Quantitative Fourier Transform Infrared Analysis for Anisidine Value and Aldehydes in Thermally Stressed Oils

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ABSTRACT: A Fourier transform infrared (FTIR) transmission-based spectroscopic method was investigated for the simultaneous monitoring of aldehyde formation and the determination of anisidine value (AV) in thermally stressed oils. Synthetic calibration standards were prepared by adding known amounts of hexanal, t-2-hexenal and t,t-2,4-decadienal to canola oil (these compounds considered representative of aldehydic compounds formed during oxidation) plus random amounts of other compounds representative of oxidation by-products. The standards were analyzed for their chemical AV. With the partial least squares (PLS) technique, an FTIR spectrometer was calibrated to predict both the concentrations of individual aldehyde types and AV, with the individual aldehyde contributions being related to the chemical AV by multiple linear regression to derive "apparent" AV values. The predictive capability of the PLS calibrations was assessed by analyzing canola oils that were thermally stressed at 120, 155, and 200°C. The apparent AV, predicted for these samples, matched the chemical AV values within ±1.65 AV units. A PLS calibration also was derived by using thermally stressed samples as calibration standards. This approach provided similar predictive accuracy as the use of synthetic calibration standards. As such, quantitative determination of AV by FTIR spectroscopy was shown to be feasible, and the synthetic calibration approach provided additional information on the aldehyde types present in a sample and allowed the use of a simple gravimetric approach for calibrating an FTIR spectrometer. This study provides the basis for the development of a rapid, automated FTIR method for the direct analysis for AV of thermally stressed fats and oils in their neat form without the use of chemical reagents. The implementation of such a method as a quality control tool would eliminate the use and disposal of hazardous solvents and reagents, required by the conventional chemical method, and drastically reduce analysis time (~2 min/sample). Possible applications include monitoring of the oxidative state of frying oils or evaluation of oxidative stability of biodegradable lubricants.
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KEY WORDS: Aldehydes, anisidine value, fats and oils, FTIR spectroscopy.

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Lipid oxidation has important economic consequences for the edible fats and oils industry because the end result is rancidity, manifesting itself by a cumulative sensory defect that is characterized by unpleasant and unacceptable off-flavors and odors. Thermal stress speeds up oxidative reactions, and, thus, lipid oxidation is of major concern in frying operations and in industrial applications, such as biodegradable lubricants or hydraulic fluids. The primary products of autoxidation, hydroperoxides, are subsequently a source of short-chain aldehydes, ketones, fatty acids, alcohols, and hydrocarbons, which contribute to the rancid off-flavors in edible oils (1). Of these compounds, aldehydes are largely considered responsible for the off-flavors in fats and oils due to their low sensory threshold values (2). As autoxidation is a complex reaction that can take many routes, depending on the lipid in question and the conditions under which the lipid is undergoing oxidation, it is difficult to unambiguously assess the oxidative state of fats and oils. In general, two measures are made, one related to the initial or primary oxidative products, i.e., hydroperoxides, plus a second measure of accumulated secondary breakdown products, usually carbonyl-type compounds (3).

A variety of chemical methods are available that attempt to monitor secondary oxidation products, including the thiobarbituric acid test, the Kreis Test, and various other methods that attempt to address both total and volatile carbonyl compounds, many of which have been reviewed by Gray (4). Some of the methods developed for carbonyl compounds have been shown to be quite sensitive, quantitative, and well-correlated to compounds associated with the development of rancidity, particularly the determination of anisidine value (AV). Based on a method originally developed by Holm *et al.* (5), and later modified to use *p*-anisidine instead of carcinogenic benzidine acetate as the reactive reagent, the AV test is a widely accepted AOCS method (6). Although the method is relatively simple, the procedure requires substantial precision and analytical time and uses relatively noxious reagents. AV is a combined measure of mostly 2-alkenals and 2,4-dienals and, to a more limited degree, saturated aldehydes, because ultraviolet (UV) absorption of the *p*-anisidine/aldehyde reaction products varies with the aldehyde type. A double bond in the carbon chain conjugated with the carbonyl double bond

increases the molar absorbance by a factor of 4–5 (6). AV is commonly used to follow the formation of aldehydic compounds in edible oils and correlates well with the development of off-flavors in lipids undergoing oxidation.

In our laboratory, work is ongoing to develop rapid automatable methods for the analysis of edible fats and oils based on Fourier transform infrared (FTIR) spectroscopy (7). To date, FTIR methods have been developed to measure iodine value and saponification number (8), cis-trans isomers (9), free fatty acids (10), peroxide value (11) and, more recently, solid fat index (12). Basic spectroscopic work has been carried out on secondary oxidation products that may be present in fats and oils, and conceptual considerations have been put forward related to the chemometric approaches that might be applied in developing appropriate quantitative FTIR methods (13). In this paper, we report on the development of FTIR calibrations that are capable of both predicting AV and assessing individual aldehyde contributions as oxidation imposed by thermal stress proceeds.

