Review Article

Perspective of vibrational spectroscopy analytical methods in on-field/official control of olives and virgin olive oil

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Vibrational spectroscopic techniques, infrared (near, mid, far) and Raman that are based on the continuous vibrational motion of the atoms in the corresponding bonds of the compounds correlate in a unique way the physical properties of materials in the gaseous, solid, or liquid state with the molecular structure. Olive industry and olive oil official control may benefit from the introduction of these powerful techniques to address quality and authenticity issues. This review focuses on evidence obtained from comparative studies among different vibrational techniques for the same set/s of samples or from the same instrumentation. It also assists the forum of discussion on what is needed for their standardization in order to find their place in the official control of the end product. The need for validation guidelines regarding the management of the instrument as well as the construction of the calibration model is highlighted. The latter depends heavily on the reference method employed. An extended list of references covering the characteristics of the techniques, advancements in instrumentation, general applications to oils and fats, and those useful for on-field and official control of olive and olive oil is also given.

Practical application: Vibrational spectroscopic techniques coupled to chemometrics can modernize olive and olive oil analysis from the field to the official control at the borders and in the market. As it occurs already for many decades in the field of other high added value commodities, i.e., milk, meat, wine, the value of the multiple type of information acquired with little sample manipulation should be recognized by all interested parties in the olive sector (stakeholders, authorities, researchers).

1 Introduction

Olive tree (*Olea europaea* L) cultivation is expanding the last 50 years all over the world as a response to the increasing demand for virgin olive oil, which, slowly but steadily, became part of any healthy diet. The interest in virgin olive oil consumption, because of its healthy constituents (monounsaturated fatty acids, tocopherols, and phenolic compounds), boosted also up the consumption of table olives and other products containing them as ingredients. Expansion of both

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Abbreviations: 3,4-DHPEA-EDA, 3,4-dihydroxyphenethyl alcohol-elenolic acid dialdehyde; **ANN,** artificial neural networks; **AOAC,** Association of Analytical Chemistry; **AOCS,** American Oil Chemist's Society; **AOTF,** Acousto-optical tunable filter; **ASCII,** American standard code for information interchange; **ATR,** attenuated total reflection; **CVA,** canonical variate analysis; **DA,** discriminant analysis; **FAEEs,** fatty acid ethyl esters; **FAMEs,** fatty acid methyl esters; **FFAs,** free fatty acids; **FID,** flame ionization detector; **FTIR,** Fourier transform infrared; **FuRES,** fuzzy rule-building expert system; **IOC,** international olive council; **IUPAC,** international union of pure and applied chemistry; **k-NN,** k-nearest neighbor; **LDA,** linear discriminant analysis; **MIR,** mid-infrared; **MS,** mass spectroscopy; **MSC,** multiplicative scatter correction; **NIR,** near infrared; **NMR,** Nuclear magnetic resonance; **OSC,** orthogonal signal correction; **PCA,** principal component analysis; **PDO,** protected designation of origin; **PGI,** protected geographical indication; **PLS,** partial least squares; **QDA,** Quadratic discriminant analysis; **RMSECV,** root mean square error of cross-validation; **RMSEP,** root mean square error of prediction; **SIMCA,** Soft independent modeling of class analogy; **SNV,** standard normal variate; **SVM,** support vector machine; **TAGs,** triacylglycerols; **TGA,** thermogravimetric analysis; **UNEQ,** unequal class model; **VOO,** virgin olive oil

cultivation and consumption to developed regions of the world such as Australia, California, and Northern Europe had an impact on the demand for high-quality products due to international competition, consumer awareness, and the strict regulations that evolved for this precious oil over the years (e.g., [1, 2]). The potential of certain producing countries (Tunisia, Turkey) to devote more land for olive orchards and the interest of China and Iran in this cultivation point out to its future beyond Spain, Italy, and Greece [3].

Currently, international statistics show that virgin olive oil is among the most expensive commercial commodities that are frequently subjected to fraudulent practices [4]. Such practices span from cases of auto-adulteration with lower quality virgin olive oil to the addition of refined olive oil, kernel olive oil, and other vegetable oils. Authentication issues include also cases of mislabeling of highly priced products, of the false denomination of origin or mislabeling cases of "monovarietal" products or even of false declaration of country of origin. The international concern is mirrored on the many published analytical advances that tackle different types of fraud. Among them, spectroscopic methods seem to present a great potential, though their contribution to the official control armory is still rather limited [5]. Moreover, virgin olive oil is highly treasured for its fruity aroma and characteristic bitter taste that are related to its freshness and absence of any type of defects due to lipolytic and oxidative deterioration. These attributes are part of the vertical legislation concerning olive oil worldwide and can be assessed either by trained panels or using instrumental means [6]. Infrared (near, mid, far) and Raman spectroscopy are techniques that correlate in a unique way the physical properties of materials in the gaseous, solid, or liquid state with the molecular structure. They are based on the continuous vibrational motion of the atoms in the corresponding bonds of the compounds. Many are the chapters in books and dedicated books on the intrinsic characteristics of vibrational spectroscopy [7–14]. Once used for the structural elucidation of organic molecules, an issue that is currently resolved using NMR, MS, and X-ray spectroscopy, their application has tipped toward providing solutions to analytical problems in various fields including that of food analysis [10–17]. Some useful review articles and book chapters have covered different aspects of the applicability of vibrational spectroscopy to edible oils and in particular to olive oil since the early 90s [18–25]. On the contrary, information on applications of vibrational spectroscopy to olives is rather limited [26]. Our review focuses on evidence obtained from comparative studies among different vibrational techniques (NIR, MIR, Raman) for the same set/s of samples or from the same instrumentation to answer different problems of olive industry and official control. It also assists the forum of discussion on what is needed for their standardization in order to be implemented in the official control of the end product. A glossary of the major terms used in this review is given at the end of the text.

2 Advances in instrumentation versus regulatory applications in the field of oils and fats

The chromatographic methods that have been used extensively in the analysis of olive oil are informative but time-consuming. Physical techniques, however, present some inherent characteristics – speed, ease of operation – that render them indispensable for a modern analyst. Their applications are steadily increasing in the course of the last 20 years and dedicated peer-reviewed journals publish manuscripts that cover all aspects of these techniques. The boost in the sales of the corresponding spectrometers and their installation in a wide range of laboratories to serve different needs prove their wide use.

