Monitoring Chemical Changes in Some Foods Using Fourier Transform Photoacoustic Spectroscopy

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ABSTRACT: The potential of Fourier transform infrared photoacoustic spectroscopy (FTIR-PAS) to characterize some common foods was studied. Lard, peanut butter, mayonnaise, and whipped topping were heated to 60 and 90 °C separately, and the spectra were obtained periodically for a period of up to 32 and 16 d, respectively. Key bands in the mid-infrared spectral region were examined to monitor changes presumably due to lipid oxidation. Spectral data were analyzed, using principal component analysis (PCA) correlation, and linear discriminate analysis (LDA) techniques with Mahalanobis distances to estimate the extent of deterioration attributable to oxidation. The PAS was found to be simple, rapid, and nondestructive. It required limited sample preparation, and proved highly desirable for analyzing low-moisture samples.

Keywords: Fourier transform infrared photoacoustic spectroscopy, lard, peanut butter, mayonnaise, whipped topping

Introduction

FOOD CONSISTS MAINLY OF CARBOHYDRATES, PROTEINS, FATS, and moisture. Each of these components has substituent groups that produce characteristic absorption bands in the mid-infrared region. Fourier transform infrared (FTIR) spectroscopy is based on the fact that substituent groups in organic compounds vibrate at frequencies associated with the mid-infrared region of the electromagnetic spectrum (wavenumbers 4000 to 400 cm^{-1}), producing characteristic absorption bands. The FTIR method has been applied to analyze foods and determine their qualitative and quantitative attributes (Van de Voort and Ismail 1991). The band of the amide group is used to measure proteins, and the bands of the carbonyl group and lipid hydrocarbon backbone are used to measure fats (Guillen and Cabo 1997). Water absorbs strongly between 3100 to 3700 cm^{-1}, and at 1640 cm^{-1} because of the stretching and bending vibrations of the O-H group (Chen and others 1998). The advantage of the FTIR technique over other methods is minimum sample preparation, rapid analysis, and nondestructive analysis, especially when coupled with a photoacoustic (PAS) accessory. The latter involves direct measurement of a photoacoustic signal produced, when infrared radiation absorbed by a sample converts into heat within the sample and transfers that heat to the gas atmosphere of the sample.

Guillen and Cabo (1997) reviewed the suitability of FTIR spectroscopy in characterizing oils and fats to determine the degree of unsaturation, free fatty acid content, saponification numbers, and solid fat content. The oxidation of edible oils was also monitored by Van de Voort and others (1994) using the FTIR method. They were able to evaluate the oxidative state of oil, or monitor the changes in oils undergoing thermal stress, by identifying common oil oxidation end-products, and relating them to representative reference compounds. Research has also shown that the changes in the oxidation process of edible oils with varying proportions of different fatty acids can be observed by studying the shift in frequency values of the major absorption bands (Guillen and Cabo 1999). The FTIR technique has been used to estimate the *trans* and *cis* double-bond content in fats and oils, as these two isomers absorb light at different frequencies (Van de Voort and others 1995; Belton and others 1988b; Ulberth and Haider 1992). Dahlberg and others (1997) classified vegetable oils by FTIR spectroscopy and could thus determine the source and properties of these oils in foods. The authors also envisioned the potential utility of FTIR as a fast method to detect adulteration in products such as cooking oils.

Van de Voort (1992) outlined the feasibility of the FTIR technique for analysis of milk, meats, juices, butter, fats, and oils. It has been reported that FTIR spectroscopy could be utilized as a quality control method for fat and moisture determination in butter and high-fat products (Van de Voort and others 1993). Those authors suggested the use of the carbonyl-stretching and water-bending vibrations in fat and water, respectively, as markers to develop a calibration standard against which the unknown could be tested. Other studies reporting the use of the FTIR spectroscopic method include: analysis of confectionery products (Belton and others 1988a), examination of cheddar cheese ripening (Chen and others 1998), retrogradation kinetics of potato starch (Van Soest and others 1994), and detection of chilling injury in zucchini squash (Buta and Wang 1993).