MATERIALS AND METHODS

Instrumentation/sample handling. The instrument used for this work was a Nicolet Impact 400 FTIR spectrometer (Nicolet Instrument Inc., Madison, WI), controlled by a 486 MHz PC run under OMNIC 1.2 software (Nicolet). The instrument was equipped with a heated sample-handling accessory (9) set to 80°C, capable of handling both fats and oils and a 100-μm NaCl transmission flow cell, loaded by aspiration. Prior to starting any analysis, isooctane was passed through the system to clean the cell and transfer lines. All samples were preheated for ~1 min to 80°C in a water bath prior to loading to minimize temperature perturbations in the cell. The instrument was continuously purged with CO_2-free dry air from a Balston dryer (Balston, Lexington, MA) to minimize water vapor and carbon dioxide interferences.

Synthetic calibration standards/chemometrics. Commercial canola oil was obtained locally and passed twice through a column of activated silica gel to remove any carbonyl compounds or other partially polar molecules that might be present. The cleaned oil was analyzed for its AV (6) to ensure that the AV was <0.3. Hexanal, *t*-2-hexenal and *t,t*-2,4-decadienal, *t*-4-hexen-3-one, hexanol, and *tert*-butylhydroperoxide (TBHP) were purchased from Aldrich Chemicals (Milwaukee, WI). A synthetic calibration matrix (Table 1) was developed by spiking the cleaned canola oil with known, random amounts of hexanal (0–26 μmol/g), hexenal (0–12 μmol/g), and decadienal (0–4 μmol/g) as representative of the aldehyde compounds to be measured. In addition, random and variable (<1%) amounts of hexenone, TBHP, hexanol, water, and oleic acid were added to the 32 canola oils spiked with the aldehydes to produce samples that were considered representative of oxidized oils (13). These standards were analyzed for their corresponding AV and served as a basis by which partial least squares (PLS) calibration models could be derived to quantitate for both individual aldehydes and AV,

based on the FTIR spectra obtained for the standards. In turn, multiple linear regression was used to relate the chemically determined AV to the known concentrations of the individual aldehydes added to the base oil.

For all standards and samples, emittance spectra were collected by co-adding 256 scans at a resolution of 4 cm^{-1}, with triangular apodization and a gain of 1.0, and ratioed against an air emittance background to produce absorbance spectra, which were stored to the hard disk for subsequent chemometric analysis. To accentuate spectral changes induced by addition of aldehydes and other components to the canola oil, the emittance spectra were also ratioed against the emittance spectrum of the starting oil to produce "differential spectra." This technique makes it possible to more readily visualize subtle spectral changes, which otherwise might be difficult to detect (14) due to the low concentrations of these components. For the development of FTIR calibrations, PLS was the chemometric method of choice (15), with variance spectra being used to determine which spectral regions exhibited changes with changes in the components of interest. The calibrations were developed with the Nicolet QuantIR software package (16), and the quality of the calibrations was evaluated and optimized by a combination of the partial residual error sum of squares test and the "leave-one-out" cross-validation approach.

The efficacy of the synthetic calibration to monitor the formation of aldehydes and predict AV was assessed by comparing the FTIR AV predictions for samples of oxidized canola oil with those obtained by the chemical method. To obtain samples that were representative of various degrees of oxidation, canola oil was thermally stressed at 120, 155, and 200°C, and the oxidation reaction was promoted by bubbling dry air through the oil while under continuous agitation. Aliquots were collected as a function of time for each temperature run, cooled, flushed with nitrogen, and stored at −20°C for subsequent FTIR analysis and chemical AV determinations. A portion of these collected samples were also used as calibration standards to develop an alternate PLS calibration model for AV determination for comparison to the synthetic calibration.

RESULTS AND DISCUSSION

Spectral analysis. Previous work on monitoring oxidation of edible oils by FTIR spectroscopy (12) indicated that it may be possible to develop and make use of synthetic calibration standards as a means of developing quantitative methods for the analysis of secondary oxidation products. Such standards are prepared by spiking a clean oil with compounds that are spectroscopically representative of oxidation products. From the point of view of developing an FTIR method to substitute for the chemical AV method, saturated, α, β-unsaturated aldehydes, and α, β, δ, γ-unsaturated aldehydes are the components of interest and were represented by hexanal, 2-hexenal, and 2,4-decadienal, respectively. The objective of this work was to use these aldehydes as a basis for developing a syn-

TABLE 1
Calibration Matrix for the Development of a Partial Least Squares Calibration[a]

