Characterization of Semisolid Fats and Edible Oils by Fourier Transform Infrared Photoacoustic Spectroscopy

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ABSTRACT: The potential of Fourier transform infrared photoacoustic spectroscopy (FTIR-PAS) for the analysis of semisolid fat and edible oil was demonstrated with butter, soybean oil, and lard as representative materials. Results of Fourier transform infrared attenuated total reflectance (FTIR-ATR) spectroscopy analysis was compared with FTIR-PAS results. The PAS technique is simple and requires no sample preparation unlike ATR. Optimal PAS instrumental parameters for obtaining quality spectra are a scanning speed of 5 kHz, number of scans of 256 scans/sample, and a resolution of 4 cm^{-1}. The PAS spectra of soybean oil and lard are similar because they have similar functional groups. Results for soybean oil compare well with those available in the literature. The ATR spectra of butter were better than those from its PAS counterpart. Functional groups corresponding to vibration mode and intensity are provided for soybean oil and lard.

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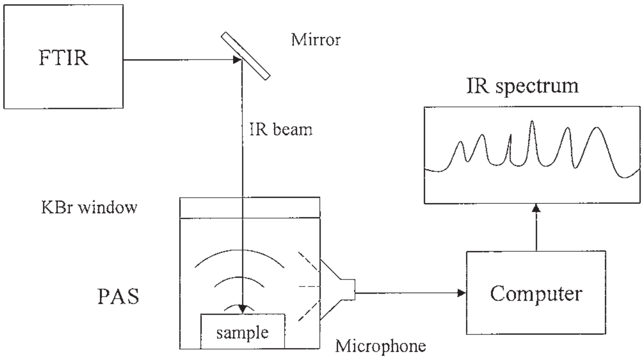
KEY WORDS: Attenuated total reflectance, butter, chemical characterization, food analysis, Fourier transform infrared photoacoustic spectroscopy, lard, soybean oil.

Fourier transform infrared (FTIR) spectroscopy has been used for quantitative and qualitative measurement of edible oils and fats (1,2). Compared with dispersive spectroscopy, the FTIR technique has a higher signal-to-noise ratio and higher resolution and accuracy in wavelength measurement (2). In conjunction with chemometrical techniques, FTIR data can be used to quantify, classify, and enumerate the difference between samples (3). FTIR has been used to determine *cis* and *trans* content, iodine value, saponification number, peroxide value, adulterations, solid fat index, and fatty acids content in oil or fat samples.

Transmission cells and attenuated total reflectance (ATR) are two of the most common FTIR accessories used for oil and fat analysis (1,2). Oil and fat samples are dissolved in CCl_4 or CS_2 and placed in a transmission cell of either NaCl or KBr for measurement. The cell should normally be much narrower (0.01 to 1 mm) than those employed in the ultraviolet and visible regions, in order to obtain clear peaks in the spectrum (4). The sample constitutes a drop of liquid pressed between two halide plates to give a layer of thickness of 0.01 mm or less. However, it is not easy to clean halide cells after testing. Halide plates or disks fog easily due to absorption of moisture from the environment and the sample. This results in contamination of the final spectrum, thereby limiting its application.

Unlike the transmission cell, the ATR measurement technique has no such limitations because liquid samples can be directly spread on the surface of the ATR crystal. However, samples that are oily have a tendency to adhere to the ATR (ZnSe) crystal surface due to hydrophobicity of the crystal and oil sample. This phenomenon is called the ATR “memory effect” (1). Adhered fat can affect the spectra of the next sample if the ATR crystal is not thoroughly washed with a suitable solvent and the background is not taken before a new measurement. ATR measurement is sensitive to change in environmental temperature (1). Although the above-mentioned techniques can be used to analyze liquid samples, they cannot be readily applied to solids analysis. FTIR techniques for solids analysis require elaborate or destructive sample preparation techniques.

