Mid-infrared Diffuse Reflectance Spectroscopy for Discriminant Analysis of Food Ingredients

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The objective was to determine if mid-infrared diffuse reflectance spectroscopy could be used to discriminate various food ingredients. Samples (n = 106) consisting of buttermilk, cheese, dehydrated onion and milk-egg powders, wheat flours, and two powdered seasonings (cheese and ranch) were scanned as neat powders using diffuse reflectance on Digilab FTS-60 and Perkin-Elmer model 2000 Fourier transform spectrometers. Discriminant analysis, using mean centering and multiplicative scatter-corrected spectra, was performed using Mahalanobis distance by principal component analysis using the averaged predicted distance or F-test indicator to select factors obtained from a one-out cross-validation analysis. Results from the two spectrometers demonstrated that discriminant analysis of food ingredient powders based on spectra of neat powders can be successfully carried out. In general, it was found that results using 4-cm^{-1} resolution spectra were somewhat superior to those based on lower 16-cm^{-1} resolution spectra.

Keywords: *Mid-infrared; DRIFTS; food ingredients; discriminant analysis*

INTRODUCTION

Unlike near-infrared reflectance spectroscopy (NIRS) which has been used extensively to quantitatively determine the composition of foods (Osborne and Fearn, 1986) and agricultural products (Williams and Norris, 1987) and for discriminant analysis of both food (Oyabu and Kubodera, 1989; Devaux et al., 1986, 1987; Downey et al., 1990) and nonfood materials (Grunenberg, 1989; Dominguez and Seymour, 1993; Peuchant et al., 1992), the use of mid-infrared spectroscopy for these same purposes has been more limited. This has been particularly true when it comes to performing diffuse reflectance mid-infrared Fourier transform spectroscopy (DRIFTS) on as-is samples (neat, not diluted with KBr). For materials, such as food ingredient powders, it has been believed that dilution with KBr or some other media (Coleman, 1993) was required to obtain spectra of the quality needed, and even then, particle size could be a considerable problem (Olinger and Griffiths, 1993a,b).

Thus, the primary use of mid-infrared spectroscopy has, until recently, been for identification of samples by qualitative analysis. These uses include utilization as detectors for instruments such as gas chromatographs (Coleman, 1993) and HPLCs (Griffith and de Haseth, 1986) and for the general identification of unknowns from their mid-infrared spectra (Colthup et al., 1990). More recently, interest has seemed to increase in utilizing mid-infrared spectroscopy in areas in which NIRS has been successful. Some of this is probably due to the design of new sampling devices, such as the diamond ATR (ASI, Millersville, MD) which makes sample handling easier (no need for KBr dilution or mulls), but is also due to the general need for rapid, non-waste-generating techniques and to the nature of mid-infrared spectra and spectroscopy. However, most of these efforts have utilized traditional mid-infrared techniques, i.e., ATR cells, KBr disks, and deposition on solid substrates such as CaF_2 disks. For example, Al-Jowder et al. (1997) used ATR to determine sample authenticity of meat products, and Kemsley et al. (1996) used the same techniques for studying adulteration of raspberry purees. Finally, a considerable amount of work using mid-infrared has also been performed with respect to coffee varieties and adulteration using a variety of techniques including: KBr disks (Suchánek et al., 1996), extracts on Si−CaF_2 disks (Dupuy et al., 1995), and DRIFTS on neat samples (Briandet et al., 1996a,b; Downey et al., 1997).

While extensive efforts have been carried out on understanding the basis for mid-infrared spectra and their interpretation (Colthup et al., 1990; Roeges, 1994), comparably little has been done in the near-infrared (NIR) spectral region. Instead of entire books and even software programs, the information available consists of a few book chapters (Murray and Williams, 1987). While there are practical reasons for this, such as the much shorter time NIRS has been extensively studied compared to the mid-infrared and the nature of the users (nonspectroscopists for the most part), there is also the inherent characteristics of the two spectral regions. While the mid-infrared spectral region consists of fundamental and overtone bands (Colthup et al., 1990), the NIR consists of overtones and combination bands whose origins can be more difficult to determine (Murray and Williams, 1987). Indeed, recent efforts have turned to utilizing correlations of mid-infrared spectra to NIR spectra to interpret NIR spectra (Barton et al., 1992; Barton and Himmelsbach, 1993).

The ability to utilize mid-infrared spectra for analysis in the same fashion as NIR spectra may bring the added

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advantage of spectral interpretability and eliminate the potential need for a second spectrometer. Since it has recently been shown that DRIFTS could be utilized to quantitatively (Reeves, 1994a,b, 1996) and qualitatively (Briandet et al., 1996a,b; Downey et al., 1997) determine the composition of neat samples (forages and coffee, respectively) with an accuracy equal to or better than that found using NIR spectra, it seemed appropriate to examine the question of utilizing mid-infrared spectra for discriminant analysis of a wider range of products. The objective of this work was to determine the feasibility of performing discriminant analysis of food ingredient powders from a variety of sources based on the mid-infrared spectra obtained by diffuse reflectance with neat samples.

METHODS

Samples. One hundred six samples consisting of 15 milk-egg (MILKE) samples collected from multiple sites around the world, 24 buttermilk powders (BUTTM) collected from across the United States, 20 (10 hard and 10 soft) wheat flours (WHEAT) from the AACC check sample program, 14 processed cheese powders (CHESP) from multiple lots at a single plant, 10 processed cheese seasonings (CHESS) also from multiple lots at a single plant, 21 processed ranch seasonings (RANCH) also from multiple lots at a single plant, and 22 regional dehydrated onion (ONION) powders (single plant, but from multiple raw material sources) were available for study. Samples were obtained either from sets of commercial samples or from production lines and thus represented very diverse sets of samples for each of the seven sample types. All samples were in a powder state and were used as-is.

