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Use of FTIR and UV–visible spectroscopy in determination of chemical characteristics of olive oils

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ABSTRACT

It was aimed to predict fatty acid ethyl ester (FAEE), wax, diacylglycerol (DAG) and color pigment contents of olive oils by using rapid and non-destructive spectroscopic techniques (FTIR and UV–vis) individually and in combination. Prediction models were constructed by using partial least squares (PLS) regression with cross and external validation. FAEEs were estimated best with FTIR + UV–Vis spectroscopy ($R^2_{cv.}$ = 0.84, $R^2_{pred.}$ = 0.90, and RPD = 3.0). PLS model with R^2_{cv} = 0.79, R^2_{pred} = 0.71, and RPD = 1.9 was obtained for the estimation of 1,2 DAG using FTIR spectral data. Major pigments, lutein, pheophytin *a* and their derivatives and total xanthophylls were quantified successfully by FTIR + UV–Vis with a range of R^2_{cv} of 0.71–0.85, R^2_{pred} of 0.70–0.84, and RPD = 1.5–2.5 values but the prediction of the rest of the pigments were poor (R^2_{cv} = 0.60–0.76, R^2_{pred}:0.42–0.62, and RPD = 1.2–1.5). Combination of two spectral data resulted in average prediction of wax content of oils ($R^2_{cal.}$ = 0.95, $R^2_{pred.}$ = 0.75, and RPD = 1.9). FTIR and UV–vis spectroscopic techniques in combination with PLS regression provided promising results for the prediction of several chemical parameters of olive oils; therefore, they could be alternatives to traditional analysis methods.

1. Introduction

Olive oil is a high profit food product due to its proven health benefits and its unique sensory characteristics. A rise in the price of this product due to increasing demand, makes olive oil quite prone to adulteration. Unfortunately, it is a very common practice to mix good quality olive oils with other vegetable oils as well as low quality olive oils such as pomace or deodorized olive oil in the market to obtain extra profit. Quality problems comprising fraudulent representation and mislabeling of olive oils cause consumers to lose confidence to this product [1].

Fraudsters continuously update their adulteration techniques as a response to new adulteration detection methods. In addition, olive oils have been started to be produced outside of traditional growth area of olives and olive oils coming from untraditional olive growth areas might have significant compositional differences compared to the limits of regulations based on European production area, even without any adulteration [2,3]. Therefore, new chemical parameters have been continuously introduced as quality indicators for olive oil. Color pigments (carotenoids, chlorophyll and derivatives), diacylglycerols (DAGs), and fatty acid ethyl esters (FAEEs) were proposed as potential quality and adulteration detection parameters [4]. Measurements of some of these compounds were already introduced as standard methods in updated regulations to overcome weaknesses of existing regulations [4]. FAAEs have been put into action by an EU 61/2011 regulation [5] as a quality parameter. FAAEs are produced as a result of enzymatic reaction of free fatty acids with low molecular weight alcohols (methanol and ethanol) under acidic conditions yielding methyl (FAMEs) and ethyl esters (FAEEs), respectively [6]. Threshold value for extra virgin olive oil lastly amended in EU 2016/2095 as FAAEs ≤ 35 mg/kg [7]. Besides being a quality parameter for olive oil, FAAEs are also considered as an indicator of adulteration with mildly refined olive oil [8].

Wax esters are formed by esterification of fatty acids to long-chain alcohols accumulated on the skin of olive fruits and they are also used as quality parameters for olive oil. Their presence in the olive oil is an indicator of olive oil purity (virgin olive oil ≤150 mg/kg) and also could be used to detect adulteration of extra virgin olive oil with olive-pomace oils [3,9].

DAGs could be found in olive oil as isomers of 1, 2, position which are the products of incomplete triacylglycerol synthesis while 1,3, DAGs are produced by enzymatic or chemical hydrolases [10]. 1,2-DAG isomers have been used as quality and freshness parameters in Australian [11] and Californian standards [12] to grade olive oil. According to both standards, olive oil could be graded as extra virgin olive oil only if it possesses ≥35% of 1,2 DAGs [3].

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Typical ratio of various pigments such as total amount of chlorophyll derivatives to total amount of carotenoids, ratio between carotenoids and lutein amount, percentage of violaxanthin and lutein as well as typical concentration range of various pigments were used for olive oil authentication [13]. It was also stated that degradation products of chlorophylls as well as carotenoids have potential in authentication of olive oils [4].

The standard analysis methods used in the determination of these chemical parameters are based on high cost wet chemistry techniques which produce waste and have long analysis time. Rapid, environmentally friendly and non-destructive spectroscopic analysis techniques such as mid-IR spectroscopy has been used to determine various important quality and/or purity parameters of olive oils such as fatty acid profile [14,15], oxidative stability, phenolic profile and total color pigments [15]. UV–vis spectroscopy has been also used in authentication studies of olive oil [16] while there are quite limited studies about its application as a quality tool for olive oil [17].

Mid-IR spectroscopy was used in the prediction of the total chlorophyll and carotenoid contents of olive oils rather than individual color pigments [15]. While a recent study successfully correlated near UV–Vis spectroscopy measurements with chromatographic results of main color pigments of olive oil [18]. However, there is no study in the literature that predicts the individual color pigment profile with FTIR and/or UV–vis spectroscopy. A preliminary study that successfully quantified FAAE content and the ratio between ethyl and methyl esters of olive oil using mid-IR spectroscopy with limited number of samples was also conducted [19]. Techniques such as near-infrared spectroscopy [20,21] and time domain reflectometry [22] were used to predict FAEE and FAME content in some recent studies. However, no studies found in the literature regarding the estimation of 1,2 DAGs in oils with mid-IR and UV–visible spectroscopic techniques.

Prediction of the several chemical parameters (DAGs, FAEEs, wax and individual pigment profile) of olive oil from UV–Vis and mid-IR spectroscopic data as well as from their fused form in combination with multivariate statistical methods is investigated in this study. As a result, these chemical parameters could be determined simultaneously with a single measurement by using the developed methodology.

2. Materials and methods

2.1. Olive oil samples

The olive oil samples were obtained from north, south, and middle part of Aegean Region of Turkey. Eighty-nine samples from two consecutive harvest years (52 samples from 2015 to 37 samples from 2016) were used in analyses. Samples were kept in dark glass bottles at refrigeration temperature (4 °C) and the head space of the bottles was flushed with nitrogen.

2.2. Chemical reagents

All reagents used in the analyses were analytical grade and obtained from Sigma-Aldrich (Germany) and Merck (Germany) unless otherwise stated.

