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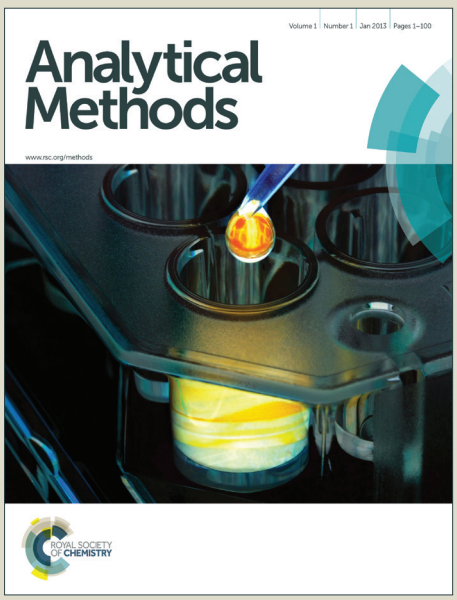

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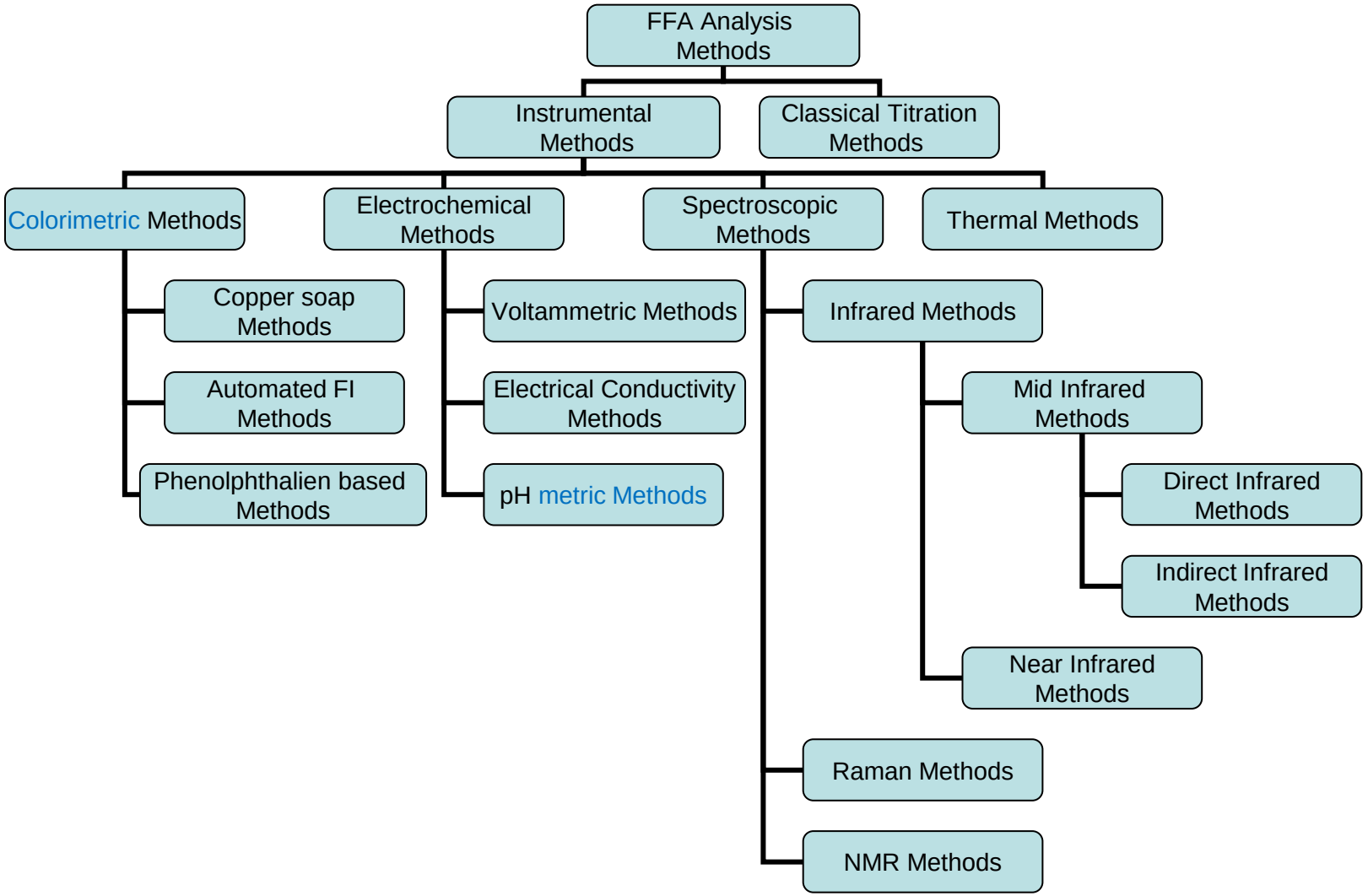
Graphical Abstract



