Supercritical Fluid Extraction Coupled to Infrared Spectroscopy for Iodine Number Analysis of Edible Oils

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A method is presented using supercritical fluid extraction coupled to infrared (IR) spectroscopy to characterize oils extracted from food samples. Supercritical CO_2 is passed through the sample and transferred directly to a high-pressure flow cell within an IR spectrometer. By monitoring the vinyllic C−H band, the amount of unsaturated fat can be determined. Using spiked samples the iodine number of the oil is shown to correlate with the absorbance intensity of the vinyllic C−H band; however, better correlation is seen with the ratio of the absorbance intensities for the vinyllic C−H and symmetric CH_2 bands.

Keywords: *Iodine number; IR; SFE; triglycerides; unsaturated fats; vegetable oils*

INTRODUCTION

Supercritical fluid extraction (SFE) is rapidly becoming an important sample preparation method in the food sciences. As a few examples, SFE has been reportedly used in removing fat from meat (King et al., 1989), fractionation of milk fat (Arul et al., 1987), removal of cholesterol from milk (Bradley, 1989) and meats (Wehling, 1991), screening for pesticides in meats (France and King, 1991; Snyder et al., 1993), and the total fat determination of foods (Lembke and Engelhardt, 1993). SFE has also the added advantage of directly coupling to other analytical methods for improvement in automation. Several papers in the literature have shown how to directly couple SFE to other analytical methods (Hawthorne et al., 1990; Kirschner and Taylor, 1993).

One such analytical method is infrared (IR) spectroscopy. In this coupled technique, termed SFE-IR, the supercritical extraction solvent, after passing through the sample matrix, is transferred directly to an FTIR spectrometer for analysis. In the literature, SFE-IR has been used to determine the caffeine content of coffee beans (Heglund et al., 1994) and the amount of finishing oil on synthetic fibers (Kirschner et al., 1994). In these papers, IR spectroscopy is used in connection with Beer's law to quantify analyte content in the samples; however, IR spectroscopy is also rich in important molecular structure information (Levine, 1970). For example, the C−H stretch bands for saturated carbons occur slightly below 3000 cm^{-1}, while the vinyllic and aromatic C−H stretch bands occur slightly above 3000 cm^{-1}. Also, the carbonyl bond has a strong, characteristic IR absorbance band. SFE-IR can be used to characterize the molecular structure of the extracted analyte.

Supercritical CO_2 is a good solvent for IR spectroscopy, especially in monitoring the C−H bands of dissolved analytes. Moreover, CO_2 is less toxic and less dangerous than the commonly used perchlorinated solvents employed in other IR-related work. Finally, for food applications, it has been shown that supercritical CO_2 is an excellent solvent for triglyceride lipids (De Filippi, 1982).

This work uses SFE-IR to characterize triglyceride fats and refined vegetable oils. These data are compared to iodine numbers determined by using the Hanus method. In traditional assay methods, the amount of unsaturated fats has been related to the iodine number. These methods are based on the addition of iodine to the carbon−carbon double bonds. Higher iodine numbers indicate higher amounts of unsaturation. Likewise, one would expect similar information from IR spectroscopy of the vinyllic C−H band. The intensity of this IR band should be proportional to the number of vinyllic C−H bonds and thus the amount of unsaturated triglyceride. This correlation has been suggested in studies employing supercritical fluid chromatography coupled to IR spectroscopy (SFC-IR) (Calvey et al., 1991).