Fourier transform infrared photoacoustic spectroscopy (FTIR-PAS) has been used for chemical and qualitative analysis, due to its advantages in nondestructive sample preparation and depth profiling (5). In FTIR-PAS (Scheme 1), the sample is placed in a stainless-steel cup and sealed in the photoacoustic cell after purging with helium gas for 5–10 min (6). A modulated infrared beam focused on the sample is absorbed and converted into heat by nonradiative deexcitation processes, which give rise to periodic temperature oscillations



SCHEME 1

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within the sample. The heat-induced thermal wave passes through the sample to the adjacent purged helium medium, causing pressure oscillations, which can be detected by a sensitive microphone as an acoustic wave. The acoustic wave is then converted into an electrical signal and a corresponding spectrum.

The depth of penetration (μ_t) of an FTIR-PAS measurement in the sample described by Rosencwaig's theory (7) is given by

$$\mu_t = \sqrt{\frac{\alpha}{\pi f}} \qquad [1]$$

where α is the sample thermal diffusivity and f is the modulation frequency of radiation. Because the sample is placed directly in the stainless-steel cup, the FTIR-PAS technique is simple and requires no sample preparation. The stainless-steel cup does not absorb the oil or fat in the sample and hence can be easily cleaned after each experiment. FTIR-PAS was used for both qualitative and quantitative analyses (5). For example, FTIR-PAS was used to determine the contents of major components in the pea seed (8). Limited research exists on the application of FTIR-PAS for food systems.

This research serves to study and demonstrate the potential of FTIR-PAS for oil and fat analysis with a specific focus on food. Because the application of PAS technique to food analysis is fairly recent, our research investigated the instrumental parameters, such as scanning speed, numbers of scans, and resolution for optimal spectra acquisition. Finally, a comparison of the results obtained from PAS and ATR was made for validation. The specific objectives were (i) to characterize lard, soybean oil and butter by the FTIR-PAS technique and (ii) to validate PAS measurement with ATR results.

EXPERIMENTAL PROCEDURES

Materials. Soybean oil (Foodhold U.S.A., Inc., Maple Heights, OH), lard (Schluderg-Kurdle Co., Inc., Baltimore, MD), and butter (Land O'Lakes, Inc., Arden Hills, MN) were used for this experiment. The samples were stored at room temperature (25°C) overnight before testing.

FTIR-PAS analysis. The FTS 6000 research-grade FTIR spectrometer (Bio-Rad Laboratories, Cambridge, MA) with a helium-purged photoacoustic (PA) detector (MTEC Photoacoustics Inc., Ames, IA) was used for collecting the PA spectra. The spectrometer was purged by an FTIR purge gas generator (model 75-52; Whatman, Inc., Haverhill, MA) to minimize CO_2 and H_2O vapor interference in the spectra. Carbon black was used for background spectra. Oil, lard, or butter was kept in a stainless-steel cup and placed in the PA detector. The PA detector was purged by helium for 10 min and then sealed. Spectra of samples in the region between 4000 and 600 cm^{-1} were obtained at a resolution of 1, 4, 8, or 16 cm^{-1} and scan numbers of 32, 64, 128, or 256 scans/sample at the scanning speed of 2.5, 5, 10, or 20 kHz. The collected spectra were processed with a Win-IR Pro™ software (Version 2.5) from Bio-Rad.

FTIR-ATR analysis. The same FTS 6000 system with a deuterated tri-glycine sulfate detector was used. A ZnSe ATR sampling accessory from Pike Technologies (Madison, WI) was used for ATR measurements. ATR spectra were collected by using 256 scans/sample at 4 cm^{-1} resolution. Before scanning each sample, the background spectrum was taken with the empty ATR crystal and stored in the computer. Liquid soybean oil sample was poured onto the ATR ZnSe crystal for measurement, while semisolid or softened butter and lard samples were pressed against the crystal to ensure close and complete contact. After each measurement, the ATR crystal was thoroughly cleaned with 1% Triton X-100 solution (Aldrich Chemical Co., Milwaukee, WI), followed by hexane (Aldrich Chemical Co.) (9). The washed crystal was then rinsed with distilled water, wiped with cotton, and dried under nitrogen gas after each measurement.

