# Rapid Quantitative Determination of Free Fatty Acids in Fats and Oils by Fourier Transform Infrared Spectroscopy

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**Rapid direct and indirect Fourier transform infrared (FTIR) spectroscopic methods were developed for the determination of free fatty acids (FFA) in fats and oils based on both transmission and attenuated total reflectance approaches, covering an analytical range of 0.2–8% FFA. Calibration curves were prepared by adding oleic acid to the oil chosen for analysis and measuring the C=O band @ 1711 $cm^{-1}$ after ratioing the sample spectrum against that of the same oil free of fatty acids. For fats and oils that may have undergone significant thermal stress or extensive oxidation, an indirect method was developed in which 1% KOH/methanol is used to extract the FFAs and convert them to their potassium salts. The carboxylate anion absorbs @ 1570 $cm^{-1}$, well away from interfering absorptions of carbonyl-containing oxidation end products that are commonly present in oxidized oils. Both approaches gave results comparable in precision and accuracy to that of the American Oil Chemists' Society reference titration method. Through macroprogramming, the FFA analysis procedure was completely automated, making it suitable for routine quality control applications. As such, the method requires no knowledge of FTIR spectroscopy on the part of the operator, and an analysis takes less than 2 min.**

KEY WORDS: Fats and oils, free fatty acids, Fourier transform infrared spectroscopy, quality control.

Fats and oils from a wide variety of sources are important to the food industry and other industrial sectors. In almost every situation, the general characterization of such oils and the monitoring of modifications they may undergo during processing are important relative to their quality, functionality and economic value (1). Typical analyses (2) that are important include iodine value (degree of unsaturation), saponification number (average molecular weight), moisture content, *cis/trans* ratio, peroxide value, thiobarbituric acid number, anisidine value and free fatty acids (FFA).

In our laboratory we have been working on the development of rapid, general-purpose quality control methods based on Fourier transform infrared (FTIR) spectroscopy (3–7). FTIR spectrometers are a major advance over conventional grating instruments, having more energy throughput, excellent wavenumber reproducibility and accuracy, extensive data manipulation capabilities (subtraction, ratioing, derivative spectra and deconvolution) and advanced chemometric software to handle calibration development (3). Some systems also have macroprogramming capabilities, useful for automating analytical procedures (8). FTIR, coupled with an attenuated total reflectance (ATR) accessory or a flow-through transmission cell (9), simplifies many of the sample handling problems commonly associated with infrared (IR) analyses and is readily amenable to routine quality control applications (10).

Oils are ideal candidates for FTIR analysis, as they are essentially single-component systems (triglycerides) and can be applied directly in their neat form to an ATR crystal or passed through a flow cell. Solid fats can also be handled in an analogous manner if the ATR crystal or flow cell is heated to above the melting point of the fat. Advantage has been taken of these features to develop a rapid ATR-based method for the direct determination of saponification number and iodine value by FTIR (11).

One of our objectives is to systematically develop rapid FTIR methods capable of replacing most of the common laborious, wet chemical American Oil Chemists' Society (AOCS) procedures and thus minimizing the environmental concerns associated with the use of organic solvents and hazardous reagents. The determination of FFAs is one of the more common analyses that fats and oils are subjected to in terms of evaluating lipolysis after extraction and of defining and monitoring refining procedures (1). The AOCS chemical method is a simple procedure based on the extraction of the FFA into ethanol and titration with a defined concentration of base to a phenolphthalein endpoint; the titer is expressed in terms of percentage of oleic acid (2). Although simple, the procedure is cumbersome, requires substantial personnel time, glassware and accurate preparation of reagents and is dependent on a visual endpoint. This paper presents a viable, utilitarian method for the determination of the FFA content of fats and oils by FTIR.

## MATERIALS AND METHODS

*FTIR.* The instrument used for this development work was a Nicolet 8210 Fourier transform infrared spectrometer (Nicolet Instrument Inc., Madison, WI) run under a DX operating system (8). Two sampling methods were assessed, one employing a transmission flow cell (100 $\mu$ $CaF_2$) and the other a horizontal ATR (40° ZnSe) plate. The flow cell was attached to a Spectra-Tech sipper unit (Spectra-Tech Ltd., Stamford, CT) to automate sample handling. The instrument was purged with dry air using a Balston dryer (Balston, Lexington, MA) to minimize water vapor and $CO_2$ interferences. Both the flow cell and ATR plate were equipped with resistance heaters (rods and tape, respectively) to allow temperature settings up to 70°C (±0.2°C), each controlled by an Omega CN4400 temperature controller (Omega Engineering, Stamford, CT). The flow cell and ATR plate were operated at ambient temperature.