Block diagram of the methods covered in the review for assessment of free fatty acids in oils and fats.

Analytical Approaches for free fatty acids assessment in oils and fats

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Abstract

Basically, free fatty acids (FFAs) are produced by hydrolysis of oils and fats. Level of FFA depends on time, temperature and moisture content because the oils and fats are exposed to various environments such as storage, processing, heating or frying. As FFAs are less stable as compared to neutral oil therefore they are more prone to oxidation and produce rancidity. Thus, FFA is a key feature linked with the quality and commercial value of oils and fats. American Oil Chemists' Society (AOCS), Association of Official Analytical Chemists (AOAC) and European Commission Regulation (EEC) have established almost identical standard methods for the assessment of FFA. These methods are based on the titration where oils or fats are needed to be dissolved in hot neutralized ethanol or ethanol/diethyl ether using phenolphthalein as an end point indicator. Titrimetric procedures are however laborious and need large amount of chemicals and solvents. Besides cost of chemicals, environmental issues further limit these procedures. In addition, accurate detection of end point especially for highly colored crude oil by colorimetric indicator is a difficult task. Despite all mentioned demerits, unfortunately

titration method is still being used in most of the edible oil industries for the determination of FFA. Due to lack of any comprehensive review on this very important topic, we have made an attempt to present a review in order to discuss various available methods with special emphasis on the instrumental methods due to their high sensitivity, accuracy and rapidity.

Keywords: Free fatty acids; vegetable oils; standard titration method; instrumental methods

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1. Introduction

Vegetable oils or fats are extracted from the oilseeds in the crude form. Various refining stages such as degumming, neutralization, dewaxing, bleaching and deodorization are followed to convert extracted crude oils to edible oils. During processing, a number of parameters such as moisture, peroxide value, free fatty acids (FFAs), saponification value (SV) and iodine value (IV) are monitored. Among these parameters FFA content is considered as a very crucial factor and linked with the quality as well as economic value of edible oils. [1] Generally FFAs are the hydrolysis product generating as a result of the oil and fat oxidation during long time storage or processing at elevated temperature and heating or frying. Initially, AOCS[2], AOAC[3] and European Commission Regulation (EEC)[4] have recommended standard methods for the determination of FFA. These methods are almost similar and based on the titration of oil (3.5-56.4 g). Oils or fats are dissolved in hot neutralized 95% ethanol (50-100 ml) or ethanol/diethyl ether (50-150 ml), against a strong base using phenolphthalein as an indicator. Although, these titrimetric methods are very simple but extremely laborious and need large amount of expensive chemicals associated with environmental issues. Furthermore, the analysis of dark crude oils in the presence of colorimetric indicator is very problematic to find out the accurate end point during titration.

To overcome such issues, a number of instrumental methods have been developed as potential alternative to classical methods for more accurate and sensitive measurement of FFA contents in the edible oils and fats. In general these methods can be categorized into three major groups:

(a) Colorimetric methods

(b) Spectroscopic methods

(c) Electrochemical methods

Generally, second and third group involves variety of sophisticated instruments with diverse approach that compete with each other in terms of speed of analysis, amenability to automation and reduction of harmful solvents. Some instrumental methods provide a significant gain in accuracy and sensitivity than classical titrimetric methods.

Several methods have been reported during the last sixty year. In 1950s, spectrophotometric technique was well addressed and several workers published plenty of articles on FFA using colorimetric methods. After that, the trend changed towards the electrochemical techniques. In last two decades, attention was however focused on use of infrared spectroscopic methods for the direct or indirect determination of FFA. These methods were claimed to be environmental friendly as either chemicals are totally avoided or require negligible amount of chemicals as well as samples. Fig. 1 shows the block diagram of the methods developed for the determination of FFA during last few decades. To the best of our knowledge there is no any comprehensive review available on FFA analysis. Therefore, present review will explore many methods which were developed in last few decades.