RESULTS AND DISCUSSION

The optimal instrumental parameters for FTIR-PAS measurement were determined after experimenting with different scan speeds, resolutions, and scan numbers. Lard samples were scanned at velocities of 2.5 kHz (0.16 cm/s), 5 kHz (0.32 cm/s), 10 kHz (0.64 cm/s), and 20 kHz (1.28 cm/s). Spectra at scan velocities of 2.5 kHz and 20 kHz are shown, respectively, in Figures 1A and 1B. The number of scans (256 scans/sample) and the resolution (4 cm^{-1}) were kept constant. Figure 1 indicates that acquisition at the lowest scan speed (2.5 kHz) provided a smoother spectrum. Considering the quality of the spectra and the time, the scan speed of 5 kHz was selected. Figures 2A and 2B show the spectra obtained at a velocity of 5 kHz and a resolution of 16 cm^{-1} for scan numbers 32 and 256, respectively. Only minimal differences in the spectra were observed for the scan numbers chosen. For

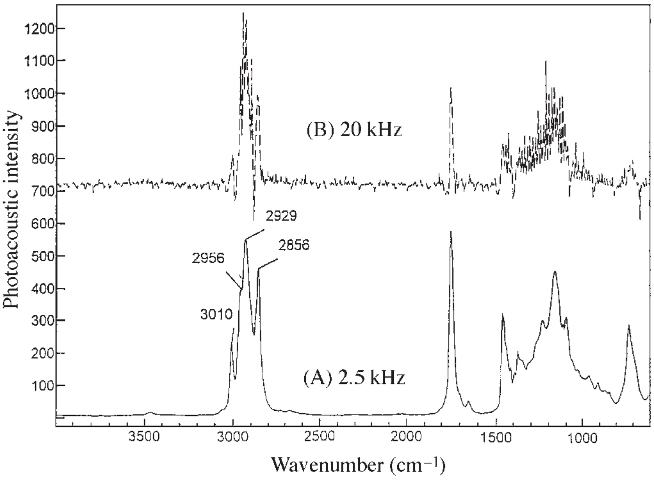

FIG. 1. Spectra of lard samples from Fourier transform infrared photoacoustic spectroscopy (FTIR-PAS) measurement at scanning speeds (velocities) of (A) 2.5 kHz (0.16 cm/s) and (B) 20 kHz (1.28 cm/s). A constant number of scans (256 scans/sample) and resolution (4 cm^{-1}) were used.

the highest accuracy, a scan number of 256 scans/sample was chosen for further measurements.

Figures 3A and 3B show the spectra of lard samples obtained at resolutions of 4 and 16 cm^{-1} at a scan speed of 5 kHz and a scan number of 256 scans/sample. Bands at 2928 cm^{-1} (methylene asymmetrical stretching band) and 2856 cm^{-1} (methylene symmetrical stretching band) are identified in the lower-resolution spectrum (Fig. 3B). However, weak bands or shoulders, such as the shoulder corresponding to 2955 cm^{-1}, caused by the methylene asymmetrical stretching band, cannot be clearly observed at 16 cm^{-1} resolution. The stretching vibration of *cis* double bonds (peak at 3007 cm^{-1}) gradually merged with the peak at 2928 cm^{-1} as the resolution was decreased (Fig. 3A and 3B). Hence, selecting a high resolution will separate the spectral band of interest from the nearby overlapping bands (10). At a resolution of 4 cm^{-1} (Fig. 3A), the weaker peaks or shoulders can be identified, such as the shoulder at 2955 cm^{-1}. Therefore, a resolution of 4 cm^{-1} was selected for further analysis because the weak peaks can be displayed in the spectrum (Fig. 3A).