2.3. Reference methods

2.3.1. Determination of fatty acid alkyl ester and wax contents

Fatty acid alkyl esters (FAAEs) as sum of ethyl (FAEEs) and methyl esters (FAMEs) are defined as a family of natural neutral lipids present in olive oils [8]. FAME and FAEE and wax contents of olive oil samples were determined according to a method by International Olive Council [23]. This method is based on the fractionation of olive oil with the addition of suitable internal standards and then direct analysis of the eluent by capillary gas chromatography (GC). Briefly, 15 g of silica gel suspended in n-hexane was placed into the glass column and was percolated with n-hexane to remove any impurities. Then, about 0.5 g of the olive oil sample was placed into a flask with the addition of internal standards as dodecyl arachidate solution for waxes and methyl heptadecanoate solution for alkyl esters together by mixing with sudan 1 indicator dye. Then, prepared sample was transferred to the chromatography column with the aid of n-hexane. Sample was percolated further with n-hexane/ethyl ether mixture (99:1) continuously until the sudan 1 color reaches to the bottom of the column. Resultant fractions were evaporated in a rotary evaporator (Heidolph Laborota-4000, Germany) at 20 °C. Fraction containing the methyl and ethyl esters as well as waxes was collected and diluted with 2 mL n-heptane. Diluted sample was filtered into a deep brown vial and then injected into GC.

GC analysis were conducted with Agilent 7890A GC-FID (USA). An HP-5 (30 m × 0.32 mm ID, 0.25 μm film, Agilent) column was used in analysis. The analytical conditions were as follows; on column inlet temperature was set to 70 °C and injection volume was 1 μL carried with hydrogen. The oven temperature was programmed as 80 °C (1 min), 20 °C/min to 140 °C (0 min), 5 °C/min to 335 °C (20 min). Detector temperature was 350 °C. The obtained peaks were further identified with GC-MS (Agilent 6890 N/5973 N Network GC/MSD System, USA) at the same conditions. The results were expressed in terms of mg/kg.

2.3.2. Determination of diacylgycerol content

A miniaturized column chromatography on a silica gel column was used to separate the isomeric DAGs as 1,2- and 1,3-isomers of C32-, C34- and C36- [24]. Firstly, olive oil sample was weighted and dissolved in 1 mL toluene. Then, it was transferred on to the prepared column with wetted silica gel while purging the flask with solvent mixture (isooctane/diisopropyl ether). Column was washed with 2 × 3.5 mL portions of the solvent mixture. DAGs were eluted with diethyl ether two times and eluate was collected in a pointed flask. Solvent was removed from the eluate with a rotary evaporator (Heidolph Laborota-4000, Germany) at 20 °C. Then, silylation reagent was added to the reaction vial containing the DAGs, and mixture was sealed and allowed to react for 20 min at room temperature. After silylation, 1 mL acetone was added into the mixture and 2 μL of the solution was used for the GC analysis. DAG isomers were identified with a GC by comparing the retention times of silylated reference standards composed of dipalmitin and distearin.

GC analysis was carried with Agilent 7890A GC-FID (USA). The column was capillary GC column as Rtx-5MS (60 m × 0.25 mm ID, 0.1 μm film, Restek, USA). Injection volume was 2 μL having 1:20 split ratio carried with hydrogen. The oven temperature was programmed to 240 °C (1 min) followed by 10 °C/min to 320 °C (16 min). Both injector and detector temperatures were set to 340 °C. The results were expressed in terms of percentages.

2.3.3. Quantification of individual chlorophylls and carotenoids

The method adapted from Ref. [25] was used to determine the pigment profiles of olive oils. Samples were extracted by the solid-phase extraction (SPE) using octadecyl (C18) disposable extraction columns (Agilent, USA). SPE column was conditioned first with methanol and then with hexane. One g of oil dissolved in 4 mL of n-hexane was injected to the column and then washed with n-hexane. Firstly, hexanic phase containing β-carotene was collected and evaluated with UV–vis spectroscopy (Shimadzu UV-2450 Spectrophotometer, Japan). Then, the remaining pigments were eluted with 5 mL acetone. The acetone phase was taken to dryness and collected in 0.3 mL of acetone for HPLC (Agilent 1200 HPLC, USA) analysis. The sample dissolved in acetone was injected into HPLC-DAD system. Separation was performed on a column packed with Waters Spherisorb S5ODS2 (25 cm × 4.6 mm ID, 5 μm particle size, Supelco, Germany) protected with a guard cartridge (3.2–4.6 mm ID, Supelco, Germany) packed with the same material as the column.

The pigments were eluted at a rate of 1 mL/min. The eluents were water + ion pair reagent as mobile phase (A) and acetone-methanol as

mobile phase (B) [26]. The gradient scheme for eluents indicated in a study in the literature [25] were as follows; initial composition as 75% (A) and 25% (B) and then (A) was decreased to 50% while (B) was increased to 50% in 10 min simultaneously and both maintained for 2.5 min. Then, (A) was further decreased to 20% in 1.5 min, (B) was increased to 80% at the same time and both maintained for 2 min. After that, (A) was lowered to 0% in 5 min while (B) was raised to 100% and both were kept constant for 14 min. After that, initial conditions were reached in 5 min. The pigments were identified simultaneously at varying wavelengths by comparing the retention times of external standards. Pheophytins *a* and *b* standards were prepared with acid treatment of chlorophyll *a and b* solutions, respectively [27]. The rest of the standards were obtained commercially. 5-point calibration curve at distinct wavelengths was obtained for each standard as follows: 410 nm for pheophytin *a* and its derivative, 430 nm for chlorophyll *a* and its derivative, 435 nm for measure pheophytin *b* and its derivative, 446 nm for lutein and its derivatives and other xanthophylls (as total xanthophylls), and 466 nm for chlorophyll *b* and derivative. The results were expressed in terms of mg/kg.

2.4. Spectroscopic methods

2.4.1. Fourier transform infrared (FTIR) spectroscopy analysis

Mid-infrared spectra (4000-650 cm^{-1}) were obtained by Perkin Elmer Spectrum 100 FTIR spectrometer (Perkin Elmer Inc., USA) possessing a deuterated tri-glycine sulphate detector (DTGS). The spectrometer is equipped with premium HATR ZnSe 45° trough plate accessory (HATR). Each spectrum was scanned 64 times with a resolution of 4 cm^{-1} and scan speed of 1 cm/s. The crystal was cleaned with hexane, ethanol and deionized water prior to each analysis.

2.4.2. Ultraviolet–visible (UV–vis) spectroscopy

A UV–visible spectrophotometer (Shimadzu UV-2450 Spectrophotometer, Japan) was used to collect the spectra of olive oil samples in a plastic cuvette from 200 to 800 nm by using air as the blank. All the measurements were taken at absorbance mode in 2.0 nm sampling interval with fast scan speed by adjusting slit width to 5.0 nm.