Most of the FTIR techniques consisted of attenuated total reflectance (ATR), diffuse reflectance, or transmission techniques, all of which require some sample preparation. More recently, FTIR photoacoustic spectroscopy (FTIR-PAS) has been used to study complex and heterogeneous materials (Jiang and others 1998). The features of the FTIR-PAS technique are nondestructive; they involve noncontact measurements and require limited sample preparation. They also have high signal-saturation limits and depth profiling capability. The principle of FTIR-PAS was originally discussed by Rosencwaig (1980). In spite of the advantages, however, the application of FTIR-PAS to study food systems is very limited. Systems studied include cheese (McQueen and others 1995a, 1995b), chocolate (Belton and others 1988a), and pea

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seeds (Letzelter and others 1995). The objective of the present study was to investigate whether the FTIR-PAS technique could be used to monitor changes in and detect deterioration of some common foods.

Materials and Methods

Sample preparation

Lard, peanut butter, mayonnaise, and whipped topping were purchased at a local retail grocery. Polystyrene disposable 60 × 15-mm Petri dishes and 43-mm aluminum weighing dishes were obtained from VWR Scientific (West Chester, Pa., U.S.A.). About 2 to 7 g of each sample were weighed into the Petri dishes and aluminum pans, and were spread uniformly. The samples in the Petri dishes were placed uncovered at 60 °C in a Model 126G Fisher Isotemp Oven (Fisher Scientific, Pittsburgh, Pa., U.S.A.), and the samples in the aluminum pans were placed at 90 °C in a Blue M oven (Blue M Electric Co., Blue Island, Ill., U.S.A.). Duplicate samples were removed from the 60 °C oven at 2, 4, 8, 16, and 32 d, and from the 90 °C oven at 2, 4, 8, and 16 d. The samples were then cooled in a desiccator for at least 30 min, then scanned by an FTIR spectrometer.

FTIR measurements

A Bio-Rad FTS 6000 FTIR spectrometer (Cambridge, Mass., U.S.A.) equipped with an MTEC Model 100® photoacoustic detector (MTEC Photoacoustics, Ames, Iowa, U.S.A.) was used. A Model 75-52 FTIR purge gas generator (Whatman, Inc., Haverhill, Mass., U.S.A.) provided carbon dioxide and moisture-free air to the system. Helium was used to purge the detector to minimize interferences due to moisture and carbon dioxide. Win-IR Pro software (Bio-Rad, Cambridge, Mass., U.S.A.) was used to optimize the FTIR parameters, collect, and process data. All spectra were obtained by averaging 256 interferograms. A reference carbon black spectrum was obtained before each set of measurements and was used as a background. This helped reduce the noise level and minimize the effects of changes in the environment.

The FTIR spectra for unheated lard and peanut butter were collected as the controls for those samples. Mayonnaise and whipped topping contain water. Presence of moisture in the sample results in masking relevant FTIR peaks which affects correct evaluation of any heat-related changes occurring in the samples. Hence, the spectra of the 2 d–60 °C heated (moisture-free) samples were chosen as the control for mayonnaise and whipped topping.

In addition to PAS, the Attenuated Total Reflectance (ATR) technique (a standard FTIR procedure applied to analyze high-moisture or liquid samples) was also used for comparison and validation. The PAS accessory was replaced by a ZnSe ATR accessory (Pike Technologies, Madison, Wis., U.S.A.). Liquid samples analyzed were mayonnaise and whipped topping. ATR spectra were collected using 256 scans/sample at 4 cm^{-1} resolution. Before scanning each sample, the background spectrum was taken with an empty ATR crystal and stored in the computer.

Chemometric operations

The Win-Das (John Wiley & Sons, West Sussex, U.K.) software was used for statistical analysis. One approach to qualitative analysis is Linear Discriminant Analysis (LDA) (Kemsley 1998). The model development, or "training phase," of LDA comprises the following steps: (1) definition of the proposed group structure, by allocating observations to 1 of a minimum of 2 groups; (2) estimation of the group centers (that is, the mean of the observations in each group); (3) calculation of the distance between each observation from all group centers; (4) comparison of the group center that is nearest to each observation with the group definitions assigned in Step 1, in order to determine the number of correct matches. The assignment success rate gives an indication of the appropriateness of the model.