Spectra. All samples were scanned as neat powders using diffuse reflectance. Samples were scanned on a Digi-Lab (BIO-RAD, Cambridge, MA) FTS-60 Fourier transform spectrometer equipped with a KBr beam splitter, ceramic source, DTGS detector, dry air purge, and diffuse reflectance attachment. In addition, a custom-made sample transport device was employed, allowing a sample area approximately 50 × 2 mm to be scanned (Reeves, 1996). Potassium bromide was used for the background spectra, and 64 coadded scans were taken for each sample from 4000 to 400 cm^{-1} at resolutions of 4 and 16 cm^{-1} (sample transport used at both resolutions).

Mid-infrared reflectance analysis was also carried out on a Perkin-Elmer (The Perkin-Elmer Corp., Norwalk, CT) model 2000 Fourier transform spectrometer from 4000 to 400 cm^{-1} (KBr beam splitter, wire coil source, DTGS detector) using a static sample cup (1 cm in diameter with an area of illumination ~ 0.86 cm in diameter). Potassium bromide was used for the background spectra, and 64 coadded scans were taken for each sample from 4000 to 400 cm^{-1} at resolutions of 4 and 16 cm^{-1} (no sample transport used for any samples).

Samples were scanned in the same order on both instruments, but the order was randomized before starting the study (same random ordering used for all instrument configurations).

Discriminant Analysis. Discriminant analysis was performed using Galactic's Discriminate program version 1.1G running under *GRAMS*/386 (Galactic Industries, Salem, NH). The method used is similar to that published by Gemperline and Shah (1990) and is based on principal component analysis and Mahalanobis distances (Mark and Tunnell, 1985). However, the method used by the Discriminate program includes an additional factor based on the spectral residuals to improve sensitivity. Finally, the Mahalanobis distances for each group of samples is normalized to the root-mean-squared groupsize (Mark, 1986). Basically, the program operates by performing a one-out cross-validation analysis for a group of samples, i.e., flours. This is performed in turn for each sample group of interest, with the resulting calibration characterizing the group in question. Unknown samples are then processed and compared to the results for the various groups in order to determine which group they best fit. The number of factors for a group were determined using the averaged predicted distance or *F*-statistic indicators (whichever indicated the greater number of factors to use) resulting from a one-out cross-validation analysis.

Table 1. Sample Distribution for Various Sample Groups

group	total	calibration set	validation set
buttermilk powders	24	16	8
cheese powders	14	10	4
cheese seasonings	10	7	3
milk-egg powders	15	10	5
onion powders	12	8	4
ranch seasonings	11	8	3
wheat flours	20	14	6
totals	106	73	33

Spectra were pretreated using mean centering and multiplicative scatter correction (*GRAMS*/386, 1994). Derivatization and averaging of spectral data points was also tried in efforts to improve the discriminant results. Data sets were analyzed using two methods: First, all samples from each group were used in a one-out cross-validation analysis to characterize each group. The resulting calibrations were then tested against all the samples. Second, every third sample of each group was set aside as part of a validation set (total of 33 samples) with the remaining samples (73 total) used to develop the discriminant models for each group. The resulting calibrations were then tested against the validation samples (calibration samples also separately tested). The number of each type of sample and the distribution between calibration and validation sets are shown in Table 1. All discriminant analyses were performed on each of the four sets of data (Digilab FTS-60 and Perkin-Elmer 2000 at 4- and 16-cm^{-1} resolutions) and unless otherwise stated used the entire spectral range.

Finally, in Tables 2−6, two distance parameters are given (HICORRECT and LOWNEGAT). The HICORRECT is the highest distance for a sample in a group from the group center. For example, if all samples fit a group perfectly then they would all have a distance of 0.00, and therefore the closer the distances to zero the better. At the other end, one wants nonmembers to have large distances with infinity being ideal. The LOWNEGAT is the closest distance of a nonmember to another group (but greater than 3.00 or it would be considered a group member and therefore an incorrect classification). The difference between the two measures gives an indication of how good the discrimination is, with the greater the difference the less likely there will be incorrect results for new samples.

RESULTS AND DISCUSSION

Samples. The objective of this work was to determine the feasibility of using mid-infrared spectroscopy to discriminate among food ingredients in a production environment. Examination of the data in Table 1 shows that a wide variety of materials were analyzed, varying from samples high in fat (~18% in cheese powders) to others very low in fat (wheat flours). At the same time, there was considerable overlap in the composition of the groups: two groups (milk-egg and buttermilk powders) were largely milk-based, there were two seasonings (cheese and ranch) which contained considerable salt, and many of the groups contained a high percentage of carbohydrates. Thus, while representing a wide range of materials, the samples still represented a challenge for discrimination. At the same time, the small number of samples contained in many of the groups combined with the diversity within the groups (see description of samples in Methods) added to the potential difficulty in developing discriminant calibrations. Finally, moisture contents were, except for the WHEAT (nominal 12% moisture), very similar for the various sample

