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Rapid Determination of Olive Oil Chlorophylls and Carotenoids by Using Visible Spectroscopy

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Abstract

The determination of total chlorophylls and total carotenoids in olive oil by using visible spectroscopy (VIS) is reported. ~~The technique proposed is rapid and non-destructive and can be used in a multi-parameter form. Moreover, it is environmentally friendly.~~ The proposed technique has been compared with the determination of these pigments by near infrared spectroscopy (NIRS) and VIS together NIRS. Several procedures for multivariate regression were tested. The reference methods used were the determination of the extinction coefficient K_{670} for total chlorophylls and K_{470} for total carotenoids. A total of 258 samples were tested. The optimization of the calibration for total chlorophylls has been set by using multiple linear regression (MLR) from the wavelengths 670-686 nm exclusively visible. Its satisfactory performance is proven from the model coefficients standard error of calibration SEC 2.63 and R^2 0.97, and the residual predictive deviation (RPD) 5.76 from the external validation. For the total carotenoids the best VIS calibration was fit by using the window of 465-475 nm and partial least squares (PLS), which provided RPD 3.68. However, the model built using the entire spectrum VIS-NIRS available (350-2500 nm) was slightly better for this last pigment, showing RPD 3.86. Hence, this study

shows the proposed VIS technique can be advantageous for the determination of total chlorophylls in olive oils while is also suitable for determining total carotenoids.

Keywords: carotenoids, chlorophylls, multivariate analysis, olive oil, visible spectroscopy.

Abbreviations

FCV, full cross internal validation; MLR, multiple linear regression; NIR, near infrared; NIRS, near infrared spectroscopy; PC_s, principal components; PCR, principal component regression; PLS, partial least squares; RPD, residual predictive deviation; SEC, standard error of calibration; SEP, standard error of performance; SLR, single linear regression; VIS, visible spectroscopy; VIS/NIRS, visible and near infrared spectroscopy; VOO, virgin olive oil; UV, ultraviolet; UV/VIS/NIR, ultraviolet, visible and near infrared;

Introduction

Olive oil can be consumed as a fruit juice, called virgin olive oil (VOO), which characterizes it and differentiates it from other plant oils. This oil, one of the main components of the Mediterranean diet, is recognized as a protector against cardiovascular diseases and cancer, due to its fatty acid composition and its content in phenolic compounds [1]. World production of olive oil ranges next to $3x10^6$ t per year, Spain being the largest producer, with an average production from the last three seasons higher to $1.4x10^6$ t [2]. A large increase in the demand for high quality VOO during recent years can be attributed not only to its particular sensory properties, but also to its potential health benefits.

Several research results have demonstrated that plant pigments play important roles in health [3]. The potential health benefit of a diet rich in carotenoids has been highlighted in multiple studies where their role as antioxidants and as agents which prevent cardiovascular diseases and degenerative eye pathologies is reported [4-5]. The provitamin A value of the carotenoids is well known. Numerous studies have also shown the anticancer activity of β-carotene and other carotenoids [6]. In addition, it has been reported that chlorophyll concentrations encountered in chlorophyll-rich green vegetables can provide substantial cancer chemoprotection, and suggested that they do so by reducing carcinogen bioavailability [7].

On the other hand, carotenoids together with polyphenols and tocopherols provide oxidative stability to olive oils [2]. An important role of chlorophylls and carotenoids in the oxidative activity of processed foodstuff, due to their antioxidant nature in the dark and pro-oxidant activity in the light, has been shown [8].

Moreover, the color of olive oil is an important visual attribute which has a decisive influence on the acceptance of the product [9]. Chlorophyll and carotenoid pigments in olive oil greatly contribute to their color, so they are commonly used for the determination of olive oil color according to several methods [10-13]. These methods, instrumental and visual, for olive oil color analysis and its relation with chlorophylls and carotenoids, have been reviewed [10]. However, none of the above methods has been tuned for the rapid and accurate determination of chlorophylls or carotenoids totals in olive oils.

Studies on the pigment composition in products derived from olives are relatively few [3]. The first high performance liquid chromatographic studies on the pigment fractions of

mono-varietal VOOs from some Spanish olive varieties were done by Mínguez et al. [14-15] and Gandul et al. [16-17]. Also the separation of olive oil chlorophyll and carotenoid pigments by thin layer chromatography and spectrophotometric identification has been described [18].