2. Discussion

2.1. Colorimetric methods

Various calorimetric methods have been reported by several researchers.

2.1.1. Copper soap method

In 1956, Ayers[5] made his first effort to determine the FFA by calorimetric method. According to his procedure, aqueous solution of copper or cobalt nitrate was used to convert FFA into water-insoluble soaps. Colored soap precipitate was extracted with chloroform and quantification was carried out by measuring absorbance at 675 and 527 nm for copper and cobalt, respectively. Although method was not proposed for FFA analysis but it provided a base to develop colorimetric methods for FFA analysis in edible oils. Later on Baker[6] proposed an improved copper salt based method which was designed particularly for FFA analysis in edible oils. The method involves the dissolution of oil in benzene followed by mixing with aqueous cupric acetate solution. The mixture was centrifuged and quantification of FFA was determined by measuring the absorption of benzene layer at 640 nm. This method showed good correlation with titrimetric method. The only limitation of this method was the dissolution of some copper salts of saturated fatty acids in benzene. In view of the inconsistencies in working wavelength of 640 vs 675 nm [5,6], Bains *et al.,*[7] examined the spectra and the effect on color of oil during the measurement. He concluded that 670 nm was the optimum wavelength for copper soaps in non-colored or colored oils. Twelve years later, Lowry and Tinsley[8] reported a substantial work to optimize the method in terms of sensitivity and reproducibility. The conclusion of his work was the stabilization of the copper soap of saturated fatty acids in benzene and improvement in the sensitivity by controlling the pH using pyridine. Due to

the practical and health concerns of benzene, various authors used alternative to benzene such as toluene [9], isooctane [10] and cyclohexane [11]. Gyrik *et al.,* [12] proposed a new analytical tool for the determination of total FFA content in thermally treated cooking oil. The method was based on the colorimetric copper soap combined with the concept of optothermal window (OW) (with 632.8 nm He - Ne laser used as a radiation source). The results obtained by this method were compared to those acquired by conventional spectroscopy; the correlation between the two methods was high for FFA concentrations exceeding 2 µmol/ml.

2.1.2. Automated FIA systems

The first automated system for FFA analysis in edible oils using copper soap method was introduced in 1980's. [9] The automation was accomplished using a flow injection analysis (FIA) system which was able to analyze up to 20 samples/h. In contrast to the previously published methods, based on organic solvents, the quantification was done in the aqueous phase. However, the system did not receive much attention, because of the complications and stability problems. Later on an improved hard ware in FIA system using a single-extraction step was reported.[13] This new system offered enhanced stability and much shorter analysis time (130 samples/h). The only shortcoming of the system was poor sensitivity. Puchades *et al.,*[14] improved the sensitivity of FIA systems through optimized operating conditions, for example pH, flow rate, tubing length/diameter, and oil/solvent proportion resulted in better analysis speed (75 samples/h) and lower limit of detection (~0.01% FFA). Despite the mentioned improvements the method did not receive much

attention due to the complications in the analysis and incompatible values as compared to the official method.

A continuous liquid-liquid extraction system using a single-channel flow-reversal injection mode was also proposed for the direct determination.[15] The results provided by the proposed method were in accordance with those obtained by a manual procedure and comparable to the standard acid-base titration method, but the sampling frequency was lower (12 samples/h).

2.1.3. Phenolphthalein based method

Due to the shortcomings of the official method, Linares *et al.,*[16] made an attempt to automate the official method. He used the same reagents as the IUPAC official method, whilst quantification was based on monitoring the decrease in the phenolphthalein (PHP) intensity at 562 nm as a result of the reaction between KOH and FFA in the oil sample. The analytical range of method was 0.15-0.81% FFA and sample throughput up to ~ 60 samples/h. Later on a modified method with similar analytical range was reported [17] using 3-7 ml of inexpensive 1-propanol as a diluent and carrier in comparison to the huge amount of solvents in official method. The sample throughput was 30-100 samples/h.