It is well known that oil and fat samples have strong C-H absorption between 3000 and 2850 cm^{-1}. Figure 1A clearly shows separate bands that correspond to C-H stretch at 2929 and 2856 cm^{-1} with a weak shoulder at 2956 cm^{-1}. Che Man and Setiowaty (11), in their FTIR transmission cell experiment on oxidized palm olein at a 2-mm transmission cell pathlength, noted that this broad band between 3000 and 2850 cm^{-1} could not be separated (11). The authors further noted that, in order to observe the strong absorbance in this region,

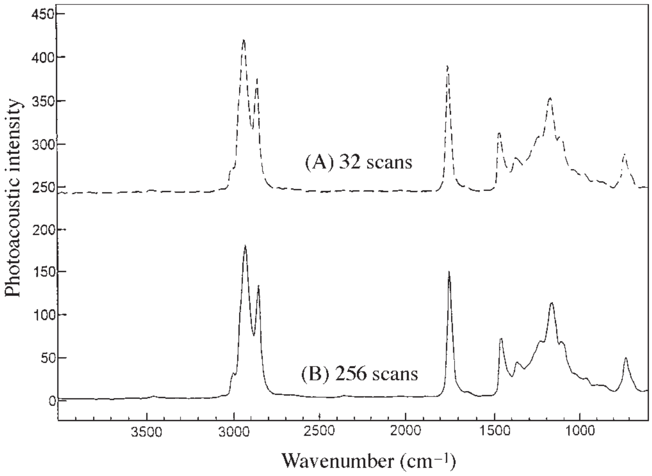

FIG. 2. Spectra of lard samples from FTIR-PAS measurement at the scanning speed of 5 kHz and a resolution of 16 cm^{-1} for scans of (A) 32 and (B) 256 scans/sample. For abbreviation see Figure 1.

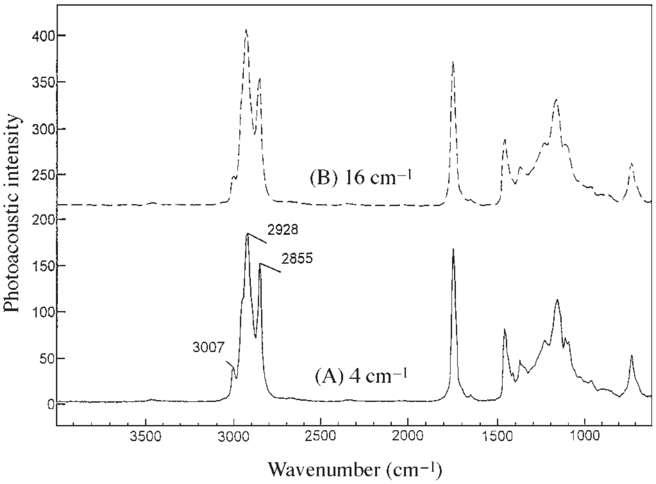

FIG. 3. Spectra of lard samples from FTIR-PAS measurement at a scanning speed of 5 kHz at 256 scans/sample with resolution of (A) 4 and (B) 16 cm^{-1}. For abbreviation see Figure 1.

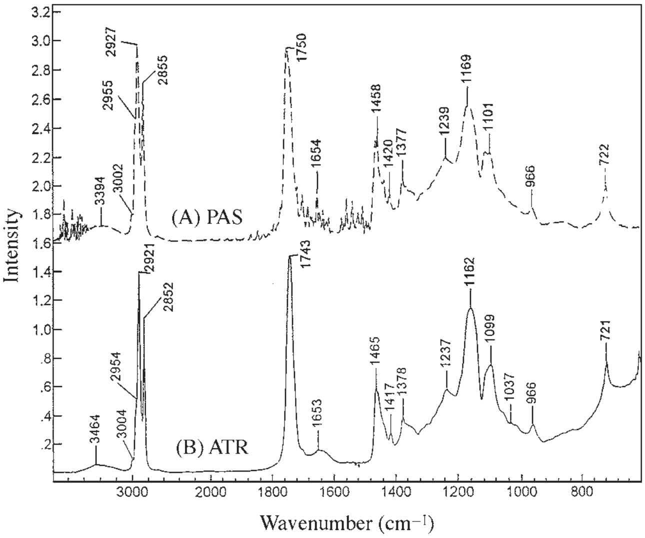

FIG. 4. Spectra of butter samples from (A) FTIR-PAS and (B) Fourier transform infrared attenuated total reflectance (FTIR-ATR) measurements at a scanning speed of 5 kHz, 256 scans/sample, and a resolution of 4 cm^{-1}. For other abbreviations see Figure 1.