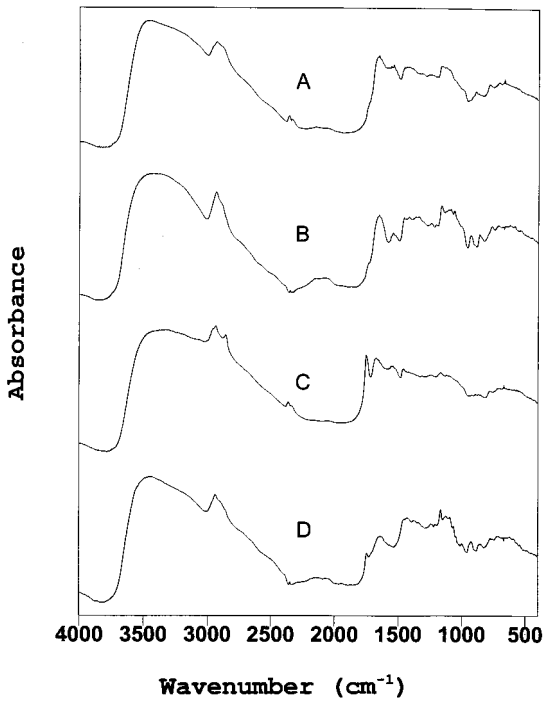

Figure 1. Mid-infrared spectra (4-cm^{-1} resolution, Digilab FTS-60 spectrometer) of typical samples of (A) milk-egg powder, (B) wheat flour, (C) cheese powder, and (D) cheese seasoning (all spectra offset on absorbance axis).

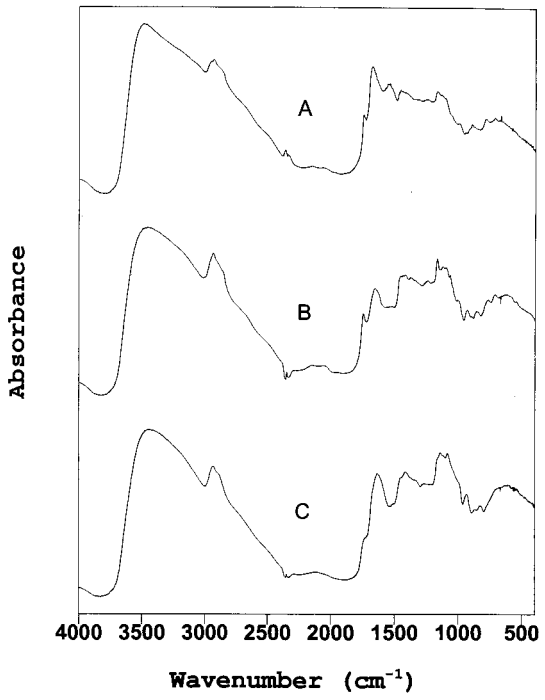

Figure 2. Mid-infrared spectra (4-cm^{-1} resolution, Digilab FTS-60 spectrometer) of typical samples of (A) buttermilk powder, (B) ranch seasoning, and (C) onion powder (all spectra offset on absorbance axis).

groups, ranging from a nominal 2.5% for the CHESP to 5.0% for the RANCH, thus minimizing moisture as a discrimination factor. Examination of PCR factors and loadings using SIMCA also showed that moisture was not the basis for the discrimination results. In summary, the samples studied provided an excellent set of data from which to determine the feasibility of discriminant analysis for food constituents in a production environment. Indeed, the diversity present in many of the sample subsets, i.e., samples from around the world or country, may well present a greater challenge than would be needed in a real production environment where samples within a subset would likely be less diverse.

Spectra. The mid-infrared spectra of typical samples for each ingredient grouping may be found in Figures 1 and 2. As shown, for the most part, each product has a unique spectrum. Even areas of the spectra which at first look similar, such as the region between 3600 and 2500 cm^{-1}, have different patterns on closer examination. Perhaps the greatest similarities occur in the regions between 3000 and 2800 cm^{-1} and again between 700 and 400 cm^{-1}. Overall, however, the spectra are different indicating that discrimination based on the spectra should not be a problem, unless the spectra of samples within groups (i.e., the MILKE or BUTTM) vary as much as the differences found between groups. Overall, the mid-infrared spectra shown appear to be more unique than the corresponding NIR spectra. This can be seen by examining the NIR spectra for four of the same samples shown in Figure 3 (Figure 3A−D corresponds to Figure 1A−D). While the NIR spectra of the WHEAT and CHESS (Figure 3B,D) look quite similar, the corresponding mid-infrared spectra (Figure 1B,D) are quite different. Also, note the greater overall detail found in the mid-infrared spectra, as compared to the corresponding NIR. It is this detail along with the larger knowledge base available that likely would

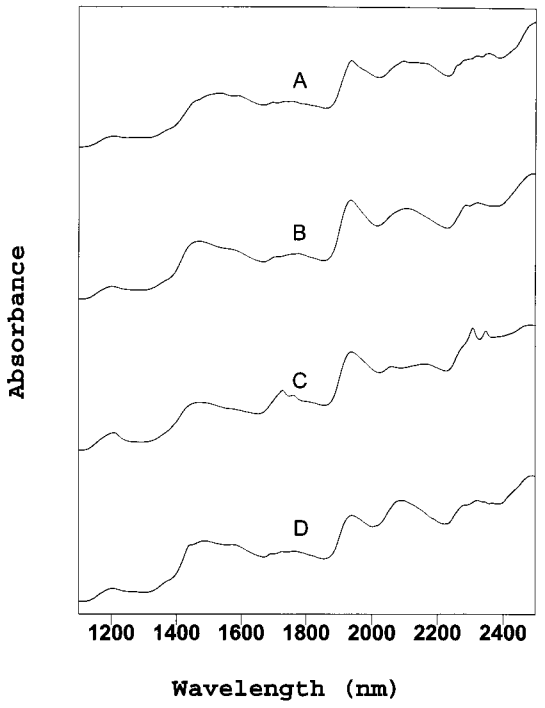

Figure 3. Near-infrared spectra (scanning monochromator, 10-nm resolution) of typical samples of (A) milk-egg powder, (B) wheat flour, (C) cheese powder, and (D) cheese seasoning (all spectra offset on absorbance axis).

make spectral interpretation in the mid-infrared a more viable option than in the NIR, although it must be noted that for complex biological samples, such as those examined here, spectral interpretation can be very difficult even in the mid-infrared. For example, efforts with spectral interpretation of modified food starches in the authors' laboratory indicated the presence of nitrogen bands in the samples when no nitrogen was present.

