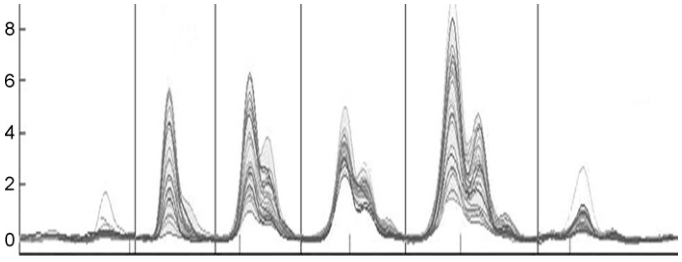

Fig. 2. HPLC–CAD chromatograms of oil samples after pretreatment of chromatographic raw data.

3.2. PLS results

Regression models were created using PLS applied to a matrix composed of 29 samples by 11,969 variables from the three calibration batches. The performance of models was evaluated by R^2. In addition, the model was also evaluated using root mean squared error of calibration (RMSEC) for calibration using leave-one-out cross validation. External validation of the model was quantified by the root-mean-squared error of prediction (RMSEP) [26,27].

Fig. 3 shows the plots of predicted versus actual concentrations of olive oil. The model of the batch of one olive oil and one sunflower oil (Fig. 3A) has a high R^2 (0.99) and a low error, RMSECV of 1.76, but the model is simple in the sense that it only models the variability of a blend of two oils. However, a more complete model is obtained when a batch of different vegetable oils and one olive oil is used. Fig. 3B shows an R^2 of 0.96 and a RMSECV of 6.79, the model predicts concentrations of olive oil in different blend samples of different vegetable oils (soybean, canola, seeds, sunflower, corn, rapeseed, grape), hence the model include more variability. Fig. 3C presents the results of different olive oils with one sunflower; the plot indicates an R^2 of 0.98 with a RMSECV of 3.74. This model shows better fit than the model in Fig. 3B, even though it uses nine different olive oils. To obtain the results for the aim of the study, the quantification of olive oil in different mixed oil samples, it was necessary to develop a model that contains all the variability, that is, four different categories and two varieties of olive oil and different types of vegetable oils. Fig. 3D shows the results for this model (batch one to three) using four latent variables, although the model presents an R^2 of 0.91, and a RMSECV 8.64, it is a good model considering that the model uses ten different vegetable oils and ten different olive oils.

