Determination of Texturized Soybean Flour in Ground Beef by Near Infrared Reflectance Spectroscopy

Lynn T. Black,* Arthur C. Eldridge, Mary E. Hockridge, and William F. Kwolek

A method is proposed to determine texturized soybean flour (TSF) in freeze-dried and defatted ground beef by computer-assisted near infrared reflectance (NIR) spectroscopy. An initial calibration set of 76 ground beef samples, spiked with known amounts of TSF, was scanned by NIR. A wavelength of 2066 was selected by regression analysis in first derivative mathematical transformation, which represents polysaccharides in soybean flour. The use of an additional wavelength proved to be of little significance in reducing the overall error. The calibration equation was tested with a set of known prediction samples to investigate the sources of variation and determine the total error associated with the method. The standard deviation of a multiple load-scan analysis was ±2.5. The least significant difference ($P = 0.05$) between two samples was 3.7. The instrument had to be calibrated with a standard prepared from the same lot of beef as that to be tested.

Soybean proteins in the form of flours, concentrates, and isolates are used in a large variety of meat products. They can be added as flours, grits, or texturized products. Partial replacement of meat proteins with soy protein not only reduces cost but also improves water and fat binding capacities and helps emulsifying properties. Government regulations control the amount of certain additives to meat products. For instance, only 3.5% texturized soybean flour (TSF) is permitted in fresh or cooked sausages, while 12.0% is permitted in spaghetti with meat balls (9 CFR 319.140, 1982; 9 CFR 319.306, 1982). Because of these regulations it is important, for control purposes, to have analytical procedures for the detection and estimation of the amounts of TSF added. Recent literature reviews (Olsman and Krol, 1978; Olsman and Hitchcock, 1980) have indicated that existing methods for determining meat additives can be divided into several categories, namely microscopic, histochemical, analysis of chemical constituents, electrophoresis, amino acid and/or peptide analysis, use of additives (TiO_2), and immunochemical procedures. The sereological, peptide, and enzyme-linked immunoabsorbant assay (ELISA) methods have been evaluated by British workers (Griffiths et al., 1981). D. J. Armstrong et al. (1982) have proposed and evaluated an electrophoretic procedure which utilizes a protein as an internal standard. However, no simple procedure has yet been developed which will solve all needs.

Since near infrared reflectance (NIR) spectroscopy has been adapted to determine moisture, protein, and oil in grains and oilseeds (Norris et al., 1978) and to determine fiber (Baker, 1983), we investigated the possibility of using NIR as a means of determining the amount of TSF added to ground beef.

EXPERIMENTAL SECTION

Sample Preparation. Fresh ground beef to be used in calibration and prediction was freeze-dried in small pieces ($^1/_2$-1-in. cubes) in a Virtis Freeze Dryer, Model 100-SRC-8, Gardiner, NY 12525. The dried sample was hexane defatted in a conventional Soxhlet extractor for 6 h. The hexane-wet material was allowed to air dry in a hood overnight to assure evaporation of remaining traces of solvent. The dried extracted ground beef was then ground in a Wiley mill to pass through a 40-mesh screen. To insure uniformity, the TSF to be added to the ground beef, was also ground to pass 40 mesh. The commercially prepared ground beef patties containing known amounts of TSF were made by grinding beef through a $^3/_8$-in. plate, followed by a 3 min mixing, then regrinding through a $^1/_8$-in. plate, forming patties, and then freezing prior to analysis. For analysis these samples were freeze-dried and defatted as previously described.

Analytical Procedure Development. The instrument used for the development of the correlations and subsequent calibration equations was a Neotec Model 6350, Mark II (Pacific Scientific Company, Silver Spring, MD). This is a full scanning instrument that gathers either reflectance or transmission absorbance data from the 1100–2500-nm (NIR) energy spectrum. The spectrophotometer is interfaced to a Nova/4 computer with 64K byte memory and a 12.5 megabyte disk drive. Original raw spectral data from defatted, freeze-dried samples of ground beef spiked with TSF were recorded as log $1/R$ (R = reflectance) and displayed on a CRT graphics terminal, as illustrated in Figure 1.