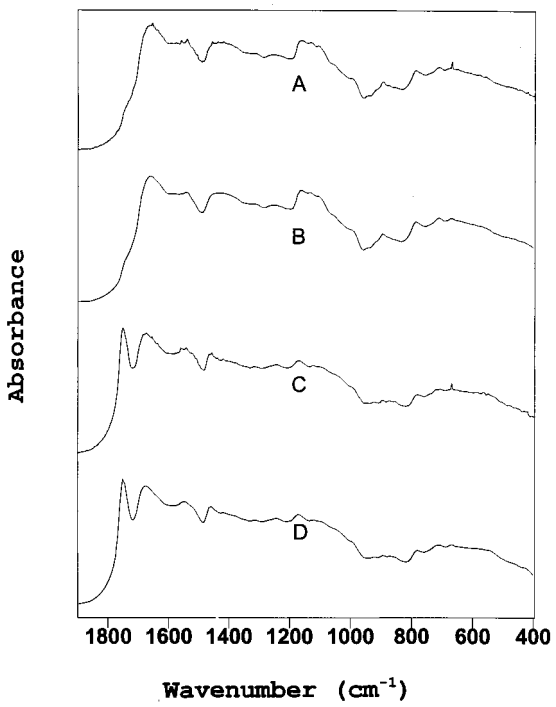

Figure 4. Mid-infrared spectra (Digilab FTS-60 spectrometer) from 1900 to 400 cm^{-1} of samples of milk-egg (A and B) and cheese (C and D) powders, at 4-cm^{-1} (A and C) and 16-cm^{-1} (B and D) resolutions (all spectra offset on absorbance axis).

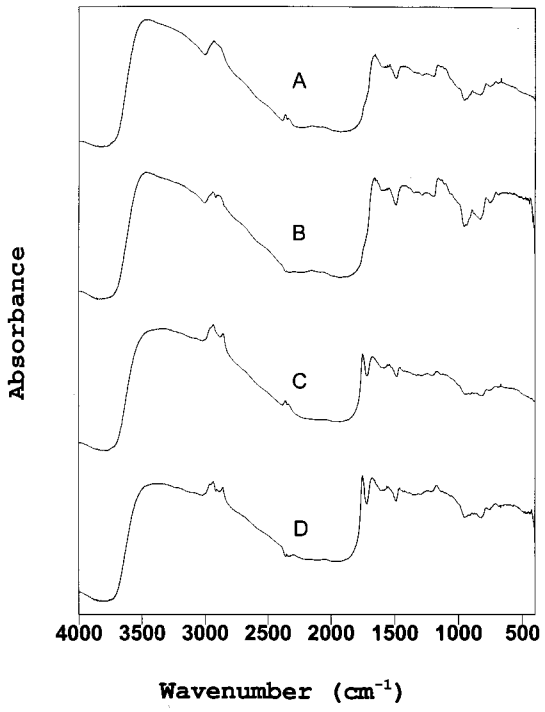

Figure 5. Mid-infrared spectra (4-cm^{-1} resolution) of samples of milk-egg (A and B) and cheese (C and D) powders from Digilab FTS-60 (A and C) and Perkin-Elmer 2000 (B and D) spectrometers (all spectra offset on absorbance axis).

In Figure 4, the 4- and 16-cm^{-1} resolution spectra (FTS-60 instrument) of MILKE and CHESP samples are shown. As can be seen, the higher resolution spectra show some finer details not apparent in the lower resolution spectra. These features do not appear to be simply due to the higher noise level of the higher resolution spectra, as many of the details occurred at the same wavenumbers for the two different samples, which would not be true of random noise in the spectra.

In Figure 5, 4-cm^{-1} resolution spectra of the MILKE and CHESP samples are shown for the FTS-60 and Perkin-Elmer 2000 instruments. Overall, the spectra appear quite similar, as might be expected. However, on close examination there are apparent differences between the spectra taken on the two spectrometers. First, the spectra taken on the Perkin-Elmer spectrometer showed a drop off in energy below 500 cm^{-1}. This is probably due to the windows (coated KBr) used to seal the Perkin-Elmer instrument, which are not present on the FTS-60 spectrometer. But there are also other differences in the form of an apparent increase in resolution for the Perkin-Elmer instrument. While the data was collected at the same resolution on both instruments, there was a difference in the number of data points collected. For the FTS-60 instrument, data was taken approximately every 2 cm^{-1} in order to achieve a resolution of 4 cm^{-1}, while on the Perkin-Elmer instrument, data was collected every 1 cm^{-1}, which appears to have resulted in an apparent increase in resolution. This can be seen in Figure 5 in the region between 3000 and 2800 cm^{-1} and elsewhere in the expanded spectra in Figure 6.

Discriminant Analysis. *FTS-60 Spectrometer: 4-cm^{-1} Resolution Spectra, Using All Available Samples in the Calibration Sets.* Results for discriminant analysis using 4-cm^{-1} resolution spectra from the FTS-60 instrument can be seen in Table 2. Results are for one-out cross-validations using all the samples in each group. The resulting calibrations were then tested against all samples from all groups. All spectra were mean-centered and multiplicative scatter-corrected.