2.5. Statistical analysis

All the statistical analyses were performed with SIMCA 14.0 software (Umetrics, Sweden). Whole FTIR (4000-650 cm^{-1}) and UV–vis (200–800 nm) spectra were used in the analysis. Partial least squares (PLS) regression was used throughout the study to construct the prediction models of the chemical parameters from FTIR and UV–vis spectra. Moreover, data fusion approach was also used to enhance the prediction ability of the PLS models by combining FTIR and converted UV–vis spectra (650-4000 cm^{-1} + 12 500-50000 cm^{-1}) in a low-level fusion. In low-level fusion, all the data from different sources were simply concatenated into a single matrix [28].

Prior to construction of calibration models by PLS regression, spectroscopic data were pre-processed to increase the prediction ability of the models by eliminating spectral variation. Mean-centering and UV-scaling were used in all of the model construction to enhance spectral signal. As pre-processing methods, first- or second-order derivative, multiplicative scattering correction (MSC), and standard normal variate (SNV) transformation were used in specific model construction [29]. The first- and second-order derivative of the spectroscopic data were calculated from moving quadratic sub-models with 15 data point long and the distance between each data point is set to 1 excluding the edge effects.

After obtaining pre-processed calibration model by splitting approximately 2/3 of the raw data (59 samples), reliability of the proposed models was checked with randomly selected external validation data set (1/3) (30 samples) as well as cross-validation. Performance of constructed models were checked by several performance parameters. Correlation coefficient (R^2) was used to reveal robustness of the corresponding models ($R^2_{cal.}$ for calibration, $R^2_{cv.}$ for cross validation, $R^2_{pred.}$ for external validation) [30]. Parameters related with error such as root mean square error of prediction (RMSEP), root mean square error of calibration (RMSEC), root mean square value of cross-validation (RMSECV) were also evaluated. As another useful parameter, number of latent variables (LVs) were also used in the model performance assessment. To obtain a robust model without overfitting, it was expected to use as few numbers of LVs as possible with high value of R^2 and low value of RMSEC/RMSEP [31].

In addition to these parameters, residual predictive deviation (RPD) and slope of the models were calculated. The RPD value for external validation models was defined as the ratio of the standard deviation of the external validation variables to RMSEP and high value indicates a better model [32]. All the statistical parameters except RPD values were calculated with SIMCA software while the RPD values were calculated according to Ozturk et al. [30].

3. Results and discussion

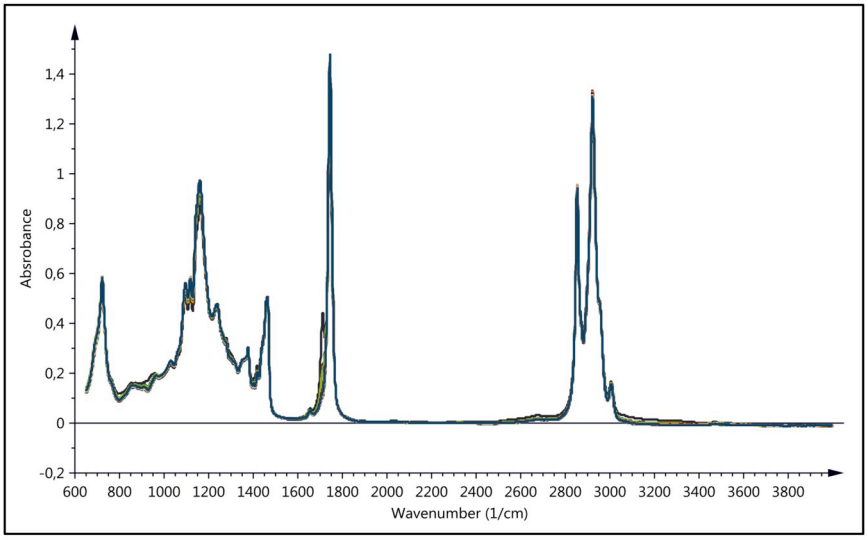
3.1. Chemical interpretation of spectral data

Raw and transformed forms of FTIR spectra of olive oil samples are shown in Fig. 1. Major peaks in the spectra and vibration modes of corresponding functional groups could be summarized as follows; band at 3009 cm^{-1} is due to C−H stretching of olefinic double bonds attributed to unsaturated fatty acid, while bands centered at 2924 and 2854 cm^{-1} known as methylene absorbance peaks are associated with antisymmetric and symmetric stretching vibrations of aliphatic C−H in $-CH_2$ and terminal $-CH_3$ groups, respectively [33]. In addition, sharp peak at about 1745 cm^{-1} known as ester peak because of C=O stretching vibration of carbonyl groups of the triacylglycerols while weak band at 1654 cm^{-1} is attributed to stretching vibration of the C=C group of *cis*-olefins [34]. Bands in fingerprint region of 1464–983 cm^{-1} are assigned to bending vibrations of $-CH_2$ and $-CH_3$ aliphatic groups as well as rocking vibrations [1,34]. Symmetric H−C−H bending at 1377 cm^{-1} could be attributed to glycerol group O−CH_2 (mono-, di- and triglycerides) [35]. CH_2 scissoring are observed at 1462 cm^{-1} whereas band between 1125 and 1095 cm^{-1} wavenumber is due to the stretching vibration of C=O ester groups and $-CH_2$ wag [34]. The last major peak located near 723 cm^{-1} could be associated with overlapping of the $(CH_2)_n$ rocking vibration and out of plane vibration (−CH wag) of *cis*-di-substituted olefins [34].

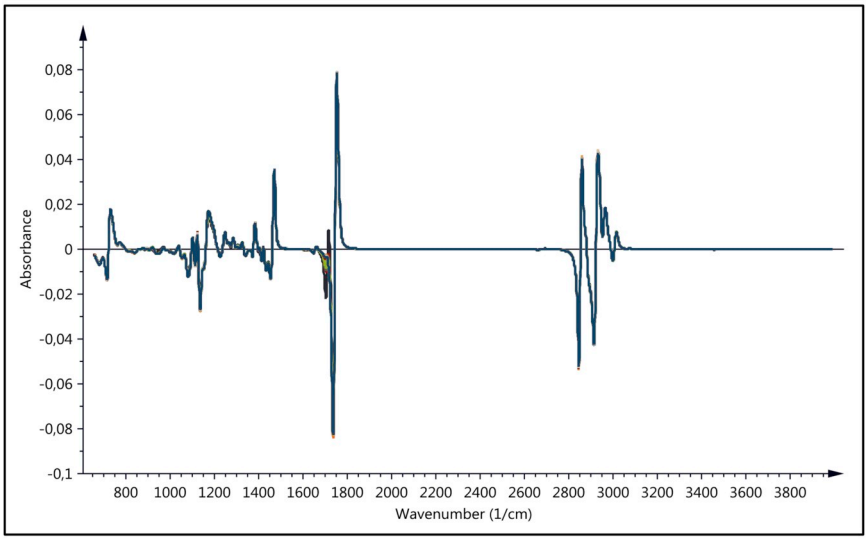
Typical UV–vis spectra of olive oil and their transformed forms are shown in Fig. 2 and they are highly correlated with pigment profile. Especially, pigments (chlorophyll and carotenoid) of olive oil dominate the light absorption between 390–720 nm. Maximum absorption for lutein, β-carotene, pheophytin a and pheophytin b were detected in following wavelengths: 486, 455, and 432 nm; 490 and 462 nm; 670 and 414; 657 and 437 nm, respectively [36].