Mínguez et al. [19] describes a simple way for the determination of overall level of carotenoids and chlorophylls in olive oils which improved analysis speed, which is a factor worth pointing out. However, it still requires the use of solvents and a considerable amount of time for data processing. Taking into account the specific extinction coefficients of these pigments, and the usual level of concentration in olive oils, it is usually necessary a previous dilution of samples in order to achieve a measurable absorbance value. However, another way of getting these measurable absorbance values could be the use of a shorter pathlength during absorbance measurements.

In the context of raising competition in the olive sector, technological innovation must play an important role by providing the necessary improvements in the sector to live up to the circumstances. On the other hand, the environmental protection concern should be taken into account. Among the different actors involved in the production and marketing of olive oil, the industry plays a key role. In the olive mill, instant olive oil characterization is required to separate the different products in different deposits, according to their characteristics, thus maintaining its quality, identity and traceability. These intensive controls of olive oil quality would be possible only with analytical systems able to provide immediately and continuously all necessary information. Using the techniques available up to date, this is not possible yet satisfactorily. The non-destructive techniques are an

alternative which is expected to provide new solutions, requiring its development and validation.

With these purposes, Sikorska et al. [20] reported the capability of the fluorescence techniques to monitor chlorophyll content in oil products. Otherwise, the interaction between pigments and near-infrared radiation (750–2500 nm) could provide useful correlations for determining olive oil chlorophylls and carotenoids. In fact, near infrared spectroscopy (NIRS) has been reported multiple times for plant pigment determination [21-27], as well the suitability of NIRS to determine the main olive oil quality parameters, such as acidity, peroxides, K270 and K232, has been shown repeatedly [28-32]. Monitoring carotenoid and chlorophyll in virgin olive oil has been reported by visible-near infrared transmittance spectroscopy (VIS-NIRS) [26], although in this study the separate analysis of the wavelengths that contribute to the predictive models of chlorophylls and carotenoids is very scarcely reported. Nevertheless, the use of the VIS spectrum only could be better than their use together with the NIR region for predictive model for these pigments. The literature on the use of visible spectroscopy for the determination of quality parameters of olive oil is very little, very recently being reported the potential of UV-Visible spectroscopy as fingerprint technique in combination with chemometrics for classification of Spanish extra virgin olive oils [33]. Thus, there are much interest on clarify the respective contributions of visible and NIR regions in predictive models for these olive oil's compounds, as the simplicity of the technique may have a significant impact in reducing the cost of the instrument used.

In the other hand, regarding multivariate methods, when the factors are few in number, are not significantly redundant and have a well-understood relationship to the responses, then multiple linear regression (MLR) can be a good way to turn data into information. However, if any of these three conditions breaks down, MLR can be inefficient or inappropriate. Partial Least Squares (PLS) is a method for constructing predictive models when the factors are many and highly collinear [34]. Principal Component Regression PCR) is also a popular method intended to overcome the problem of multicollinearity which arises with spectral data [35]. The difference between PLS and PCR is usually quite small, but PLS [36] will usually give results comparable to PCR-results using fewer components [37].

~~For this purpose~~To answer the above questions, here is reported a ~~study on~~method for determination of chlorophylls total and carotenoids total in olive oil by using VIS multivariate models. ~~t~~The contribution of the spectral regions ultraviolet (UV, 350-400 nm), VIS (400-779 nm) and NIR (780-2500 nm) to predictive models of total chlorophylls and total carotenoids of olive oil have been studied. ~~from the spectral regions ultraviolet (UV, 350-400 nm), VIS (400-779 nm) and NIR (780-2500 nm) is reported. Chlorophylls total and carotenoids total in olive oil have been determined by using VIS multivariate models, which performance were better than that obtained from single wavelengths. A short optical pathlength of 5 mm was used for the purpose of getting measurable absorbance values without olive oil dilution. The procedures Single Linear Regression (SLR), Multiple Linear Regression (MLR), Principal Component Regression (PCR) and Partial Least Squares (PLS) were tested. The technique proposed is truly rapid and non destructive, it does not require the use of solvents or reagents, it is environmentally friendly and can be~~

~~used in a multi parameter form. On the other hand, it is potentially less prone to experimental errors, because there is neither sample weighing nor volume adjustment.~~

Materials and methods

Virgin olive oils

The development of predictive models was carried out based on spectra acquisition and reference analysis of VOO extracted in the Instituto de la Grasa (CSIC), from samples provided by a research project, taken in different olive groves of the provinces of Seville and Huelva. All the samples were extracted by a lab mill MC2 (Ingeniería y Sistemas, S.L., Spain). The VOO samples amounted to 258, and were composed of the varieties Picual, Arbequina and Manzanilla in equal quantities. The first two are quantitatively important cultivars in the production of olive oil in Spain. Manzanilla cv. is for table olives, but each campaign a small part of its production is used for olive oil extraction, with specific quality characteristics highly valued. The reason of including different olive cultivars in the study is to assure diversity in sample pigments composition. Olive oil samples were stored at 4ºC until spectra acquisition.