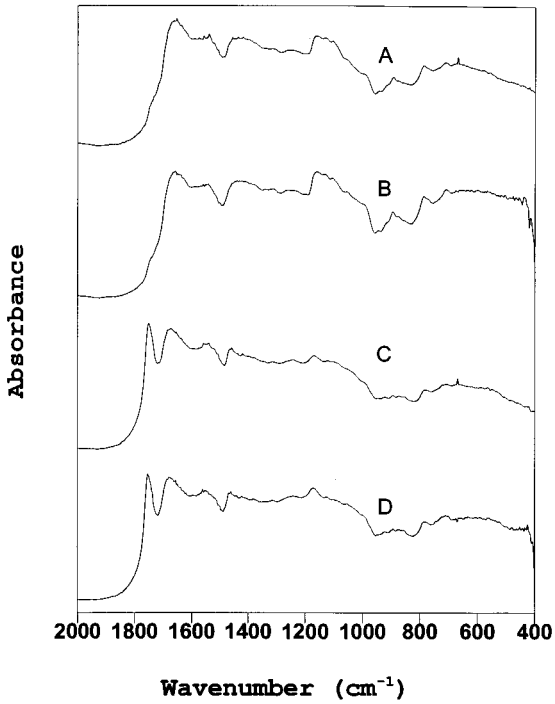

Figure 6. Expanded (2000−400 cm^{-1} only) mid-infrared spectra (4-cm^{-1} resolution) of samples of milk-egg (A and B) and cheese (C and D) powders from Digilab FTS-60 (A and C) and Perkin-Elmer 2000 (B and D) spectrometers (all spectra offset on absorbance axis).

As can be seen by the results in Table 2, using spectra pretreated only by mean centering and multiplicative scatter correcting resulted in calibrations which correctly classified all samples. The use of derivatives or

Table 2. Discriminant Results Using Spectra (4-cm^{-1} Resolution and All Samples within Each Group) from Digilab FTS-60 Spectrometer[a]

	absorbance spectra, no derivative				
group[b]	CORRECT[c]	INCORRECT[d]	FALSEPOSIT[e]	HICORRECT[f]	LOWNEGAT[g]
BUTTM	24	0	0	1.41	8.97
CHESP	14	0	0	1.14	49.18
CHESS	10	0	0	1.31	18.01
MILKE	15	0	0	1.21	9.10
ONION	12	0	0	1.33	21.52
RANCH	11	0	0	1.35	10.48
WHEAT	20	0	0	1.24	35.37

[a] All spectra were mean-centered and multiplicative scatter-corrected. [b] BUTTM, buttermilk powders; CHESP, cheese powders; CHESS, cheese seasonings; MILKE, milk-egg powders; ONION, onion powders; RANCH, ranch seasoning; WHEAT, wheat flours. [c] Samples within the specified group correctly placed in that group when testing using calibrations from all groups. [d] Samples within the specified group not correctly placed in that group when testing using calibrations from all groups. [e] Samples from another group placed in specified group when testing using calibrations from all groups. [f] Farthest distance from group of any correctly placed sample within the specified group when testing using calibrations from all groups. [g] Lowest distance from group of any sample correctly identified as not being a member of the group.

Kubelka Munk spectra (derivatized and nonderivatized) were not of any additional benefit (data not shown). These results contrast with NIR results, where the use of derivatives improved calibrations (Reeves and Zapf, unpublished data).

A detailed exploration of the effect of derivatizing, mean-centering, and multiplicative scatter-correcting spectra showed that the use of first derivatives with gaps of 8, 16, or 32 data points or a second derivative with a gap of 32 data points resulted in calibrations which correctly classified the samples but were not of any additional benefit, while the use of second derivatives with gaps of 8 or 16 data points resulted in some false positives. Other derivatives with either more narrow or very wide gaps were also tried (data not shown) but were not found to be of further benefit.

Finally, the results (data not shown) obtained when spectral data points were averaged (decreased resolution and noise) showed that averaging data points had virtually no effect on the resulting calibration accuracy. The only readily apparent change was a decrease in the distance of the closest non-MILKE sample to the MILKE group (LOWNEGAT) from approximately 9 (using the full spectra) to approximately 7 when averaging data points.

FTS-60 Spectrometer: 4-cm^{-1} Resolution Spectra, Using Two-Thirds of Samples in the Calibration Sets and One-Third as Validation Set. The application of calibrations developed using two-thirds of the total samples to the remaining one-third of the samples (independent validation set) gave the results in Table 3. A single validation sample of the CHESS group was incorrectly identified with a distance of 3.31, just outside the limit of 3.0 recommended by Galactic for inclusion but still well-separated from the lowest distance (LOWNEGAT) of a nongroup member (5.01). It should also be noted that while the Galactic software recommends a distance of 3.0 or less for inclusion, it also states that work by Mark and Tunnell (1985) has shown that, while a distance of 3.0 may be theoretically justified, in reality, distances as great as 10−15 can work better depending on the samples in question.

Adjusting the number of factors used for the various calibrations gave the results in the bottom of Table 3. Although there were two incorrectly classified samples, with distances of 3.92 (ONION) and 4.09 (RANCH) from their proper groups, comparisons to the distances for nongroup members (13.26 for ONION and 14.30 for RANCH) shows that all samples could be correctly classified by slightly increasing the distance limit (for inclusion in those groups) with no danger of including a nonmember in a group (false positive). Overall, these results were better than that achieved using NIR spectra, although for unknown reasons, the low negative distances were often much greater for NIR spectra (Reeves and Zapf, unpublished data). Averaging data points and derivatization (data not shown) made no difference in the calibration/validation results shown here.

While adjusting the number of factors used, to improve the results, is not conventional, it does demonstrate that accurate discrimination was feasible. In a production environment, experience over time would show whether the number of factors used should be more or less than indicated by the indicators used here. There are a wide variety of indicator functions available, many of which would select more factors than utilized in this study. Also, with small data sets, one is more limited in how many factors can be used.