The progress in instrumentation of vibrational spectroscopic techniques is continuous. What once was cutting-edge technology has now become easily available, whereas new applications, unknown 20 years ago, are on the brink of commercialization. Instrumental advances in sources, lasers, optics, and detectors combined with fast Fourier transformation allow the collection of thousands of infrared spectra within a few minutes or Raman spectra of samples in the picogram regime within a few hundred milliseconds. Moreover, the availability of pico- and femtosecond tunable laser sources has made acquisition of various nonlinear spectroscopic effects via the excitation of short-lived vibrational states a routine [7]. Sources of ultrafast infrared pulses readily available make feasible the study of very fast motions of molecular systems on the timescale on which they actually occur (ultrafast IR). This enables the real-time monitoring of many reactions [27]. Numerous accessories and various sampling facilities that can be automatically recognized upon connection followed by appropriate alignment of optics, all easy to be handled without special training make the analysis of almost any sample a simple task. In addition, coupling with auto-samplers adds to the high through-put character of the techniques. The manufacture of spectrometers containing an array of beam splitters and detectors that can be used interchangeably may increase sensitivity, resolution, and provide the ability to change from one infrared module to another. Thus, the amount of acquired information is increased, while the capital cost of purchasing different platforms is decreased [28]. Besides, a new generation of interferometers with an angle lower than 90° facilitates the use of cheaper beam splitters, reducing the cost even more [8]. Further on, the on-site/on-line examination can be carried out with portable equipment (e.g., Acousto-optical tunable filter-based spectrometer, AOTF) and miniature infrared sensors. Coupling of vibrational spectrometers to GC, HPLC, or TGA can be valuable for complementary identification, increase in sensitivity and/or improved quantification [8, 20]. These improvements were determined by the parallel progress in the computational efficiency power over the past 20 years

that made possible the automation of the spectrometer components, as well as storage and processing of long data sets. A characteristic example is the vibrational spectroscopic imaging device, which can obtain up to 100 000 individual pixel spectra through special microscopes and convert them to pseudo-color images or the 2-dimensional correlation spectroscopy [7, 29]. Software that can read the output of instruments from different vendors facilitates performance comparisons [30]. The availability of plentiful routines for data processing and analysis through the instrument software offers sophisticated statistical treatments in a friendly way even to the non-expert. At the same time, interferences such as those from atmospheric water and carbon dioxide can be also removed [31, 32]. The ability to extract pretreated spectra as ASCII files offer the possibility to the most trained researchers of using algorithms and routines from sophisticated software such as Matlab to obtain valuable qualitative and/or quantitative information. All of these continuous instrumentation and computation advancements seem to contribute to the serious delay in the standardization of vibrational analytical procedures for regulatory purposes. For example, as it can be seen in Table 1, recommended methods of analysis in the field of oils and fats, and related foodstuffs using vibrational techniques are limited and mainly focus on total *trans* fatty acid content in fats and oils. All but one do not involve chemometrics. However, vibrational spectroscopy, as any other high-throughput technique, spectroscopic or chromatographic, generates a multiplicity of information that is not fully exploited without the proper statistical treatment of the spectra. As it was recently stated "Costs and the regulatory requirements alone will push us to use spectroscopic methods of analysis. We must choose the best region for specific measurements. We must accept the advantages of process control along with the initial cost of implementation..." [33]. This is currently recognized in a method under evaluation by AOCS for the determination of total *trans*, total saturated, total monounsaturated, and total polyunsaturated fatty acid content, which involves PLS1 analysis [34]. This procedure, if accepted, may be proved to be the key to open the entrance for the adoption of vibrational spectroscopy methods coupled to chemometrics in olive and olive oil analysis.

3 Applications to olive on-field control

The quality of the oil is directly related to the olive fruit characteristics at harvesting. The drupe of this fruit consists of the exocarp (skin), the mesocarp (flesh), and the endocarp (pit). The one or two seeds are shielded in a woody shell. Olive fruit growth and development lasts for 4–5 months. Optimum stage of harvesting depends on whether the olives are for milling (virgin olive oil) or for processing (table olives), and is strongly related to local climatic conditions and cultivation treatments. During maturation, the chemical composition changes significantly regarding the primary and secondary metabolites [35, 36]. Different olive cultivars are used for the production of the two major products of the olive industry. In both cases, quality of the end product depends on that of the raw fruits. The latter should be healthy, without infestation and bruises, and free of contamination. Characterization of cultivar seems to be a rather complex issue taking into account homonyms and synonyms within a certain country or among countries, as well as the fact that crossing between cultivars is a frequent agricultural practice for improvement [37–39]. Different modes of vibrational spectroscopy have been employed to answer quality control needs that arise from these traits of the olive fruit. Applications to intact olive fruit are mainly those using NIR spectroscopy and have been very recently reviewed by Stella et al. [26]. NIR spectroscopy presents an inherent advantage over MIR-ATR regarding sample preparation [40–42]. Raman spectroscopy though applicable to paste and intact fruit remains at present of limited use [43, 44]. NIR spectroscopy entered the agricultural and

Table 1. Recommended methods of analysis in the field of oils by international bodies

Body	Method	Method number
Mid-infrared spectroscopy		
AOAC	Total isolated *trans* unsaturated fatty acids in fats and oils. ATR-FTIR spectroscopy	2000.10-2000
	Isolated *trans* isomers in margarine and shortening	965.34-1997
	Isolated *trans* unsaturated fatty acid content in partially hydrogenated fats. Infrared spectrophotometric method	994.14-1998
AOCS	*trans* isomers (isolated) by FTIR	Cd 14-95 (09)
	trans isomers in Triglycerides by ATR/FTIR	Cd 14d-99 (09)
	Negative second derivative infrared spectroscopic method for the rapid (5 min) determination of total isolated *trans* fat	Cd 14e-09
IUPAC	Determination of content of isolated *trans*-unsaturated compounds by infrared spectrophotometry	2.207
Near-infrared spectroscopy		
AOCS	Determination of Iodine value by pre-calibrated FT-NIR with disposable vials	Cd 1e-01 (09)

food sector decades ago in the form of dedicated instrumentation for specific purposes (milk scan, wine scan, olive scan). Information on the specific characteristics of the particular instrumentation is provided by the manufacturers [45–47]. We present as a case study, the potential of a portable miniature NIR spectrometer developed by a specific company, which has been successfully applied for the examination of grape quality [48] and can also be applied for on-field predictions of characteristics that are detrimental to the olive oil industry (e.g., oil content, concentration of phenolic compounds). AOTF technology for the generation of monochromatic beams acts as an electronically tunable spectral bandpass filter. It is a miniature device with no moving parts, and the transmitted wavelength is only determined by the applied radio frequency. The latter is generated with digital precision. Thus, once calibration is made in the factory there is no need for recalibration upon use. Among its technical specifications of interest is that (i) the wavelength of operation can be as narrow as 1 nm; (ii) the transmission efficiency may be up to 98%; whereas (iii) the intensity of the filtered light is precisely and rapidly adjusted by varying the radio frequency power and due to the crystal of tellurium dioxide the wavelength repeatability error is less than ± 0.05 nm. An advantage for industrial application is that even with the addition of the optical fiber the whole system is air-tight keeping, thus, optical and electrical components protected from dust and corrosive vapors and, once programmed, it needs no further supervision [49–51].