For Step 3 in this analysis, Mahalanobis distance (D_M) as defined by the matrix equation (Kemsley 1998) was used. D_M is one of the most widely used parameters in discriminant analysis. In vector notation, the Mahalanobis distance, D_M between the j^{th} observation x_j and the k^{th} group center μ_k is given by:

$$D_M = (\{x_j - \mu_k\}[W^{-1}]\{x_j - \mu_k\}^T)^{0.5} \quad (1)$$

where x_j and μ_k are both $(1 \times r)$ row vectors, and W is the average "within groups" covariance matrix calculated separately for each group, and T is the transpose. If there are g groups, then W is given by:

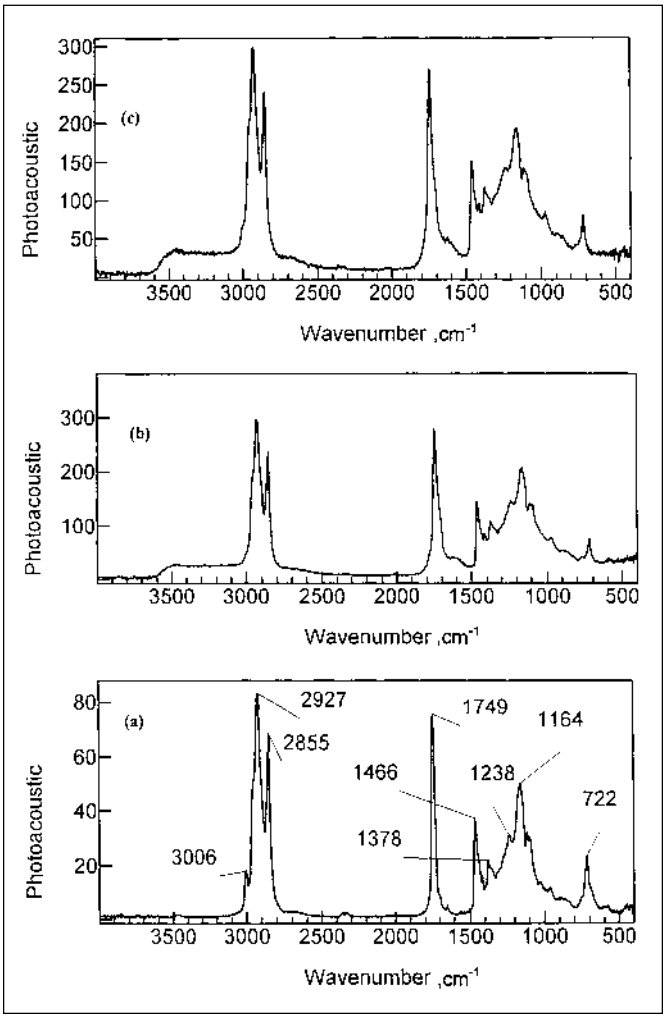

Figure 1—FTIR-PAS spectra of lard: (a) unheated sample; (b) sample heated at 90 °C for 16 d; (c) sample heated at 60 °C for 32 d

$$W = \frac{\sum_{k=1}^{E}(n_k - 1)S_k}{n - g} \quad (2)$$

Here n is the number of observations and S_k is the covariance matrix of group k.

Linear discriminant analysis was applied to calculate the Mahalanobis distances using principal component analysis (PCA) scores of the original data. The PCA transformation method was applied to compress the data into training sets. When the data compression is used as a precursor to LDA, the x_j in the above equation, and the expressions for the other distances, represent transformed observations, comprising PC scores. The derived quantities, such as group centers and covariance matrices, are calculated from the transformed observations, and the assignment of success rate obtained in Step 4 gives an indication of how well the model fits the data.

Results and Discussion

THE FTIR SPECTRA OF ALL THE SAMPLES SHOWED WELL-RESOLVED peaks, except in the case of high-moisture samples. Table 1 shows the major ingredients present in the samples with their respective moisture contents. Vibrational bands of water overlap with those of the solutes, resulting in broad bands that cannot usually be deconvoluted into their constituents. The peaks are representative of the chemical groups of the components present in the samples. Lard, peanut butter, mayonnaise, and whipped topping were used for deterioration studies. Heating temperatures used were 60 and 90 °C. FTIR-PAS spectra of samples heated for different days were collected. Figure 1 to 4 compare the spectra of unheated samples with the spectra of 16 and 32-d samples

Table 1—Major ingredients of the samples

Sample	Moisture (% d.b.)[1]	Ingredients
Lard	~0.5	Lard
Peanut butter	0.82	Peanuts, sugar, hydrogenated vegetable oils, corn syrup
Mayonnaise	12.8	Soybean oil, vinegar, eggs, corn syrup, water
Whipped topping	54.6	Water, corn syrup, partially hydrogenated vegetable oil, sugar, milk casein