By definition, the iodine number is the number of grams of elemental iodine absorbed by 100 g of fat, oil, or wax (Hendricks, 1977). For a pure triglyceride, the iodine number, I, can be calculated as

$$I = 25381(d/M) \tag{1}$$

where d is the number of carbon−carbon double bonds in the molecule and M is the molecular weight of the triglyceride. In the case of triolein, d is 3 and M is 885.40, giving an iodine number of 86. Now the value for d is proportional to the number of carbon−carbon double bonds per mole of the triglyceride, while the ratio d/M is proportional to the number of carbon−carbon double bonds per gram of the triglyceride. According to Beer's law, the IR molar absorptivity of the vinyllic C−H band should be proportional to d. But by measuring the IR absorbance of the vinyllic C−H band in reference to the weight of the triglyceride, one would measure a coefficient, ϵ(H−C═C), that would be proportional to the ratio d/M. According to this simple model and eq 1, the measured absorptivity coefficient, ϵ(H−C═C), should be linearly proportional to the iodine number of the triglyceride:

$$I \propto \epsilon(\text{H}-\text{C}=\text{C}) \tag{2}$$

In this paper this relationship is verified with data collected from refined vegetable oils. Also, an improvement is presented to this formula that incorporates another IR band for internal calibration.

An actual SFE-IR analysis is performed directly on corn tortilla chips. The collected IR spectrum is presented. The iodine number of the extracted oil is

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estimated using the improved calibration curve determined from the spiked-oil samples. Since these extractions are dynamic, that is, the supercritical fluid is continuously flowing during the entire extraction, an extraction kinetic profile can be easily generated by monitoring the effluent spectrum as a function of time. SFE-IR is also nondestructive, so the analytes could be collected and assayed later by using other analytical techniques.

MATERIALS AND METHODS

Apparatus. The SFE-IR interface used in this work was based on a high-pressure IR flow cell manufactured by The Foxboro Co. (Foxboro, MA). The cell windows were fabricated from zinc selenide. The flow cell's optical path length was 10 mm and had an internal volume of 200 μL. The high-pressure flow cell, rated up to 500 atm, was connected to the SFE extractor through a 304L stainless steel capillary transfer line. This transfer line allows the supercritical fluid effluent from the extractor to flow to the high-pressure IR flow cell. The capillary tubing is 1 m long with a 100-μm i.d. and an internal volume of less than 8 μL. This tubing is heated by passing an electrical current through it. Temperature control of the heated transfer line is achieved by monitoring its temperature from the electrical resistance of the tubing, using the same technology as the Isco, Inc. (Lincoln, NE), coaxially heated capillary restrictor. The stainless steel capillary tube serves as both the heating element and the temperature sensor. The transfer line is thermally and electrically insulated with a woven fiberglass sleeve. The pressure in the flow cell and the flow of the fluid were maintained by an Isco 1.5 mL/min coaxially heated capillary restrictor.

Although the flow cell was not originally designed to be heated, it was quickly discovered that it must be when fats and waxes are extracted to prevent window fogging. The flow cell was modified by attaching two Watlow (St. Louis, MO) 25-W, 24-V cartridge heaters onto the top and bottom of the flow cell body. A K-type thermocouple was attached to the front window nut of the flow cell. The temperature of the flow cell was controlled by a Fuji Electric (Tokyo, Japan) PYZ4 temperature controller with supporting electronics.

The Isco SFX 2-10 extractor with an Isco 260D syringe pump was used for the SFE portion of the instrument system. The SFE extract effluent was monitored by FTIR spectroscopy, using a Bomem (Quebec, Canada) M100 FTIR spectrometer or a Nicolet (Madison, WI) MagnaIR 750 FTIR spectrometer, both with a resolution of 4 cm^{-1}. Even though the high-pressure IR flow cell was designed especially for the Foxboro spectrometers, it was easily installed in both the Bomem and Nicolet FTIR spectrometers with minor optical realignment. Due mainly to the thick zinc selenide windows and the 4-mm-diameter aperture, the empty flow cell transmits 5% of the total IR light. The zinc selenide windows cut off IR transmission below 800 cm^{-1}; however, since the CO_2 also absorbs strongly in this region, it is not a limitation. The flow cell was electrically and thermally isolated from the spectrometer to prevent interference with the instrument. A schematic of the complete SFE-IR instrument system can be found in Figure 1.