Instrumentation

Spectral acquisition was carried out using an UV/VIS/NIR Labspec Pro (Analytical Spectral Devices Inc., Boulder). Labspec Pro is equipped with three detectors. The detector for the ultraviolet and visible range (350-1000nm) is a fixed reflective holographic diode array. The wavelength range 1000-1800 nm is covered by a holographic fast scanner InGaAs detector cooled at -25ºC. The same device before mentioned coupled with a high

order blocking filter operates over an 1800-2500 nm interval. The instrument is equipped with internal shutters and automatic offset correction, the scanning speed being 100 ms. The spectrometer can be used with a spectrophotometric cuvette accessory for transmittance measurement, joined by fiber optic connectors to the light source spectrometer by one side of the accessory and to a detector on the opposite side.

Spectral acquisition

The performance of the models built including the NIR spectrum together with the visible is to be assessed. Hence, the temperature of the sample should be considered since it plays an important role on the NIR radiation it reflects and absorb [348], and it constitutes a decisive factor in NIR spectroscopy. Therefore, the samples were taken from 4 ºC storage and placed in a laboratory at approximately 25 ºC for 18 h before processing. Prior to the recording of spectra, 125 mL containers of the samples were placed in a thermostated water bath fixed at 33 °C for 30 min, verifying temperature stability. The spectra acquisition was performed nondestructively in transmittance mode with each virgin olive oil sample, without further sample preparation, for two replicates of each sample. For the purpose of avoiding the use of cleaning solvents, the quartz cuvette was cleaned after each spectrum acquisition using oil excess from the next sample.

The whole spectrum comprised in the interval 350-2500 nm was acquired, each spectral variable corresponding to a 1 nm interval. The spectrometer was configured for continuous acquisition of 50 spectra, recorded their average as representative spectrum of the sample. Scans were performed in a Hellma quartz spectrophotometric cuvette with 5 mm path length. Indico Pro software (Analytical Spectral Devices Inc., Boulder) was used for this

purpose. The acquisition process required for each sample was less than a minute, all steps included.

Reference analysis

Clorophyll total and carotenoids total analyses in olive oils samples were determined through the method described by Mínguez et al. [19]. Briefly, the chlorophyll and the carotenoid fractions were evaluated from the absorption spectrum at 670 nm and 470 nm respectively, from the pigment extract in cyclohexane (Merck for spectroscopy, Darmstadt, Germany), using the same solvent as blank reference. The absorption maximum at 670 nm in the spectrum of the total extract is due exclusively to the presence of the chlorophyll fraction. As pheophytin "a" is the major component of this fraction, the group of chlorophyll derivatives can be evaluated as if all were pheophytin "a", after calculating the coefficient of specific extinction in cyclohexane. The value in ethyl ether is $\varepsilon_o = 613$ nm [19].

The spectrum of the carotenoid fraction takes the form of the dominant pigment, lutein. Thus, it is possible to evaluate the yellow pigments in the total spectrum as if they are all lutein [19]. The maximum at 470 nm is chosen after obtaining the corresponding coefficient of extinction because it is a zone without interference from the pheophytin "a". This value is reported $\varepsilon_o = 2000$ [19].

For the procedure, 7.5 g oil were weighed exactly, dissolved in cyclohexane and taken to a final volume of 25 mL. Once the absorption spectrum was obtained, the chlorophyll total

and carotenoids total fractions were deduced at 670 nm and 470 nm respectively, and expressed as $mg.Kg^{-1}$. All the samples were measured with two replicates.

Chemometry and calibration procedure

Prior to calibrations the transmittance data were transformed to absorbance and mean normalized. Savitzsky-Golay derivatives first and second were tested to see if they improve the performance of the models. The UV, VIS and NIR spectral regions were tested to prove the advantages these can provide, either using exclusively the NIR spectrum (1100-2500 nm), or by using the visible spectrum (400-1099 nm) together with UV (350-399 nm) and NIR.