Applications of NIR-AOTF, and particularly of the hand-held miniature model, to acquire useful information on olive drupe is presented in Table 2 [39, 52–58]. In all of them, the measurements were taken on the intact fruit. Only in the work described by Allouche et al. [54], olive paste was used because their purpose was to optimize the milling process via on-line monitoring using NIR as an on-line sensor. Interestingly, the publications are coming from a limited number of laboratories, two in Spain [39, 52, 54, 55] and one in Italy [53, 56–58]. The spectral range in all of the applications was the same and coincided with one of the four specific spectral range options provided by the manufacturer. The respective range covers almost the array of wavelengths (1100–2500 nm or 9090–4000 cm^{-1}) of the NIR spectrum, where the most important bands for olive are located according to the literature [19]. Some not important differences among groups were found for the selected values of other parameters in the instrument set up, namely, that of resolution/increment of spectra recording. It seems that once selected were then kept the same in all studies of the group. Spectra were recorded using the highest resolution set-up; the narrowest increment (1 nm) or spectral resolution (2 nm) of the instrument was employed. The number of scans varied though from five to fifty per drupe. Similarly, the selection of scans made by each group was different but once selected remained the same in all their studies. No explanations were given for the selection of this parameter, except from that by Bellincorto et al. [53], who commented that "10 spectra per

average represent a good compromise between speed of acquisition and signal quality of the spectrum." Considering that the signal quality is important to identify subtle details among samples and the fact that 50 scans per drupe can be collected within only 5 s [39], a low number of scans is not recommended and this point needs standardization. This should also apply when other NIR instruments are used considering that even lower scans (e.g., two) have been reported [59] and it is not clear whether such a selection is related to manufacturer instructions or not. Furthermore, the number of fruits making a representative sample should be defined since in some of the studies no information is provided, whereas in other 10–20 drupes are reported. A close inspection of the publications aiming at predicting the oil content, indicated the use of the same statistical software and rather similar spectral handling to construct the PLS models, at least in [39, 52, 55]. Consequently, it was considered interesting to compare those results in order to have an estimate on the robustness of the methodology granted that the pool of samples differs in number, characteristics, and harvesting dates. The comparison of the findings shows that adequate linearity was obtained, $r = 0.83$–0.98 regardless of the experimental design, however, the RMSECV values, a regular criterion of evaluation in these studies, varied up to 70-fold (0.276–19.5). The largest deviation was evidenced in the study of Fernandez–Espinoza [55], who covered five olive cultivation areas and used two large groups of samples (group 1, 202 samples of different maturity and group 2, 458 mature samples) separately or combined ($n = 656$). The author pointed out that a great number of samples and inclusion of samples varying in maturity influence negatively the performance of the model. This was not, however, in agreement with the findings of Bellincorto et al. [53], who studied 192 samples of different maturity and did not obtain significant differences among the models constructed for the individual groups of samples ($n = 48$–75) and their pool ($r = 0.959$–0.995, RMSECV $= 0.276$–1.413). Limitations in the applicability of the technique are discussed by a group of researchers [57] regarding the contents of individual phenolic compounds – oleuropein, verbascoside, 3,4-DHPEA-EDA – and total phenols, who examined fruits from different cultivars and also the respective pool of samples. A few years later [58], they employed the PLS models constructed in their first publication [57] to analyze data for the same constituents for fruits from other cultivars. In both publications, they consider the need to improve the robustness of the predicting models by increasing the number of samples. However, their follow-up publication indicated that cultivar seems to be a limiting factor and they stated that "the substantial reduction in r^2 and the ∼2-fold increase in the error of RMSEP… highlighted the necessity of developing specific models for each cultivar to improve the predictability of NIR." It is important to note that they correlated the presence of phenols with bands in the region 1650–1750 cm^{-1}. Clearly, the

Table 2. Applications of NIR-AOTF to the examination of intact olive drupe or its paste

Application/reference	Spectra recording	Samples	Sample handling	Chemometrics
		Proximate olive composition and morphological characteristics		
Oil/moisture content/[39]*	1100–2300 nm/at 1 nm interval/50 spectra per drupe	256 samples in total (two samples of five drupes/tree): 144 samples on-tree in the field (Trial 1) from Arbequina, Picual and two selections from a breeding program (Sel1 and Sel2) – 29th September–3rd December); 112 samples in the laboratory (Trial 2) from Arbequina, Verdial de Huévar, Manzanilla de Sevilla, Picual (16th September–12th December)	Intact	PLS-full cross validation using various spectra pre-processing
Oil, moisture/[52]*	1100–2300 nm/at 1 nm interval/50 spectra per drupe	359 samples (ten drupes/tree) in total from two progenies plots [genotypes from 2004/05 crosses between Arbequina × Arbosana, Picual × Koroneiki, Manzanilla de Sevilla open pollination and wild (Olea europaea L ssp. europaea var. sylvestris) open pollination; genotypes from crosses between Sikitita × Arbosana] and two selection plots (genotypes selected in progenies from crosses in 1998/1999).	Intact	PLS-full cross validation [two calibration models were developed, one for the progenies (117 samples) and one from the selections (134 samples). External validation with 66 samples from progenies and 42 from selections]
Oil content/[53]**	1100–2300 nm/2 nm wavelength increment/2 × 10 spectra per drupe	192 samples (20 drupes/sample) in total from "Leccino" cv, "Frantoio" cv and "Arbequina" cv (25th of October 2010 or later)	Intact	PCA, PLS-cross validation using total sample set
Pulp/stone ratio, extractability index, moisture, and oil contents/[54]*	1100–2500 nm/resolution at 2 nm/ten scans per paste	210–630 samples (November–January/four consecutive crop seasons)	Olive paste	Artificial neural networks
Moisture, oil content/[55]***	1000–2300 nm in diffuse reflectance mode/1 nm wavelength increment/five scans per drupe	668 samples (50 drupes/sample) in total. 465 samples of different maturity stage olives and 203 samples of mature olives of Hojiblanca, Picual and Arbequina cv. (January 2011–February 2012). All from five olive cultivation areas of Southern Spain (Andalucia) and Southern Portugal (Alentejo)	Intact	PCA, LDA, PLS using cross-validation and test-set validation
		Textural characteristics		
Infestation by Bactrocera oleae/[56]**	1100–2300 nm in diffuse reflectance mode/resolution at 2 nm/2 × 10 scans per drupe	896 drupes (Olea europaea L., cv. canino) free from visual external impact damage and/or decay (local farm in Central Italy at the end of October)	Intact	Genetic algorithm for feature selection in combination with LDA, QDA, or kNN
		Minor compound composition		
Total phenols, verbascoside	1100 – 2300 nm/2 nm	450 drupes in total. 180 drupes	Intact	PLS-cross validation for

(*Continued*)

Table 2. (*Continued*)