[1]d.b. = dry basis

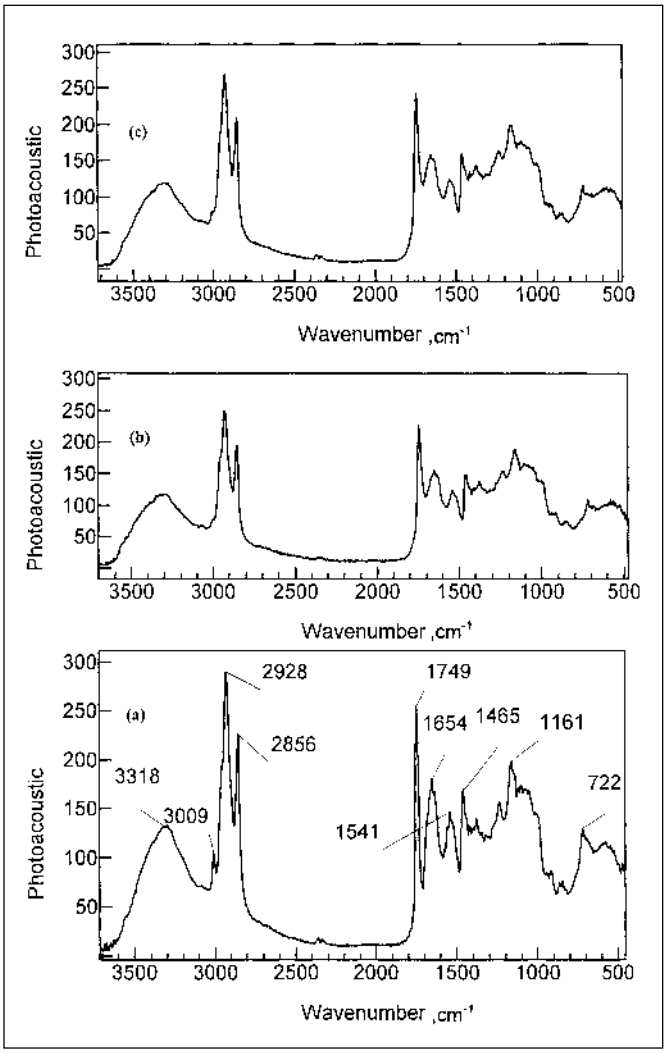

Figure 2—FTIR-PAS spectra of peanut butter: (a) unheated sample; (b) sample heated at 90 °C for 16 d; (c) sample heated at 60 °C for 32 d

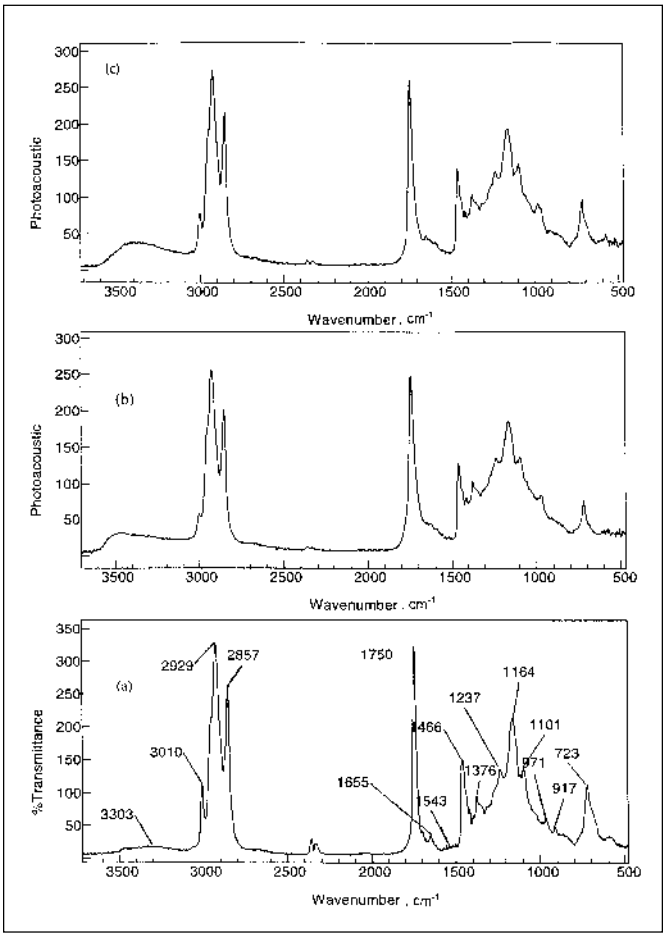

Figure 3—FTIR-PAS spectra of mayonnaise: (a) unheated sample; (b) sample heated at 90 °C for 16 d; (c) sample heated at 60 °C for 32 d

maintained at 90 and 60 °C. Comparisons of the PAS and ATR spectra) are then made (Figure 1 to 4, and 5).