Reagents. The triglycerides (tripalmitin, tristearin, triolein, and trilinolein, 99% purity) and refined vegetable oils (olive, peanut, sesame, corn, soybean, and safflower) were purchased from Sigma Chemical Co. (St. Louis, MO). A sample of palm oil was obtained from the Midwest USDA Labs (Peoria, IL). Boiled linseed oil was purchased at a local hardware store. The iodine numbers for each of the refined vegetable oils were determined according to the Hanus method (AOAC 920.158) by Midwest Laboratories, Inc. (Omaha, NE). The iodine numbers for the pure triglycerides were calculated according to eq 1. The pelletized diatomite (as Isco WetSupport) and other SFE accessories were provided by Isco. The SFE-grade CO_2 was purchased from Air Products and Chemicals, Inc. (Allentown, PA). All solvents were purchased from Aldrich Chemical Co. (Milwaukee, WI).

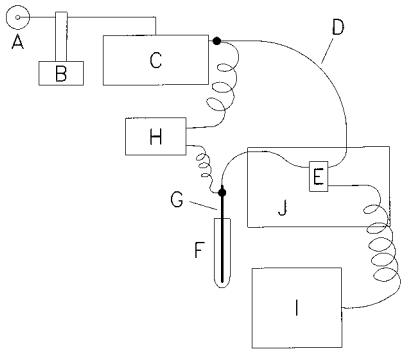

Figure 1. Schematic of SFE-IR system: (A) SFE-CO_2 tank, (B) Isco 260D syringe pump, (C) Isco SFX 2-10 extractor, (D) heated transfer line, (E) 10-mm SFE-IR flow cell, (F) analyte collection trap, (G) Isco coaxial heated restrictor, (H) Isco coaxial heater controller, (I) flow cell temperature controller, (J) FTIR spectrometer.

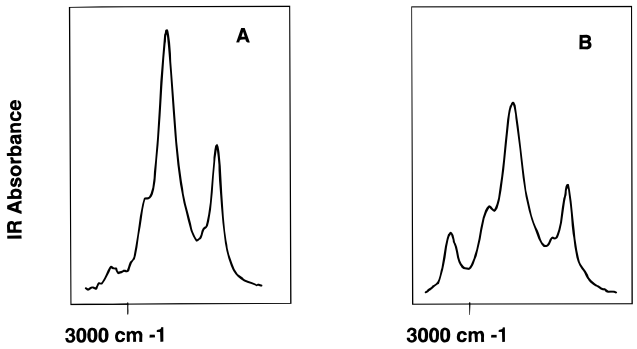

Figure 2. C−H infrared bands for vegetable oils: (A) palm oil, iodine number = 50; (B) linseed oil, iodine number = 173.

Procedure. The main goal of this work is to demonstrate the correlation between iodine numbers and data collected by SFE-IR. Samples were prepared by spiking precise amounts (ranging from 0.15 to 1.2 mg) of the vegetable oils or pure triglycerides onto diatomite, an inert matrix. These oil-spiked samples were extracted with supercritical CO_2 at 400 atm and 80 °C flowing at 1.5 mL/min (compressed fluid). During the extraction, fresh CO_2 continually flowed through the sample and was delivered by the heated transfer line to the heated SFE-IR flow cell, while the Bomem M100 FTIR spectrometer recorded the extract spectrum. The temperatures of the transfer line, IR flow cell, and coaxial restrictor were maintained at 80 °C. The total extraction time was 7 min, but the extract effluent was only monitored by the FTIR spectrometer for the first 5 min of the extraction. The final 2 min of the extraction was intended to thoroughly purge the transfer line and flow cell to prevent carryover into the next run.

Since these oils are quite viscous, solutions prepared in tetrachloroethene were used to improve volumetric dispensing. This lot of tetrachloroethene was free of any hydrocarbon contamination and transparent in the IR regions for the C−H stretch and carbonyl bands. Solutions, typically 80 mg of oil/mL of solution, of each vegetable oil or triglyceride were prepared and volumetrically spiked at different precise amounts onto diatomite in 2.5-mL stainless steel sample cartridges. Seven sample cartridges were prepared for each oil, containing 2.0, 4.0, 6.0, 8.0, 10.0, 12.0, and 14.0 μL of the solution, respectively. A blank sample cartridge containing only diatomite but no oil was also prepared for collecting the background IR spectrum.