Partial Least Squares (PLS) was used for the selection of the specific spectral variables which should be included in the models. The procedure of selection consisted on performing several consecutive cycles of elimination of variables whose contribution to the model is null. It was made by selecting those variables whose spectral correlation coefficients with the parameter analyzed were closer to zero. This elimination was carried out by using the 'Mark with rectangle' option on the regression coefficients graph of The Unscrumbler. Variable selection ended in the last cycle which improved the statistical model R^2 and R^2_{CV}. Model fitness was assessed by its standard error of calibration (SEC) and proximity between R^2 and R^2_{CV}, independently from the model validation procedure below described.

The methods Principal Component Regression (PCR), Multiple Linear Regression (MLR) and Single Linear Regression (SLR) were tested. ~~Single linear regression (SLR), multi linear regression (MLR), principal component regression (PCR), and partial least squares~~

~~(PLS) [35]~~ PLS, PCR, MLR and SLR models were obtained using The Unscrumbler (CAMO Software AS, Norway). The full cross internal validation (FCV) procedure was employed. The number of principal components (PC_s) was fixed in the minimum to maximize the explanation of the parameter by the model. It was set for each calibration after tests parting from the number initial of PC_s ten.

~~The UV, VIS and NIR spectral regions were tested to prove the advantages these can provides, either using exclusively the NIR spectrum (1100-2500 nm), or by using the visible spectrum (400-1099 nm) together with UV (350-399 nm) and NIR. Additionally, the selection of the specific spectral variables which should be included in the models was conducted. The procedure of selection consisted on performing several consecutive cycles of elimination of variables whose contribution to the model is null. It was made by selecting those variables whose spectral correlation coefficients with the parameter analyzed were closer to zero. This elimination was carried out by using the 'Mark with rectangle' option on the regression coefficients graph of The Unscrumbler. Variable selection ended in the last cycle which improved the statistical model R^2 and R^2_{CV}. Model fitness was assessed by its standard error of calibration (SEC) and proximity between R^2 and R^2_{CV}, independently from the model validation procedure below described.~~

Model performance assessment

External validation exercises were conducted for both parameter analyzed. With this purpose, one fifth of the samples available was reserved and constituted a validation set. The calibration models previously built were used to predict the K_{670} and K_{470} extinction

coefficients, corresponding to the chlorophyll total and carotenoids total contents respectively, on the validation set.

The model performance was assessed mainly by the residual predictive deviation (RPD), described as the ratio of the standard deviation (σ) of the reference data from the validation set to the standard error of performance (SEP) [~~36~~39].

Results and discussion

Olive oil visible spectrum

The assignment of the major visible absorption bands of olive oil has been described by Moyano et al. [9]. Olive oil visible spectra characteristic of the Picual, Manzanilla and Arbequina are shown in Figure 1. An initial peak appeared close to 420 nm. This area corresponds to the absorption by olive oil of wavelength dark blue colored light, which could be due mainly to carotenoids, as well as to pheophytin a, pheophorbide a and pyropheophytin a. A second peak near 460 nm was found, corresponding to the absorption of the blue light, characteristic of carotenoids. Finally, a third peak was observed approximately at 670 nm, coinciding with the chlorophyll absorption.

Figure 1

Population characterization

The values from the parameters studied resulting from the reference analysis, corresponding to the calibration and external validation sets, are included in Table 1. A wide variation ranges ~~in~~ regarding the olive quality parameters studied were integrated into

the calibrations, as can be seen. The chlorophyll total and carotenoids total contents average from the calibration set analyzed were 17.23±15.83 and 11.08±7.46 mg.Kg^{-1}, respectively.

Spectral Variable Analysis and Chemometry

The tests carried out using Savitzsky-Golay derivatives first and second didn't improve the fit of the models, whereas the absorbance data mean normalized provided the best outcomes. The visible window of 670-686 nm gave the best performance for predicting chlorophyll total, as result from the tests with different wavelength intervals, as is detailed below. On the contrary, the entire spectrum available (350-2500 nm) brought the best yields for carotenoids total. As is shown in Table 2, the PLS models built exclusively from NIR region provided R^2 0.56 and 0.62 for the predictive models of chlorophylls and carotenoids respectively, values which are too low for intend to use these models for analysis.