FTS-60 Spectrometer: 16-cm^{-1} Resolution Spectra, Using All Available Samples in the Calibration Sets. The results using 16-cm^{-1} resolution spectra from the FTS-60 instrument in a one-out cross-validation using all the samples in each group may be seen in Table 4. Unlike the results using 4-cm^{-1} spectra (Table 2), all samples were not correctly classified, with two BUTTM samples also identified (false positives) as being MILKE samples. Trial and error manipulation of the number of factors used for the MILKE calibration was able to correct the calibration (data not shown), but this was not necessary when using 4-cm^{-1} resolution spectra from the same instrument or when averaging the 4-cm^{-1} resolution spectra to give the same effective resolution (data not shown). Also, derivatization was of no additional benefit.

It is interesting that the results using 16-cm^{-1} resolution spectra were not as good as those achieved using the higher resolution 4-cm^{-1} spectra, even when the 4-cm^{-1} spectra were averaged to give the same or even fewer number of spectral data points [1870, 468, 234, and 117, respectively, for the full and averaged (4, 8, or 16 data points) 4-cm^{-1} spectra versus 468 for the 16-cm^{-1} spectra]. While averaging spectral data points reduces the noise in the spectra, this would not seem to be involved, since the 16-cm^{-1} spectra would be significantly lower in noise than the 4-cm^{-1} spectra.

Since it is not a question of noise, and since there is no question that when averaging the 4-cm^{-1} spectra

Table 3. Discriminant Analysis with Two-Thirds of Each Group as a Calibration Set and One-Third as the Validation Set[a]

group[b]	CORRECT[c]	INCORRECT[d]	FALSEPOSIT[e]	HICORRECT[f]	LOWNEGAT[g]
		Calibration Set			
BUTTM	16	0	0	1.25	5.02
CHESP	10	0	0	1.29	8.46
CHESS	7	0	0	1.18	4.34
MILKE	10	0	0	1.28	5.50
ONION	8	0	0	1.55	8.25
RANCH	8	0	0	1.67	6.14
WHEAT	14	0	0	1.47	10.02
		Validation Set			
BUTTM	8	0	0	2.23	4.58
CHESP	4	0	0	1.15	8.44
CHESS	2	1 (3.31)	0	2.64	5.01
MILKE	5	0	0	2.91	11.56
ONION	4	0	0	1.84	7.47
RANCH	3	0	0	1.12	6.55
WHEAT	6	0	0	1.80	9.71
		Best Achievable for Calibration Set			
BUTTM	16	0	0	1.25	5.02
CHESP	10	0	0	1.29	8.46
CHESS	7	0	0	1.09	16.95
MILKE	10	0	0	1.28	5.50
ONION	8	0	0	1.32	11.57
RANCH	8	0	0	1.32	14.67
WHEAT	14	0	0	1.47	30.93
		Best Achievable for Validation Set			
BUTTM	8	0	0	2.23	4.58
CHESP	4	0	0	1.15	8.44
CHESS	3	0	0	1.63	19.90
MILKE	5	0	0	2.91	11.56
ONION	3	1 (3.92)	0	1.90	13.26
RANCH	2	1 (4.09)	0	1.20	14.30
WHEAT	6	0	0	1.80	32.62

[a] 4-cm^{-1} resolution spectra from Digilab FTS-60 instrument, mean-centered and multiplicative scatter-corrected. [b] BUTTM, buttermilk powders; CHESP, cheese powders; CHESS, cheese seasonings; MILKE, milk-egg powders; ONION, onion powders; RANCH, ranch seasoning; WHEAT, wheat flours. [c] Samples within the specified group correctly placed in that group when testing using calibrations from all groups. [d] Samples within the specified group not correctly placed in that group when testing using calibrations from all groups (numbers in parentheses are distances from group). [e] Samples from another group placed in specified group when testing using calibrations from all groups. [f] Farthest distance from group of any correctly placed sample within the specified group when testing using calibrations from all groups. [g] Lowest distance from group of any sample correctly identified as not being a member of the group.

Table 4. Discriminant Results Using Spectra (16-cm^{-1} Resolution and All Samples within Each Group) from Digilab FTS-60 Spectrometer[a]

	absorbance spectra, no derivative				
group[b]	CORRECT[c]	INCORRECT[d]	FALSEPOSIT[e]	HICORRECT[f]	LOWNEGAT[g]
BUTTM	24	0	2	1.29	4.46
CHESP	14	0	0	1.34	21.29
CHESS	10	0	0	1.15	5.96
MILKE	15	0	0	1.44	6.77
ONION	12	0	0	1.39	11.43
RANCH	11	0	0	1.62	7.62
WHEAT	20	0	0	1.29	18.20

[a] All spectra mean-centered and multiplicative scatter-corrected. [b] BUTTM, buttermilk powders; CHESP, cheese powders; CHESS, cheese seasonings; MILKE, milk-egg powders; ONION, onion powders; RANCH, ranch seasoning; WHEAT, wheat flours. [c] Samples within the specified group correctly placed in that group when testing using calibrations from all groups. [d] Samples within the specified group not correctly placed in that group when testing using calibrations from all groups. [e] Samples from another group placed in specified group when testing using calibrations from all groups. [f] Farthest distance from group of any correctly placed sample within the specified group when testing using calibrations from all groups. [g] Lowest distance from group of any sample correctly identified as not being a member of the group.

down to only 117 data points the resolution of the resulting spectra is less than that of the nonaveraged 16-cm^{-1} data, the question remains as to why the difference. One possible explanation may lie in the way the samples were scanned. The higher resolution scan of a sample at 4 cm^{-1} takes approximately 4 times as long as for 16-cm^{-1} resolution for the same number of scans. The longer scan time can result in the scan being more representative of the sample as a whole. With a static sample cup, this means that a single spot on the sample cup is illuminated for a longer time and can result in burning or damage to the sample. This has been personally observed in the form of darkening of some samples during other studies. While the transport device reduces the chance of overheating by continually moving the sample under the illumination spot (Reeves, 1996), it also means that different places on the sample cup, and thus different subfractions of the sample, are being scanned over the time period used for scanning. For example, if only a single scan from 4000 to 400 cm^{-1} were made, and the transport was moved at a velocity high enough to move the 50-mm sample path length