*Spectra acquisition.* Prior to calibration or analysis, the flow cell was initially cleaned by passing a 1% solution of Triton X-100 (Sigma Chemical, St. Louis, MO) through the cell, followed by water and propanol rinses. Samples were pumped into the cell with the sipper, each sample purging the previous one.

For the ATR system, the crystal surface was thoroughly cleaned with a tissue soaked with Triton X-100 solution, followed by water and propanol rinses. ATR analysis was

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carried out by applying the sample onto the crystal, recording the FTIR spectrum and then wiping the crystal clean with propanol. All spectra were collected by co-adding 128 scans at a resolution of 4 $cm^{-1}$ and a gain of 2.0.

*Direct method—fresh oils.* Olive oil was used as the matrix to develop FFA calibrations. The oil was extracted three times with 0.01N KOH in a separatory funnel to remove all FFAs, the water layer was separated and the oil was dried over anhydrous sodium sulfate. Laboratory-grade oleic acid (BDH Chemicals, Toronto, Ontario, Canada) was added to the clean olive oil in precise amounts to cover a range of 0–8%.

A reference background single-beam spectrum of the fatty acid-free olive oil was recorded and stored to disk. The single-beam spectrum of each oleic acid-spiked olive oil standard was ratioed against the clean olive oil single-beam background spectrum to produce an absorbance spectrum of oleic acid, the spectral features of the oil having been ratioed out. The absorbance spectrum was baseline-corrected by using 1850 and 1580 $cm^{-1}$ as anchor points and the peak height of the oleic acid carboxyl C=O absorption band was measured @ 1711 $cm^{-1}$. A standard curve was obtained by regressing the FFA contents of the standards against the recorded peak heights.

The same procedure was followed for the acquisition of the spectra of samples, except that the single-beam background spectrum employed was that of a fatty acid-free sample of the same type of oil as that being analyzed.

*Indirect method—oxidized/thermally stressed oils.* For oxidized/thermally stressed oils, an indirect FTIR method, based on a simple 1% KOH/MeOH extraction, was investigated to overcome potential interfering absorbances from autoxidation products. Methanol was dried over molecular sieves (type 4A, J.T. Baker Chemical Co., Phillipsburg, NJ) prior to use. The extraction procedure consisted of adding KOH/anhydrous MeOH to oil in a 1:1 ratio (w/w) in a 10-mL screw-capped test tube and shaking thoroughly for 1 min. The sample was centrifuged for 2 min in a benchtop centrifuge to ensure complete phase separation, and the upper MeOH layer was pumped directly into the $CaF_2$ flow cell. The single-beam spectrum of the methanol solution containing the FFAs was ratioed against the background single-beam spectrum of 1% KOH/MeOH. The resulting absorbance spectrum was baseline-corrected by using 1550 and 1612 $cm^{-1}$ as anchor points and the absorbance of the ionized carboxyl group ($COO^-$) of oleic acid was measured @ 1570 $cm^{-1}$. Calibration for this procedure simply involved preparation of a set of calibration standards by adding various amounts of oleic acid to 1% KOH/MeOH, ratioing the single-beam spectra recorded for these standards to that of KOH/MeOH and plotting absorbance @ 1570 $cm^{-1}$ *vs.* the concentration added.

*Analysis.* To test the efficacy of both the direct and indirect methods, olive oil was saponified with NaOH to produce a mixture of FFAs. The saponified oil was acidified to pH 5 with HCl to regenerate the FFAs from the salts and was washed three times with water in a separatory funnel to remove any salt and residual soaps, the water was separated and the fatty acid mixture was dried over anhydrous sodium sulfate. The FFA mixture was added (w/w) to a selection of commercial oils (olive, peanut and sesame), and these samples were then analyzed by the AOCS titrimetric procedure. These samples were also analyzed by the direct FTIR procedure, with the FFA content predicted from the calibration equations derived from the oleic acid/olive oil standard curves. A parallel comparative experiment was carried out by the indirect method with FFA-spiked samples of both regular olive oil and the same oil stressed thermally to induce oxidation.