Chlorophylls

The tests to determinate the wavelengths with an optimal contribution to the total chlorophylls predictive calibration ~~procedure of multivariate analysis~~ indicated the most ~~appropriate~~ suitable ~~for total chlorophylls~~ was ~~MLR (Ch_1) using~~ the VIS window 670-686 nm. The best procedure of multivariate analysis using this window was MLR (Ch_1), as proven from its statistics R^2 0.97 and SEC 2.63, shown in Table 2. PLS (Ch_2) and PCR (Ch_3) using the same wavelengths showed lower performance, and very similar between both according their statistics, with R^2 0.96 for both and SEC 3.20 and 3.22 (Table 2). Interestingly, both PLS and PCR, did provide R2 slightly lower only than MLR, but their SEC were 21.7% and 22.7% higher, hence they were worst. The calibration developed

using VIS and NIR regions, by using the procedure previously described for selection of spectral variables (Ch_4), did not improve statistical indicators of calibration neither the predictive performance compared to Ch_1, as it was proved by the data shown in Table 2, with SEC 3.13. Particularly noteworthy is the predictive model obtained by SLR (Ch_5) with the only variable corresponding to a spectral wavelength of 670 nm, the same used in the K_{670} reference, showed low efficacy with SEC 5.71. This fact emphasizes that using one only wavelength is not a better approach for determining chlorophylls by visible spectroscopy than using the multivariate method with the window 670-686 nm. Likewise, the models built using MLR with the window restricted to 670-672 nm spectral variables were no better (data not shown). On the other hand, the calibration built exclusively using NIR (Ch_6) gave the worst performance among the methods assessed, as evidenced by their SEC 10.47 and R^2 0.56 values (Table 2). These data reveal that chlorophyll content of the olive oil does not appear to be significant in the NIR spectrum. The good fit among prediction and measurement from the data used for the chlorophyll total calibration Ch_1 using the VIS window 670-686 nm is shown in Figure 2.

The assessment of the models was mainly carried out by using them to determine the chlorophyll total content of a set of 54 samples, which was initially reserved for this purpose. The RPD of each multivariate procedure from the same validation set, proves the results referred above. The dispersion plot of this prediction is demonstrated in Figure 2 (Vch_1). As it's evidenced by the RPD value 5.76, shown in Table 2, the predictive model performance was very satisfactory. This ratio, the most widely used for determining the acceptability of a model, must reach the threshold of 3 for this purpose [36], which was fairly exceeded.

VIS/NIR transmittance spectroscopy has been reported successful for on-line monitoring carotenoid and chlorophyll pigments in virgin olive oil [26], where the separate analysis of the wavelengths contributing to the predictive models of chlorophylls and carotenoids was very scarce. The PLS model carried out in the present work including NIR together VIS regions (Ch_4), similarly to the work is reported above [26], provides RPD 4.05 which shows is valid for practical use and agrees with the study referred. However, its performance was clearly lower to the model built using wavelengths VIS only.

Figure 2

The spectral windows more suitable for build the models were slightly different among the different multivariate analysis methods (data not shown), that highlights the interaction between the variables and the model method. Hence, it reveals that most appropriate spectral variables to predict a certain parameter may depend on the nature of the predictive model.

Carotenoids

The tests to determinate the wavelengths with an optimal contribution to the total carotenoids predictive calibration revealed the most suitable was the VIS window 465-475 nm. The PLS (Ca_1) and PCR (Ca_2) models using this~~e visible wavelengths of 465-475 nm window developed using PLS (Ca_1) and PCR (Ca_2)~~ showed statistics SEC 2.28 and 2.30 and R^2 0.91 and 0.90, which are very similar, and RPD 3.68 from the external validations both (Table 2). These results demonstrate that using this window of the visible spectrum is suitable to determine total carotenoids. The calibration developed using MLR with the same wavelengths 465-475 nm (Ca_3) showed statistics similar to those from PLS and PCR,

although the SEP 2.11 and RPD 3.31 from the external validation were worst. This gospel agrees with the possibility of deleting factors, thus reducing dimensionality, what constitutes the major advantage of PCR regarding MLR. The possible advantage of PLS over PCR is that it incorporates more information in the model-building phase [35], and reflected actually on the R^2 and SEC of the predictive models of total carotenoids built by PLS, slightly better than that of PCR.

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Nevertheless, the best performance for predicting the total carotenoids of the olive oils was brought by the PLS model built from the entire spectrum available of 350-2500 nm (Ca_4), being proven for the calibration SEC 1.74 and R^2 0.95 and RPD 3.86 from the external validation exercises, shown in Table 2. As in the case of predicting total chlorophyll, this result agrees with the previously referred study [26], which reported as successful the on-line monitoring of the total carotenoids of virgin olive oil using VIS/NIRS.