During the first 5 min of the spiked-oil extraction, the oil-laden supercritical CO_2 flowed from the sample through the SFE-IR flow cell while the Bomem FTIR spectrometer collected 85 scans. These scans were co-added together to form a single spectrum. This co-addition of the FTIR scans integrates the IR absorbance signal through time for each SFE-IR extraction. Figure 2 shows two example spectra collected for the palm and linseed oils in the region of the C−H bands.

Before any oil sample extraction was run, a single-beam background spectrum was collected as a reference. The background FTIR spectrum was collected under the same conditions as the oil extraction spectra; however, only pure CO_2 flowed through the SFE-IR flow cell. During a background run, the blank sample cartridge was extracted while the Bomem FTIR collected 85 scans. The background spectrum removes most of the effects due to the IR absorbance of the flow cell windows and the CO_2 fluid.

After the sample spectrum was collected for each spiked-oil extraction, the IR absorbance data were obtained from the peak height (i.e. intensity maximum) analysis of the C−H bands. The Bomem spectral analysis software gave the peak height values in units of absorbance units (AU) but not corrected for baseline. To estimate the baseline, the absorbance value in the flat region around 3100 cm^{-1} was used. This baseline estimate was subtracted from the peak height values for the C−H bands, giving baseline-corrected peak heights. These corrected values were used to generate the Beer plots. The error due to this baseline estimate is a constant offset, so it only affects the *y*-intercept of the plots and not the slope. This is the reason that some of the Beer plots do not have a *y*-intercept of exactly zero. Only information from the slope is used in this work. The slopes of these Beer plots are interpreted to be the absorptivity coefficients.

Peak height analysis was used instead of peak area analysis since peak height is more in conformity to Beer's law. Peak height is in units of AU, while peak area is in units of AU·cm^{-1}. In some applications peak area analysis may be more appropriate, but in this case a stricter adherence to Beer's law was desired. The peak height value from the SFE-IR sample spectrum is the average IR absorbance signal integrated over the first 5 min of the dynamic extraction. This procedure is analogous to the integration through time of an eluting peak in chromatography for a single-wavelength detector.

To demonstrate the sufficiency of the spiked-oil extraction conditions, a kinetic experiment on spiked triolein was performed using SFE-IR. First, an 80 mg/mL solution of triolein in tetrachloroethene was prepared. Next a single-beam background spectrum of five scans (ca. 20 s) was collected on the Bomem FTIR with the SFE-IR flow cell installed. A 2.5-mL stainless steel SFE sample cartridge filled with diatomite but containing no triolein was dynamically extracted with CO_2 at 400 atm, 80 °C, and 1.5 mL/min. The transfer line, SFE-IR flow cell, and coaxial restrictor were also at 80 °C. This supercritical fluid was allowed to flow through the flow cell as the FTIR scanned. After the background spectrum was collected, another 2.5-mL stainless steel SFE sample cartridge filled with diatomite was spiked with 10.0 μL of the above triolein solution. This 0.8-mg triolein-spiked sample was dynamically extracted with CO_2 at the same conditions for 5 min while spectra were collected. Since the Bomem software did not support the collection of chemigrams, five consecutive sample spectra were collected during the 5-min dynamic extraction. Each sample spectrum was started at the beginning of each minute during the extraction. Each spectrum is the co-addition of five scans, giving the average IR absorbance signal over the first 20 s of each minute.

A bar graph representation of this kinetic profile is presented in Figure 3. The baseline-corrected peak heights for the vinyllic C−H and symmetric C−H bands are shown. The error bars estimate the baseline noise for each spectra. The kinetic profile for this extraction shows that it is near completion after 5 min. The absorbance signal for the weak vinyllic C−H band drops below the noise level after the fourth minute of the extraction. From a signal-processing viewpoint, the IR information from the extraction is complete within 5 min. Finally, the absorbance signals for all of the IR bands are well below 0.5 AU. According to the Bomem FTIR Operator's Manual, signals above 0.5 AU can cause deviations from Beer's law.