3.2. Prediction of FAMEs, FAEEs, FAAEs and waxes by PLS regression

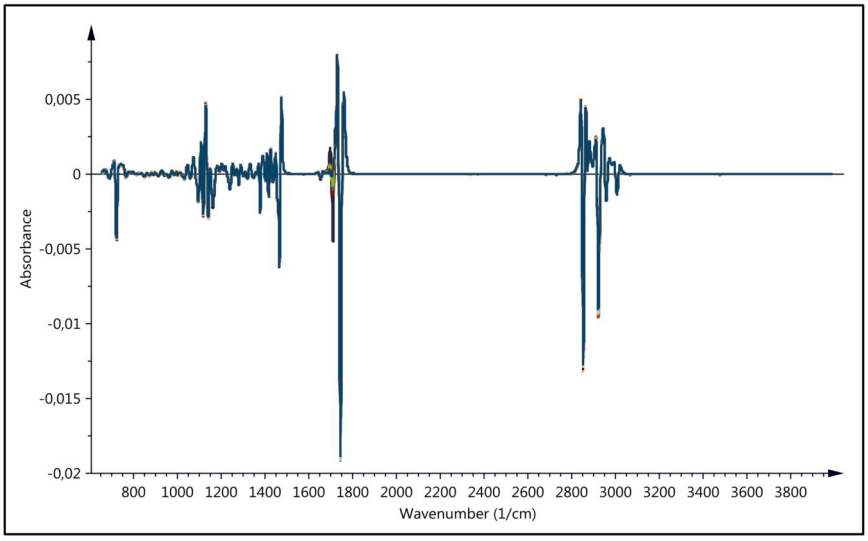
FAAE and wax contents of the olive oil samples were quantified with the reference methods. Then, PLS regression analysis of FTIR, UV–vis, and fused spectral data was performed to predict FAAEs including FAMEs and FAEEs as well as wax content of olive oils. Results of the measured chemical parameters of the olive oil samples are briefly mentioned since this is a study which aims to predict the amounts of these parameters from spectroscopic data rather than a chemical characterization investigation. Range of chemical parameters in these types of studies are quite important since a wide range of measured components provides construction of robust models and different grades of olive oils (free fatty acid range: 0.2–4.97%) from various geographic origins and two harvest years were used as samples to provide the necessary variability of chemical composition. Ranges and



(a)



(b)



(c)

Fig. 1. (a) Raw, (b) first derivative and (c) second derivative FTIR spectra of olive oil samples.

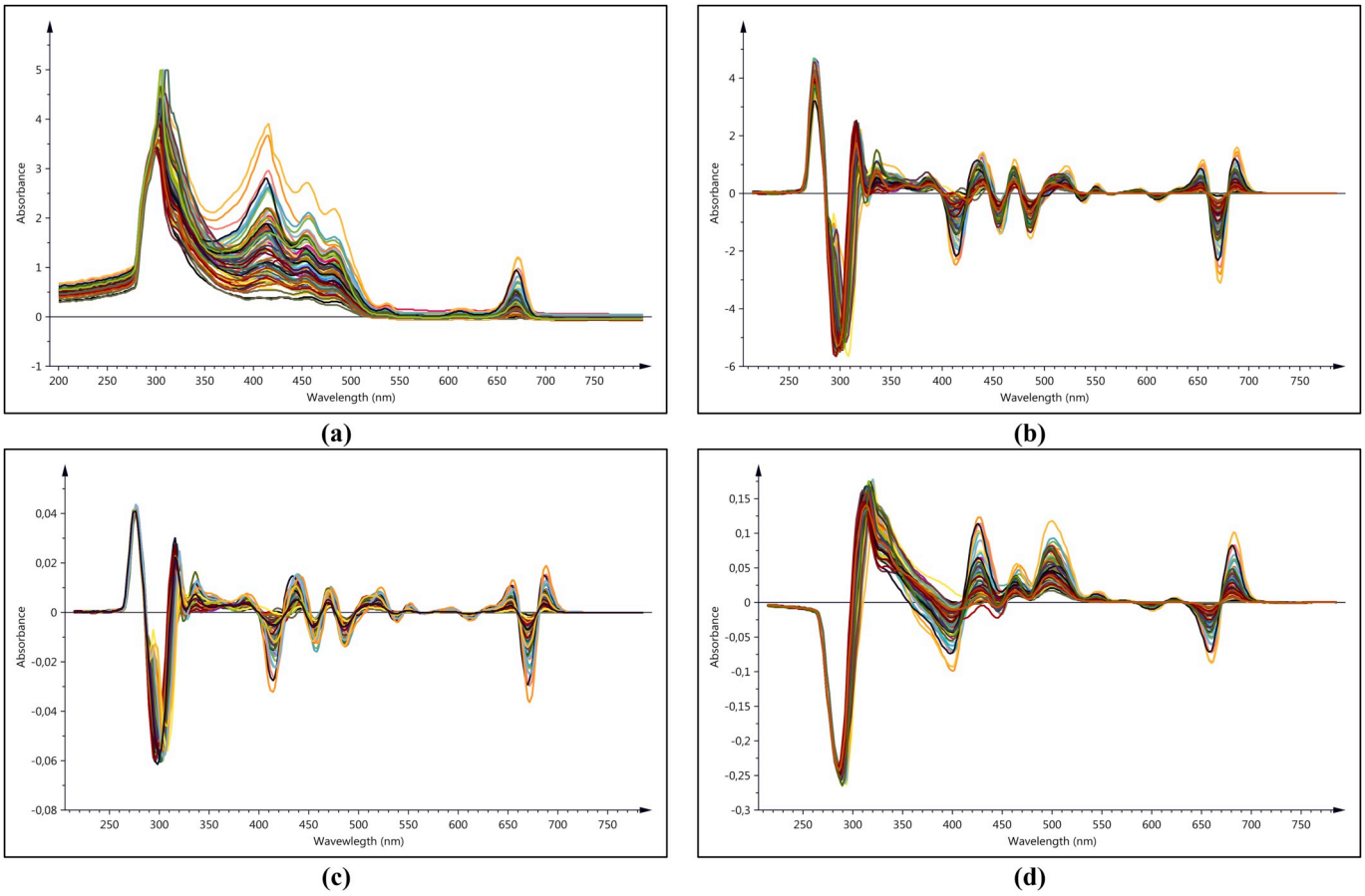


Fig. 2. (a) Raw, (b) first derivative, (c) second derivative and (d) second derivative + MSC + SNV of UV–vis spectra of olive oil samples.

means of the reference data are presented in Table 1. These values are comparable with the ranges found in the literature [20,21]. Second-order derivative of FTIR spectra is shown in Fig. 1c and, second order derivative + MSC + SNV were used in alkyl ester and wax prediction from UV–vis (Fig. 2d) and FTIR + UV–vis spectral data since they resulted in the development of the best models.