Again we must emphasize that models constructed by SLR (Ca_5) from the single spectral variable 470 nm used in the reference method showed no better performance than using the wavelengths 465-475 nm, that is demonstrated by the SEP 2.63 and RPD 2.66 from the external validation and by the calibration R^2 0.81 and SEC 3.27, included in Table 2. As well, the PLS model developed from exclusively NIR region for predicting total carotenoids provided the worst outcome, with R^2 0.62, value which is too low for intend to practical use. The good fit among predictions and measurements from the calibration for total carotenoids from the exclusively visible window of 465-475 nm is shown in Figure 3 (Ca_1) and the same from the external validation (Vca_1). The PLS model VIS/NIRS from

350-2500 nm (Ca_4) and the corresponding validation (Vca_4) are shown in Figure 4. As can be seen, both models showed performance fairly similar.

Figure 3

Conclusions

Multivariate models set in this work from the wavelength intervals 670-686 nm for total chlorophylls and 465-475 nm for total carotenoids clearly improved those built by SLR from the single wavelengths used in the reference methods. None of the wavelengths from the spectrum NIR or UV regions assessed in this study provided any improvement in the performance of the multivariate model for total chlorophylls.

The spectral variables used in the models were very few, allowing the use of VIS standard spectrophotometers, therefore relatively inexpensive. The technique proposed is truly rapid and non-destructive, and easy to use also. Moreover, it is potentially less prone to experimental errors, because there is neither sample weighing nor volume adjustment. Another major advantage is the possibility of a multi-parametric determination of chlorophylls and carotenoids from a single measurement. Last but not least, it should be noted that the technique proposed is environmentally friendly, avoiding a considerable consumption of solvents or reagents, which aids in avoiding environmental costs. In summary, the proposed technique can be advantageous for the determination of total chlorophylls and carotenoids.

~~This paper has analyzed the respective contribution of UV, NIR and VIS spectrum in predictive models of chlorophylls total and carotenoids total. None of the wavelengths from~~

~~the spectrum NIR or UV regions assessed in this study provided any improvement in the performance of the multivariate model for total chlorophylls. The exclusively visible window of 670-686 nm gave the best model for this pigment. It must be noted that predictive models visible may have the advantage of having less influence on the spectrum of the temperature, both environmental as of the samples.~~

~~The models using the exclusively visible wavelengths of 465-475 nm, either using PLS or PCR, were successful for measuring total carotenoids. The model VIS/NIRS for total carotenoids using the entire spectrum available showed performance slightly higher in the external validation exercise.~~

~~It should be pointed out that multivariate models set from the wavelength intervals used in this work both total chlorophylls and total carotenoids clearly improved those built by SLR from the single wavelengths used in the reference methods. The spectral variables used in the models were very few, which allows for the use of VIS standard spectrophotometers, therefore relatively inexpensive. Another major advantage is the possibility of a multi-parametric determination of chlorophylls and carotenoids from a single measurement. Both factors may contribute significantly to determining the features of the equipment and adapting them to reasonable economic parameters. Also, the ease in use of the technique has to be highlighted. Last but not least, it should be noted that the technique proposed is environmentally friendly, avoiding a considerable consumption of solvents, which aids in avoiding environmental costs. In summary, the proposed technique can be advantageous for the determination of total chlorophylls and carotenoids.~~

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Figure captions

Figure 1. Example of olive oil visible spectrum from Picual (P) Manzanilla (M) and Arbequina (A).

Figure 2. Predictive model VIS for total chlorophylls (Ch_1) and external validation (Vch_1).

Figure 3. Predictive model VIS for total carotenoids (Ca_1) and external validation (Vca_1).

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Figure 4. Predictive model VIS/NIRS for total carotenoids (Ca_4) and external validation (Vca_4).

	Total chlorophylls		Total carotenoids	
	Calibration	*Validation*	*Calibration*	*Validation*
N[a]	205	53	205	50
Range	1.39-88.13	1.47-74.35	2.05-38.53	2.62-36.16
$\overline{X}$[b]	17.23	15.58	11.08	10.39
σ[c]	15.83	13.99	7.46	6.99

Table 1. Statistics of chlorophylls and carotenoids from the calibration and validation sets. [a]N, size; [b]$\overline{X}$, mean (mg Kg^{-1}); [c]σ, standard deviation (mg Kg^{-1}).