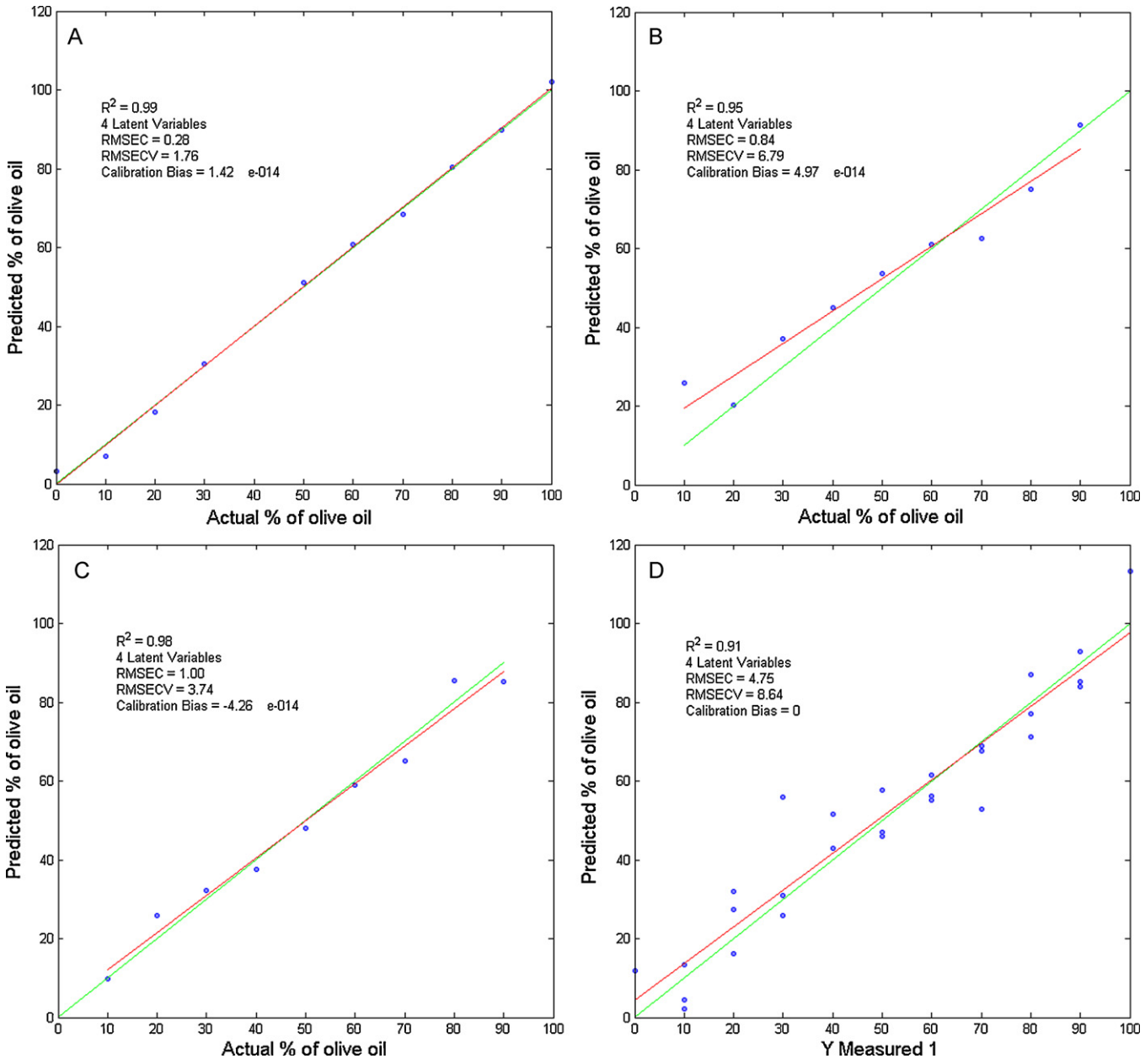

Fig. 3. Predicted % of olive oil in different batches. See Table 1 for details of oil sample composition. (A) Batch 1; (B) batch 2; (C) batch 3 (D) batches 1–3.

3.3. iPLS results

In order to investigate if the results could be improved, variable selection was applied. Using iPLS to find, if exists, a subset of peaks that give a lower RMSECV and better R^2 than the full-chromatogram. The iPLS approach splits the data into a number of intervals and calculates local PLS model for each interval [28]. The models were constructed using up to four latent variables. The matrix used in the model was 29 samples by 601 variables. The results did not improve dramatically; RMSECV decreased from 8.64 to 7.79 (Fig. 4B). Fig. 4A shows the two segments that are considered in the model, which are the segments that presented the lowest RMSECV. The segments are two of the four groups of peaks characteristic of olive oil. Olive oil is characterized by four major ECN peaks: 44, 46, 48 and 50. The ECN40 is not present in this types of oils, and ECN42 (trilinolein LLL) is present in trace amounts, unlike some vegetable oils that are characterized by high concentrations of LLL.

In the first segment are the TAGs with ECN44, which are present in olive oil and in vegetable oils, the second segment is the peak of triolein, the TAG most abundant presents in olive oil, therefore the variable selection confirm that both peaks are important to quantify olive oil [29].

The original PLS model (Fig. 3D) was validated with ten samples (Table 1), including blends of four different types of vegetable oils and two varieties of olive oils at five different percentages. The selection of the vegetable oils was due to the common blends that are presented in food stuffs, like potato chips or bakery products [24]. In Fig. 5 are shown the results obtained for the training set and the validation set. The RMSEP obtained for the validation set was 10% (Fig. 5).