Both the calibration and analysis procedures developed for FFA analysis (ATR or flow cell) for the direct and/or indirect method were programmed as macros on the Nicolet 8210 system. The operator would simply have to follow instructional prompts to operate the instrument (5); only the regression required to obtain the calibration equations would have to be performed outside the macro program. Below is a simplified outline of the macro used to automate the analysis. Regression analysis and statistical evaluation of the data were carried out with Statgraphics (STCS Inc., Rockville, MD), a general-purpose statistical analysis and graphics package. The accuracy and reproducibility of the methods were evaluated in terms of mean difference (MD) and standard deviation of the difference (SDD) as advocated by the Association of Official Analytical Chemists (12).

[1] ENTER DATE:
[2] ENTER OPERATOR NAME:
[3] ENTER PRODUCT NAME:
[4] ENTER PRODUCT CODE #:

[5] HAVE YOU CALIBRATED THE INSTRUMENT? Y/N
[6] IF YES GO TO [19]

[7] **CALIBRATION ROUTINE**
[8] PUMP PROPANOL THROUGH CELL TO CLEAN
[9] PUMP IN CLEAN REFERENCE OIL
[10] HOW MANY STANDARDS ARE THERE? "X"
[11] SCAN; STORE SINGLE-BEAM FILE TO DISK AS EMFR
[12] x=1
[13] PUMP IN CALIBRATION STANDARD x
[14] SCAN TO OBTAIN SINGLE-BEAM FILE EMFx
[15] RATIO EMFx TO EMFR, BASELINE CORRECT AND MEASURE ABSORBANCE @1711 = "ABS"
[16] PRINT: STANDARD "x" HAS AN ABSORBANCE OF "ABS"
[17] IF x<X GOTO [13]
[18] CALIBRATION ROUTINE COMPLETE—CARRY OUT LINEAR REGRESSION OF ABSORBANCE VS % OLEIC ACID ADDED TO THE OIL TO OBTAIN SLOPE "S" AND INTERCEPT "I"

[19] **ANALYSIS ROUTINE**
[20] ENTER SLOPE OBTAINED FROM REGRESSION "S"
[21] ENTER INTERCEPT OBTAINED FROM LINEAR REGRESSION "I"
[22] PUMP PROPANOL THROUGH CELL TO CLEAN
[23] PUMP IN CLEAN REFERENCE OIL
[24] HOW MANY SAMPLES ARE THERE? COUNT=X
[25] SCAN; STORE SINGLE-BEAM FILE TO DISK AS EMFR
[26] x=1
[27] PUMP IN SAMPLE x
[28] SCAN TO OBTAIN SINGLE-BEAM FILE EMFx
[29] RATIO EMFx TO EMFR, BASELINE CORRECT AND MEASURE ABSORBANCE @1711; "ABS"
[30] FFA = (ABS*S)+I
[31] PRINT: SAMPLE "x" CONTAINS "FFA" % FREE FATTY ACIDS
[32] IF x<X GOTO [27]
[33] END OF ANALYSIS

## RESULTS

*Calibration.* Figure 1a presents an overlaid series of ATR spectra of olive oil, containing ~1-8% oleic acid, ratioed against an air background, illustrating the complete oil spectrum and its major bands. The triglyceride ester-linkage (COOR) band @ 1748 $cm^{-1}$ is off-scale because the 40° ZnSe crystal has a greater depth of penetration than conventional 45 or 60° ZnSe ATR crystals. The C=O absorption of oleic acid dimers (13) lies on the shoulder of the COOR band and is discernible as a band rising @ 1711 $cm^{-1}$ with increasing concentrations of FFA. The absorption band due to the monomers, which is observed @ 1760 $cm^{-1}$ in $CS_2$ solution (13), was not detected. Figure 1b illustrates the same set of spectra after ratioing against the spectrum of fatty acid-free olive oil, illustrating that the spectral contribution of the oil has been eliminated, leaving only the spectrum of oleic acid. The noise in the 1760-1725 $cm^{-1}$ region of these spectra is due to the highly absorbing COOR group not allowing adequate energy to reach the detector to be digitized and ratioed properly. In the transmission mode, where the practical path length required is ~100 $\mu$ (*vs.* an effective path length of ~45 $\mu$ on a 40° ATR crystal in this region of the spectrum) to allow viscous oils to flow at a reasonable rate, the oleic acid band sits on a shoulder of ~0.8 absorbance units, and the ability to accurately ratio out the spectrum of the base oil at 1711 $cm^{-1}$ was thus a concern. However, ratioing under these conditions was accurate and consistent as long as sufficient (~128) scans were taken.