To determine that the spiked-oil extraction is exhaustive in 5 min, extractions were performed at the same conditions but assayed gravimetrically. Approximately 25 mg of neat (not in solution) vegetable oil was spiked on diatomite in a 2.5-mL

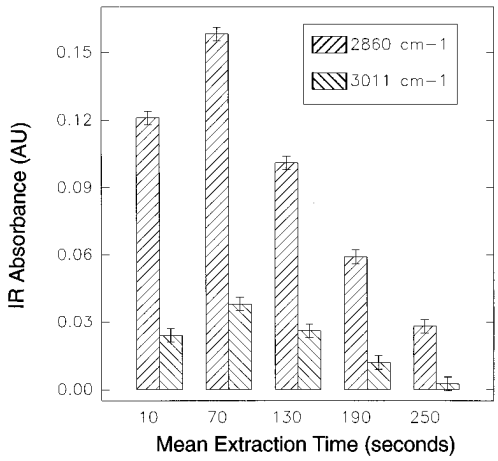

Figure 3. SFE-IR kinetic profile for the extraction of spiked triolein on diatomite. Error bars indicate baseline noise.

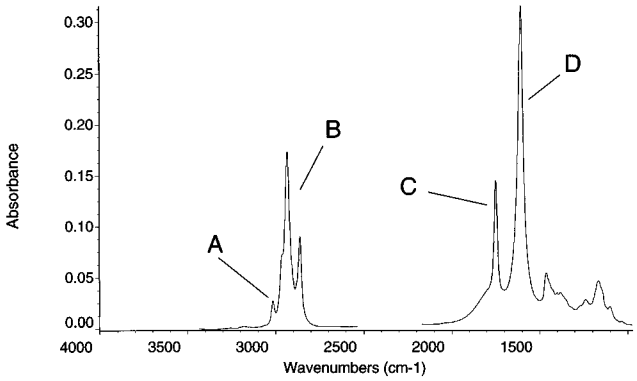

Figure 4. SFE-IR spectrum of corn tortilla chips: (A) vinyllic C−H band, H−C=C, unsaturated fat; (B) C−H bands, fat; (C) C=O band, fat; (D) water band.

sample cartridge. This amount is over 20 times that typically used in the other spiked-oil experiments. This larger sample size was used to reduce as much as possible the error from weighing. Each cartridge was weighed with a precision of ±0.3 mg, before and after the extraction. For an experiment of four replicates ($n = 4$) using sesame oil, 94 ± 4% of the spiked oil was extracted from the cartridge. Since the diatomite was pre-extracted and dry, the problems caused by water for these types of assays are not a concern.

In addition to the spiked-oil extractions, oils from corn tortilla chips were extracted by SFE-IR to collect a spectrum from a real sample. One gram of corn chips was crushed and added to a 10-mL disposable (high-temperature crystalline polymer) SFE sample cartridge. The chips were dynamically extracted with CO_2 at the same conditions as above, except the extraction time was extended to 20 min. Since the sample cartridge volume increased by a factor of 4, the extraction time was increased proportionally. The Nicolet MagnaIR 750 was used, and it scanned the supercritical extract effluent during the 20-min dynamic extraction (1000 scans). A background spectrum was first collected using an empty sample cartridge. Only one corn-chip extraction was performed. The spectrum (the co-addition of 1000 scans) for this run is presented in Figure 4.

RESULTS AND DISCUSSION

Figure 4 presents the IR spectrum of oil extracted from corn tortilla chips. It is included in this paper to show a spectrum collected by SFE-IR for a real food sample. From this spectrum, the characteristic water band at 1600 cm^{-1}, the fat carbonyl band at 1750 cm^{-1}, the fat C−H bands slightly below 3000 cm^{-1}, and the

unsaturated fat vinyllic C−H band slightly above 3000 cm^{-1} are all quite visible. In addition, the signal-to-noise ratio is good. Gaps in the spectrum at 2200−2525 and 3450−3900 cm^{-1} are due to the strong absorbance bands of the CO_2. The total fat content could be determined from the intensity of the fat carbonyl band without interference from water. However, for this paper, we are not interested in quantifying the total fat content but characterizing the fat. The intensity of the fat vinyllic C−H band gives information on the degree of unsaturation of the extracted fat.