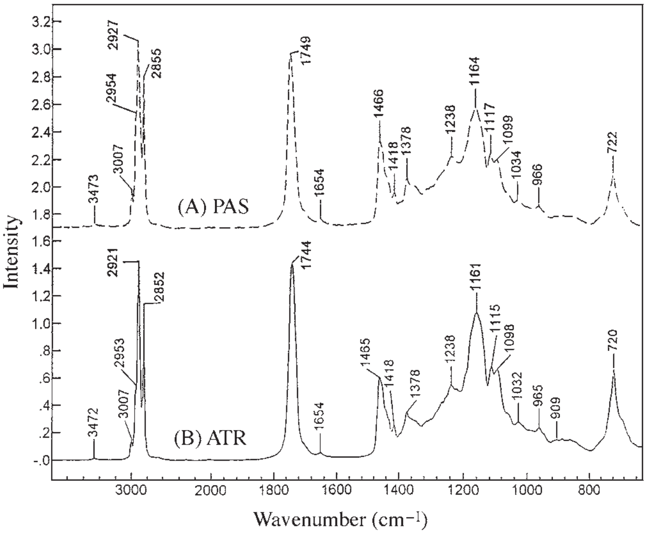

FIG. 5. Spectra of lard samples from (A) FTIR-PAS and (B) FTIR-ATR measurements at a scanning speed of 5 kHz, 256 scans/sample, and a resolution of 4 cm^{-1}. For abbreviations see Figures 1 and 4.

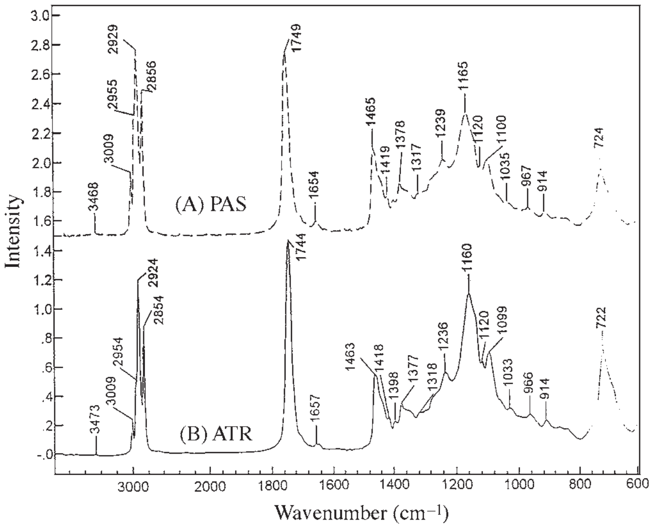

FIG. 6. Spectra of soybean oil samples from (A) FTIR-PAS and (B) FTIR-ATR measurements at a scanning speed of 5 kHz, 256 scans/sample, and a resolution of 4 cm^{-1}. List of Table TABLE 1. Frequencies, functional groups, vibration mode, and intensity of soybean oil and lard in FTIR-PAS spectra. For abbreviations see Figures 1 and 4.

the pathlength of the transmission cell has to be small (about 0.01 mm). Our research demonstrates the potential of PAS for characterizing highly absorbing samples with no sample preparation. The spectra obtained are similar to those obtained by Guillén and Cabo (12).

Spectra of butter, lard, and soybean oil samples obtained from FTIR-PAS and FTIR-ATR methods are compared in Figures 4–6, respectively. Figure 4 shows clearly that the moisture in butter (14.8% moisture content) has a major effect on the PAS measurement. The moisture in butter vaporized due to heat generated by IR radiation and contaminated the helium-purged environment of the PAS system. It is well known that water vapor has many strong bands in the mid-infrared. They appear as spikes in two regions: one between 3700 and 3100 cm^{-1} (O-H stretching vibration) and another centered at 1640 cm^{-1} (O-H bending vibration). Furthermore, the water vapor bands can overlap with the bands of other components in the sample. Figure 4A shows that amide A (3300 cm^{-1}), amide B (3100 cm^{-1}), and amide I (1650 cm^{-1}) (13) bands, caused by the absorption of protein, are obscured due to the presence of water vapor from the moist sample in the photoacoustic cell. Figure 4B is the ATR spectrum of butter. As expected, the ATR method is preferable to PAS when analyzing liquid samples with high moisture content.