A direct measurement of FFA in virgin olive oil without dilution was reported using similar system.[18] The system was equipped with an optical fiber beam connected to the spectrophotometer set at 562 nm. The system had the same sensitivity as the previous methods with a sample throughput of 12-60 samples/h. Afterward an indirect titrimetric method was developed based on the complete extraction of acids from oil into reagent (0.05 mol dm^{-3} triethanolamine in the mixture of 50 % H2O + 50 % 2-PrOH) and indirect titration of acids in BH^+ form against aqueous alkali in the presence of a PHP indicator.[19]

The benefits of proposed method included the lack of toxic solvents, analysis at room temperature and no need for preliminary neutralization of acid admixtures.

Saad *et al.,*[20] reported a single and two-line manifold FI methods using PHP and bromothymol blue (BTB) as indicators. The method was based on the monitoring of the changes of absorbance of the indicators at 562 nm for PHP and 627 for BTB. Various FI parameters were optimized such as carrier and reagent concentration, length of reaction coil, flow-rate, injected volume and size of mixing chamber. The single-line manifold with PHP as indicator was recommended for the determination of samples with acidity higher than 0.4, while for lower acidities (< 0.4) a two-line manifold with BTB was recommended. The method was linear over the range 0.4-10.0 FFA for single-line manifold and 0.11-0.50 FFA for the two-line manifold. Sample throughputs were 35-74 and 21-46 samples/h for single-line and two-line manifolds, respectively.

A non-aqueous single-line manifold system was built by modification of an HPLC for FIA.[21] Oil samples were injected without pre-treatment into a *n*-propanol solution containing KOH and PHP. The linear concentration range was calculated as 0.09–1.50 and 0.07–1.40 FFA% for corn and sunflower oils, respectively. The results were comparable with those obtained by the AOCS method. The benefits of proposed method were its simplicity and high sample throughput.

Makahleh and Saad[22] reported a single line FIA method attached with capacitively coupled contactless conductivity detector (C^4D). Methanol mixed with 1.5 mM sodium acetate (pH 8) 80:20 (v/v) was used as a carrier stream at a flow rate of 1.0 mL min^{-1}.

Good agreement was found between the standard non-aqueous titrimetry method and the proposed method. The proposed method offers high sampling rate of 40–60 sample/h and considerably low cost automated system that needs minimum human intervention over long periods of time. Recently a visual and green titrimetric method was proposed using PHP as an indicator.[23] The sample was dissolved in a water–ethanol mixture (1:1 v/v) and titrated with a 0.02 mol L^{-1} aqueous NaOH solution. The results obtained by proposed approach were comparable with AOCS titration method.

2.1.4. Miscellaneous spectrophotometric methods

A new approach for the FFA determination in non aqueous medium without titration was reported in the literature.[24,25] The method utilized phenol red as fatty acid indicator, which was solubilized in reverse micelles formed by AOT [sodiumbis(2-ethylhexyl) sulfosuccinate] in isooctane. Quantification was achieved by monitoring the changes in absorbance at 560 nm (disappearance of the red color). Although the method was economical (small amounts of samples and solvents) but found to be lacking in accuracy. Therefore, no any additional efforts were made for its improvement. Rejeb and Gargouri [26] reported a new method for the determination of olive oil acidity. The method was based on the measurement of polyunsaturated free fatty acids reacting with lipoxygenase. Hydroperoxy-fatty acids enzymatically formed were easily determined by UV spectrophotometry at 234nm. The proposed method was successfully applied for the determination of acidity in olive oil samples and good agreement was obtained between the proposed enzymatic method and classical titration method. This method was accurate, simple, sensitive, inexpensive, and reliable for acidity determination of olive oil with a

high sample throughput. Recently, Fedosov *et al.,*[27] developed a novel microtitration method for determining the FFA in small samples of oil (5–150 mg). The method was based on the optical signal of pyranine (aqueous pK 7.3), which changed its absorbance and fluorescence. All reactants were dissolved in a medium with universal solubility, which allowed accurate optical measurements on conventional equipment. Titration curves for FFA standards stipulated the selection of the neutralization point. Comparison of fluorescence and absorbance of pyranine pointed to somewhat better ‘‘signal to noise’’ properties of the fluorescent signal when working with heavily pigmented oil samples. Blind examination of different experimental mixtures (FFA = 0.15–40 %) revealed a good correspondence between the pyranine method and official method. Furthermore, it was reported that the small size of optical cells could allow further minimization of the pyranine assay if adapting it to an automated procedure.