As visual support for this idea, Figure 2 presents the C−H band spectra for palm and linseed oils. Palm oil has a low unsaturated fat content, while linseed oil has a high unsaturated fat content. These spectra were obtained during the spiked-oil experiments with palm and linseed oils. The spectra presented in Figure 2 have the same absorbance scales and are for 1-mg oil samples. The intensity of the vinyllic C−H band (>3000 cm^{-1}) increases from palm oil to linseed oil. Also interesting to note are the intensities of the other C−H bands (<3000 cm^{-1}), which decrease from palm oil to linseed oil. This is not surprising since the number of saturated carbons in the triglyceride molecules is lower for the more unsaturated linseed oil.

Before verification of eq 2 was attempted, the sufficiency of the SFE conditions (CO_2 at 400 atm and 80 °C flowing at 1.5 mL/min for 5 min) was tested. First, a kinetic profile for the extraction of triolein was collected by SFE-IR. This information is presented in Figure 3. The extraction appears to be nearly complete at 5 min. The signal from the weak vinyllic C−H band is already below the noise level after 4 min. To determine if the above extraction conditions are exhaustive, 25 mg of neat sesame oil was extracted from dry diatomite and assayed gravimetrically. According to these data, the extraction is 94 ± 4% complete. In the spiked-oil extractions used to verify eq 2, the oil sample sizes were up to 1.2 mg, which is $^1/_{20}$ the amount of oil used in the above experiment. It is expected that these smaller sample size extractions should be more complete since there is less opportunity of saturating the CO_2 solvent. Since the signal from the vinyllic C−H band is extremely weak after 4 min and the extraction is over 90% complete by 5 min, recording the IR spectrum of the extract effluent during the first 5 min is considered adequate.

For quantitative verification of eq 2, SFE-IR data were collected for four pure triglycerides (tripalmitin, tristearin, triolein, and trilinolein) and for eight refined vegetable oils (palm, olive, peanut, sesame, corn, soybean, safflower, and boiled linseed oils). From these data Beer plots for the C−H stretch IR bands were prepared. Figure 5 presents the Beer plot for the sesame oil sample as an example. These plots were analyzed by linear regression using Jandel Scientific (San Rafael, CA) SigmaPlot 5.0 to determine the IR absorptivity coefficient, ϵ, for each band. The value for ϵ is determined from the slope of each linear plot: IR absorbance (AU) versus oil weight (mg). The triglyceride data are presented in Table 1, while the refined vegetable oil data are presented Table 2. Each table also includes the iodine numbers.

As can be seen from Table 1, tripalmitin (C16:0) and tristearin (C18:0), both saturated triglycerides, have no observable absorbance band around 3011 and 3017 cm^{-1}. This is to be expected since carbon−carbon double bonds are absent in both. Triolein (C18:1), a monounsaturated triglyceride commonly found in edible oils, has

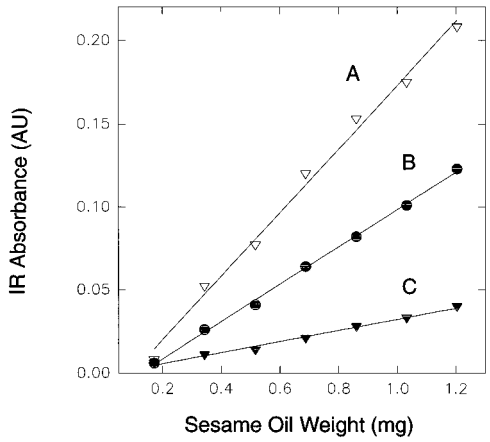

Figure 5. SFE-IR Beer plots for sesame oil: (A) asymmetric CH_2 band; (B) symmetric CH_2 band; (C) vinyllic C−H band, H−C=C.