A typical plot of absorbance @ 1711 $cm^{-1}$ *vs.* oleic acid concentration (0-8%) for measurements in the flow cell is presented in Figure 2, and the corresponding equation obtained from linear regression of these data is:

$$FFA = 0.2383 + 6.2787A \quad [1]$$

with $R^2 = 0.998$ and SE = 0.149% where FFA = % FFA as oleic acid, A = absorbance, $R^2$ = correlation coefficient and SE = standard error.

Careful examination of the plot indicated that the relationship is not linear but has a slight curvature. The nonlinear effect may be attributed to the monomer-dimer equilibrium, which is not accounted for in Equation 1 because only the absorption by dimers is measured and related directly to the FFA content. Because this equilibrium is shifted far to the right (14), the curvature produced is not excessive, and the error associated with it can be minimized by calibrating and regressing the data over selected ranges (*i.e.,* 0-2, 2-5 and 5-8%) and then applying the appropriate linear equation based on the absorbance result obtained.

The preferred calibration approach is to use nonlinear regression, and it was found that the following equation fit the data well:

$$FFA = -0.044 + 4.9594A + 1.5446A^{1/2} \quad [2]$$

with $R^2 = 0.999$ and SE = 0.074%.

Calibrating the system for ATR produced a similar curvilinear plot, and the respective linear and nonlinear calibration equations obtained were:

$$FFA = 0.130 + 16.686A \quad [3]$$

with $R^2 = 0.999$ and SE = 0.124%;

$$FFA = -0.176 + 12.014A - 3.3414A^{1/2} \quad [4]$$

with $R^2 = 0.999$ and SE = 0.079%.

When lower concentrations (0-1%) were examined by both ATR and transmission, it was found that ATR reached its limit of detection near 0.2%, while the transmission method could detect amounts as low as 0.05% FFA when calibrated over this range.

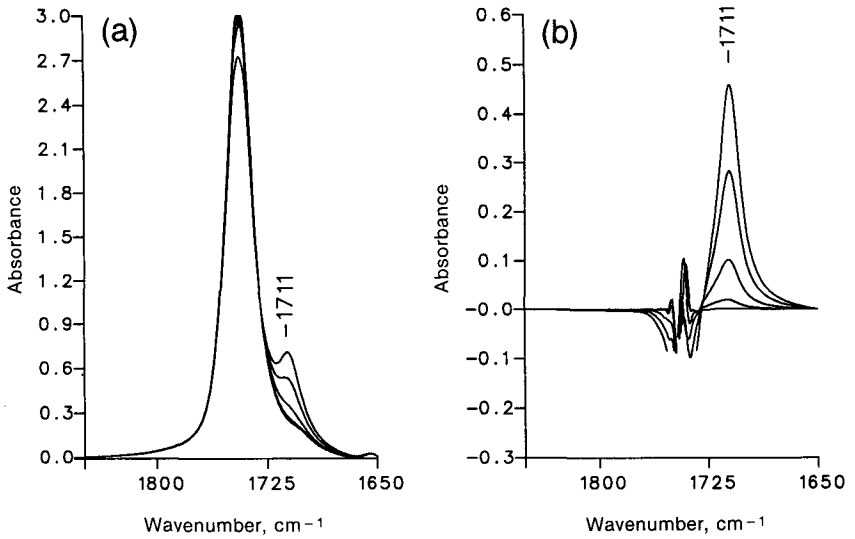


**FIG. 1. (a) Overlaid attenuated total reflectance spectra of olive oil spiked with oleic acid ratioed against the spectrum of air; (b) overlaid spectra of the same samples ratioed against the spectrum of olive oil.**