Sample	Hexanal	Hexenal	Decadienal	Water	Ketone	FFA	ROOH	ROH	AV
1	1.320	0.190	0.003	+[b]	+	+	+	+	0.88
2	2.090	2.080	0.570		+	+	+		13.80
3	1.000	0.990	0.270				+	+	7.48
4	0.035	0.051	0.002		+		+		0.15
5	3.670	2.890	0.150		+		+		9.75
6	25.600	5.200	0.312	+			+	+	23.52
7	0.490	1.170	0.014		+		+		1.91
8	0.000	0.000	0.000						0.29
9	0.000	0.000	0.000	+		+	+		1.00
10	1.310	0.500	0.320	+	+		+		7.82
11	1.680	5.380	0.720		+	+	+		26.09
12	1.300	0.750	1.220	+		+	+		23.93
13	1.490	4.710	2.380		+	+	+	+	48.92
14	1.230	9.910	3.020		+	+	+	+	65.57
15	10.90	11.100	0.170		+	+	+	+	31.99
16	1.400	3.210	1.100	+	+	+	+	+	25.42
17	8.690	5.070	0.110		+	+	+	+	15.44
18	1.290	0.160	0.068					+	2.20
19	0.990	0.657	0.076	+			+	+	2.61
20	3.720	8.170	0.317	+		+	+	+	23.69
21	9.550	1.560	0.145		+	+	+	+	8.88
22	0.087	1.340	0.062	+		+	+		4.95
23	5.310	0.583	0.197		+		+		8.83
24	6.000	6.330	0.378		+	+	+		21.37
25	13.500	4.920	3.470	+	+		+	+	68.04
26	1.100	0.411	0.104	+	+	+	+		2.89
27	0.000	0.000	0.000				+	+	0.00
28	0.615	4.960	1.510		+	+	+	+	32.79
29	3.380	1.230	0.868	+	+		+	+	17.01
30	0.990	0.370	0.094	+	+	+	+		2.60
31	0.861	6.940	2.110		+	+	+	+	45.90
32	1.420	4.470	2.260		+	+	+	+	46.47

[a]With hexanal, hexenal, and decadienal (μmol/g) as the components of interest; the balance of the other components are potentially interfering substances. FFA, free fatty acid; AV, anisidine value. [b]+ Indicates component added to oil.

thetic calibration matrix, from which a PLS calibration model could be derived to quantitatively predict both AV and aldehyde content of thermally stressed oils. In a real system undergoing oxidation, products other than aldehydes are formed that may have an effect on quantitation and thus must be represented in the calibration model to account for their spectral contributions and/or interferences. Such compounds include hydroperoxides, alcohols, free fatty acids, water, and possibly ketones. The characteristic IR absorptions associated with various compounds that are representative of oxidation products in oil have been determined in a previous study and are summarized in Table 2. The influence of each of these compounds on the measurement of the aldehyde components of interest was investigated by adding each component individually to a clean oil sample and to a bulk oil spiked with the three aldehydes. Analysis of these spectra indicated that the presence of hydroperoxides and alcohols caused an apparent broadening of the strong triglyceride ester linkage absorption, centered at ~1748 cm⁻¹. In the differential spectra relative to a clean oil, this broadening manifests itself as a peak around 1725 cm⁻¹, with the maximum appearing at slightly lower frequencies for hydroperoxide as compared to alcohol. The effects of both hydroperoxides and alcohols were concentration-dependent, with the band shifting to slightly lower frequencies as more hydroperoxide or alcohol was added. The appearance of this peak in the differential spectrum was attributed to hydrogen bonding of alcohols and hydroperoxides with the triglyceride ester carbonyl groups. These observations are of consequence because broadening of the triglyceride ester linkage absorption obscures the carbonyl absorption of saturated aldehydes, centered at 1727 cm⁻¹, effectively eliminating the carbonyl absorption as a primary means of measuring saturated aldehydes. Fortunately, saturated aldehydes have unique but weak signals in the CH stretching region (2820–2700 cm⁻¹), which provides an alternate means of making a measurement of their contribution. The carbonyl absorptions of hexenal and decadienal (1697 and 1689 cm⁻¹) appeared to be far enough away from the broadened triglyceride band to be used for quantitation.

Based on this assessment, a synthetic calibration matrix was devised by adding random amounts of the three aldehydes (hexanal, hexenal, and decadienal) to a clean canola oil plus random amounts of TBHP, hexanol, water, oleic acid, and hexenone as per Table 1. Figure 1 presents the resulting

TABLE 2
Vibrations of Functional Groups Associated with Compounds That May Be Formed During the Process of Oxidation