Application/reference	Spectra recording	Samples	Sample handling	Chemometrics
oleuropein 3,4-DHPEA-EDA/ [57]**	wavelength increment/ 2×10 spectra per drupe	(Moraiolo cv), 180 drupes (Dolce di Andria cv) and 90 drupes (Nocellara Etnea cv) – [23/9/2010–10/11/2010 in three sets of 54 samples; six sets of samples/stage; five (for cv. Nocellara Etnea) or ten (for cv. Moraiolo and Dolce di Andria) drupes/sample set]		calibration and prediction for each cultivar
Total chlorophylls, carotenoids, anthocyanins, phenols, rutin, verbascoside oleuropein 3,4- DHPEA-EDA, firmness/[58]**	1100–2300 nm/2 nm wavelength increment/ 2×10 spectra per drupe	33 samples in total from Leccino cv, Leucocarpa cv and Buscionetto cv	Intact	PCA, PLS-cross validation, total sample set for calibration and validation

Different superscripts *,**,*** denote different institutes.

technique merits further examination and optimization toward standardization because it can be used for multiple determinations at high speed and requires no sample preparation. Who will undertake the initiative to start a collaborative study on this issue; the manufacturers, the researchers, or an interested body like International Olive Council? As indicated in Table 2 and the preceded discussion, the points that possibly need optimization and standardization can be distinguished to trouble-free ones, i.e., the number of olives that should be measured, the number of scans per sample, and those that are rather puzzling, i.e., the steps of the chemometric analysis in order to build up, validate, and update the prediction model, and also whether or not cultivar is a limiting factor for the application of this fast procedure. Furthermore, it is noted that the values used for the calibration were obtained with non-standardized methods. This fact introduces a certain bias that affects the precision of the constructed models. There are also studies with similar objectives employing other NIR instrumentation, which are covered by the recent review by Stella et al. [26] on which similar observations could be made. The use of AOTF spectrometer has a true potential for the on-field olive quality control, the analytical procedure is rather straightforward, the instrumental sources of error are minimal and its standardization relies on the choice of spectra manipulation.

4 Applications to virgin olive oil (VOO) authenticity issues of interest in the official control

There are many research methods and applications of vibrational spectroscopy to olive oil covering different aspects (oxidative deterioration, classification, adulteration,

etc.). We focus on authenticity issues taking into account what Aparicio et al. [5] reported recently about the current analytical challenges to address them. Except of those that remain the same over the decades (e.g., addition of common seed oil rich in unsaturated fatty acids or the addition of low to high VOO categories), others have to do with the addition of new generation of adulterants (hazelnut oils, deodorized olive oil) and mainly with issues related to the geographical traceability of VOOs (country, region, county, PDO, PGI). The so far reported applications of vibrational spectroscopy in the area of virgin olive oil authenticity are many, easy to list out but difficult to compare. In our review, we present and discuss as case studies those papers that present data using more than one vibrational technique for the same set or sets of data, and also those papers that make comparisons between a vibrational technique and a conventional one. We tried to identify the merits of each of them in revealing information taking into account speed, sample pretreatment steps, cost, analyst expertise, capital, and running costs of the procedure in order to answer the question "is there space or need for vibrational spectroscopy techniques in the official control of virgin olive oil?" Table 3 presents these applications [60–72].

Yang and Irudayaraj [60] were the first, who attempted to compare the vibrational techniques on their performance toward detection of adulteration of virgin olive oil and concomitantly the only to apply FTIR-photoacoustic spectroscopy for such a purpose. The adulterant was olive pomace oil. Application of all the techniques showed that they were of comparable potency regarding detection of the level of adulteration. Raman presented the lowest SEP value, whereas the highest one, almost 3.8-fold, was obtained with FTIR-photoacoustic spectroscopy. It is worth mentioning that the recorded spectra by both MIR techniques were

Table 3. Applications of vibrational techniques for quantification of constituents or adulterants in olive oil and olive oil classification (cultivar, geographical origin)

Application/reference	Techniques	Samples	Chemometrics	Main findings
Addition of olive pomace oil to extra virgin olive oil/ [60]	Vis/NIR; FTIR-ATR; FTIR-PAS; FT-Raman	Extra virgin olive oil mixed with olive pomace oil (0–100% in 5% increments/ 21 different concentrations in total). Each experiment was replicated four times. 75% of samples consisted the calibration set and 25% the validation	Spectra pre-processing: MSC; quantification: PLS	Comparison of the prediction models from the different spectroscopic methods indicated that FT-Raman spectroscopy gave the highest correlation ($r^2 = 0.997$) with the lowest prediction error (SEP = 1.72%). FTIR-ATR $r^2 = 0.991$/ SEP = 3.28%; FTIR-PAS $r^2 = 0.99$/SEP = 4.51%; Vis/NIR $r^2 = 0.99$/ SEP = 3.27%
Discrimination among ten different edible oils and fats/[61]	FT-IR; FT-NIR; FT-Raman; Titlometric (Iodine number)	Commercial samples of butter, lard, cod liver oil, extra virgin olive oil, corn oil, peanut oil, canola oil, soybean oil, safflower oil, and coconut oil	Spectra pre-processing: area normalization; classification: PCA-CVA or LDA, PLS-CVA or LDA	FTIR spectroscopy was found to be the most superior for discrimination and classification of edible oil and fats followed by the FT-Raman method. The FT-NIR method, although capable of discriminating and classifying oils and fats, was found to be less efficient than the other two spectroscopic methods
Quantification of extra-virgin olive oil adulteration with soybean oil/[62]	FT-NIR; FT-MIR (using KBr windows); FT-Raman	One representative extra-virgin olive oil sample by mixing equal volumes of the four different commercial extra-virgin olive oils and one representative soybean oil sample prepared in the same manner. Adulterated olive oil samples were prepared in volumetric flasks of 10.0 mL from the mixing of the two oils in the range 0.0–30.0 and 70.0–100.0% (increments of 1.5%). Other six samples, with percentages of 40, 45, 50, 55, 60, and 65% of soybean oil addition (48 samples consisted the calibration set and 12 samples the validation set)	Spectra pre-processing: scaling centered in the average; quantification: PLS	The NIR and Raman techniques show good PLS prediction models for quantification of the addition of soybean oil in olive oil and can be used independently. MIR has presented a greater dispersion of data. This effect was attributed to: (i) the skeletal vibration bands of lipid structures which are mainly in the range from 800 to 1300 cm^{-1}, so they are concentrated in a small part of the complete MIR spectrum (400–4000 cm^{-1}); (ii) the difficulty of homogenizing the oil drop placed on the surface of the KBr window can also influence the presented results
French olive oil classification/[63]	FT-NIR; FT-MIR (ATR diamond)	411 commercial virgin olive oil samples from the French Inter-Professional	Spectra pre-processing: MSC and normalization of each IR and NIR spectrum	The number of components was determined by using

(Continued)

Table 3. (*Continued*)