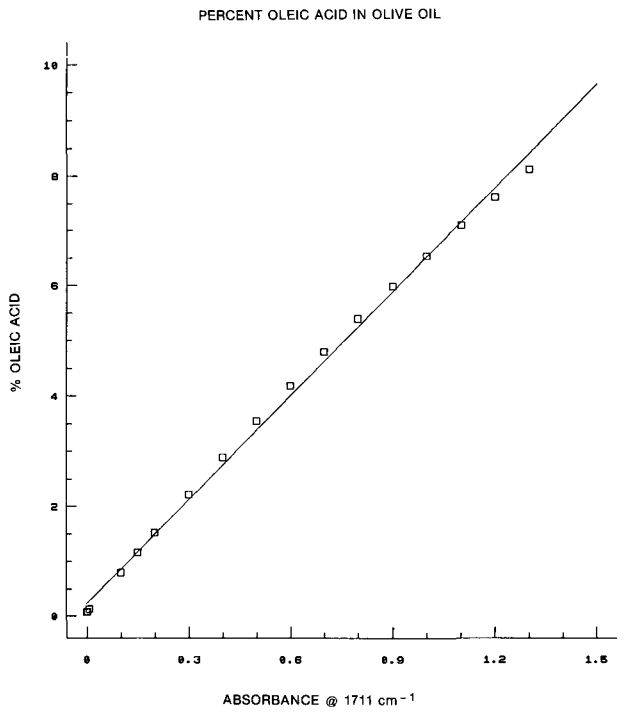


**FIG. 2. A plot of percentage of oleic acid *vs.* absorbance @ 1711 $cm^{-1}$ obtained from a 100 $\mu$ $CaF_2$ flow cell over the range of 0.02–8.0% free fatty acids.**

These calibration curves are not applicable to fats and oils that have been extensively oxidized or thermally stressed. Oxidative breakdown products such as hydroperoxides, aldehydes and ketones, as well as FFAs, are commonly present in such samples (15,16), with aldehydes and ketones having absorption bands in the 1730–1670 $cm^{-1}$ region. The effect of the presence of these absorption bands on the determination of FFAs is illustrated in the bottom panel of Figure 3, which shows a broad tail on the ester carbonyl peak extending to ~1670 $cm^{-1}$. This envelope includes contributions from saturated and unsaturated aldehydes as well as FFA. Thus, the presence of aldehydes and ketones in significant amounts can cause errors in the measurement of the FFA content, especially at lower levels (<2.0%), with the relative error decreasing as higher concentrations of FFAs are reached. To overcome this limitation, an alternate, indirect analytical method was developed based on the extraction of FFA from oils with 1% KOH/MeOH. This method simultaneously extracts the FFAs and converts them to their ionized potassium salts, which absorb at 1570 $cm^{-1}$ rather than 1711 $cm^{-1}$ (top panel, Fig. 3). Thus, although oxidation products as well as a small amount of oil are extracted into the methanol phase together with the FFAs, the shift of the FFA peak upon ionization of the FFAs eliminates spectral interferences from oxidation products and allows the unambiguous measurement of FFAs. The standard curve obtained for oleic acid (0.2–2%) in 1% KOH/MeOH