Total chlorophylls					Total carotenoids				
	Calibration		*Validation*			*Calibration*		*Validation*	
Model	R^2	SEC	SEP	RPD	Model	R^2	SEC	SEP	RPD
Ch_1-MLR	0.97	2.63	2.43	5.76	Ca_1 -PLS	0.91	2.28	1.90	3.68
Ch_2-PLS	0.96	3.20	2.73	5.12	Ca_2 -PCR	0.90	2.30	1.90	3.68
Ch_3-PCR	0.96	3.22	2.72	5.14	Ca_3 -MLR	0.91	2.19	2.11	3.31
Ch_4-PLS[a]	0.96	3.13	3.51	4.05	Ca_4 -PLS[(1)]	0.95	1.74	1.81	3.86
Ch_5-SLR	0.87	5.71	4.27	3.28	Ca_5 -SLR	0.81	3.27	2.63	2.66
Ch_6-PLS[b]	0.56	10.47			Ca_6 -PLS[(2)]	0.62	4.58		

Table 2. Statistics of the models built by Multiple Linear Regression (MLR), Principal Component Regression (PCR), Partial Least Squares (PLS), Simple Linear Regression (SLR), PLS from UV, VIS and NIR regions (PLS[a]) and PLS from exclusively NIR region (PLS[b]). R^2, model squared coefficient of calibration; SEC, standard error of calibration; SEP, standard error of performance; RPD, residual predictive deviation.

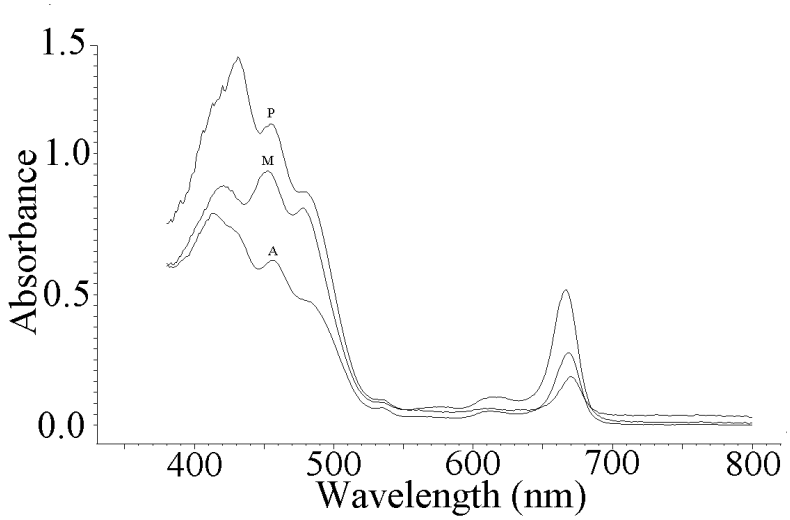

Figure 1

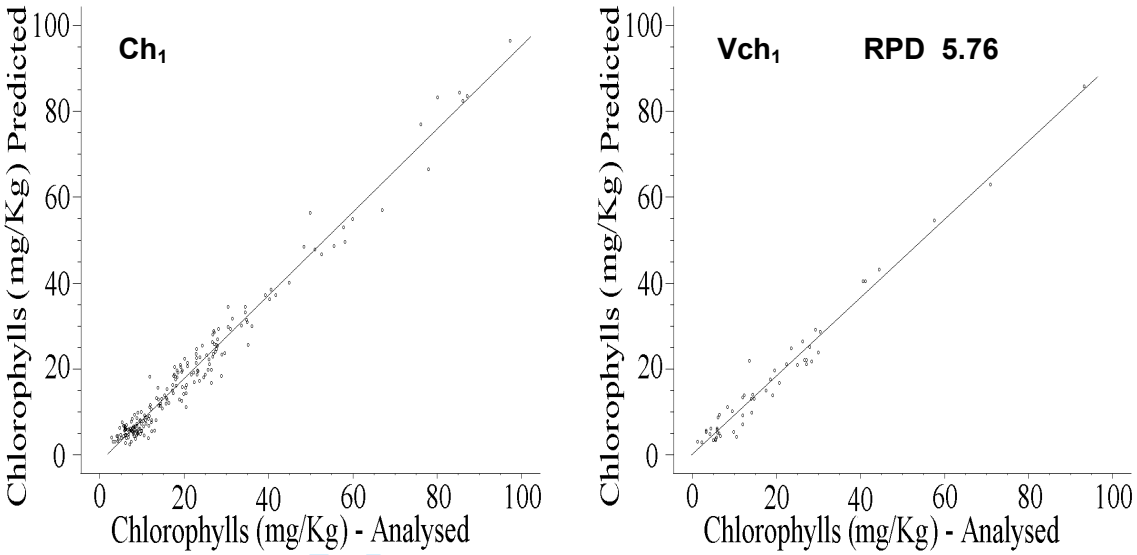

Figure 2. Predictive model VIS for total chlorophylls (Ch_1) and external validation (Vch_1).