a vinyllic C−H band at 3011 cm^{-1}. Trilinolein (C18:2), a polyunsaturated triglyceride also commonly found in edible oils, has a vinyllic C−H band at 3017 cm^{-1}. The intensity of the vinyllic C−H band increases with the iodine number (calculated by eq 1) for each triglyceride. Also note that the intensities of the two other C−H bands, the symmetric and asymmetric CH_2 bands, decrease with an increase in iodine number. As stated earlier, this is to be expected since the intensity of these two bands depends on the number of saturated carbons in the sample. So as the iodine number increases for triglycerides of similar molecular weights, the number of saturated carbons decreases, thus reducing the intensity of these bands.

Table 2 shows similar trends for the refined vegetable oils. As the iodine number increases, the intensity of the vinyllic C−H band also increases. The intensities of the symmetric and asymmetric CH_2 bands tend to decrease with increase in iodine number, except for the sesame oil sample. A possible explanation for the sesame oil deviation may be suggested by its saponification number. This particular sesame oil sample has a saponification number of 167, while the other vegetable oils have saponification numbers ranging from 191 to 197. A lower saponification number indicates longer fatty acid chains, which may contain more saturated carbons, resulting in more intense CH_2 bands. (These oil samples were assayed for saponification number according to official method AOCS Db 8-48 by Midwest Laboratories, Inc., in Omaha, NE.)

Figure 6 presents a plot of iodine number versus the vinyllic C−H absorptivity coefficient. Using data from Tables 1 and 2, a correlation between the iodine numbers and the intensities of the vinyllic C−H band can be seen; however, not all of the data points lie exactly on a straight line. It appears to be slightly nonlinear. From the error bars (based on first standard deviations), the deviations of three data points may be significant. Also, the correlation coefficient, r, for this plot is 0.983. It appears from these data that the simple model for eq 2 is only an approximation.

A practical problem with simply correlating iodine numbers with vinyllic C−H band intensities is that the vinyllic C−H absorptivity coefficient is dependent on instrument parameters, such as the optical path length of the IR flow cell. A dimensionless parameter would be more useful and portable. One solution is to correlate the iodine number with a ratio of band intensities by using another lipid-related IR band as an internal

Table 1. SFE-IR Triglyceride Data

		AU/mg		
triglyceride	iodine number[a]	ϵ(symCH_2),[b] 2860 cm^{-1}	ϵ(asymCH_2), 2930 cm^{-1}	ϵ(H−C═C), >3000 cm^{-1}
tripalmitin	0 ± 1	0.1381 ± 0.0053	0.2138 ± 0.0163	0
tristearin	0 ± 1	0.1335 ± 0.0046	0.2142 ± 0.0130	0
triolein	86.0 ± 0.9	0.1187 ± 0.0051	0.2035 ± 0.0088	0.0268 ± 0.0021
trilinolein	173.2 ± 1.7	0.0817 ± 0.0022	0.1422 ± 0.0048	0.0450 ± 0.0016

[a] Iodine numbers determined by eq 1; uncertainty based on reagent purity. [b] Uncertainty is estimated standard deviation with 68% confidence.

Table 2. SFE-IR Vegetable Oil Data

		AU/mg		
oil	iodine number, Hanus method (AOAC 920.158)	ϵ(symCH_2), 2860 cm^{-1}	ϵ(asymCH_2), 2930 cm^{-1}	ϵ(H-C═C), >3000 cm^{-1}
palm	50.6 ± 1.8	0.1152 ± 0.0056	0.1913 ± 0.0146	0.0186 ± 0.0017
olive	81.0 ± 2.0	0.1093 ± 0.0067	0.1828 ± 0.0131	0.0264 ± 0.0038
peanut	91.7 ± 1.5	0.1074 ± 0.0065	0.1820 ± 0.0101	0.0270 ± 0.0019
sesame	103.0 ± 2.1	0.1125 ± 0.0020	0.1914 ± 0.0069	0.0332 ± 0.0014
corn	124.2 ± 2.6	0.0996 ± 0.0027	0.1681 ± 0.0070	0.0345 ± 0.0024
soybean	125.3 ± 1.5	0.1022 ± 0.0079	0.1809 ± 0.0124	0.0368 ± 0.0037
safflower	143.2 ± 3.8	0.0961 ± 0.0025	0.1637 ± 0.0062	0.0398 ± 0.0019
linseed	172.8 ± 5.1	0.0814 ± 0.0048	0.1357 ± 0.0069	0.0422 ± 0.0024