Compound	Vibration	Peak maximum	Relative abs[a]
Water	v **OH**	3650 and 3550 cm^{-1}	NA
Water	δ **HOH**	1625 cm^{-1}	NA
Hexanol	v **ROH**	3569 cm^{-1}	0.06
t-Butyl hydroperoxide	v **ROOH**	3447 cm^{-1}	0.04
Hexanal	v **RHC=O**	2810 and 2712 cm^{-1}	0.02 and 0.03
Hexanal	v **RHC=O**	1726 cm^{-1}	0.20
Hexenal[b]	v **RHC=O**	2805 and 2725 cm^{-1}	0.03 and 0.03
Hexenal	v **RHC=O**	1697 cm^{-1}	1.00
Hexenal	v **RC=CH**–HC=O	1640 cm^{-1}	0.10
Hexenal	δ **RC=CH**–HC=O	974 cm^{-1}	0.25
2,4-Decadienal[b]	v **RHC=O**	2805 and 2734 cm^{-1}	0.03 and 0.02
2,4-Decadienal	v **RHC=O**	1689 cm^{-1}	0.81
2,4-Decadienal	v **RC=CH**–HC=O	1642 cm^{-1}	0.52
2,4-Decadienal	δ **RC=CH**–HC=O	987 cm^{-1}	0.27
4-Hexen-3-one[b]	v **RC(=O)HC=CHR**	1703 and 1679 cm^{-1}	0.23 and 0.38
4-Hexen-3-one	v **RC(=O)HC=CHR**	1635 cm^{-1}	0.32
4-Hexen-3-one	δ **RC(=O)HC=CH**–R	972 cm^{-1}	0.29
Oleic acid	v **RCOOH**	3310 cm^{-1}	0.03
Oleic acid	v **RC(=O)OH**	1711 cm^{-1}	0.25

[a]Relative to a value of 1 for the strongest absorption band. NA, not applicable.
[b]All double bonds in the *trans* form.

differential spectra of selected synthetic calibration standards, showing the spectral variation introduced in the four major regions of interest (OH, CH, C=O and *trans*). In the OH region, water, alcohol, and hydroperoxide absorptions are observed, the large broad band being a combination of OH and OOH absorption bands. In the CH region, one can discern the weak CH absorptions of the aldehydes that have been added to the system. In the C=O region, from left to right, the higher-frequency band is a result of broadening of the triglyc-

FIG. 1. Differential spectra of selected synthetic calibration standards used to model an oxidizing canola oil system, illustrating the OH, CH, C=O, and *trans* regions (A–D, respectively).

eride ester linkage absorption by hydrogen bonding as described above, masking the C=O absorption of saturated aldehydes; the next bimodal band is a combination of the C=O absorptions of the unsaturated aldehydes present, while the absorption in the 1650–1640 cm^{-1} range is due to both water and conjugated aldehydes. In the *trans* region, the absorption band observed is a superposition of the absorptions of isolated and conjugated *trans* bonds of the 2-alkenals and 2,4-dienals, respectively.
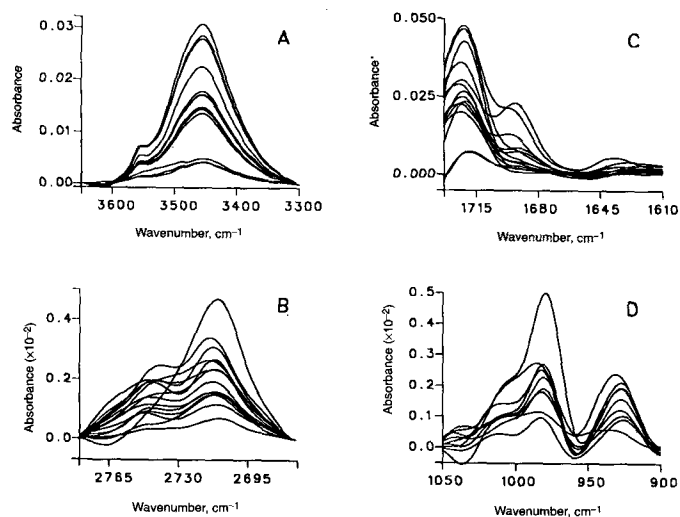
Spectroscopy of thermally stressed oils. As an oil undergoes oxidation, the reaction involves the uptake of oxygen *via* the formation of hydroperoxides, which subsequently break down by various mechanisms to produce a variety of shorter-chain aldehydes, fatty acids, and alcohols. Figure 2 shows differential spectra obtained as canola oil undergoes oxidation at 155°C. The regions examined parallel Figure 1 for the synthetic calibration standards. In the OH region, it is apparent that extensive formation of hydroperoxides is taking place, with some formation of alcohols in the later stages of oxidation. In the CH region, a broad band develops, attributed to formation of aldehydes. In the C=O region, a substantive peak develops at ~1725 cm^{-1}, a frequency characteristic of the formation of saturated aldehydes; however, based on our evaluation of the synthetic calibration standards, the formation of hydroperoxides and alcohols would also give rise to a band at this position due to hydrogen-bonding effects. Subsequent treatment of samples, taken during the early stages of oxidation, with triphenylphosphine, which quantitatively converts hydroperoxides to alcohols, led to a clear shift of this band
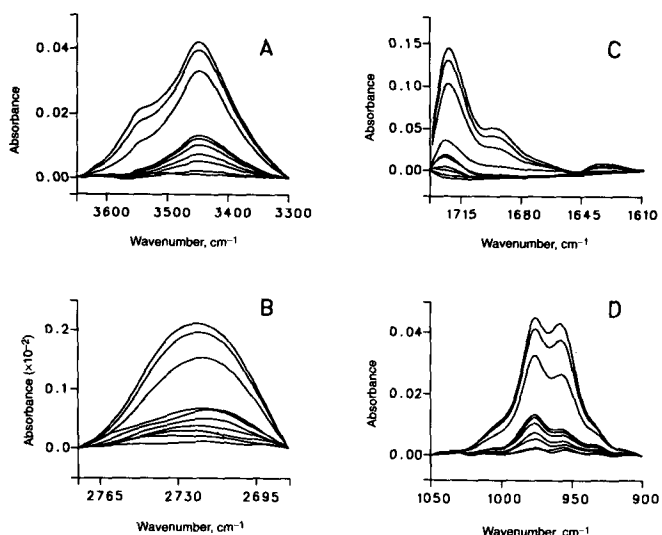
FIG. 2. Differential spectra of a time course of canola oil undergoing oxidation at 155°C, illustrating the OH, CH, C=O, and *trans* regions (A–D, respectively).