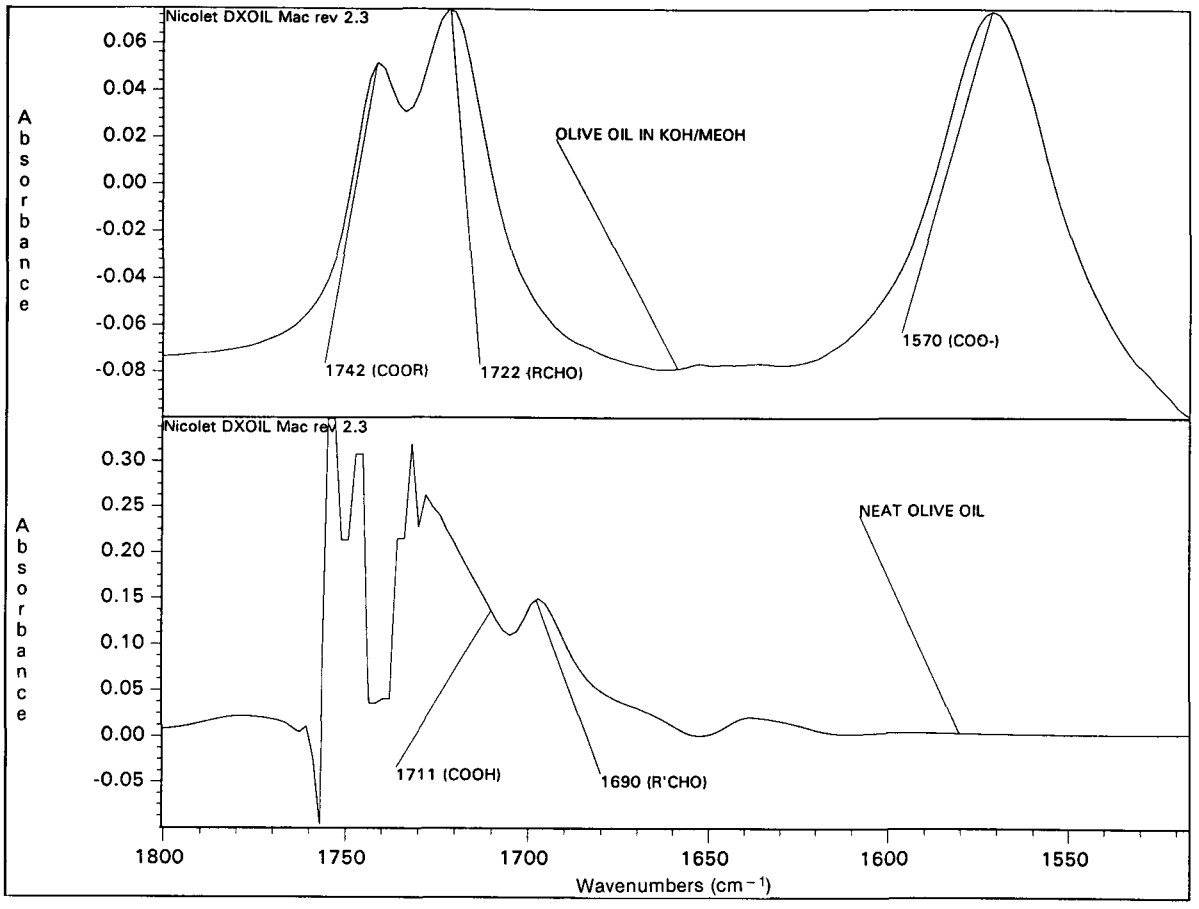


**FIG. 3. Bottom: Transmission Fourier transform infrared spectra of thermally stressed oil ratioed against the spectrum of fresh oil. Top: Spectrum of the 1% KOH/MeOH extract. RCHO and R′CHO represent saturated and unsaturated aldehydes, respectively.**

with the 100 $\mu$ transmission cell produced the following calibration equation:

$$FFA = -0.030 + 7.576A \quad [5]$$

with $R^2 = 0.995$ and SE = 0.024%. No nonlinear effects were observed in this standard curve because the potential for dimer formation *via* hydrogen bonding is destroyed when the salt is formed.

*Analysis.* FFAs prepared by the saponification of olive oil were added to a set of vegetable oils and analyzed by the AOCS chemical method and by transmission FTIR. The indirect method was assessed by spiking thermally stressed and regular olive oil with the FFAs. The macro programs written for controlling the instrument and incorporating calibration Equations 2 and 5 for converting absorbance data to %FFA were used for these analyses. Table 1 presents representative data obtained for duplicate analyses by the direct method with the 100 $\mu$ $CaF_2$ flow cell.

The data in Table 1 indicate that the chemical method has an accuracy of ±0.05% relative to the amount of FFA added to the oils, with a reproducibility of ±0.02%. FTIR is not quite as accurate (±0.13%) as the chemical method. The reproducibility of the FTIR procedure is similar to that of the AOCS titration method. Comparison of all the data by a two-way analysis indicated that there were no significant differences between any of the data sets ($P < 0.001$). These results indicate that the direct FTIR method generally performs as well as the titration method and is independent of the oil analyzed. The latter method potentially has more sources of experimental error, *i.e.*, standardization of base, weighing and endpoint determination, than the direct FTIR method, which is a simpler procedure with fewer manipulative steps. The ATR method performed similarly, except that concentrations below 0.2% could not be measured (data not presented).

Table 2 presents data for the indirect method assessed on both regular and thermally stressed olive oil spiked with FFAs and analyzed by transmission FTIR. The reproducibility is similar to that of the direct method while the accuracy is somewhat better, even though the data include the composite error of the extraction and FTIR measurement steps. The method is simple and works well; however, it is important to standardize the procedure to obtain consistent results. Furthermore, there is a possibility of some saponification taking place at the interface if the samples are left standing for more than 24 h: a mean increase in FFA content of 0.098 and 0.156% was obtained for the regular and oxidized oils, respectively (Table 2) when the samples were left to stand overnight prior to separation of the oil and MeOH phases and FTIR analysis. ATR can also be used for the indirect method; however, the result may be somewhat more variable if care is not taken to avoid evaporation of MeOH, which can be minimized by covering the ATR crystal. The overall results of both the direct and indirect methods indicate that the reproducibility of the FTIR method is excellent and that its accuracy is on a par with that of the reference procedure.