Application/reference	Techniques	Samples	Chemometrics	Main findings
		Olive Oil association (AFIDOL) from five successive crops (2003/2004–2007/2008): "Haute-Provence" ($n = 50$), "Nice" ($n = 57$), "Nimes" ($n = 39$), and "Nyons" ($n = 49$), Aix-en-Provence ($n = 104$), Vallée des Baux ($n = 112$)	to unit length, Spectra fusion, and mean centering; classification: FuRES, PLS-DA	bootstrap Latin partitions (BLPs) in the signal correction routine and maximizing the average projected difference resolution (PDR). The same approach was used to select the number of latent variables in the PLS-DA evaluation and perfect classification was obtained
Quantitative analysis of selected fatty acids and triacylglycerols/identification of the Registered Designation of Origin (RDO) of extra virgin olive oils/[64]	FT-NIR; FT-MIR (ATR diamond); GC (FAMEs); HPLC (TAGs)	Commercial virgin olive oil samples ($n = 412$) from the French Inter-Professional Olive Oil Association (AFIDOL) from five successive crops (2003/2004–2007/2008): "Haute-Provence" ($n = 50$), "Nice" ($n = 57$), "Nimes" ($n = 39$), and "Nyons" ($n = 49$), Aix-en-Provence (104), Vallée des Baux (112). 275 samples consisted calibration set and 137 the prediction set)	Spectra pre-processing: mean centering, and absorbance normalization; quantification: PLS or PLS after PCA (H-PLS); classification: PLS-DA or H-PLS-DA	MIR spectra give better results than NIR spectra. The model built on the concatenated matrix (NIR + MIR) does not improve the results. The use of H-PLS improved the results obtained in the NIR and MIR spectral ranges separately. In this case, the reduction of the data allows better fitting for the models. For the determination of the geographical origins, as the results obtained in the MIR range were very good, the concatenated method or the H-PLS method does not improve the results. The introduction of NIR data does not allow an increase in the discriminated power of the models
Cultivar discrimination/[65]	FT-NIR; FT-MIR (ATR 11 reflections)	82 monovarietal extra virgin olive oils; 27 Casaliva, 28 Leccino, and 27 Frantoio cultivars grown in three different sites (Puegnago, Follonica, and Mirto) representative of Italian olive growing regions	Spectra pre-processing: removal of segments without absorbance, SNV, 1st derivative, SELECT or Wavelet transform; classification: LDA, PCA-LDA	There was a positive effect of the fusion of NIR and MIR spectra, in fact, the prediction abilities always increased after coupling the two types of information, using either feature selection or data compression
Varietal discrimination/[66]	FT-NIR; FT-MIR (ATR); Titlometric (Acidity, peroxide value); spectroscopic ($K_{232/270}$, total chlorophyll, total carotenoid); GC (FAMEs); HPLC (tocopherols)	82 monovarietal extra virgin olive oils from three single-cultivars: "Casaliva," "Leccino," and "Frantoio" (27 from Casaliva, 28 from Leccino, and 27 from Frantoio)	Spectra pre-processing: SNV, first derivative using the Savitzky–Golay method after removing spectra segments with no absorption. The spectra were also fused; classification: LDA, SIMCA	The best results were obtained by MIR spectroscopy; in fact, the percentages of correct classification and prediction were 94.2 and 86.6%, respectively. Also in this case, "Leccino" was the cultivar best classified

(*Continued*)

Table 3. (*Continued*)

Application/reference	Techniques	Samples	Chemometrics	Main findings
Characterization of PDO olive oil Chianti/[67]	NIR; FT-MIR (ATR 11 reflections); UV–vis; GC (FAMEs according to EU Reg. 796/2002)	57 samples divided into two classes according to their geographical origin	Spectra pre-processing: first derivative, SNV; SELECT algorithm for variable selection; classification: PCA, UNEQ, SIMCA; quantification: PLS (oleic and linoleic acid content) validation using both cross-validation procedure and external validation	achieving a correct prediction rate of 93%. In class-modeling, the results obtained using the spectroscopic data were better than those obtained with the fatty acid composition (selective information). NIR and UV–vis spectroscopy provided the best results in obtaining class-models efficient to characterize the Chianti Classico PDO olive oils. Combining all the information slightly improved the UNEQ and SIMCA results. Too small improvement to justify the combination of UV–vis, NIR, and MIR. As far as PLS is concerned, the results did not improve upon combination. NIR spectroscopy appeared as the most powerful to predict the content of oleic and linoleic acids
Addition of seed oils and refined olive oils to virgin olive oil/[68]	FTIR-ATR (horizontal); FT-Raman	140 spectra formed the database "pure oils" comprised of seven different groups: 36 extra-virgin olive oils, 10 refined olive oils, 28 sunflower oils, 18 rapeseed oils, 9 peanut oils, 21 soybean oils, and 18 corn oils. The training set consisted of 84 spectra, the tuning set of 27 spectra, and the test set of 29 spectra. The database was chosen after preliminary data processing (PCA). Outliers were removed. 11 extra-virgin olive oils adulterated with five olive oils ($n = 275$); five extra-virgin olive oils adulterated with five seed oils at levels 5, 15, 25, 35, and 45% w/w ($n = 125$)	Spectra pre-processing: area- normalization, baseline-correction; classification: PCA-LDA; PCA-ANN	FTIR better than Raman at classifying oil samples and detecting adulteration; no additional benefit from combining the two data sets. For the infrared data, 100% of the samples were correctly classified. For the Raman data, the best prediction was 93.1%. The adulteration detection limit provided by PLS and ANN, when applied to the infrared data, was 5% for each of the adulterants used (the minimum adulterant level). For the Raman data, the detection limit was 45% when the adulterant was refined olive oil and 5% for the other adulterants
Addition of Hazel nut Oil/[69]	FT-MIR (ATR horizontal with 6 reflections);	189 (training set), and 44 samples (blind samples).	Spectra pre-processing: smoothing, first-derivative.	A complete discrimination between olive and hazelnut

(*Continued*)

Table 3. (*Continued*)