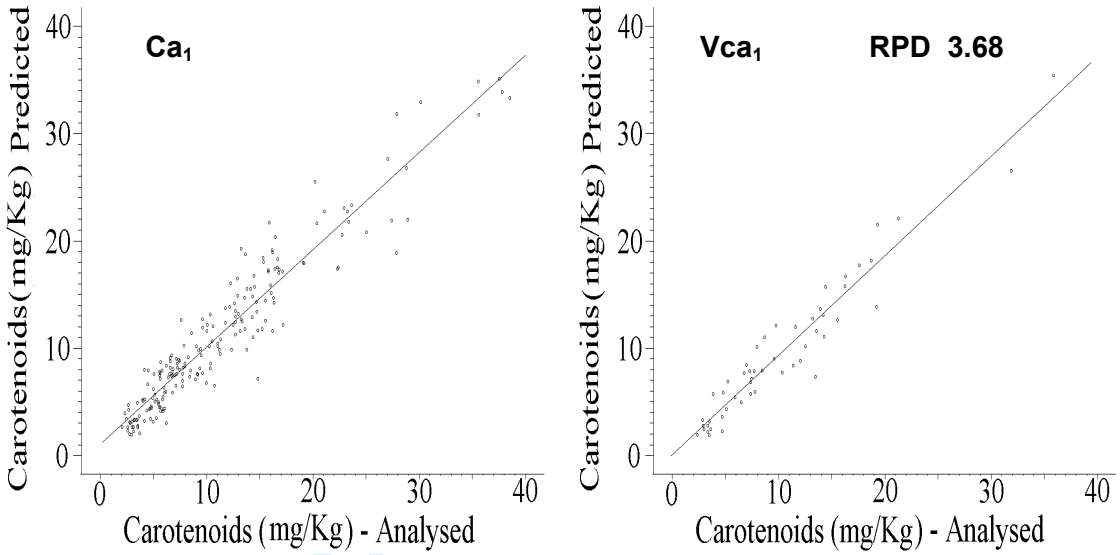

Figure 3

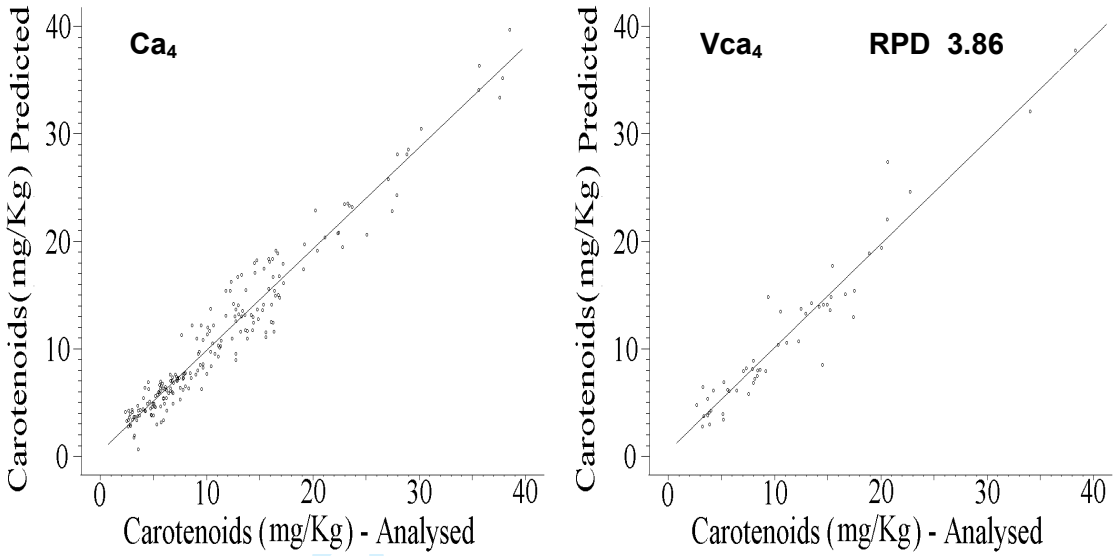

Figure 4