Statistical parameters of regression models for each spectroscopic approach are listed in Table 2. FTIR spectral data were found successful in quantification of FAMEs (3.14–539.04 mg/kg) with 3 LVs explaining 99.4% and 92.6% of the total variance in the calibration and prediction data set, respectively. In addition, $R^2_{cal.}$ and $R^2_{pred.}$ have high values of 0.99 and 0.93, respectively also with a high RPD value (3.1) required for a successful prediction model. Moreover, RMSEC and RMSEP values (6.06 and 16.97, respectively) were reasonable when compared with the range and magnitude of FAMEs.

UV–vis spectra of olive oil samples were also used to predict FAMEs. The regression models showed that UV–vis spectra were not that successful compared to FTIR spectral data in prediction with lower statistical values ($R^2_{cal.}$ = 0.72, $R^2_{cv.}$ = 0.60, $R^2_{pred.}$ = 0.47, and RPD = 1.3) including 2 LVs which explains 71.8% and 46.8% of calibration and prediction sets, respectively.

Combination of FTIR and UV–vis spectra were also used to investigate if there was any improvement of the constructed models. It was observed that FTIR + UV–vis data provided similar prediction ability on determination of FAMEs content of olive oils compared to FTIR spectroscopy alone (Table 2). FAMEs could be predicted well with robust model parameters ($R^2_{pred.}$ = 0.91 and RPD = 2.9). There is no study in the literature related with direct determination of total methyl ester content of olive oils with FTIR and/or UV–vis spectroscopy while some other studies applied other methods such as near-infrared (NIR) spectroscopy and time domain reflectometry (TDR). A recent study showed that NIR spectroscopy in combination with PLS regression could predict FAMEs content of olive oils quite successfully with high R^2 value for both calibration (0.95) and validation (0.92) set [21]. Also, TDR was found as a promising method in quantification of FAMEs content of olive oils with PLS regression model having good R^2 value for both calibration (0.996) and external validation (0.905) [22].

FAEE is a chemical parameter that is used in regulations about the quality of olive oil [7] and it was also predicted using different spectral techniques. It was found that FTIR was successful in predicting total ethyl esters (1.66–243.59 mg/kg) found in olive oil samples with 4 LVs explaining 99.4% and 87.7% of calibration and prediction models, respectively. R^2_{cal} of 0.99., $R^2_{cv.}$ of 0.85 and $R^2_{pred.}$ of 0.88 were determined and these values indicate good prediction ability (Table 2). The model performance was also supported by tolerable error values of RMSE for calibration (4.92), cross validation (27.43), and prediction (23.64) with robust RPD of 2.8 and slope of 0.99 values.

The regression models developed using UV–vis spectra for the prediction of FAEEs content have average statistical values ($R^2_{cal.}$ = 0.77, $R^2_{pred.}$ = 0.78, and RPD = 2.1) with 3 LVs which explains 76.8% and 77.7% of calibration and prediction sets, orderly (Table 2).

Combination of FTIR + UV–vis spectral data performs well and is slightly better than FTIR in quantification of FAEEs with higher $R^2_{pred.}$ = 0.90 and RPD = 3.0 as well as lower RMSEP value of 21.98 (Table 2). The FTIR and fused data findings were comparable with the literature in which NIR spectroscopy and TDR were used. Two different studies conducted by NIR spectroscopy reveals that NIR could be used in FAEEs prediction promisingly [20,21]. PLS model parameters in a study using NIR spectroscopy [21] resulted in $R^2_{pred.}$ = 0.88 and 0.89 values and generated models in another study with NIR spectroscopy had $R^2_{cv.}$ = 0.73 and RPD = 1.92 [20]. TDR provided a robust PLS regression model for alkyl ester determination ($R^2_{pred.}$ = 0.923) with very

Table 1
Range and mean of reference chemical parameters of olive oil samples.

Measured parameters	Range	Mean	Standard deviation
Fatty acid alkyl esters and wax (mg/kg)			
FAMEs	3.14–539.04	46.44	68.89
FAEEs	1.66–243.59	48.45	62.14
FAAEs	6.94–659.00	94.88	120.42
Waxes	5.26–89.59	26.96	17.84
Diacylglycerols (%)			
C34 1,2	6.33–12.57	8.89	1.50
C34 1,3	5.97–18.65	11.71	2.51
C36 1,2	20.77–55.72	34.96	7.52
C36 1,3	25.40–55.37	44.21	6.44
Total 1,2	28.14–68.39	43.90	8.77
Total 1,3	31.61–71.86	56.10	8.77
Ratio	0.39–2.16	0.83	0.33
Color pigments (mg/kg)			
Pheophytin *a*	0.16–19.21	5.89	3.53
Pheophytin *a* der.	0.03–2.59	0.80	0.49
Chlorophyll *a*	0.01–0.26	0.04	0.04
Chlorophyll *a* der.	0.00–0.12	0.04	0.03
Pheophytin *b*	0.04–0.65	0.17	0.12
Pheophytin *b* der.	0.02–0.73	0.17	0.14
Total Xanthophyll	0.24–3.35	0.98	0.57
Lutein	0.60–6.29	2.28	1.25
Lutein der.	0.06–1.35	0.39	0.28
Lutein second der.	0.05–1.38	0.26	0.18
Chlorophyll *b*	0.10–1.70	0.51	0.36
Chlorophyll *b* der.	0.03–0.39	0.12	0.09
***β*-carotene**	0.66–6.79	3.18	1.29

limited number of samples [22].