TABLE 1

**Results of Duplicate Analyses of Oils Spiked with an FFA Mixture by the AOCS Reference (CHEM) and the Direct FTIR Method[a]**

| Oil | SPIKE | CHEM1 | CHEM2 | FTIR1 | FTIR2 |
|---|---|---|---|---|---|
| Olive0 | 0.000 | 0.00 | 0.00 | 0.000 | 0.000 |
| Olive1 | 0.062 | 0.03 | 0.04 | 0.103 | 0.113 |
| Olive2 | 0.498 | 0.53 | 0.50 | 0.419 | 0.425 |
| Olive3 | 2.000 | 2.08 | 2.09 | 1.871 | 1.888 |
| Olive4 | 5.040 | 5.08 | 5.08 | 5.108 | 5.177 |
| Olive5 | 8.090 | 8.16 | 8.18 | 7.992 | 8.018 |
| Peanut0 | 0.000 | 0.00 | 0.00 | 0.000 | 0.000 |
| Peanut1 | 0.063 | 0.05 | 0.06 | 0.118 | 0.131 |
| Peanut2 | 0.501 | 0.52 | 0.50 | 0.431 | 0.430 |
| Peanut3 | 1.990 | 2.05 | 2.07 | 1.849 | 1.844 |
| Peanut4 | 5.040 | 5.10 | 5.10 | 4.943 | 4.951 |
| Peanut5 | 8.090 | 8.18 | 8.14 | 7.940 | 7.938 |
| Sesame0 | 0.000 | 0.00 | 0.00 | 0.000 | 0.000 |
| Sesame1 | 0.060 | 0.07 | 0.08 | 0.115 | 0.124 |
| Sesame2 | 0.499 | 0.51 | 0.54 | 0.426 | 0.427 |
| Sesame3 | 2.018 | 2.13 | 2.14 | 1.916 | 1.914 |
| Sesame4 | 5.020 | 5.00 | 5.04 | 5.214 | 5.281 |
| Sesame5 | 8.040 | 8.22 | 8.19 | 8.084 | 8.114 |
| $MD_r$[1] | | | −0.002 | | −0.013 |
| $SDD_r$ | | | 0.021 | | 0.022 |
| $MD_a$[2] | | −0.046[b] | | 0.031[c] | 0.059[d] |
| $SDD_a$ | | 0.057 | | 0.100 | 0.127 |

[a]Abbreviations: MD = mean difference; SDD = standard deviation of the difference; r = reproducibility; a = accuracy; FTIR, Fourier transform infrared; FFA, free fatty acids; AOCS, American Oil Chemists' Society.

[b–d]Comparisons of spike to mean chemical; mean chemical to mean FTIR; and spike to mean FTIR.

TABLE 2

**Results of Duplicate Analyses of Oxidized and Regular Olive Oils Spiked with an FFA Mixture and Analyzed by the Indirect KOH/MeOH FTIR Method[a]**

| | Oxidized | | | Regular | | |
|---|---|---|---|---|---|---|
| Oil | SPIKE | FTIR1 | FTIR2 | SPIKE | FTIR1 | FTIR2 |
| Olive1 | 0.221 | 0.235 | 0.233 | 0.219 | 0.253 | 0.255 |
| Olive2 | 0.521 | 0.515 | 0.507 | 0.496 | 0.481 | 0.489 |
| Olive3 | 1.101 | 1.101 | 1.097 | 0.946 | 0.922 | 0.941 |
| Olive4 | 1.581 | 1.531 | 1.564 | 1.472 | 1.420 | 1.463 |
| Olive5 | 1.997 | 1.902 | 1.874 | 1.926 | 1.790 | 1.868 |
| $MD_r$ | | | 0.002 | | | −0.033 |
| $SDD_r$ | | | 0.022 | | | 0.033 |
| $MD_a$ | | | 0.003 | | | −0.000 |
| $SDD_a$ | | | 0.005 | | | 0.050 |

[a]See Table 1 footnotes for Abbreviations.