2.2. Electrochemical methods

2.2.1. Voltammetric technique

Voltammetry is an important class of electrochemical methods widely used in analytical chemistry and various industrial processes. In voltammetry, information about an analyte is obtained by measuring the current as the potential is varied.[28] It is commonly used for compounds that are readily oxidized/reduced to provide highly selective and sensitive quantitative methods. Electrochemically FFA are weakly active and can be reduced or oxidized. The voltammetric behavior of quinone in the presence of acids in buffered protic solvents was first reported by Takamura and Hayakawa.[29] It was reported that reduced form of quinone (dissolved in ethanol) gave a very strong voltammetric signal in presence of acids. For FFA analysis in edible oils the behavior of quinone has been

utilized by various authors[30-34] to develop HPLC and FIA methods. The FIA system coupled with voltammetric detection had high sensitivity, better reproducibility, low solvent consumption and excellent throughput of 60 samples/h. Unfortunately this technique did not receive much attention. Li *et al.,*[35] proposed a new method using polypyrole-modified electrode in linear potential sweep voltammetric mode. In this method, the behavior of naphtoquinone in the presence of FFA was investigated in ethanol/1,2-dichloroethane (3:1) solution containing 0.1 M $LiClO_4$. A linear calibration graph was obtained in the range of 5.0×10^{-6}–6×10^{-3} M for FFA ($R = 0.993$), with sensitivity of 2.41×10^{-2} A L/mol and a limit of detection 1.2×10^{-6} M. The method was accurate, superior in sensitivity and capable to analyze 45 samples/h.

2.2.2. Electrical Conductivity method

Recently a new approach for the determination of FFA in edible oils based on the electrical conductivity (EC) value change of a 0.04 M potassium hydroxide (KOH) solution during KOH–FFA mixed reaction was reported.[36] The EC value changes of the KOH solution layers were determined, and the FFA was determined from calibration plot. Various parameters were optimized like the concentration of KOH solution, holding time, the types of edible oils, setting temperature, and the ratio of oil to KOH solution (*m*/*v*). The method was validated using standard addition and the AOCS method. The results and analytical performance of the EC procedure was better than that of the AOCS method.

2.2.3. pH-metric methods

In general the low polarity and high viscosity of oils have made it impractical to use pH metric methods for the determination of FFA. Lapshina *et al.,*[37] carried out pH metric measurements by extracting FFA from oil into a polar solvent. In order to facilitate extraction, the author suggested the use of a weak base triethanolamine (TEA) in an appropriate solvent mixture (diethyl ether 80%, chloroform 19% and water 1%) to convert carboxylic acids into their salts. Although the method was workable but showed poor reproducibility because of instability of the electrode and the basic reagent in the solvent mixture. Later on the method was modified by replacing the triethanolamine with a 1:1 (v:v) mixture of iso-propanol and water containing 0.2 M TEA and 0.02 M KNO_3.[38-41] Rather than extracting FFA, this solvent mixture effectively makes an emulsion in the range of pH measurement. Soon after, Velasco-Arjona and Luque de Castro [42] proposed a fully automated method using robotic station for the determination of AV in olive oil without titration based on pH measurements of emulsion. The excellent correlation with potentiometric titration demonstrated the convenience of the automated method. This method was able to analyze 15 samples/h. Various publications on this approach were reported for acidity measurements in oilseeds.[43-45] A new concept of flow titration was proposed using sequential injection analysis (SIA) with a diode array spectrophotometric detector linked with a multivariate curve resolution-alternating least squares (MCR-ALS) in the presence of alizarine indicator.[46] Total waste generated in this method was 3.33 ml (0.41 ml of sample, 0.25 ml of indicator and 3 ml of carrier). The results of the proposed method compared well with tritrimetric method and the frequency of the analysis was 12 samples/h.