Total alkyl ester (FAMEs + FAEEs) content of olive oils was also determined by FTIR spectroscopy in combination with PLS regression. Oil samples used in this study has a wide range of FAAE content (6.94–659.00 mg/kg). As in other parameters FTIR provided successful quantification of FAAEs. Constructed PLS model contains 3 LVs explaining 98.9%, 87.3%, and 95.7% of the total variation of calibration, cross validation, prediction sets, respectively. Additionally, the model possesses quite high regression coefficients (0.99, 0.87, 0.96) and RPD (4.1) value with a very reliable slope (0.99) (Table 2). The obtained results are in accordance with the finding of a study in literature in which FTIR spectroscopy was applied to the limited number of olive oil samples with narrow FAAE range but still good $R^2_{cal.}$ of 0.98 was obtained in prediction of FAMEs + FAEEs [19]. However, UV–vis spectroscopy was not as good as FTIR spectroscopy for FAAEs determination, and it only provided average prediction power with $R^2_{cal.}$ = 0.78, $R^2_{pred.}$ = 0.74, and RPD = 1.9 values. On the other hand, combination of FTIR and UV–vis resulted in a robust prediction model ($R^2_{cal.}$ = 0.96, $R^2_{pred.}$ = 0.96, and RPD = 3.4). NIR spectroscopy [20,21] and TDR [22] have been also used in quantification of total alkyl esters with promising results. In the present study, variable importance for the projection (VIP) values were determined for FTIR and UV–vis models to see the importance of variable effect on methyl, ethyl, and alkyl esters prediction model explanation. It was observed that in FTIR related models the bands between 1700 and 1800 cm^{-1} and fingerprint region (1464–983 cm^{-1}) have the highest VIP values and the observed peaks could be attributed to the stretching of C=O as typical of esters and contain distinct peaks correlated with the amount of methyl ester and ethyl ester, respectively [33,35]. Also, VIP values of the constructed models with UV–vis revealed that peaks between 200 and 300 nm comprising absorption of conjugated dienes and trienes were important.

Total wax content (5.26–89.59 mg/kg) was also estimated with FTIR and the obtained PLS model possessed average quantification power with $R^2_{cal.}$ = 0.99, $R^2_{pred.}$ = 0.71, and RPD = 1.7 (Table 2). While, UV–vis spectral data were not good enough to estimate total wax content because of low R^2 and other statistical parameters (Table 2). However, FTIR + UV–vis data allowed better prediction of wax content of olive oils compared to only FTIR spectroscopy. FTIR + UV–vis spectra have average prediction power for total wax quantification with tolerable statistical parameters ($R^2_{cal.}$ = 0.95, $R^2_{pred.}$ = 0.75, and RPD = 1.9) (Table 2). Despite low prediction ability of the proposed model, it could still be used for screening purposes of olive oil quality to distinguish low, medium and high values of waxes. To the best of our knowledge there is no comparable literature that predicts wax content of olive oils with any spectroscopic techniques. However, TDR was used unsuccessfully in the same type of investigation [22].

3.3. Prediction of DAGs content by PLS regression

Ranges and means of DAG content of the olive oil samples are shown in Table 1. C32 values for 1,2 and 1,3 DAG isomers were also quantified but they were in negligible amounts (data not shown). Similar ranges of DAG content of Turkish olive oils obtained from 4 distinct olive cultivars were reported [37].

According to the Australian and Californian standards, total 1,2% DAG content is a representative parameter for the quality of olive oil. Consequently, it was focused on total 1,2 DAG% (28.14–68.39%) in this investigation rather than other individual DAGs. Model parameters for each spectroscopic technique and their combination are given in Table 3. PLS model developed with first order derivative of FTIR spectral data (Fig. 1b) for the prediction of total 1,2 DAG content have 5 LVs which explains 98.6%, 79.2%, and 70.9% of total variations with respect to calibration, cross-validation and external validation models.

Table 2
Statistical parameters of PLS regression models for prediction of fatty acid alkyl ester and wax contents of olive oils by different spectroscopic methods.

Method	Parameter (mg/kg)	Transformation	LVs	R^2 cal.	R^2 cv.	R^2 pred.	RMSEC	RMSECV	RMSEP	RPD	Slope
FTIR	FAMEs	2nd order derivative	3	0.99	0.87	0.93	6.06	41.63	16.97	3.1	0.99
	FAEEs	2nd order derivative	4	0.99	0.85	0.88	4.92	27.43	23.64	2.8	0.99
	FAAEs	2nd order derivative	3	0.99	0.87	0.96	13.73	60.10	26.69	4.1	0.99
	Waxes	2nd order derivative	4	0.99	0.77	0.71	1.80	9.45	11.70	1.7	0.99
UV–vis	FAMEs	2nd order + MSC + SNV	2	0.72	0.60	0.47	24.41	28.54	77.86	1.3	0.72
	FAEEs	2nd order + MSC + SNV	3	0.77	0.63	0.78	30.62	37.55	30.49	2.1	0.77
	FAAEs	2nd order + MSC + SNV	3	0.78	0.61	0.74	59.27	78.49	61.07	1.9	0.78
	Waxes	2nd order + MSC + SNV	2	0.71	0.60	0.61	10.32	11.95	11.01	1.4	0.71
FTIR + UV–vis	FAMEs	2nd order + MSC + SNV	3	0.99	0.89	0.91	5.39	40.39	17.83	2.9	0.99
	FAEEs	2nd order + MSC + SNV	4	0.99	0.84	0.90	5.76	26.52	21.98	3.0	0.99
	FAAEs	2nd order + MSC + SNV	2	0.96	0.86	0.96	26.78	54.85	32.45	3.4	0.96
	Waxes	2nd order + MSC + SNV	3	0.95	0.64	0.75	3.90	10.74	9.75	1.9	0.95

Table 3
Statistical parameters of PLS regression models for prediction of DAGs by different spectroscopic methods.