from 1725 to 1727 cm^{-1}, confirming that this band is largely due to hydrogen-bonding effects. The shoulder developing between 1705 and 1680 cm^{-1} shows the formation of unsaturated aldehydes, and the peak on the far right is due to the formation of α, β, δ, γ-unsaturated aldehydes (decadienal type). Although not clearly discernible in Figure 2C, there is also evidence of decreasing absorption at 1654 cm^{-1} owing to the loss of *cis* double bonds. In the *trans* region, the broad bimodal band (960–990 cm^{-1}) demonstrates the formation of both isolated and conjugated *trans* double bonds with time.

Both commonalities and differences are evident when comparing these spectra (Fig. 2) with those of the calibration standards (Fig. 1). Major differences are observed in the *trans* absorption region because changes in this region in the process of oxidation are due to both the formation of *trans*-containing hydroperoxide moieties and their breakdown to unsaturated aldehydes. In addition, the concomitant loss of *cis* double bonds gives rise to the negative absorptions observed at 1654 cm^{-1} (C=C stretching band). Because the spectral effects of *cis* to *trans* isomerization of the hydroperoxides, formed during oxidation, cannot be modeled readily by synthetic calibration standards, the *trans* absorption region and the C=C stretching band are not suitable for quantitation in the synthetic calibration approach. What is common to both systems is the C=O region (1710–1680 cm^{-1}) to measure the unsaturated and conjugated unsaturated aldehyde contributions and the relatively weak CH bands by which to determine the saturated aldehyde contributions.

Development of calibration models. The determination of AV is predicated on the reaction of *p*-anisidine with aldehydes and the measurement of the absorbance of the reaction products in the UV portion of the spectrum. The extinction coefficients of these products depend on the aldehyde type. FTIR spectroscopy is capable of measuring the spectral changes associated with appearance or loss of a variety of

functional groups (12) and, as such, could provide an alternate means of determining AV. The basic premise in developing a synthetic calibration is that the IR spectral features of the aldehydes contributing to the chemical AV can be adequately mimicked using selected aldehydes, if interferences of other oxidation products that may be formed as autoxidation proceeds, such as moisture, free fatty acids, alcohols, and hydroperoxides (13), are taken into account, The chemometric method of choice for developing a multicomponent calibration is PLS (15), as it is capable of accounting for interfering compounds that are external to the measures in question, as long as the calibration standards are spectrally representative of the samples to be analyzed in the spectral regions associated with the components of interest. One way of assessing the representativeness of the calibration set is by examining the variance spectrum, which is indicative of the spectral variability relative to the mean spectrum. Figure 3 compares the variance spectrum of the calibration standards to those obtained for canola oil thermally stressed at 120, 155, and 200°C in the four spectral regions of interest. The variance spectra of the thermally stressed oils are generally similar to that of the calibration standards, although differences in the product distribution for each temperature condition are apparent. The 120°C run formed mostly hydroperoxides; the 155°C run formed hydroperoxides, some alcohol, and aldehydes; and the 200°C run formed largely alcohols and aldehydes. In the carbonyl region, the correlation between the hydrogen bonding band and hydroperoxide/alcohol formation is apparent in these variance spectra because there is a shift of this band to lower frequencies when hydroperoxides are dominant. These variance spectra indicate that the unsaturated aldehyde carbonyl region (1710–1680 cm^{-1}) shows increased variability as temperature rises, although the saturated alde-
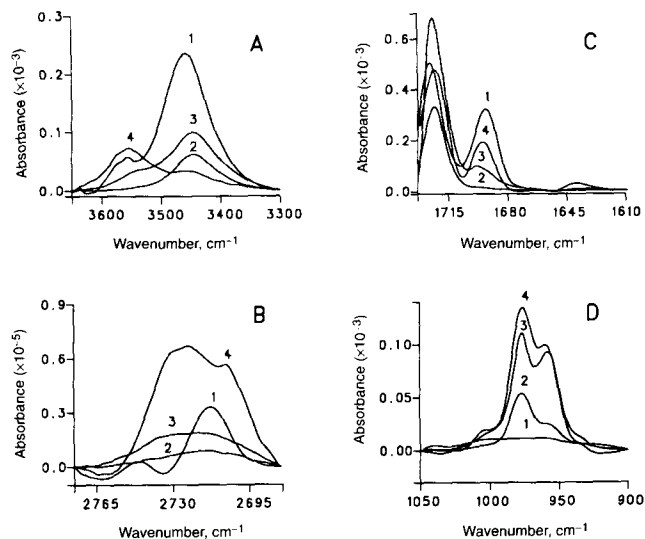