Calibration of the NIR instrument was accomplished by scanning 30 samples, each from the same lot, of freshly prepared ground beef which had been freeze-dried, defatted, and spiked with 0–30% TSF. These percentages reflect the actual amounts of TSF in defatted and freeze-dried ground beef. The solids content of fresh ground beef is approximately 18%, therefore a range of 0–30% of defatted and freeze-dried ground beef would represent a range of 0–5.4% TSF relative to the total untreated fresh ground beef. Reflectance spectra were taken for each sample by using the average of 50 scans taken at 700 wavelengths (1100–2500 nm, every 2 nm). The spectral data and corresponding soy values were stored in the computer. A mathematical transformation was then performed on all spectral data to obtain the first derivative of log $1/R$ spectra $d(\log 1/R)/d\lambda$ for all samples. Figure 1 shows an example of log $1/R$ and first derivative for a sample containing TSF.

To evaluate all possible primary calibration wavelengths for TSF in ground beef, the first derivative spectral data and their respective TSF percentages were subjected to multiple regression analysis with existing instrumental software. Figure 2 is a plot of correlation coefficients for the first derivatives of log $1/R$ spectra data vs. NIR

Northern Regional Research Center, Agricultural Research Service, U.S. Department of Agriculture, Peoria, Illinois (L.T.B., A.C.E., and M.E.H.), and Mathematical Statistician, USDA, Agricultural Research Service, North Central Region, stationed at the Northern Regional Research Center (W.F.K.).

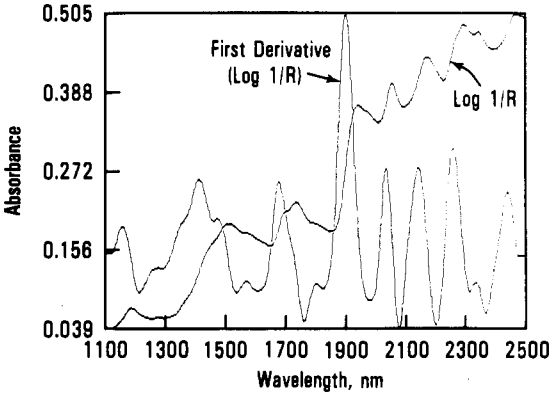

Figure 1. Typical log $1/R$ and mathematically transformed d(log $1/R$)/dλ spectral data for TSF extended ground beef.

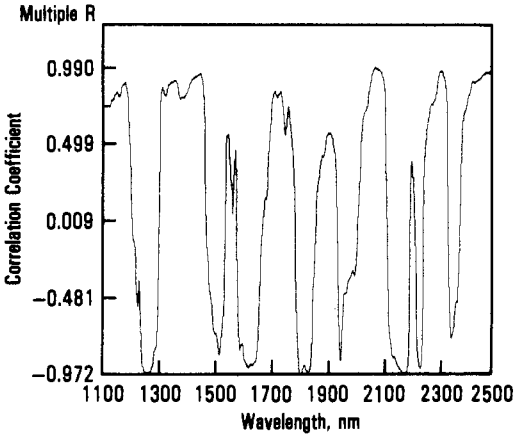

Figure 2. Correlation coefficient vs. wavelength using d(log $1/R$)/dλ to correlate with TSF content of ground beef.

Table I. Calibration Equation Components for TSF in Ground Beef

data file: beef/soy contains 30 samples standard error = 1.43 multiple correlation r = 0.988		
constants[a]	wavelength	correlation (r)
$K(0)$ = 111.511		
$K(1)$ = 4546.84	2066	0.988

[a] Model used: $Y = K(0) + K(1)X$ where X is an observation at wavelength i.

wavelength. The highest correlation occurred at a wavelength of λ 2066, with a correlation of r = 0.99 and a standard error (SE) of 1.43 (Table I). One of the capabilities of the computer's regression program is to create and save a calibration equation which is representative of the data for prediction of unknown samples. The NIR band responsible for the high correlation at λ 2066 is probably due to an OH stretch/band combination caused by the presence of sugars and polysaccharides in TSF.