2.3. Spectroscopic methods

2.3.1. Infrared methods

2.3.1.1. Direct Infrared methods

The possibility of utilizing FTIR spectroscopy for FFA analysis has been examined by number of scientists over the last two decades. Lanser *et al.,*[47] first time reported a semi quantitative method for the analysis of FFA in soybean oils by computer-assisted FTIR using deconvolution technique. He collected the spectra of oils using transmission cell, and observed the spectral changes in the carbonyl region in the range 2000-1600 cm^{1}, and correlated to the FFA content obtained by the standard AOCS method. The method was based on developing a calibration plot using oleic acid as standard into FFA-free soybean oil. A major restriction to make the method more quantitative was the spectral variation of ester band among various oils, because the carboxylic acid C=O absorption appears on the shoulder of triglyceride ester linkage absorption.

Later on an accurate method was developed based on the measurement of the carboxylic acid C=O band at 1711 cm^{-1} using transmission approach, covering an analytical range of 0.2–8% FFA.[48] To avoid the matrix effects noted earlier[47], the ratioed out calibration and sample spectra against the spectrum of a FFA-free oil of the same type of oil were analyzed rather than a background spectrum of air. This approach gave results comparable in precision and accuracy to that of the AOCS reference titration method. Through macroprogramming the method was completely automated, this approach takes an analysis time less than 2 min.

After the above preliminary studies, a new procedure was reported for FFA in olive oil based on attenuated total reflectance (ATR) FTIR spectroscopic measurements using partial least-squares regression (PLSR).[49] The developed method was applicable to samples of different categories of olive oil with analysis time of 5 min/sample. Che Man *et al.,*[50-51] described transmission FTIR spectroscopic methods using 200 μm NaCl windows for low level of FFA contents in palm olein (PO) and 100 μm BaF_2 windows for higher FFA in crude palm oil (CPO). The PLS calibration models were developed to correlate FTIR and chemical method. The developed method drastically reduced the analysis time to less than 2 min/sample. Verleyen *et al.,*[52] followed up more rigorously research on the work previously reported[47] and developed effective calibrations for different oils (corn, soybean, sunflower, palm, palm kernel, and coconut oils). It was concluded the calibration models developed for the six oils differed significantly and indicated the need to develop a calibration for each specific oil or fat.

Inon *et al.,*[53] developed the chemometric based method using PLS multivariate calibration and net analyte signal (NAS) preprocessing for the determination of FFA in commercial olive oil samples of different types and origins. The limit of detection (LOD), sensitivity and selectivity of the methodology developed were evaluated in terms of the net analyte signal and found LOD value of 0.072%, with sensitivity of 0.077 in terms of per unit concentration.

Due to the practical limitations of transmission spectroscopy for the quantification of higher FFA, a simple and direct methodology was developed for the determination of higher FFA contents (>30) by single bounce (SB) ATR FTIR spectroscopy in poultry feed lipids[54], deodorizer distillates and crude oils.[55] The accuracies of the methods were

checked by comparison to a conventional AOCS titrimetric procedure. Yu *et al.,*[56-57] reported a new approach for the automated determination of FFA in edible oils using spectral reconstitution (SR) technique. The data obtained by this technique was accurate and reproducible. The developed automated method was capable of analysing ~90 samples/h; a rate appropriate with the throughput required by commercial contract or high-volume process control laboratories.

2.3.1.2. Indirect infrared methods

For fats and oils that may have undergone significant thermal stress or extensive oxidation, an indirect FTIR spectroscopic method was developed for FFA based on ATR approach in order to remove the matrix effect.[48] The 1% KOH/methanol was used to extract the FFA and convert them to their potassium salts. The carboxylate anion absorbs at 1570 cm^{-1}, well away from interfering absorptions of carbonyl commonly present in oxidized oils. To overcome the matrix effects the indirect method was more sensitive and accurate than the direct determination of FFA, although the method complicated the analysis. An automated flow injection system was developed for the rapid determination of the FFA content in olive, sunflower, and corn oils.[58] The method was based on the indirect approach[48] to avoid the matrix effects linked with direct measurements of carboxylic acid band at 1711 cm^{-1}. This automated method was able to analyze ~40 samples/h.