FIG. 3. Variance spectra of the synthetic calibration set (1) and the variance spectra obtained from representative samples taken over the time courses of three temperature runs for 120 (2), for 155 (3), and for 200°C (4), illustrating the OH, CH, C=O, and *trans* regions (A–D, respectively).

hyde region (CH) only shows evidence of substantive change at 200°C.

Two PLS calibration models were developed based on the synthetic calibration set, one using the amounts of hexanal, hexenal, and decadienal added as the concentration data and the other using the chemical AV of the synthetic calibration standards. Both calibrations were optimized by the "leave-one-out" cross-validation procedure to minimize the prediction error and provide an estimate of the overall accuracy of the predictions. Linear regression of the optimized cross-validation FTIR results, obtained for the individual aldehydes and AV, vs. the amounts of aldehyde added to the oils and their chemical AV values, respectively, produced the equations presented in Table 3. Figure 4 presents a cross-validation plot of FTIR AV vs. chemical AV, which is generally representative of the other plots. All the plots were linear, with the slopes and correlation coefficients being close to 1.0, although the performance of the hexanal predictions was somewhat poorer, likely because these predictions are based on the relatively weak absorptions in the C–H stretching region. As structured, these PLS-based synthetic calibration models provide the means to measure either the amount of each type of aldehyde formed or the overall AV of a sample.

Of particular interest is the relationship between the individual aldehyde predictions and the chemical AV. It is well known that the AV test measures predominantly unsaturated aldehydes and is particularly sensitive to conjugated aldehydes, especially dienals (6). Therefore, the AV test is considered an indirect measure of carbonyl compounds in general, and its overall response is a function of the types of aldehydes present. Because the spiked aldehyde concentrations added to the clean canola oil are known, along with their corresponding AV, it is possible to determine the relative contribution of each aldehyde class to the chemical AV by multiple regression analysis. The equation derived by relating AV to each aldehyde class after forcing the regression through the origin was:

$$AV_c = 0.34S + 2.16U + 15.36C \quad r^2 = 0.997 \quad SE = 1.37 \quad [1]$$

where AV_c = chemical AV, S = saturated aldehydes (μmol/g), U = monounsaturated aldehydes (μmol/g), and C = conjugated diunsaturated aldehydes (μmol/g).

TABLE 3
Regression Equations Obtained from Cross-Validation Plots for the Individual Aldehyde PLS Calibrations and for the PLS Calibration Based Directly on AV[a]

Component	Calibration number	Region (cm⁻¹)	Slope	Intercept	r^2	SE[b]
Hexanal	I	2790–2670	0.926	–0.104	0.890	1.76
Hexenal	II	1710–1670	1.000	0.004	0.996	0.18
Decadienal	III	1710–1670	1.005	–0.005	0.996	0.06
AV	IV	2790–2670	1.006	0.417	0.998	0.99
		1710–1670				