The spectra of lard samples obtained from PAS (Fig. 5A) and ATR (Fig. 5B) measurements are similar. The same phenomenon can also be observed in the spectra of soybean oil obtained from PAS (Fig. 6A) and ATR (Fig. 7b). The spectra of lard (Fig. 5) and soybean oil (Fig. 6) obtained from PAS and ATR techniques are similar because they have similar functional groups. These fats essentially constitute fatty triglyceride esters with different substitution patterns, lengths, and degrees of unsaturation of the acyl groups and other minor components.

In the FTIR-PAS method, the lard is directly placed in the sample cup, and the measurement is taken nondestructively. In the FTIR-ATR method, the lard cannot be analyzed as is. The sample has to be either dissolved in a solution medium or heated sufficiently to allow it to adhere or spread uniformly on the ATR crystal. Other methods of IR measurements, such as halide disk (11,12) or AgCl disc (14), require sample preparation. Furthermore, the background spectrum needed for the PAS method is taken only once during setup, while a

TABLE 1
Frequencies, Functional Groups, Vibration Mode, and Intensity of Soybean Oil and Lard in FTIR-PAS Spectra[a]

			Intensity	
Wavenumber (cm^{-1})	Functional group	Mode of vibration	Soybean oil	lard
3468	-C=O (ester)	Overtone	W	W
3009	=C-H (*cis*-)	Stretching	M	W
2955	-C-H (CH_3)	Stretching (asym.)	W	W
2929	-C-H (CH_2)	Stretching (asym.)	S	S
2856	-C-H (CH_2)	Stretching (sym.)	S	S
1749	-C=O (ester)	Stretching	S	S
1654	-C=C- (*cis*-)	Stretching	W	W
1465	-C-H (CH_2, CH_3)	Bending (scissoring)	S	S
1419	=C-H (*cis*-)	Bending (rocking)	W	W
1378	-C-H (CH_3)	Bending (sym.)	M	M
1239	-C-O, -CH_2-	Stretching, bending	W	W
1165	-C-O, -CH_2-	Stretching, bending	S	S
1120	-C-O	Stretching	W	
1100	-C-O	Stretching	W	W
1035	-C-O	Stretching	W	W
967	-HC=CH- (*trans*-)	Bending out of plane	W	W
914	-HC=CH- (*cis*-)	Bending out of plane	W	W
724	-$(CH_2)_n$-, -HC=CH- (*cis*-)	Bending (rocking)	M	M

[a] FTIR-PAS, Fourier transform infrared photoacoustic spectroscopy: W, weak band; M, moderate band; S, strong band.

background spectrum for ATR is necessary after each measurement to minimize the memory effect (1). Another advantage of the PAS method is that cleaning the PAS sample cup is much easier than cleaning the ATR ZnSe crystal. Thus, FTIR-PAS can be a simple and powerful technique for edible oil and semisolid fat characterization.

FTIR spectra in the mid-infrared region comprise fundamental and characteristic bands whose frequencies and intensities can clearly determine the relevant functional (chemically reactive) groups in biological materials. The change in intensity of these bands can provide information on qualitative and quantitative aspects of the sample. For example, the absorption band at 967 cm^{-1} has been widely used to determine *trans* unsaturated fatty acid content in oils (2). The frequencies of characteristic oil and fat bands in the PAS spectra are labeled in Figures 4–6 and the assignment of functional groups, their vibration mode, and intensity obtained by Guillén and Cabo (15) are listed in Table 1. The functional groups and the corresponding frequencies for soybean oil from the PAS spectra correspond with the results of Guillén and Cabo (15).

The simplicity of sampling and the nondestructive nature of analysis are some of the unique advantages of the PAS method over the conventional ATR and transmission methods. Results show that, while the ATR method is a proven technique for high-moisture samples, the PAS method has tremendous potential for analyzing oils, solids, and low-moisture samples.

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