A transmission spectroscopic method using potassium phthalimide (K-phthal) as a weak base to convert the FFA present in oils to their carboxylate salts without causing oil

saponification was reported.[59] The method was automatable, and capable of analyzing about 60 samples/h. Later on a sensitive and rapid methodology for the quantification of a low levels (<0.005%) of FFA in edible oils was described using sodium hydrogen cyanamide (NaHNCN) to convert FFA to their carboxylate salts.[60-61] The feasibility of employing a portable variable filter array (VFA) IR spectrometer equipped with a transmission flow cell for FFA analysis was reported by Li *et al.*[62] The indirect approach was employed to convert the FFA to their salts as described earlier. [60-61] Based on this measurement, a VFA-IR spectrometer provided an economical instrumental means for at-line monitoring of FFA levels in crude and refined edible oils capable of analyzing ~20–30 samples/h. Aryee *et al.,*[63] determined FFA content in fish oils employing same approach. It was reported that FTIR method is a flexible and viable instrumental alternative to the standard titrimetric method.

2.3.2. Near infrared methods

The near infrared (NIR) methods for the analysis of FFA in oils and fats were reported before the mid infrared (MIR) methods. Frankel *et al.,*[64] used NIR spectroscopy for the first time to determine the quality of the soybeans and its oil stored at different moisture levels. He observed that NIR analysis at 2260 nm showed a good correlation coefficient of 0.864 with titratable FFA. Furthermore, it was reported that NIR analysis is most suitable and rapid to evaluate hydrolytic deterioration in stored soybeans. This methodology was used to evaluate the factors affecting the food quality of soybeans for domestic and foreign markets.

After the initial work of Frankel *et al*.,[64] there was no any further work carried out on NIR for ten years. Majority of the methods were developed on mid IR range. In 1997, a direct method for the determination of FFA in fish oil was reported.[65] PLS and multiple linear regression (MLR) was used for calibration and described that first derivative mathematical treatment provided better results as compared to the second derivative and *N*-point smoothing for NIR spectra. The calibration equation for FFA obtained from PLS was found better as compared to the MLR.

CheMan *et al.,*[66] developed a calibration for the determination of FFA in crude palm oil and its fractions based on the NIR reflectance approach. For the preparation of FFA calibration, oil was hydrolyzed with 0.15% (w/w) lipase in an incubator at 60°C (200 rpm). The optimized calibration models were constructed with MLR analysis based on C=O overtone regions from 1850–2050 nm. Calibrations were validated with an independent set of 8–10 samples. The developed method was rapid, and its accuracy was generally good for raw-material quality control. The method was capable to analyze 12 samples/h. The hydrolytic degradation of lipids in fish oil was monitored in transflectance mode by an NIR monochromator instrument in the range of 1100–2500 nm.[67] For FFA analysis PLS regression calibration model was performed. The accuracy of the developed model was tested using a validation set. It was reported that NIR spectroscopy with PLS regression could be successfully used to monitor hydrolytic degradation of lipids in the fish oil stored under industrial conditions.

A novel method was developed for the estimation of FFA content directly in high-oleic sunflower seeds.[68] For the calibration a sample set of different varieties from the harvest of 2004 and 2005 was used. The developed NIR spectroscopic calibration was calculated with a modified PLS algorithm, standard normal variate (SNV), detrend scatter correction and the 2nd derivative of the spectra of ground sunflower seeds. The results obtained by this method demonstrated the efficiency and cost effectiveness of the NIR method for the evaluation of FFA content in sunflower seeds.

Ng *et al.,*[69] described a transmission based method using PLS and forward stepwise multiple linear regression (FSMLR) techniques for FFA in frying oils. PLS model provided improved results compared to FSMLR model when derivative as spectral treatment used in the region of 700-1100 nm. The best correlations between the NIR and wet chemical method for FFA was 0.943. Where as in the longer wavelength region (1100-2500 nm) FSMLR model was better than PLS model. The developed method was capable to analyze 20 samples/h. Many authors used NIR calibration for FFA quantification in the oxidation [70] and interesterification of oil [71] to replace the traditional methods. Furthermore it was reported that NIR spectroscopy might be adapted for real-time quality control purposes. Recently, Yu *et al.,*[72] reported an automatic determination of acid value in edible oils by NIR spectrometer coupled with continuous samples injection cell using edible oils as raw materials. The R^2 and the RMSEP of the calibration for acid value were 0.9873 and 0.114 mg/g in the range of 5500-4600 cm^{-1} with baseline correction point at 6524 cm^{-1} and 4823 cm^{-1} and SNV. By this method analysis rate could be achieved as 90 samples/h.