Area-normalization of the spectroscopic data was done to compensate for gross differences in spectral response that are caused by physical effects, rather than compositional properties of the samples. To study lipid oxidation, the spectral range should include information on the functional groups related to lipids and other related constituents, while excluding regions dominated by noise or other components such as water and protein, which are not directly related to oxidation. The spectral range between 2700 to 3100 cm^{-1} (the most representative of bands related to lipids) was selected for oxidation studies using LDA. Equation 1 was used to calculate the Mahalanobis distances between unheated and heated samples, using the selected spectral region by LDA with 2 principal component scores (Table 2). The reassignment success rate was more than 95% with 2 principal component scores in all cases, which shows a successful LDA model (Kemsley 1998).

Table 2—Mahalanobis distances of heated samples from unheated samples

Drying time, d	Lard		Peanut butter		Mayonnaise		Whipped topping	
	60°C	90°C	60°C	90°C	60°C	90°C	60°C	90°C
0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
2	5.0	5.7	4.9	1.6	8.4	13.4	23.7	31.5
4	6.8	13.0	7.9	2.0	8.8	13.8	25.1	33.2
8	11.4	18.2	8.6	10.5	9.0	18.4	27.4	36.1
16	20.5	24.3	19.2	17.0	10.9	33.2	28.5	36.1
32	37.4		64.0		29.5		29.2	

Lard

Figure 1 shows the FTIR-PAS spectra of the unheated and heated lard. It illustrates the dominant spectral features associated with oils and fats. Three distinct peaks at 2927, 2855 and 1749 cm^{-1} could be observed. The first represents the asymmetrical, and the second represents the symmetrical stretching modes of the methylene group (-C-H(CH_2)) of the fatty acid backbone (Safar and others 1994). The peak at 1749 cm^{-1} is a result of the absorption of the ester carbonyl group (C = O) of the triglycerides. Unsaturated fatty acids present in lard gave a small peak at around 3006 cm^{-1} which is characteristic of the *cis* double-bond stretching absorption (Van de Voort and others 1995). The peaks at 1466 and 1378 cm^{-1} are due to the bending vibrations of the CH_2 and CH_3 aliphatic groups of the fatty acids, whereas the bands at 1238 and 1164 cm^{-1} are related to the C-O stretching of esters (McDonald and Mossoba 1997). The sharp peak at 722 cm^{-1} is associated with the rocking vibration of the methylene group (Guillen and Cabo 1999). A distinct change in the spectra of lipids (2700 to 3100 cm^{-1}) in lard at 60 and 90 °C with drying time was noticed from the LDA results, which otherwise was not discernible by visual examination. Table 2