Application/reference	Techniques	Samples	Chemometrics	Main findings
	FT-Raman	The admixtures of the training samples were prepared with refined and lampante virgin olive oils from several geographical origins (Greece, Italy, Morocco, Spain, Tunisia, and Turkey) and two kinds of hazelnut oils (refined and crude) from France, Italy, Spain, and Turkey. The percentage of hazelnut oil in olive oil was 2–20% (2, 5, 8, 11, 14, 15, 17, and 20%). Spectra recording on intact oil or the unsaponifiable fraction after extraction	Univariate statistics was used to carry out the repeatability study (relative standard deviation <12%), classification: stepwise LDA	oils is possible. Adulteration can be detected if the presence of hazelnut oil in olive oil is >8% and if the blends are of Turkish olive and hazelnut oils. The limit of detection is higher (15%) when the blends are of edible oils from diverse geographical origins. The best results were obtained with FT-MIR and the analysis of the unsaponified fraction
Characterisation and classification of Italian virgin olive oils/[70]	NIR; FT-MIR (ATR horizontal, 11 reflections); Titlometric (acidity, peroxide value), spectroscopic ($K_{232/270}$ and ΔK)	119 Italian extra virgin olive oils: 60 monovarietal extra virgin olive oils obtained by the single-cultivars Casaliva, Leccino and Frantoio (20 from Lombardy, 19 from Tuscany and 21 from Calabria) and 59 industrial extra virgin olive oils (20 from Lombardy, 20 from Tuscany and 19 from Calabria) obtained from a mixture of cultivars characteristic of each geographical area	Spectra pre-processing: –; classification: PCA, LDA, PLS-DA, SIMCA	Overall, NIR spectroscopy was able to better classify industrial oils, allowing a correct classification of about 90% on the basis of geographical origin, while the MIR technique was able to classify both monovarietal and industrial olive oils, allowing a higher correct classification of samples (>95%). Also, the SIMCA method, more conservative than LDA and PLS-DA techniques, was able to classify correctly MIR spectra on the basis of geographical origin
Tracing the origin of extra virgin olive oils/[71]	NIR; FT-MIR (ATR horizontal 10 reflections)	20 extra virgin olive oil samples from the PDO area of Sabina (13 of them from 2009 harvest and 7 from 2010) and 37 samples (22 from 2009 harvest and 15 of 2010) from other origins (other areas of Italy or Mediterranean countries)	Spectra pre-processing: MIR (comparison of different approaches): subtraction of a linear or a quadratic baseline, MSC, 1st and 2d derivative, and the combination of MSC with the subtraction of a quadratic baseline or with 1st or 2d derivative. The flat regions of the signal (2000–2500 cm^{-1} and 3200–4000 cm^{-1}) were eliminated prior to the statistical analysis. NIR (comparison of different approaches): MSC,	Both spectroscopic techniques (mid- and near-infrared), after proper signal pretreatments, resulted in models with high accuracy, as evaluated on an external test set. However, among the two, NIR spectroscopy provided the better results, as processing of the corresponding spectral data by PLS-DA allowed to correctly predict the origin of 100% validation sample the discriminant information was linked to

(Continued)

Table 3. (*Continued*)

Application/reference	Techniques	Samples	Chemometrics	Main findings
			detrending, 1st and 2d derivatives and the combinations of MSC with each of the other three; classification: PLS-DA, SIMCA	the absorption and reflection bands of the most characteristic moieties present in olive oils (carbonyl and aliphatic CH stretching, bending and combination bands). The harvesting year of the samples can have an effect on the spectroscopic fingerprint of olive oils (as evidenced both by PLS-DA and SIMCA) but that, at the same time, it is still possible to obtain discriminant classification models that can be transferable to a high extent from year to year. The discriminant information is really linked to the geographical origin and not to conservation treatments
Geographical origin of Italian olive oil (Ligurian and non-Ligurian)/[72]	NIR; FT-MIR (ATR 11 reflections)	Extra virgin olive oil samples (210 Ligurian and 700 non-Ligurian) collected by marketing or regulatory bodies/from three consecutive harvests (2005–2007)	Spectra pre-processing: Mean centering (only for SVM); classification: PLS-DA, SVM, GENOPT-SVM have been tested and statistically compared using McNemar's statistical test	For the two datasets, SVM with optimized pre-processing give models with higher accuracy than the one obtained with PLS-DA on pre-processed data. In the case of the NIR data set, most of this accuracy improvement (86.3% compared with 82.8% for PLS-DA) occurred using only a single pre-processing step. For the FTIR dataset, three optimized pre-processing steps are required to obtain SVM model with significant accuracy improvement (82.2%) compared to the one obtained with PLS-DA (78.6%)

almost identical. Furthermore, using Raman, the simplest model was obtained since only one factor was necessary for its construction, whereas with the other techniques 4–13 factors were required. The same researchers, a few years later, compared FT-MIR, FT-NIR, and FT-Raman techniques using a single instrument with the appropriate addition of accessories and detectors to examine their efficiency in classifying various edible fats, including extra virgin olive

oil [61]. From the data in the figures, it can be deduced that more than ten samples were used in each category. Upon comparison, it was evidenced that FT-MIR provided better classification rate of samples (98.9%) employing PLS-CVA on the part of the spectra (1400–1800 cm^{-1}) that contained vibrations related to C=C bond. The respective factors employed for discrimination were only three, whereas using the other techniques 5–10 were required to obtain

classification rates higher than 84%. FT-Raman and FT-NIR combined with PLS-CVA were slightly less efficient (94 and 93%, respectively) and no improvement was observed when only a part of the spectrum covering vibrations of C=C bond was employed. Since then, a comparative study of the infrared and Raman techniques was only recently made by Mendes et al. [62] who tried to quantify the adulteration of extra virgin olive oil with soybean oil. The respective group used different instruments but of the same manufacturer and analyzed the whole spectrum in each case. GC analysis of fatty acids was carried out only for the pure oils and not for the adulterated mixtures. So, that it is not possible to extract information for comparison between the official GC method and the spectroscopic ones. The predicted models presented comparable r values (>0.992); MIR spectroscopy predicted values were the most distant from the true ones in comparison to those obtained using NIR and Raman. Such a difference was reflected in the calculated RMSECV and RMSEP values. Despite this, univariate paired t-test comparison of predicted values versus the true ones showed no statistical difference for each method (p-values were 0.103/NIR, 0.108/MIR, and 0.117/Raman) at 95% level of confidence, suggesting their equal potency. NIR and Raman are more advantageous over MIR spectroscopy because the measurements in the former two are carried out directly in the glass vial containing the sample. In this way, no cleaning of the cells or windows between samples is necessary as it is in MIR spectroscopy.

Other studies reported in Table 3 [63–72] presented data obtained by only two vibrational techniques. In some of them, data from other types of analytical techniques were also given. In most of the cases, data were obtained by NIR and MIR spectrometers. Few were the studies discussing the performance of infrared versus Raman spectroscopy. Harrington et al. [63], using a large number of commercial samples ($n = 411$) of different cultivars, geographical origin, and from five consecutive years (2003/2004–2007/2008) showed that absolute classification could be obtained using the information from the combined NIR and MIR spectra of the respective samples. The aim was to introduce a novel chemometric approach that may significantly reduce the processing time of data without compromising accuracy. This was feasible with the aid of the significantly faster PC-OSC approach combined either with PLS2-DA or fuzzy rule-building expert system. The details of the study are limited and no attempt was made to examine whether such an approach provides better results than the analysis of NIR or MIR spectra, separately. Such an attempt was published a year later by some researchers of the same group, together with new collaborators [64], who analyzed the data (full spectrum) of the same pool of samples using unexpectedly another statistical approach for classification and not making reference to their own previous one for comparison. In the same study, the data from the two infrared techniques separately or in combination were also examined toward the prediction of the concentration of certain fatty acids (16:0, 18:0, 18:1, 18:2, 18:3) or triacylglycerols (LOL, LOO, PLO, OOO, POO, POP). The corresponding experimental values were obtained using GC and HPLC analyses, respectively. The experimental approach followed in the two studies is illustrated in Fig. 1. On the basis of the quantitative results obtained, it was evidenced that the analysis of MIR spectra provided slightly better predictions both for the levels of fatty acids and triacylglycerols than NIR. Data fusion did not necessarily improve the prediction unless variable reduction using PCA was preceded (H-PLS). This was better observed using the triacylglycerol content.