Method	Parameter	Transformation	LVs	R^2 cal.	R^2 cv.	R^2 pred.	RMSEC	RMSECV	RMSEP	RPD	Slope
	(%)										
FTIR	C34 1,2	1st order derivative	3	0.88	0.62	0.66	0.55	1.07	0.81	1.7	0.88
	C34 1,3	1st order derivative	5	0.99	0.83	0.80	0.31	1.26	1.03	2.2	0.99
	C36 1,2	1st order derivative	5	0.99	0.79	0.73	0.94	4.29	3.59	1.9	0.99
	C36 1,3	1st order derivative	5	0.98	0.77	0.66	0.89	4.02	3.42	1.7	0.98
	Total 1,2	1st order derivative	5	0.99	0.79	0.71	1.13	5.09	4.29	1.9	0.99
	Total 1,3	1st order derivative	5	0.99	0.79	0.71	1.13	5.09	4.29	1.9	0.99
	Ratio	1st order derivative	5	0.99	0.82	0.40	0.03	0.16	0.29	1.3	0.99
UV–vis	C34 1,2	1st order derivative	2	0.40	0.27	0.31	1.18	1.27	1.28	1.2	0.40
	C34 1,3	1st order derivative	2	0.66	0.58	0.65	1.52	1.65	1.51	1.6	0.66
	C36 1,2	1st order derivative	2	0.62	0.56	0.54	4.73	4.97	5.17	1.5	0.62
	C36 1,3	1st order derivative	2	0.52	0.44	0.47	4.51	4.71	4.84	1.4	0.52
	Total 1,2	1st order derivative	2	0.59	0.52	0.51	5.74	5.99	6.26	1.4	0.59
	Total 1,3	1st order derivative	2	0.59	0.52	0.51	5.74	5.99	6.26	1.4	0.59
	Ratio	1st order derivative	2	0.53	0.43	0.51	0.23	0.24	0.24	1.4	0.53
FTIR + UV–vis	C34 1,2	1st order derivative	3	0.88	0.60	0.69	0.55	1.11	0.77	1.8	0.88
	C34 1,3	1st order derivative	6	0.99	0.88	0.83	0.20	1.22	0.95	2.4	0.99
	C36 1,2	1st order derivative	4	0.98	0.76	0.66	1.16	4.51	4.04	1.7	0.98
	C36 1,3	1st order derivative	6	0.99	0.82	0.56	0.48	4.01	4.03	1.5	0.99
	Total 1,2	1st order derivative	4	0.98	0.74	0.64	1.41	5.39	4.78	1.7	0.98
	Total 1,3	1st order derivative	4	0.98	0.74	0.64	1.41	5.39	4.78	1.7	0.98
	Ratio	1st order derivative	6	0.99	0.80	0.36	0.02	0.17	0.30	1.3	0.99

$R^2_{cal.}$ (0.99), $R^2_{cv.}$ (0.79) and $R^2_{pred.}$ (0.71) values further confirmed the goodness of the models for 1,2% DAGs from chemical data. Close RMSEC (1.13), RMSECV (5.09), and RMSEP (4.29) values indicate a robust model with no over fitting. Slope of the calibration curve (0.99) is good for high reliability with RPD value of 1.9. For the other individual DAG parameters similar performance values were obtained ($R^2_{cal.}$ = 0.88–0.99, $R^2_{cv.}$ = 0.62–0.83, $R^2_{pred.}$ = 0.40–0.80, and RPD = 1.3–2.2). The highest VIP value of the corresponding model was around 1360 cm^{-1} accompanied with 3500 cm^{-1} which are highly correlated with diglyceryl compounds. Thus, FTIR spectroscopy could be used for screening of olive oil quality according to a threshold value of 35 mg/kg for 1,2 DAGs.

However, first order derivative of UV–vis spectroscopy (Fig. 2b) and FTIR + UV–vis combinations were not successful compared to FTIR spectral data alone in predicting total 1,2 DAGs content with lower performance parameters, $R^2_{pred.}$ = 0.51 and RPD = 1.4 for UV–vis and $R^2_{pred.}$ = 0.64 and RPD = 1.7 for fused data. Negligible contribution of UV–vis spectrum to the generated models of DAGs could be because of no relation of pigmented compounds with DAG content [21].

In the literature, there is no study about quantification of DAGs by FTIR spectroscopy. However, NMR spectroscopy have been used in qualitative and quantitative analysis of the diglyceride content [38]. Nevertheless, NMR study was based on direct determination of target compounds rather than prediction of them.

3.4. Prediction of chlorophyll and carotenoid content by PLS regression

Details about concentration ranges of the pigments in olive oil samples are provided in Table 1. However, it might not be very easy to compare the results with the literature since pigment concentration is variable depending on cultivar, geographic origin, maturity of olives, climate and storage conditions [18]. In the present study, pheophytin a (0.16–19.21 mg/kg), total xanthophylls (0.24–3.35 mg/kg), lutein (0.60–6.29 mg/kg), and β-carotene (0.66–6.79 mg/kg) were determined as major pigments while the rest of the pigments have lower quantities (Table 1).

Statistical parameters for prediction models developed for chlorophylls and carotenoids using FTIR, UV–vis and their combination data are listed in Table 4. Second-order derivative of each spectroscopic data was used in individual chlorophyll and carotenoid predictions. Second derivative of UV–vis spectroscopy (Fig. 2c) was more successful compared to second derivative of FTIR (Fig. 1c) in prediction of individual color pigments. FTIR measurement might not be sensitive enough to small amounts of pigments present in olive oil; therefore, predictive power of the models developed with data from this spectroscopic technique might be low. However, data fusion improves the prediction ability of UV–vis spectroscopy. In addition, reliable prediction models for β-carotene with any studied spectroscopic techniques could not be obtained. The range of β-carotene concentrations for the studied samples was very limited and multivariate regression techniques generally provide better models with samples having wider concentration ranges.

UV–vis spectroscopy provided relatively promising results in prediction of individual pigments. The best regression models were obtained for the pigments with the highest concentrations, lutein and its derivative, pheophytin *a* and its derivative, and total xanthophylls. As can be seen from Table 4, regression coefficients $R^2_{cal.}$, $R^2_{pred.}$ and RPD values were found in the range of (0.62–0.86), (0.65–0.84), and (1.7–2.5), respectively indicating successful prediction. In addition, constructed models were not overfitted since they have close and low error values for each parameter. According to a study in the literature near-UV-vis spectra of olive oils were also highly correlated with the main pigments of olive oil [36]. The highest VIP values of the proposed models for the present study were around 450 and 480 nm for lutein and its derivative and also around 670 nm for pheophytin *a* and its derivative which are comparable with the previous study.

UV–vis spectral data provided moderate prediction for the rest of the pigments. The reason for lower prediction ability than that of major pigments could be because of the lower amount of these pigments in olive oil. These pigments includes chlorophyll *a* and its derivative ($R^2_{pred.}$ = 0.66 and 0.46, RPD = 1.1 and 1.3, respectively), pheophytin *b* and its derivative ($R^2_{pred.}$ = 0.55 and 0.61, RPD = 1.5 and 1.2, respectively), lutein second derivative ($R^2_{pred.}$ = 0.66 and RPD = 1.5), chlorophyll *b* and its derivative ($R^2_{pred.}$ = 0.67 and 0.60, RPD = 1.4 and 1.6, respectively). One recent study in the literature successfully correlated four main pigments of olive oil, β-carotene, lutein, pheophytin *a* and pheophytin *b* with near-UV-vis spectroscopy using very limited number of samples [18]. Fluorescence spectroscopy was also used in successful determination of chlorophyll *a and b* and pheophytins *a* and *b* content of 42 olive oil samples in combination with PLS regression [39].

Data fusion approach was found slightly better, in general, on prediction of major pigments compared to UV–vis alone. The statistics

Table 4
Statistical parameters of PLS regression models for prediction of individual color pigments of olive oil by different spectroscopic methods.