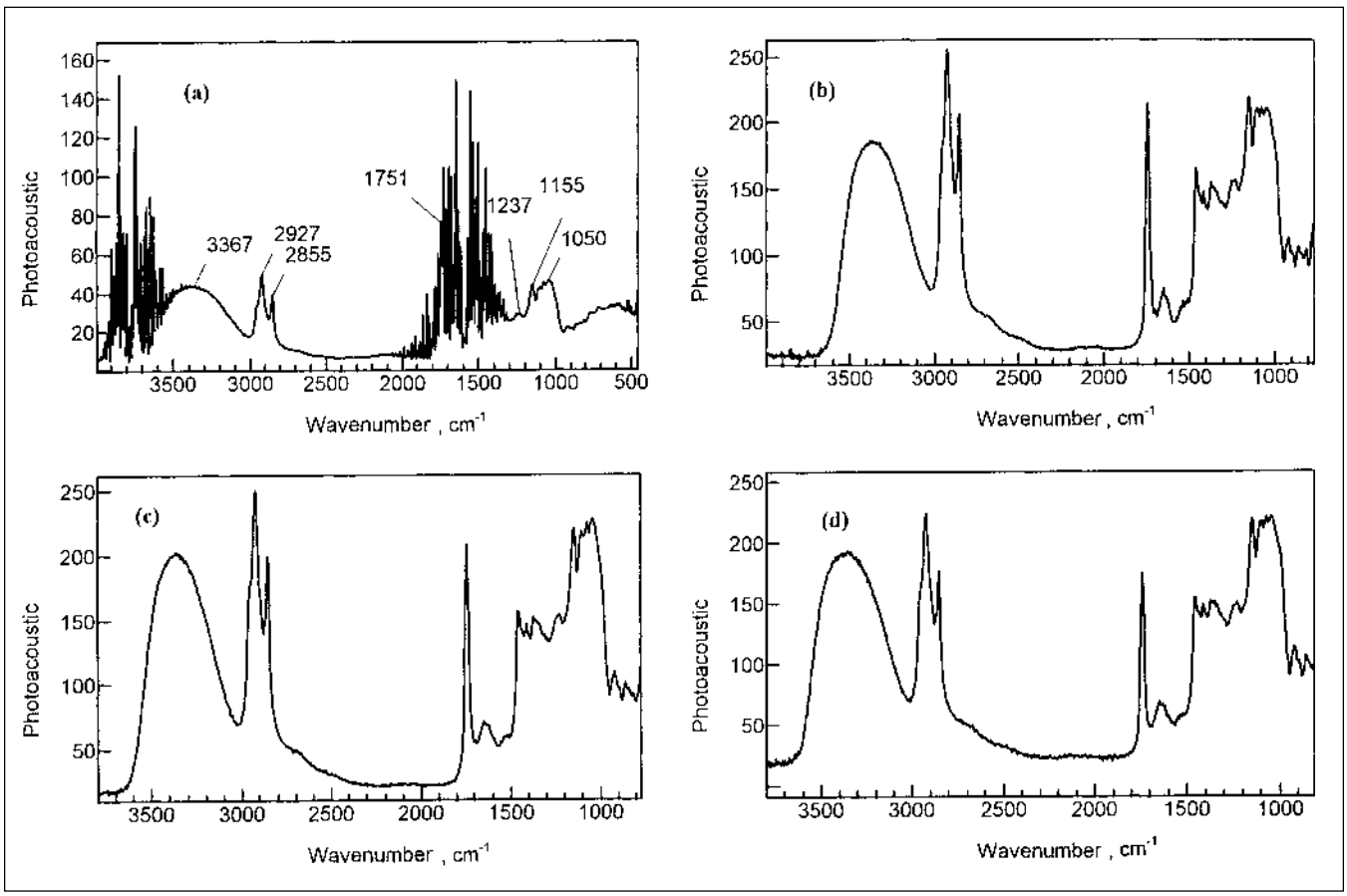

Figure 4—FTIR-PAS spectra of whipped topping: (a) unheated sample; (b) sample heated at 60 °C for 2 d; (c) sample heated at 90 °C for 16 d; (d) sample heated at 60 °C for 32 d

shows that the D_M values at 60 °C increase with drying time, from 0.0 at day 0 to 37.4 at day 32. A similar trend was observed at 90 °C. The distances between the spectra of unheated and heated samples (5.7 to 24.3) show that the extent of oxidation is high at 90 °C. As oxidation proceeds, hydroperoxides are formed along with secondary oxidation products, such as aldehydes and ketones (Van de Voort and others 1994). The slight broad peak present in heated samples around 3444 cm^{-1} can be attributed to the OH stretching vibration of hydroperoxides. The *cis* double-bond stretching vibration band near 3006 cm^{-1} (proven to be related to oil composition) decreases at a rate that is basically dependent on the nature of sample. It is evident in the FTIR spectrum of the heated sample where the *cis* double bond-stretching peak at 3006 cm^{-1} disappears in the heated lard, due to the occurrence of isomerization of oils in the sample (Figure 1b and 1c). The loss of the *cis* double-bond is characteristic of lipids undergoing oxidation under moderate conditions (Van de Voort and others 1994). Aldehydes exhibit bands in the 1730 to 1680-cm^{-1} region owing to the high absorptivity of their carbonyl groups (McDonald and Mossoba 1997), but they overlap with the strong band of the fat carbonyl group at 1749 cm^{-1}. Under oxidative conditions, this band begins to decrease with drying time.

Peanut butter

Figure 2 shows the FTIR spectra of the unheated and heated peanut butter. The peaks in the spectra correlate well with the functional groups of the ingredients present in the sample. The prominent broad peak at about 3318 cm^{-1} is due to the N-H stretching of the amide group (Amide A) of the sample's proteins. Generally, water absorbs in the same region of the spectrum as both amide proteins, but that possibility can be eliminated because peanut butter does not contain appreciable amounts of water (Table 1). Other protein-related peaks are also prominent in the FTIR spectra of peanut butter. The peak at 1654 cm^{-1} (Amide I) represents the carbonyl and C-N stretching of the amide groups, and the peak at 1541 cm^{-1} relates to a combination of the C-C and C-N stretching, and N-H and C-O bending vibrations of the amide group.