Table 5. Discriminant Analysis with Two-Thirds of Each Group as a Calibration Set and One-Third as the Validation Set[a]

group[b]	CORRECT[c]	INCORRECT[d]	FALSEPOSIT[e]	HICORRECT[f]	LOWNEGAT[g]
			Calibration Set		
BUTTM	16	0	0	1.50	9.97
CHESP	10	0	0	1.41	14.99
CHESS	7	0	0	1.21	5.38
MILKE	10	0	0	1.39	4.96
ONION	8	0	0	1.32	14.80
RANCH	8	0	0	1.19	6.89
WHEAT	14	0	0	1.29	24.07
			Validation Set		
BUTTM	8	0	0	2.17	7.39
CHESP	4	0	0	1.27	15.74
CHESS	2	1	0	1.63	9.41
MILKE	4	1	0	2.85	7.04
ONION	3	1	0	1.94	15.30
RANCH	3	0	0	2.01	8.91
WHEAT	4	2	0	1.74	30.79

[a] 16-cm^{-1} resolution spectra from Digilab FTS-60 instrument, mean-centered and multiplicative scatter-corrected. [b] BUTTM, buttermilk powders; CHESP, cheese powders; CHESS, cheese seasonings; MILKE, milk-egg powders; ONION, onion powders; RANCH, ranch seasoning; WHEAT, wheat flours. [c] Samples within the specified group correctly placed in that group when testing using calibrations from all groups. [d] Samples within the specified group not correctly placed in that group when testing using calibrations from all groups. [e] Samples from another group placed in specified group when testing using calibrations from all groups. [f] Farthest distance from group of any correctly placed sample within the specified group when testing using calibrations from all groups. [g] Lowest distance from group of any sample correctly identified as not being a member of the group.

during the time it took for the single scan, then the data collected at 4000 cm^{-1} would be for the sample at the front of the sample cell and that at 2200 cm^{-1} for the sample at the middle, etc. At the other extreme, a step scan spectrometer coupled to a stepped transport device could be set up to make a complete scan for each step the transport and sample moved. For a completely homogeneous sample, the results would be expected to be the same for the two extremes, but manufactured samples, such as those studied here, are rarely that homogeneous, and when combined with the effects of particle size differences and variations in the composition of particles and penetration depth of the mid-infrared radiation (Olinger and Griffiths, 1993a,b), scanning different fractions of the total sample at different frequencies might result in an effect similar to that caused by sample heterogeneity. Since the scans taken at 4 cm^{-1} took longer than the same number of scans at 16 cm^{-1}, the result would be that the higher resolution data would see a more representative subsample and therefore might result in better calibrations as seen here. More comprehensive experiments will be needed to determine the conditions and samples for which resolution and/or scanning protocol are critical for discriminant analysis using mid-infrared spectra.

FTS-60 Spectrometer: 16-cm^{-1} Resolution Spectra, Using Two-Thirds of Samples in the Calibration Sets and One-Third as a Validation Set. The results when using an independent data validation set for 16-cm^{-1} spectra are presented in Table 5. As can be seen, results were not as good as those found using 4-cm^{-1} resolution spectra with five samples incorrectly classified, as opposed to one with the higher resolution spectra (Table 3). Efforts to improve the results, by trial and error changes in the number of calibration factors used, reduced the number of misclassified samples to one with a distance of 3.27 from the CHESS group. Thus, like the calibrations based on 4-cm^{-1} resolution spectra, determining the correct number of factors to use produced calibrations where all samples were separated into the proper groups, although the separation between samples in a group and those outside (high distance for correctly classified samples versus low distance for samples not in the group) was overall less for the 16-cm^{-1} spectra. Finally, as with the 4-cm^{-1} resolution spectra, averaging spectral data points was not found to be of any help.

Perkin-Elmer Model 2000 Spectrometer: 4-cm^{-1} Resolution Spectra, Using All Available Samples in the Calibration Sets. The results using 4-cm^{-1} resolution spectra from the Perkin-Elmer 2000 spectrometer in a one-out cross-validation using all the samples in each group may be seen in Table 6. As with the FTS-60 spectra, all samples were correctly classified using mean-centered and multiplicative scatter-corrected data, and the additional application of derivatives resulted in a degradation of the results (data not shown). Other than slightly higher distance values for samples not in a group (LOWNEGAT), there was little difference between these results and the results achieved using the FTS-60 spectrometer with the transport device (Table 2).

Perkin-Elmer Model 2000 Spectrometer: 16-cm^{-1} Resolution Spectra, Using All Available Samples in the Calibration Sets. As shown in Table 6, the results using 16-cm^{-1} resolution spectra from the Perkin-Elmer model 2000 instrument were very similar to that achieved with the 4-cm^{-1} spectra with all samples properly classified using mean-centered and multiplicative scatter-corrected data. Further treatment with derivatives was again of no additional benefit (data not shown). It is interesting that, for the Perkin-Elmer 2000 data, using the lower resolution spectra did not result in the increase in incorrectly classified samples that occurred for the FTS-60 data. In addition to using a static cell, as discussed in the methods, the Perkin-Elmer 2000 instrument also collected data every 1 cm^{-1}, whereas the FTS-60 instrument collected data at a rate approximately one-half the designated resolution. As discussed in the section on spectra, this appeared to result in some sort of increased resolution, and this may explain the results: the lower resolution Perkin-Elmer spectra appearing as though they were actually recorded at a higher resolution with reduced noise and thus producing higher quality calibrations. In any case, the results showed that quality calibrations were attainable