[a]PLS, partial least squares; AV, anisidine value. [b]SE for individual aldehydes are in μmol/g.
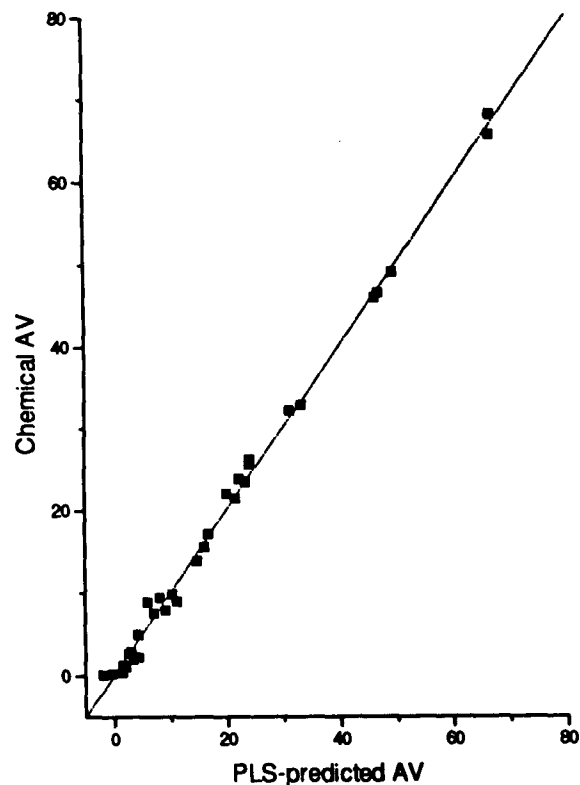

FIG. 4. Cross-validation plot of chemical vs. Fourier transform infrared-predicted anisidine value (AV), obtained from the partial least squares (PLS) calibration model based on synthetic calibration standards.

The coefficients in Equation 1 indicate that the relative UV response of the three aldehyde types (S, U, and C) is ~1:6:45 for the AOCS AV method vs. 1:6:11 reported by Holm *et al.* (5), who used benzidine acetate as the reagent and based their measurements on heptanal, 2-nonenal, and 2,4-hexadienal. The SE obtained from the multiple regression can be considered a measure of the accuracy of the chemical AV method because it relates AV to gravimetrically added aldehyde concentrations. Equation 1 allows FTIR-predicted aldehyde concentrations to be converted to AV; for the sake of clarity, the AV values determined by this approach are termed "apparent" AV. The predicted aldehyde concentrations of the calibration standards obtained from cross-validation were transformed to "apparent" AV (AV_{app}) values and regressed against the chemical AV, yielding the following equation:

$$AV_c = -0.70 + 1.031AV_{app} \quad r^2 = 0.994 \quad SE = 1.48 \quad [2]$$

The SE obtained from this regression is slightly higher than that for the calibration based directly on AV (Table 3, calibration IV), due to the fact that there is an accumulation of errors from the three individual aldehyde determinations. However, both the direct and indirect approaches to determining AV are satisfactory, the errors being comparable to those associated with the chemical AV determination (Eq. 1).

Calibration assessment. In thermally stressed oils the actual amounts of various aldehyde types formed are unknown, although the chemical AV determination does provide a gen-

eral indicator of the total amount of carbonyl compounds in the oil. To determine whether the synthetic calibration approach based on aldehydes is a viable means of determining AV in thermally stressed oils, the aldehyde concentrations in selected samples, taken from three temperature runs (120, 155, and 200°C), were determined by FTIR by using the PLS calibration models and were converted to "apparent" AV values with Equation 1. The apparent AV values obtained were then regressed against the actual chemical AV values determined for these samples (Fig. 5), yielding the following equation:

$$AV_c = 1.08 + 0.953\,AV_{app} \quad r^2 = 0.994 \quad SE = 1.65 \quad [3]$$

There is clearly a good relationship between the "apparent" AV predictions and the results obtained by the standard chemical method. The PLS calibration model based solely on the chemical AV measurements (Table 3, calibration IV), as opposed to the individual aldehyde concentrations, produced similar results:

$$AV_c = 1.48 + 0.961\,AV_p \quad r^2 = 0.994 \quad SE = 1.67 \quad [4]$$

where AV_p = PLS-predicted AV. In both Equations 3 and 4, the SE is only slightly higher than that of Equation 1, which is indicative of the accuracy of the chemical method. These results imply that a generalized synthetic calibration is a satisfactory model of the wide compositional variability of "real" systems undergoing oxidation under different temperature conditions.
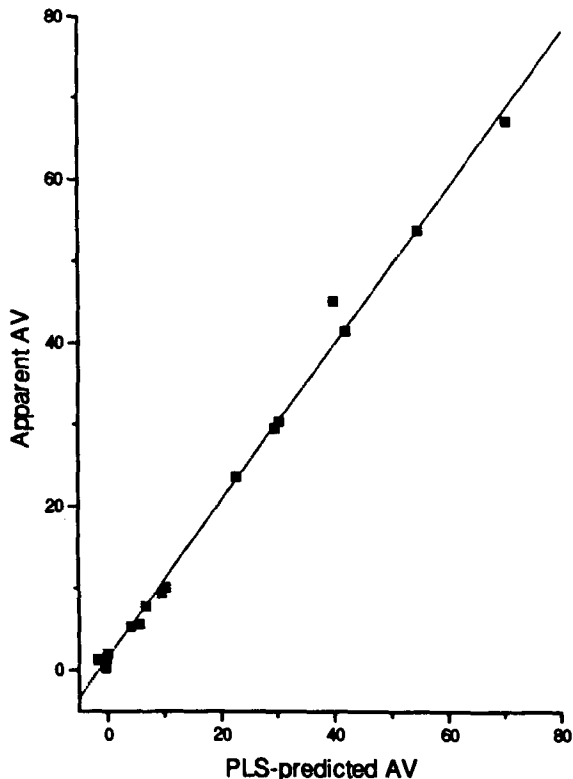