2.3.3. Raman spectrometry

Fourier transform (FT) Raman spectrometry in combination with PLS regression was used for direct reagent-free determination of FFA content in olive oils and olives. Oils were directly investigated in a simple flow cell, while olives were measured in a dedicated sample cup, which was rotated eccentrically to the horizontal laser beam during spectrum acquisition. External and internal (leave-one-out) validation were used to assess the predictive ability of the PLS calibration models for FFA content in oil and olives in the range 0.20–6.14 and 0.15–3.79%, respectively.[73] The results obtained by the proposed procedures could be used for screening of good quality olives before processing, as well as, for the on-line control of the production of oil.

El-Abassy [74] reported a visible Raman spectroscopic method for determining the FFA in extra virgin olive oil using PLS. For the calibration, oleic acid was used to increase the FFA content in extra virgin olive oil up to 0.80%. The calibration curve of actual FFA% obtained by titration versus predicted values based on the Raman spectral regions 1600-945 cm^{-1} provided a robust and precise model with high accuracy for the prediction of the FFA%. Although the methods were simple and accurate, but high cost of the instrument restricted the use of this technique in the routine analysis.

2.3.4. Nuclear magnetic resonance spectroscopy

Nuclear magnetic resonance (NMR) spectroscopy is increasingly applied to the analysis of food and food components. In the field of edible oils and fats, NMR provides useful information on the lipid class composition (the relative amount of triacylglycerols, diacylglycerols, FFA, sterols, phospholipids, and other minor components). The determination of FFA by NMR was first studied on the lipids extracted from albacore

tuna *(Thunnus alalunga)* using high-resolution ^{13}C NMR spectroscopy.[75] FFA carbonyl resonances were detected at the lower field of the ^{13}C NMR carbonyl region and used for its quantitative determination. NMR data agreed with those obtained using UV spectrophotometry, showed that NMR is a suitable method to follow lipolytic alterations. It was mentioned the method could be used to monitor the changes in fish lipids and oils during processing or storage. Sun and Moreira[76] studied the correlation between NMR proton relaxation times and the increase in FFA in soybean oil degradation. Soybean oil was degraded by heating only and by frying corn tortilla dough for 10 min/h up to 50 h. Both AOCS and NMR methods were used to determine the extent of oil degradation. Results showed that FFA increased as heating time increased. The longitudinal relaxation time (T_1) and the transverse relaxation time (T_2) decreased as oil degradation increased. There was a linear relationship between NMR and AOCS measurements. The number of methods reported on ^{13}C NMR[77,78], ^{31}P NMR[79], and ^{1}H NMR for FFA in vegetable oils.[78, 80] Although the developed methods were easy, rapid and simple and could be used as a promising tool for online quality control, but did not received much attention from the industry due to the high price of the NMR equipment and technical skills.

2.4. Thermogravimetric methods

Thermometric methods are also titrimetric methods that use the change of temperature resulting from a chemical reaction to determine the end point of the titration. The principle is the same as that for potentiometric auto-titration; the only difference is the type of sensor (detector) usually thermistors are used. Vaughan and Swithenbank[81] reported first considerable advances in determining the end point of the acid/base reaction in non-aqueous medium. A novel titrimetric procedure has been reported for the

determination of FFA in edible oils using an automated thermometric titration with KOH in isopropanol as titrant.[82] The end point was indicated by the strongly exothermic base-catalyzed reaction between acetone and chloroform. The procedure was accurate, sensitive, easy to use, fast, and highly reproducible but did not get much attention.

3. Conclusion

The above described instrumental techniques proposed considerable merits over classical titration methods in terms of speed, automation and reduction of costly as well as toxic chemicals. Calorimetric methods have poor sensitivity and need frequent calibration. Electrochemical methods require titrant standardization while accuracy and reproducibility depends on the conditions of the electrodes. Optimization of several parameters is also very essential. The discussion reflects that none of the methods described provides specificity to FFA measurement as all are based on measuring total acidity rather than FFA. Among the spectroscopic methods, FTIR methods have proved to be more beneficial in terms of simplicity, economy, environment friendly nature and speed of analysis, especially when using direct approach for the determination of FFA in vegetable oils. However, selection of the method for FFA determination depends on the availability of equipment, skilled worker, purpose of study and number of samples.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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