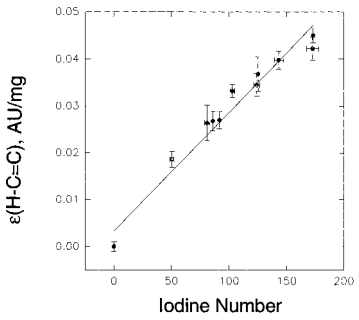

Figure 6. Plot of ϵ(H−C═C) vs iodine number.

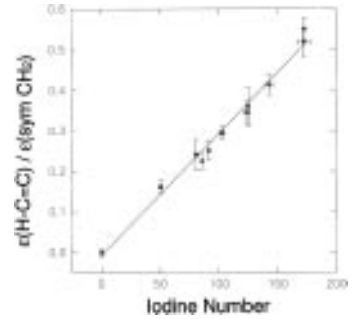

Figure 7. Plot of ϵ(H−C═C)/ϵ(sym CH_2) vs iodine number.

reference. Figure 7 presents a plot of iodine number versus the ratio ϵ(H−C═C)/ϵ(symCH_2) using data from Tables 1 and 2. The symmetric CH_2 band was chosen since it appears to have a negative correlation to the iodine number. Also, its absorptivity coefficients have lower uncertainty as compared to the asymmetric CH_2 band. Note that the data points based on the ratio of the C−H bands fit a straight line better than those based on the vinyllic C−H band alone. From the error bars in Figure 7, these deviations are less significant than those using the vinyllic C−H band information alone. The correlation coefficient, *r*, for this plot is 0.995, an improvement over the plot in Figure 6. The plot of iodine number versus the ratio ϵ(H−C═C)/ϵ-(asymCH_2) is similar to Figure 7 (Liescheski and Macomber, 1995).

According to Figure 7, the iodine number can be determined by multiplying the value of the ratio for vinyllic C−H versus symmetric CH_2 bands by 330 ± 10, the value for the slope of the plot. For example, in the spectrum from the corn tortilla chips (Figure 4), the estimated ratio of the intensities of the vinyllic C−H band versus the symmetric CH_2 band is 0.31 ± 0.02. Using the slope information from Figure 7, the iodine number of the extracted oil is estimated to be 102 ± 8. Even though these particular chips were not analyzed according to the standard methods, this number is reasonable for corn oil. According to *The Merck Index*, 9th ed., (2510), the iodine number for corn oil can range from 109 to 133. Finally, the slope information from Figure 7 is less instrument dependent (i.e. more portable) than that from Figure 6, but the effects of flow cell temperature and solvent strength (i.e. pressure) are unknown. These effects are expected to be small though.

CONCLUSION

SFE-IR shows promise in characterizing edible oils and fats extracted from solid foods. SFE-IR data can correlate well with iodine numbers, indicating the amount of unsaturated fat. Although the intensity of the vinyllic C−H band correlates ($r = 0.983$) with iodine numbers for pure triglycerides and refined vegetable oils, the ratio ϵ(H−C═C)/ϵ(symCH_2) has a better correlation ($r = 0.995$). This ratio also has the added benefit of using the symmetric CH_2 band as an internal reference, thus removing the effects of instrumental parameters, such as the flow cell optical path length. Finally, preliminary data suggest that SFE-IR may be used to determine saponification numbers (Liescheski and Macomber, 1995). Using SFE-IR, one can quickly determine the unsaturated fat content of a food sample without the use of perchlorinated solvents or freons.

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