FIG. 5. "Apparent" AV of thermally stressed canola oil samples, determined from Equation 1 by using PLS-determined aldehyde concentrations, plotted against their chemical AV. Abbreviations as in Figure 4.

We have also investigated an alternative approach, based on the use of thermally stressed samples from the three temperature runs as calibration standards, by using the identical spectral regions for calibration as in the three aldehyde PLS calibration models. To avoid building in any time correlations, the samples selected for calibration from the three temperature runs were quantitatively blended in known random proportions to obtain a range of AV values. The regression of the cross-validation results of the AV PLS model against the calculated chemical AV produced the following equation:

$$AV_c = 1.33 + 0.961\,AV_p \quad r^2 = 0.998 \quad SE = 1.25 \quad [5]$$

Subsequent validation experiments, based on a separate series of temperature runs, produced a linear relationship for IR-predicted AV vs. chemical AV of the form:

$$AV_c = 0.55 + 0.983\,AV_p \quad r^2 = 0.998 \quad SE = 1.21 \quad [6]$$

These results indicate that AV can be measured with good accuracy by FTIR spectroscopy when the calibration is based on either synthetic calibration standards or representative oxidized samples that have been mixed randomly to break any time correlations.

Calibration implementation. Because a synthetic calibration approach had been successfully applied to the determination of peroxide value (11), the present study was undertaken to develop a "universal" synthetic calibration for AV determination of thermally stressed oils, including the ability to quantitate individual aldehyde types that contribute to the overall AV. AV determination is somewhat more complex than peroxide value because three components are considered rather than one, with the additional complication of hydrogen bonding obscuring one of the key quantitative regions (hexanal carbonyl @ 1727 cm^{-1}). The results indicate that the synthetic calibration approach works well, as does a more conventional calibration approach with oxidized samples. A key advantage of the synthetic calibration approach is that one has the ability to monitor the changes in individual aldehyde types, as well as AV, with good accuracy. Furthermore, as the relationship between the concentrations of the aldehyde standards and AV has been established (Eq. 1), an FTIR spectrometer can be calibrated for the determination of AV without carrying out chemical analyses of the calibration standards; i.e., with the availability of this relationship, the primary method against which the FTIR spectrometer is calibrated becomes gravimetrically based. A drawback of the synthetic calibration approach is the need to prepare a relatively large number of calibration standards (~30) to ensure that the synthetic calibration matrix adequately models the concentrations and ranges of the interfering components that may be present in oxidized oils (specifically hydroperoxides and alcohols). Accordingly, in some quality control situations, where only AV is of interest, without the benefit of following the formation of individual aldehyde types, it may be more convenient to develop an AV calibration based on samples representative of the oxidation process being monitored, eliminating the need for laboratory preparation of synthetic

calibration standards. With a frying oil as an example, relatively large samples (~50 mL) can be taken over a time course and inspected by differential spectroscopy to select samples for AV analysis, using the changes in the OH, CH, and C=O regions as a basis for selecting samples that have undergone significant spectral changes. Subsequently, the samples selected are analyzed for AV by the chemical method and then mixed quantitatively in a random fashion, so that any correlations between aldehydes and other oxidation products are not built into the calibration set. A PLS calibration model is then developed to predict AV directly. Approached in this manner, the development of a calibration requires a minimum amount of chemical analysis, and the calibration model reflects the AV response for the oil system in question under the oxidative conditions of interest.

This preliminary study indicates that FTIR spectroscopy can be used as a means of determining the oxidative state of an oil, as measured by AV. The rationale for investigating the development of an FTIR method for AV determinations is that it allows for the rapid and routine determination of this parameter and provides for the possibility of monitoring the relative amounts of various aldehyde types being formed, if of interest. As indicated in a number of our previous publications, the analysis can be automated by integrating the calibrations developed into preprogrammed analytical routines, which are used to drive an FTIR spectrometer equipped with a heated sample-handling accessory that allows the analysis to be carried out directly on neat samples (fats or oils). The FTIR method serves as a means of reducing both analytical time and reagent use and disposal problems associated with the chemical AV method. It would be particularly useful in monitoring oil quality in snack food frying operations and for other potential applications, including the evaluation of the oxidative stability of vegetable oil-based lubricants and hydraulic fluids.

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