RESULTS AND DISCUSSION

Due to the heterogeneous nature of ground beef, uniform loading of an NIR sample cup without some type of previous homogenization was nearly impossible. For this initial study the samples were freeze-dried, defatted, and ground through a 10-mesh screen which produced a very uniform, although somewhat coarse, fibrous material. Although federal regulations allow for the addition of several percent of hydrated TSF to meats, when samples are freeze-dried and defatted the relative content of the TSF is much higher. For initial evaluation a 30-sample calibration set, ranging from 0–30% soy flour in ground beef, was scanned by NIR. Two mathematical transformations of the log $1/R$ data were examined. Due to the coarse nature of these samples, the initial evaluation was performed on the second derivative of log $1/R$ data in an effort to reduce the negative effects of coarse particle size (Norris et al., 1984). Examination of the correlation coefficient vs. wavelength plot for 30 samples of beef and soy flour indicated the presence of several highly correlating wavelengths. However, many of these correlations were greatly diminished, either after the addition of more samples to the calibration set or by examination of different sets. In addition, many of these highly correlating wavelengths were found ineffective during prediction. One of the wavelengths that did predict with consistency occurred at λ 2320, which represents C–H/CH_2 stretch/deformation combination caused by the presence of plant cellulosic material present in TSF. The calibration equation constants generated by λ 2320 were exceedingly high, indicating poor sensitivity to TSF.

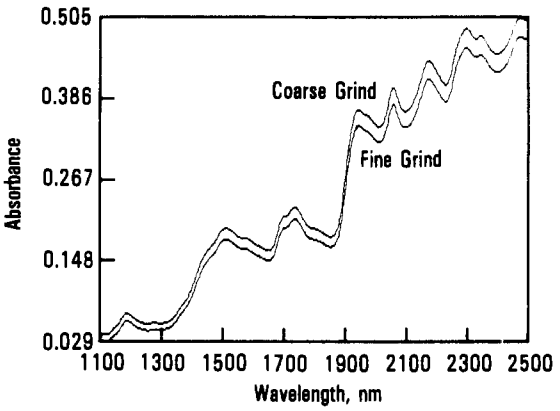

Figure 3. Typical log $1/R$ spectral data for ground beef with mesh sizes 10 and 40.

The highest correlation observed in data form d(log $1/R$)/dλ occurred at λ 2066, with a correlation, r = 0.99 and SE = 1.43 (SE is the standard error of the estimate). This 30-sample calibration set was increased to 76 samples with a subsequent change in the correlation, r = 0.91 and SE = 3.30. A helping term at λ 1540 was added to the calibration equation which was obtained by linear summation through the use of the computer's multiple regression program. This second wavelength improved the overall correlation and SE only slightly, therefore the final equation that was selected contained only one wavelength term.

To demonstrate the importance of controlling grind size, the above set of calibration samples was ground in a Wiley mill to pass through a 40-mesh screen. These samples, differing only in grind size, were then rescanned by NIR (Figure 3). When the calibration equation from the coarsely ground set was used to predict the data from the finely ground set, there was a predictably constant positive bias of about 4%. The effect of grind size could also be observed by comparing the spectra of ground beef which had been spiked with TSF ground through 40 mesh vs. TSF ground through 100 mesh (Figure 4).

The NIR calibration described here, unlike many others, is not based on results from a primary laboratory method, but on absolute or known values from laboratory prepared samples. Thus the errors outlined here are virtually all associated with NIR.