In addition to the protein peaks, the FTIR spectrum of peanut butter also shows peaks associated with fats, specifically the distinct peaks at 2928, 2856, and 1749 cm^{-1} (as noted above for lard in Figure 2). Peaks corresponding to the stretching of C-O groups of the fatty acid esters at 1161 cm^{-1}, and a sharp peak at 722 cm^{-1} associated with the rocking vibration of the methylene group, are also prominent. Furthermore, a small peak at 3009 cm^{-1}, representing the *cis* double-bond stretching absorption, is also present. The bands in the 904 to 1153 cm^{-1} region are assigned to C-O and C-C stretching modes and those around 1199 to 1474 cm^{-1} regions are due to the bending modes of O-C-H, C-C-H, and C-O-H angles (Hineno 1977). The band caused by the absorption of soluble starch at 1650 cm^{-1} is obscured, due to the presence of moisture.

Although the peanut butter sample visibly deteriorated within 2 d of heating, D_M values in Table 2 show that deterio-

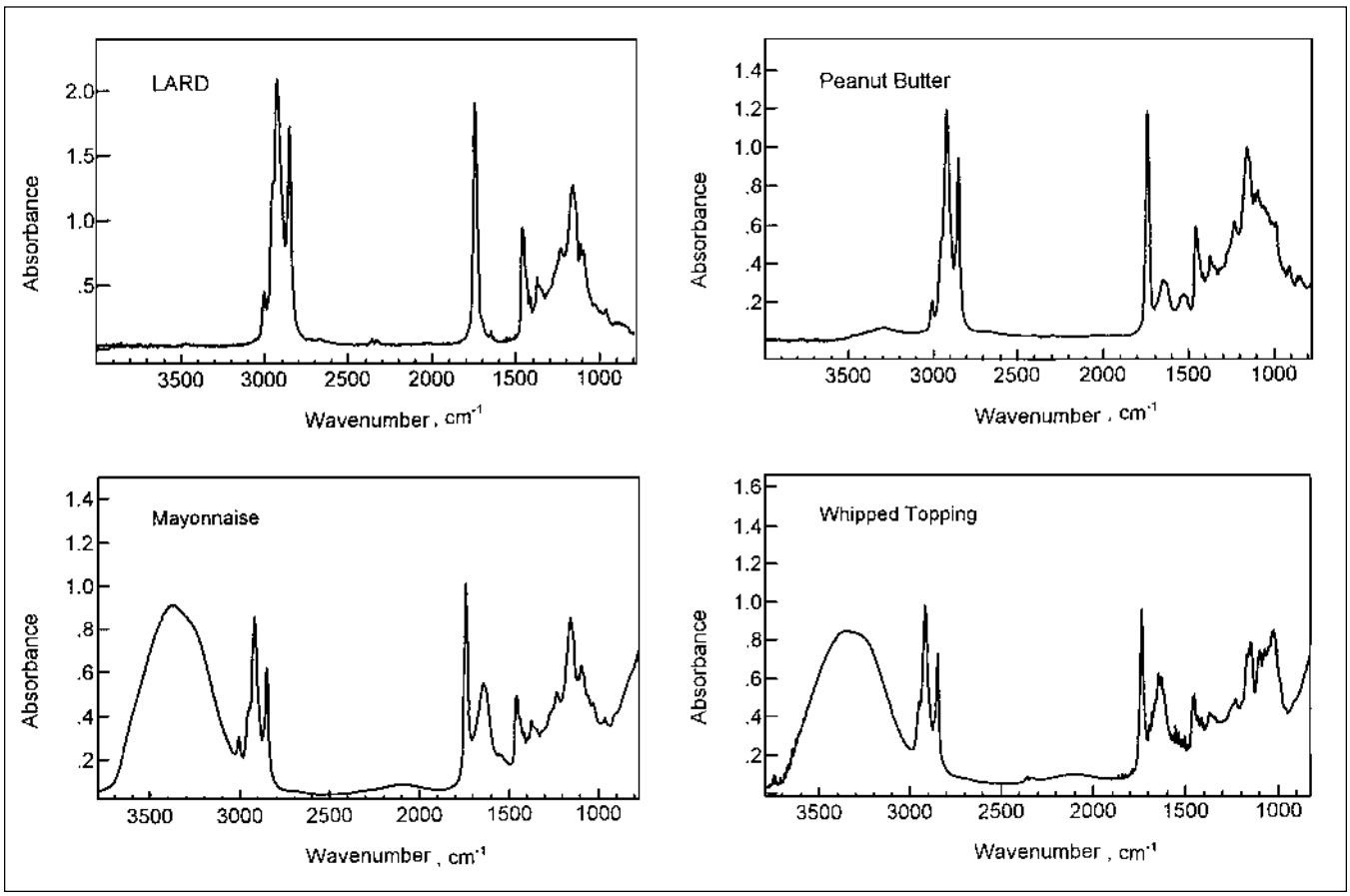

Figure 5—ATR spectra of (a) lard, (b) peanut butter, (c) mayonnaise, (d) whipped topping at 0 d