The classification approach adopted showed that MIR was superior to NIR (three misclassified out of 137 samples vs. 24), equally efficient to the H-PLS approach (three
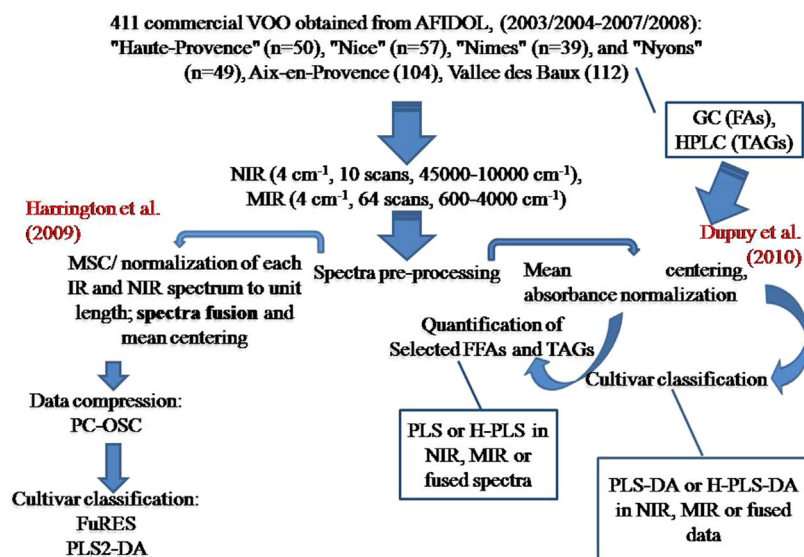
Figure 1. Schematic representation of vibrational spectroscopy procedures developed for olive cultivar classification and quantification of selected FFAs and TAGs in olive oils ($n = 411$, 2003/2004–2007/2008) based on Refs. [63, 64].

misclassified out of 137 samples) and slightly inferior to that after data fusion (one misclassification). These findings imply that even in the study of Harrington et al. [63] the success of classification could be rather attributed to the contribution of MIR spectra despite the different statistical approach adopted.

Data reduction approaches aim to remove variables with low information and balance difference in the size of observations and variables, which affect model precision. In this sense, it is interesting to examine the results presented in two recent publications of another research group for the classification of 82 monovarietal extra virgin olive oils derived from three major Italian olive cultivars. Thus, Cassale et al. [65] submitted first for publication the results from chemometric strategies. These strategies included feature selection or data compression to the separate or combined NIR and MIR spectra. The authors used two spectrometers of the same manufacturer for their studies and after typical spectra pretreatments, they employed either the "select" algorithm as a feature selection technique or the "wavelet transform" algorithm for data compression. A third approach, namely application of PCA prior to LDA analysis was used as the reference strategy. In the first case, the classification efficiency was higher on the basis of MIR spectra analysis (86.2%) in comparison to that based on NIR spectra (81.7%). The analysis of the combined spectra provided slightly better results (88.6%). Data compression had as a consequence to retain the 75% of the variance. A similar trend on the performance of separate or combined spectral information was observed, however, the classification efficiency was poorer (∼56–72%) than that obtained with feature selection. The usefulness of such an approach was further supported by the application of PCA-LDA since the classification efficiency did not exceed the 66%. Despite the promising findings the authors stressed, what is a general need for method standardization, "that in order to produce a robust model more samples covering different harvesting years, geographical areas or even Mediterranean countries would be required." In addition, the authors did not use any chemical indices to compare the performance of the spectroscopic technique with. In the follow-up study of the same group [66], the authors compared the select-LDA approach with that of SIMCA, a well-known classification technique. This time, they did not fuse the data sets but they included and compared the classification efficiency of each data set to that obtained using official and other criteria (see Table 3). Using all the chemical indices the classification efficiency was not higher than 65–66% regardless the statistical treatment. Using MIR or NIR data the classification efficiency was superior to the former (86.6 and 83%, respectively). Furthermore, select-LDA was more proficient than SIMCA, since with the latter the success was not higher than 65.8% irrespectively of the technique used.

It is worthy to mention that infrared spectroscopy per se or in combination with visible absorption and coupled to chemometrics was found to be a promising tool to address issues of auto-adulteration. Useful information can be provided for the adulteration with deodorized olive oil, an adulterant of rising interest. More specifically, Mignani et al. [73] relying on Vis-NIR (400–1700 nm) spectra recording with an integrating sphere that contained the oil sample and optical fibers for illumination/detection that provided scattering insensitive measurements, showed that this was feasible with an RMSEP value of 4%. In another study [74], the MIR spectrum and particularly the region 2839.0–912.3 cm^{-1}, free from poor signal-to-noise ratio, was proposed as more suitable to construct a PLS model that could quantify down to 29.3 mg/kg the Σ (FAMEs + FAEEs) and predict adequately the FAEEs/FAMEs ratio. The model was constructed using a set of cheap commercial extra virgin olive oils previously characterized with regard to the specific indices with the aid of GC-FID [75]. The value of the first index normally cannot exceed 75 mg/kg for an extra virgin olive oil or in case it is estimated in the range 75–150 mg/kg, the second index should be less or equal to 1.5 [75]. If findings do not satisfy these criteria then the possibility of adulteration with low-quality olive oils (deodorized or from oils obtained from overripe, damaged, or poorly preserved olives) is high [74]. Nevertheless, the authors stressed the need for further confirmation of their findings through the examination of larger sets of samples in terms of number and Σ (FAMEs + FAEEs) content.

The findings on detection and quantification of adulteration of the studies included in Table 3 rely mainly on elaborated mixtures of oils of known identity and composition. Nevertheless, in real life, the adulterated samples are a conundrum. The adulterant used is of unknown history, which implies that among others it may have been stored under unsuitable conditions (exposure to temperature, oxygen, light) promoting uncontrolled oxidation for a long time. The same applies for the base olive oil used for adulteration. It is more probable that the perpetrator has used low-quality products. Oxidative changes take place even when the prolonged oil storage is under ambient conditions (dark, room temperature) and the limits set for official criteria are still satisfied because changes in MIR spectra are more pronounced [76]. Oxidation as shown by Nenadis et al. [76], Guillen and Cabo [77–79], and Navarra et al. [80] causes changes in the absorbance intensity or the area of bands related to unsaturation, changes in the region of oxidation products such as peroxides or even a shifting in wave numbers. Vibrational techniques do not rely on separation and determination of individual constituents to comment on the changes in composition. This inherent characteristic that may affect the performance of the various PLS models is not studied thoroughly yet. Especially, models that rely on band selection are expected to be more influenced according to Barton and Himmelsbach [33]. Information on shifts and another type of changes in the spectrum of olive oil or potential adulterants exposed at

temperatures 60–190°C have been presented in a number of publications not only for MIR but also for NIR and Raman techniques [81–87].