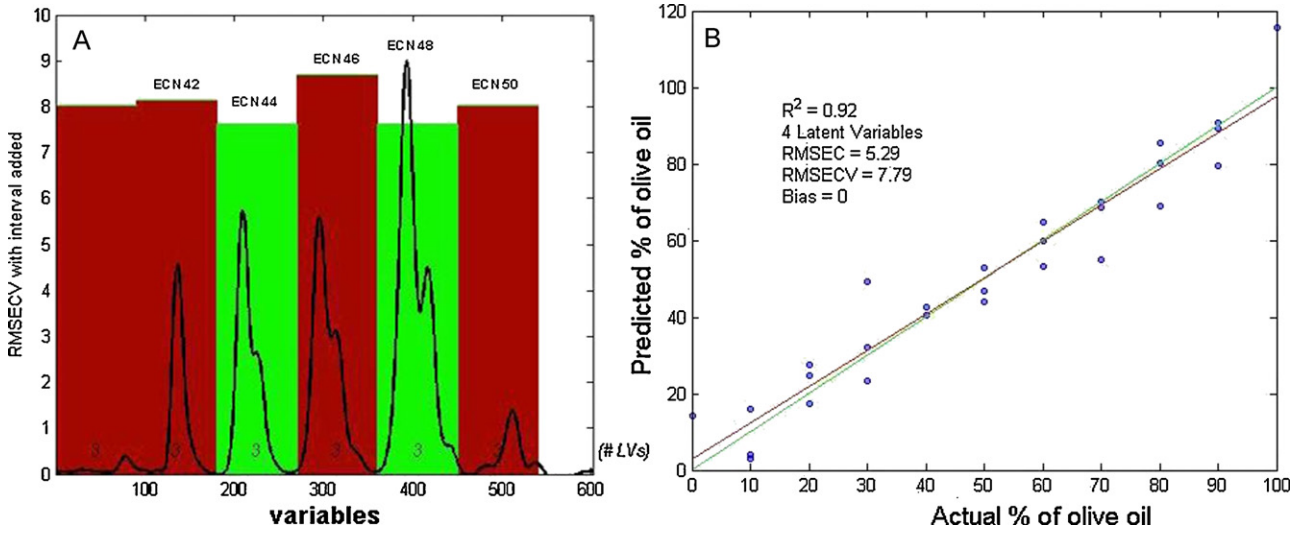

Fig. 4. iPLS using all the oil samples; (A) selected interval for the four batches, (B) predicted values using the selected intervals.

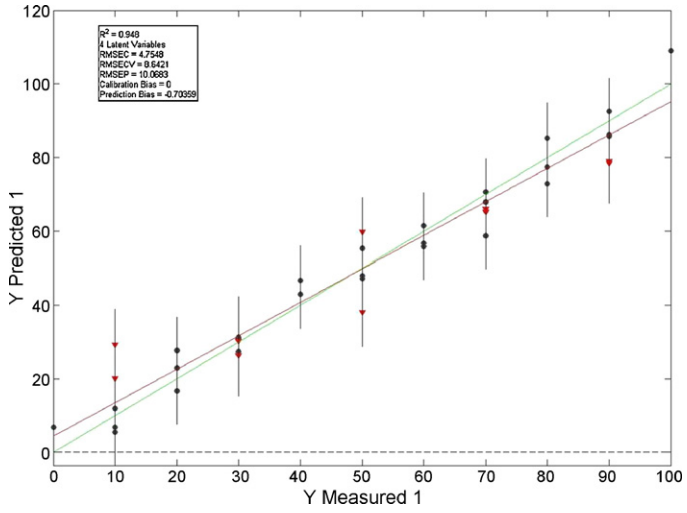

Fig. 5. External validation using PLS model with 10 vegetable oils and 10 olive oils (●, training set; ▼, validation set).

4. Conclusions

Olive oil was able to quantify in edible oils blends using HPLC–CAD, applying chemometric tools (PLS and iPLS) to the full chromatogram. Different categories of olive oils (extra-virgin, virgin, refined and pomace) and various vegetable oils (sunflower, rapeseed, corn, soybean, canola, seed and grape seed) were used at different concentrations. As a result, using the whole variability of the samples, the PLS obtained a low RMSECV of 8.6%. Also iPLS was applied; using two peak segments obtaining also, low RMSECV of 7.8% a slight improvement in RMSECV and also providing a chemically meaningful selection of peaks. The external validation was applied to the PLS model obtaining a 10% of RMSEP.

The proposed method provides a way to quantify olive oils using the full TAG chromatogram, chromatographic fingerprint, instead of using the information derived from the signal profiles.

The results obtained show the ability to establish and quantify the presence of various vegetable oils blended with different varieties and types of olive oils with errors not exceeding 10%.

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