## DISCUSSION

The AOCS FFA method is predicated on the measurement of total titratable acidity, expressed by convention as percentage of oleic acid. The FTIR approach directly mimics this procedure, spectrally measuring carboxyl groups rather than utilizing a stoichiometric chemical reaction. By calibrating the method with oleic acid, the results obtained are expressed directly in terms of the reference method. A similar, but less straightforward, approach has been taken by other workers (17), who described the FTIR determination of the FFA content of soybean oil. Their analysis required the application of spectral deconvolution techniques because they did not ratio the spectra of samples against that of a reference oil, and therefore, the FFA peak was overlapped by the much stronger ester linkage carbonyl band. The sampling technique employed by these workers, involving pressing the oil in its neat form between two KBr plates, would not be convenient for routine use. The transmission flow cell method employed in the present study is more convenient from a sample handling perspective; in addition, the use of a sipper unit automates sample handling, allowing multiple samples to be analyzed sequentially, avoids a cleaning step between samples and is suitable for both the direct and the indirect method. ATR is also an excellent technique, working well for FFA concentrations of >0.2%, and serves more as a general purpose accessory that is suitable for on-demand analyses where a flow cell would be limiting. Both approaches allow one to obtain an analysis in less than 2 min.

The indirect method need only be considered in circumstances when low concentrations of FFA have to be determined in oxidized or thermally stressed oils, where the direct method would give erroneous results due to spectral interferences arising from the presence of aldehydes and ketones. The one additional step required is straightforward; however, care must be taken that the extraction is carried out with a 1:1 (w/w) oil/methanol ratio (±0.0010 g) to maintain the theoretical accuracy of 0.02% FFA. Alternatively, any deviations from this ratio can be corrected for if the exact weights of oil and methanol are recorded. The upper limit for FFA determinations by the KOH/MeOH extraction is ~3% due to solubility limitations. The indirect method also provides the possibility of evaluating the amount of residual water in fats and oils after processing. Calibration for this determination would simply require preparing a set of standards containing 0.2–2% water in anhydrous MeOH and relating their moisture content to the height of the water absorption peak at 1650 $cm^{-1}$ in their FTIR spectra.

In circumstances where the sample in question is a solid fat, the direct and indirect method can still be used. For analyses of such products by the direct method, the fat should first be melted and passed warm into a heated flow cell or placed on a heated ATR plate. The input and outlet lines on the flow cell require wrapping with heating tape to prevent solidification of fat in the lines. Calibrations have to be carried out at the same temperature, and the cell temperature has to be controlled to within 1°C, as fluctuations in cell temperature change the refractive index/density of the oil and the effective pathlength of the flow cell or ATR sampling accessory. For the more general case, where both fats and oils are to be analyzed, it is best to carry out all work at 70°C, a temperature suitable for melting most fats. For the indirect method, the extraction can be carried out at 70°C in a screw-capped test tube to avoid MeOH evaporation, with the fat maintained in the liquid state. After extraction, the sample can be run warm through the heated flow cell; if the ATR method is employed, the sample must be cooled prior to analysis to crystallize the fat and avoid evaporative loss of methanol.

One of the unique benefits derived from the methods developed is the advantage of being able to program and automate the method. This ability converts the FTIR instrument into a utilitarian quality control tool that can be used in the lab or plant for routine analyses. The operator need not have any knowledge of FTIR spectroscopy and simply follows menu-driven prompts specific to the analysis in question, *i.e.*, present sample, pump and the results are printed out. The calibration procedure is just as simple and only involves the preparation of serial dilutions of oleic acid in an appropriate oil for the direct method or in 1% KOH/MeOH for the indirect method. The calibrations are stable and can be verified through the use of a bulk check sample with a known FFA content.

The methods described provide a simple and direct means of determining the FFA content of fats and oils, giving results comparable to the AOCS reference method. The automation of the method by macroprogramming makes it suitable for routine quality control analysis, providing FFA results in less than 2 min.

## ACKNOWLEDGMENTS

The authors thank the Natural Sciences and Engineering Research Council of Canada for funding this research and Nicolet Instrument for providing the 8210 spectrometer. Thanks also go to L. Drouin and E. Despland for technical assistance.

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[Received September 8, 1992; accepted January 11, 1993]