ration is gradual with drying time at both 60 ° and 90 °C. Lipid oxidation and possible isomerization caused changes in spectrum, which included the disappearance of the *cis* double-bond stretching peak at 3009 cm^{-1} (Figure 2). Another change observed in the heated sample is the decrease in the peak corresponding to 722 cm^{-1}, visibly prominent in the unheated sample (Figure 2).

Mayonnaise

The bands representing the various functional groups of the sample components are not clearly visible due to the presence of moisture in the unheated sample; hence, the 2 d-60 °C heated moisture-free sample of mayonnaise was used to characterize the peaks (Figure 3a). Peaks due to the proteins (about 3303, 1655, and 1543 cm^{-1}), fats (about 2929, 2857, 1750, 1466, 1376, 1237, and 1164 cm^{-1}), and carbohydrates (about 970 to 1153 cm^{-1}) can be observed. In addition to the above components, mayonnaise has a significant amount of water (Table 1). But since after 2 d the sample was relatively moisture-free, the peaks between 3100 to 3500 cm^{-1} can therefore be attributed to protein vibrations.

Figure 3 shows the spectra of heated mayonnaise samples. The region between 3100 to 3500 cm^{-1} shifted toward 3444 cm^{-1}, which indicated the presence of hydroperoxides resulting from oil oxidation. It can also be observed that the relative height of the *cis* double-bond stretching peak at 3010 cm^{-1} has decreased. This further indicates oil oxidation and isomerization. Peaks at 1655 and 1543 cm^{-1} (representing the absorption of the protein amide groups) are only vaguely discernible in the spectrum of the heated sample. The D_M values for mayonnaise given in Table 2 indicate that deterioration increased with drying time at both 60 ° and 90 °C.

Whipped topping

FTIR spectra of the whipped topping's unheated sample, control sample (2 d, 60 °C heated), plus the 16 d at 90 °C and 32 d at 60 °C heated sample are shown in Figure 4. The unheated sample shows the strong effect of high moisture on the sample. The 2-d spectrum shows peaks corresponding to proteins, fats, and carbohydrates that are associated with the different functional groups present in the molecules of the sample components (as detailed above). On the 2nd day at 90 °C, the D_M value was 31.5 while the value at 60 °C was 23.7. The D_M values slightly increased from 2 d to 32 d of heating, whereas no visual differences can be seen in these spectra. The major change here appeared to be loss of moisture between 0 d and 2 d.

PAS and ATR Comparison

The ATR spectra of unheated lard, peanut butter, mayonnaise, and whipped topping are given in Figure 5. The ATR spectra of these samples clearly display the distinct peaks corresponding to the fat- and protein-related functional groups. A comparison of Figure 5 with the unheated spectra of mayonnaise and whipped topping (Figure 4a) demonstrates the effect of moisture on the PAS spectra. The effect of moisture can be clearly seen in the regions between 3800 and 3300 cm^{-1} (O-H stretch) and around 1640 cm^{-1} (O-H bend) in the PAS spectra of the respective high-moisture samples. Although PAS is a very powerful nondestructive analysis tool, the more traditional ATR method is still preferable to PAS for analyzing high-moisture products. One of the key advantages of FTIR-PAS is the depth- profiling capability for nondestructive evaluation of successive layers below the surface (Irudayaraj and Yang 2000).

Conclusions

STUDIES CONDUCTED DEMONSTRATED THE POTENTIAL OF FTIR-PAS as a rapid and nondestructive tool for monitoring changes attributable to oxidation of some common low-moisture foods. Mahalanobis distances were measured between heated and unheated samples, using LDA to estimate the deterioration due to lipid oxidation. Results indicated that the deterioration was greater after 2 d of drying at 60 °C and 90 °C. FTIR-PAS combined with LDA and PCA correlation can be used to monitor quantitative and qualitative changes in specific food components during processing and storage.

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