Instrumental specifications affect to a variable extent spectrum recording. This effect can be critical for method transferability but few are the studies dealing with this aspect [88–91]. A good example that can be used as a guide in the application of vibrational techniques to olive oil for official control is coming from the field of dairy products. In the work described by Grelet et al. [91], a procedure of standardization for the milk MIR spectra recorded with different FTIR instruments is proposed. Validation guidelines based on distillation of ideas and best practices from experts in the scientific community, like those proposed for NIR in the pharmaceutical industry [92] or those for the development and the validation of NIR prediction models and their related instrument management by AOCS [93], which should be extended to MIR and Raman instrumentation, could be useful for olive oil official control. Still, errors do not necessarily derive from vibrational methods but can be equally due to weaknesses of the methods used for calibration, in particular if these are research methods. Even reference methods should be carefully studied since the error may be larger than expected, affecting consequently negatively the calibration. Last but not least, knowledge on chemometrics should be followed by "analytical skills in wet laboratory assays"…because "this technology is not a case where you can leave the driving to someone else" [33].

Overall, it seems that there is "space and need" for the introduction of vibrational spectroscopy in the olive oil official control as it is illustrated in Fig. 2. Nevertheless, any proper implementation needs a clear cut frame of how chemometrics should be applied to vibrational spectroscopy data.

5 Future perspectives

Vibrational spectroscopic techniques (NIR, MIR, Raman) present a great potential for the analysis of any food item and fit well with the concepts of foodomics [94]. The comparison does not point out one of them as the most applicable to olive oil analysis. Each of them has specific pros and cons, and all of them require the use of a high number of samples for the development of robust calibration models. Taken into account that calibration is a tedious procedure, it is of utmost importance that these models can be transferable from instrument to instrument. Transferable data can be piled up to form open access spectral databanks that can be used to build up statistical models using chemometrics. Coupling with chemometrics is an intrinsic characteristic of any vibrational spectroscopic procedure. All these can be challenges for international bodies, such as the IOC, and regulatory agencies that have opened in the past consultation

on methods improvement in the olive sector [95, 96]. These steps are a prerequisite in order these "all green" analytical techniques to find their place in the official control armory.

6 Glossary

6.1 Virgin olive oils

Virgin olive oils are the oils obtained from the fruit of the olive tree (*Olea europaea* L.) solely by mechanical or other physical means under conditions, particularly thermal conditions, that do not lead to alterations in the oil, and which have not undergone any treatment other than washing, decantation, centrifugation, and filtration [97].

6.2 Edible commercial categories of virgin olive oil in the EU

Two categories are foreseen, the "extra virgin olive oil" and the "virgin olive oil" where the former is any virgin olive oil that has a free acidity, expressed as oleic acid, of not more than 0.8 g per 100 g, a zero median of the defect and a median of the fruity attribute higher than zero, and the latter is any virgin olive oil that has a free acidity, expressed as oleic acid, of not more than 2 g per 100 g, a median of the defect less than or equal to 3.5 and a median of the fruity attribute higher than zero [98].

6.3 Vibrational spectroscopy

The field of spectroscopy that refers to infrared (IR) absorption spectroscopy and Raman scattering (including
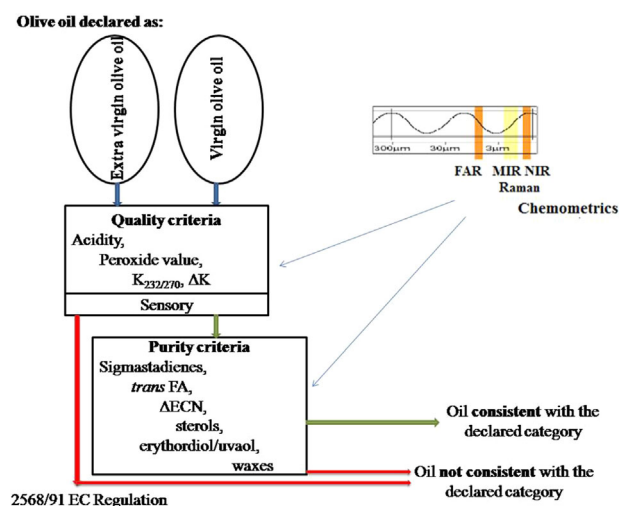
Figure 2. Illustration of proposed entry of vibrational spectroscopy techniques in the olive oil official control for the assessment of its quality and authenticity.

several variants of these two major categories) to probe the continuous vibrational motion about their equilibrium position which undergoes atoms in molecules [7].

6.4 Infrared spectroscopy

The spectroscopic technique observing vibrational transitions of molecules through absorption of infrared (IR) radiation (800–1 000 000 nm or 14 000–10 cm^{-1}). The prerequisite of IR activity is the change in dipole moment of the molecule during a given normal mode of vibration [99].

6.4.1 NIR spectrum

This is the first spectral region (800–2500 nm or 12 500–4000 cm^{-1}) exhibiting absorption bands related to molecule vibrations. It is characterized by harmonics and combination bands [100].

6.4.2 MIR spectrum

This is the main region of vibrational spectroscopy (2500–25 000 nm or 4000–400 cm^{-1}; the region 1500–500 cm^{-1} known as fingerprint). It retains information, allowing organic molecules to be identified, and the structure and conformation of molecules to be characterized [100].

6.5 Raman spectroscopy

It is the spectroscopic technique involving a scattering process that arises when the incident light excites molecules in the sample, which subsequently scatter the light. Most of this scattered light is at the same wavelength as the incident light, but some is scattered at a different wavelength. The process leading to this "inelastic" scatter is called the Raman effect. For vibrational activity in Raman spectrum that lies in a similar region to MIR, a prerequisite is a change in the electronic polarizability of the analyte molecule [10, 99].

6.6 Fourier transformation

It is a mathematical algorithm that extracts appropriately weighted harmonic components from a general periodic waveform. It assists accurate revealing of the spectrum from the recorded interferogram [7].

6.7 Chemometrics

It is a chemical discipline that uses mathematics, statistics, and formal logic (i) to design or select optimal experimental procedures; (ii) to provide maximum relevant chemical information by analyzing chemical data; and (iii) to obtain knowledge about chemical systems [101].

6.8 Foodomics

A new discipline that studies both the food and nutritional domain by applying and combining cutting-edge analytical technologies and multivariate data analysis [94].

The authors have declared no conflict of interest.

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