Table 6. Discriminant Results Using Spectra from Perkin-Elmer 2000 Spectrometer[a]

group[c]	CORRECT[d]	INCORRECT[e]	FALSEPOSIT[f]	HICORRECT[g]	LOWNEGAT[h]
All Samples in Calibration Sets					
No Derivative (4-cm^{-1} resolution)[b]					
BUTTM	24	0	0	1.36	6.21
CHESP	14	0	0	1.38	68.36
CHESS	10	0	0	1.49	22.32
MILKE	15	0	0	1.19	5.16
ONION	12	0	0	1.26	27.21
RANCH	11	0	0	1.28	10.83
WHEAT	20	0	0	1.24	51.40
No Derivative (16-cm^{-1} resolution)[b]					
BUTTM	24	0	0	1.14	9.41
CHESP	14	0	0	1.34	66.47
CHESS	10	0	0	1.50	13.22
MILKE	15	0	0	1.21	15.01
ONION	12	0	0	1.17	24.80
RANCH	11	0	0	1.48	10.17
WHEAT	20	0	0	1.38	39.83
Split Two-Thirds as Calibration Sets, One-Third as Validation Set					
Calibration Set (4-cm^{-1} spectra)[i]					
BUTTM	16	0	2	1.45	3.02
CHESP	10	0	0	1.34	33.82
CHESS	7	0	0	1.33	10.66
MILKE	10	0	0	1.37	7.86
ONION	8	0	0	1.54	8.73
RANCH	8	0	0	1.13	13.48
WHEAT	14	0	0	1.34	26.32
Validation Set (4-cm^{-1} spectra)[i]					
BUTTM	7	1	1	2.58	3.02
CHESP	4	0	0	0.98	32.68
CHESS	3	0	0	2.85	15.50
MILKE	5	0	0	1.83	8.38
ONION	3	1	0	1.32	8.00
RANCH	2	1	0	1.07	14.09
WHEAT	5	1	0	2.69	26.49

[a] All spectra mean-centered and multiplicative scatter-corrected. [b] All samples within each group used. [c] BUTTM, buttermilk powders; CHESP, cheese powders; CHESS, cheese seasonings; MILKE, milk-egg powders; ONION, onion powders; RANCH, ranch seasoning; WHEAT, wheat flours. [d] Samples within the specified group correctly placed in that group when testing using calibrations from all groups. [e] Samples within the specified group not correctly placed in that group when testing using calibrations from all groups. [f] Samples from another group placed in specified group when testing using calibrations from all groups. [g] Farthest distance from group of any correctly placed sample within the specified group when testing using calibrations from all groups. [h] Lowest distance from group of any sample correctly identified as not being a member of the group. [i] Two-thirds of samples in each group used in the calibration sets and remaining one-third used as the validation set.

based on spectra collected on the Perkin-Elmer spectrometer using a static cup.

Perkin-Elmer Model 2000 Spectrometer: 4-cm^{-1} Resolution Spectra, Using Two-Thirds of Samples in the Calibration Sets and One-Third as a Validation Set. Table 6 also contains the results using an independent validation set for 4-cm^{-1} spectra generated on the Perkin-Elmer spectrometer. As with the other data sets, dividing the samples into a calibration set and testing on an independent validation led to an increase in incorrectly classified samples. Adjusting the number of factors used (data not shown) eliminated the false positives in both the calibration and validation sets and resulted in validation set results containing five samples misclassified with distances of 3.52 and 5.38 (MILKE), 7.14 (BUTTM), 4.44 (ONION), and 3.58 (RANCH), all well separated from nongroup samples, but not as good as found using the 4-cm^{-1} resolution spectra from the FTS-60 instrument, where only two misclassified samples (based on a distance of 3.0 for proper classification) were produced after factor adjustment (Table 3). Finally, the results using a validation set and 16-cm^{-1} data were similar to those found with 4-cm^{-1} spectra, in that manipulation of calibration factors was necessary to achieve the best classification (data not shown).

Math Pretreatments in General. In addition to the efforts shown in the various tables, other efforts at treating the spectra were investigated. These include the use of standard normal variate with and without detrend, variance scaling in combination with mean centering, limiting the spectral range used, and other derivatives. While all combinations were not tried on all the different data sets, the ones tried were not found to be of any added benefit. It is possible that a particular combination may help under some circumstances, but in general, it appears that for mid-infrared spectra only mean centering and multiplicative scatter correction need be used to develop accurate discriminant calibrations. This is in contrast to NIR spectra (Reeves and Zapf, unpublished data) and to quantitative analysis using mid-infrared spectra (Reeves, 1996), where derivatives were found to be very beneficial in both cases and variance scaling was useful for quantitative work in both regions.

CONCLUSIONS

Results using mid-infrared spectra from two different makes of Fourier transform spectrometers demonstrated that discriminant analysis of neat powders of food ingredients can be successfully carried out. In

general, it was found that results using 4-cm^{-1} resolution spectra were somewhat superior to those based on lower resolution 16-cm^{-1}, but good results could be obtained at either resolution. While correct classifications could be achieved using factor selection based on the average predicted distance or *F*-test indicators when using one-out cross-validations using all available samples, when an independent validation set was used (one-third of total samples), adjustment of the number of factors to use was required for the best results. This may be due to the specific subset of samples in the calibration sets and/or the diversity of the samples used within a set which resulted in differences between samples in the two sets. In conclusion, efforts have demonstrated the feasibility of discriminant analysis using mid-infrared spectra of neat food ingredient powders.

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