The accuracy of the NIR method was established relative to knowns by scanning a set of 30 beef samples which had been spiked with 0–30% TSF. The data in Figure 5 illustrate that a high correlation (r = 0.98) exists between

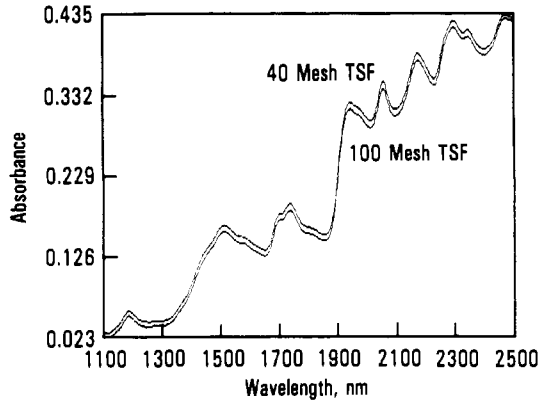

Figure 4. Typical log $1/R$ spectral data for ground beef (db/df) containing 15% TSF ground to 40 and 100 mesh.

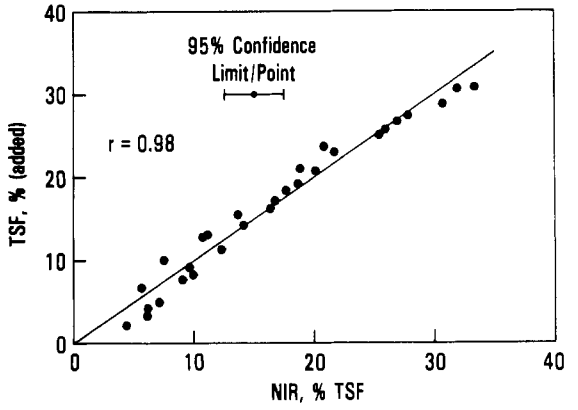

Figure 5. Relationship between NIR and laboratory prepared TSF extended ground beef.

Table II. Precision Estimates for NIR Analysis: TSF in Ground Beef

source of variation	TSF, %
	standard deviation
rotation σ_R^2	0.9
rotation + loading $\sigma_R^2 + \sigma_L^2$	2.6
	LSD (p = 0.05)
between rotations	2.5
between loadings	7.3
	95% confidence limits
1 loading	5.1
2 loadings	3.6
4 loadings	2.5

the NIR and the known levels of TSF. As can be seen in Table II, the 95% confidence limit of any single NIR value is ±5.1%. This value can be reduced to ±2.5 by increasing the amount of sample averaging to four, above which accuracy increases only slightly and limits to practicality begin to be exceeded.

To determine the precision or repeatability of NIR, three spectra of each sample of the above set of 30 samples were collected. Two of these three spectra were of the same load, the third was of a different load. This experiment was repeated on 3 different days. The least significant difference (LSD) between single loadings is 7.3 (Table II) while the LSD between repeat readings on the same load is 2.5. The maximum day-to-day instrumental deviation based on the data from the above experiment was s = 0.19. Since 30 observations were taken on each day, the precision associated with these values is high. Thus, day-to-day differences are significant at the 99% level. However, this day-to-day instrumental variation appears to be of little practical importance, especially when compared to other sources of error.

Table III. Texturized Soy Flour in Ground Beef by NIR (%)[a]

week/source	added TSF, %	calibration within lot	calibration between lots
1A	21.8	21.8	20.8
1B	22.1	21.6	20.1
1C	21.5	21.6	17.1
1D	21.2	22.0	24.5
1E	21.1	21.3	21.9
2A	21.7	21.5	29.3
2B	21.4	21.5	33.0
2C	21.4	21.3	14.6
2D	21.2	21.5	27.8
2E	21.3	21.2	27.6
3A	21.6	21.2	15.6
3B	21.5	21.7	19.4
3C	21.5	21.1	17.8
3D	21.8	21.3	19.1
3E	21.3	21.2	13.1
mean	21.5	21.4	21.4
std dev		0.27	5.58

[a] Average of 6 values: 2 samples at each approximate level of 8, 20, and 36%.