Method	Parameter ((mg/kg))	Transformation	LVs	R^2 cal.	R^2 cv.	R^2 pred.	RMSEC	RMSECV	RMSEP	RPD	Slope
FTIR	Pheophytin *a*	2nd order derivative	5	0.99	0.72	0.18	0.19	2.42	3.60	1.1	0.99
	Pheophytin *a* der.	2nd order derivative	5	0.99	0.70	0.03	0.03	0.32	0.57	1.0	0.99
	Chlorophyll *a*	2nd order derivative	5	0.99	0.75	0.18	0.00	0.03	0.02	0.9	0.99
	Chlorophyll *a* der.	2nd order derivative	5	0.99	0.66	0.36	0.00	0.02	0.02	1.2	0.99
	Pheophytin *b*	2nd order derivative	5	0.99	0.71	0.04	0.01	0.10	0.13	1.0	0.99
	Pheophytin *b* der.	2nd order derivative	3	0.94	0.49	0.00	0.03	0.12	0.15	0.9	0.94
	Total Xanthophyll	2nd order derivative	4	0.99	0.61	0.46	0.07	0.41	0.37	1.2	0.99
	Lutein	2nd order derivative	5	0.99	0.75	0.41	0.08	0.71	1.27	1.2	0.99
	Lutein der.	2nd order derivative	4	0.96	0.54	0.49	0.02	0.20	0.21	1.4	0.96
	Lutein second der.	2nd order derivative	5	0.99	0.79	0.60	0.01	0.12	0.12	1.5	0.99
	Chlorophyll *b*	2nd order derivative	3	0.96	0.72	0.24	0.07	0.21	0.37	1.1	0.96
	Chlorophyll *b* der.	2nd order derivative	5	0.99	0.68	0.30	0.01	0.07	0.08	1.2	0.99
UV–vis	Pheophytin *a*	2nd order derivative	2	0.77	0.64	0.75	1.61	1.99	2.00	2.0	0.77
	Pheophytin *a* der.	2nd order derivative	1	0.62	0.60	0.65	0.28	0.28	0.34	1.7	0.62
	Chlorophyll *a*	2nd order derivative	3	0.69	0.45	0.66	0.02	0.03	0.02	1.1	0.69
	Chlorophyll *a* der.	2nd order derivative	3	0.75	0.56	0.46	0.01	0.02	0.02	1.3	0.75
	Pheophytin *b*	2nd order derivative	2	0.67	0.55	0.55	0.07	0.08	0.08	1.5	0.67
	Pheophytin *b* der.	2nd order derivative	3	0.86	0.67	0.61	0.06	0.09	0.09	1.2	0.86
	Total Xanthophyll	2nd order derivative	3	0.86	0.78	0.84	0.22	0.28	0.22	2.5	0.86
	Lutein	2nd order derivative	3	0.84	0.74	0.76	0.55	0.67	0.57	2.0	0.84
	Lutein der.	2nd order derivative	3	0.83	0.72	0.83	0.13	0.16	0.10	2.3	0.83
	Lutein second der.	2nd order derivative	2	0.55	0.38	0.66	0.14	0.16	0.09	1.5	0.55
	Chlorophyll *b*	2nd order derivative	4	0.83	0.65	0.67	0.17	0.24	0.16	1.4	0.83
	Chlorophyll *b* der.	2nd order derivative	3	0.72	0.51	0.60	0.05	0.06	0.06	1.6	0.72
FTIR + UV–vis	Pheophytin *a*	2nd order derivative	5	0.99	0.80	0.76	0.26	1.87	2.08	1.9	0.99
	Pheophytin *a* der.	2nd order derivative	5	0.99	0.82	0.77	0.04	0.28	0.26	1.8	0.99
	Chlorophyll *a*	2nd order derivative	4	0.99	0.69	0.56	0.00	0.03	0.02	1.4	0.99
	Chlorophyll *a* der.	2nd order derivative	5	0.99	0.69	0.53	0.00	0.02	0.02	1.4	0.99
	Pheophytin *b*	2nd order derivative	3	0.97	0.60	0.57	0.02	0.08	0.11	1.4	0.97
	Pheophytin *b* der.	2nd order derivative	4	0.99	0.65	0.62	0.02	0.10	0.07	1.5	0.99
	Total Xanthophyll	2nd order derivative	4	0.99	0.85	0.84	0.06	0.28	0.22	2.5	0.99
	Lutein	2nd order derivative	3	0.96	0.71	0.70	0.22	0.65	1.05	1.5	0.96
	Lutein der.	2nd order derivative	4	0.99	0.78	0.76	0.02	0.16	0.16	1.8	0.99
	Lutein second der.	2nd order derivative	5	0.99	0.76	0.82	0.01	0.13	0.07	2.0	0.99
	Chlorophyll *b*	2nd order derivative	3	0.96	0.76	0.54	0.07	0.21	0.31	1.3	0.96
	Chlorophyll *b* der.	2nd order derivative	3	0.95	0.66	0.42	0.02	0.06	0.09	1.2	0.95

presented in Table 4 showed that major pigments (pheophytin *a*, total xanthophyll, and lutein) including their derivatives (pheophytin *a* der., lutein der., and lutein second der.) were successfully predicted with higher $R^2_{cal.} \geq 0.96$ and higher in range of $R^2_{cv.} = 0.71–0.85$ and $R^2_{pred.} = 0.70–0.84$ compared to UV–vis. However, minor pigments (chlorophyll *a*, pheophytin *b*, and chlorophyll *b*) with their derivatives were not predicted that successfully with lower model performance parameters ($R^2_{cv.} = 0.60–0.76$, $R^2_{pred.} = 0.42–0.62$).

4. Conclusions

Several chemical quality parameters of olive oils including FAEE, DAGs and chlorophyll and carotenoid pigments were predicted from FTIR and UV–vis spectral data as well as their combination using multivariate regression. The results showed that FTIR + UV–vis and FTIR could be used to predict not only FAAEs but also FAMEs and FAAEs content of olive oil successfully. Moreover, FTIR + UV–vis spectroscopy could quantify wax esters less accurately. Only FTIR spectroscopy was found as a promising alternative to wet chemical method based on tedious and expensive extraction as well as derivatization steps for determination of DAG content of olive oils. The other examined parameters were individual pigment contents of olive oil which are especially important for authenticity studies. Both UV–vis and FTIR + UV–vis spectroscopy had good prediction ability for quantification of major pigments as well as their derivatives while moderate prediction was obtained for minor pigments and their derivatives.

This study showed that spectroscopic techniques offered advantages over classical methods in determination of several chemical quality parameters of olive oils since they are faster, relatively cheaper and environmentally friendly compared to wet chemical methods.

Conflicts of interest

Authors declare no conflict of interest.

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