After the initial findings indicated that NIR might be suitable for measuring TSF in ground beef, several small sets of samples were compared over a 3-month period. These preliminary comparisons tended to indicate that determinations by NIR would be independent of ground beef lots. However, subsequent work has shown that while occasionally different lots of ground beef are very similar, substantial inconsistencies may exist between a single source on different days or between different sources. To determine the extent of this variation, a lot of fresh ground beef was obtained from each of five different sources on the same day of the week. Five more lots were obtained from the same sources exactly one week later and again after two weeks. These 15 lots were then freeze-dried and defatted as described earlier. A portion of each of these lots was spiked with TSF at the levels of 8, 20, and 36%, in duplicate, thus creating 6 samples/lot or a total of 90 ground beef samples containing known amounts of TSF. Each of these samples was subjected to NIR analysis, with an individual average being calculated from each of the 6 values obtained from each lot. The NIR values shown in Table III were obtained by two methods. First, a calibration equation was created from the 6 representative samples from each of the 15 beef lots (15 calibrations). Each within-lot calibration equation was then used to determine predicted TSF values for each lot of beef. The excellent agreement between the added TSF laboratory values and those obtained through NIR prediction would therefore be expected. The standard deviation based on deviation of predicted TSF values from known values was s = 0.27. Secondly, the average TSF values based on NIR values between lots were calculated with a calibration equation generated from all 90 data points representing all lots. Results indicated the presence of substantial variation, s = 5.58. Clearly there are large significant deviations between lots. The most probable cause of between-lot variation is that inherent differences in ground beef affect the overall reflectance pattern. These findings indicate the necessity of preparing a calibration equation from the same beef lot as that to be analyzed, eliminating variation due to lot differences. While this constant requirement for recalibration based on variation between beef lots may ultimately be a disadvantage, it will eliminate the need, that most NIR calibration equations have, for

Table IV. Composition of Commercially Prepared Extended Beef Patties

sample	lean beef (10% fat)	fat beef (50% fat)	water	TSF	TSF, %[a] added	TSF, %[a] NIR anal.
1	56.75	38.75	3.21	1.79	10.9	9.8[b]
						10.2[c]
						10.3[d]
2	50.00	40.00	6.43	3.57	20.6	21.5
						21.6
						21.4
3	37.50	42.50	12.86	7.14	40.1	37.0
						36.3
						36.0
4	25.00	45.00	19.29	10.71	56.0	53.2
						55.9
						55.8

[a] Fat free, dry basis. [b] Sample cup, single load. [c] Sample cup, reload same sample. [d] Rescan previous sample load, rotational change.

periodic routine adjustments to remove bias and also to eliminate day-to-day instrumental variation.

To test this method on actual commercial TSF-extended ground beef, a small set of samples of fresh beef patties containing known amounts of TSF were prepared in a commercial meat processing plant (Table IV). As previously outlined, these samples and sufficient quantities of unextended beef from the same source which would be used to create a companion calibration set were freeze-dried and defatted. These samples, which ranged from approximately 2–10% TSF in the original patties, (10.9–56% TSF in the freeze-dried, defatted material) were run in triplicate (load, reload, and reread) by NIR. Based on the 95% confidence limits of 3.6 for 2 single loads, these TSF values appear to be within the limits of error expected, based on the data previously described. The development of these analytical correlations did require the use of the previously mentioned 6350 research model NIR spectrometer; however, it is important to note that once a wavelength has been established, this analysis can be performed on a much less expensive, but equally accurate and rapid instrument with filtered optics.

CONCLUSIONS

Although the present study indicates the necessity of calibrating the NIR with beef from the same lot as that to be tested, the method can be successfully used to provide an indication of the level of addition of TSF to beef. The determination of TSF in other meats has been given a limited examination. Chicken, fish and pork, each mixed with added TSF, have been examined by NIR. Correlation plots of each meat indicate that satisfactory results could be obtained within the parameters outlined in this method. It seems quite feasible that the level of TSF or other extenders could easily be determined in most meats and their products by NIR.

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