Soy Lecithin Phospholipid Determination by Fourier Transform Infrared Spectroscopy and the Acid Digest/Arseno-Molybdate Method: A Comparative Study

J.M. Nzai and A. Proctor*

Department of Food Science, University of Arkansas,
Fayetteville, Arkansas 72704

ABSTRACT: A study was conducted to determine the accuracy and precision of phospholipid analysis by a simple Fourier transform infrared spectroscopy (FTIR) method relative to the conventional phospholipid phosphorus analysis by the acid digest/arseno-molybdate method by Bartlett. Commercial soy lecithins of known concentrations of phospholipid were prepared and the phospholipid content measured by the FTIR and Bartlett methods. The coefficients of determination and of variances using the two methods were determined. The coefficient of determination for the FTIR method was >0.976 while that for the Bartlett method was ~0.821. The coefficients of variances (CV) for 1–20% phospholipid concentration range using 10 replicate samples were found to lie between 3.59 and 9.45% for the FTIR method, while the Bartlett method had much higher CV for the same range and replicates (8.95 to 48.73%), signifying the higher accuracy and precision of the FTIR compared to the Bartlett method in the determination of the actual percentage of phospholipid. The Bartlett method gave no significant difference in the phospholipid levels at smaller concentrations, indicating its limitation in accurately determining percentage phospholipid of samples at low concentrations. The one-way analysis of variance at the 1–20% phospholipid concentration range showed that there were significant differences in the mean percentage phospholipid levels for the FTIR data, which was therefore able to distinguish samples with small differences in phospholipid levels. The FTIR method gave consistently reliable results within the range chosen (1–20% phospholipid content). FTIR is a fast, simple, and reliable analytical tool for quantitative phospholipid analysis.

Paper no. J8935 in *JAOCS 76*, 61–66 (January 1999).

KEY WORDS: Bartlett method, Fourier transform infrared spectroscopy, lecithin, phospholipid analysis, phospholipids.

Phospholipids are 0.1 to 1.8% of the total lipid content of soy oil (1). Phospholipids are responsible for losses in neutral lipids in oil processing during neutralization, and they contribute to the discoloration of the oil during deodorization and steam distillation (2). Removal of oil phospholipids results in the subsequent removal of iron and copper and improves oil oxidative and flavor stability. Therefore, accurate measurement of phospholipids in vegetable oils is important to determine oil quality.

The method by Bartlett (3) is widely used to determine edible oil phospholipid phosphorus by ashing of the oil followed by colorimetric determination of the phosphatide phosphorus as a blue or yellow phospho-molybdate complex measured by absorbance at 830 nm. However, the method is not specific for phospholipids since the colorimetric reaction also includes other forms of oil phosphorus. This method is also time-consuming, tedious, and often inaccurate, and it requires careful reagent addition in order to get reproducible results. Therefore, there are opportunities for the development of automated instrumental methods to improve analytical speed, accuracy, and efficiency of analysis. Such alternative methods would be particularly well received if they addressed environmental concerns regarding the use of large volumes of solvents and reagents in quality control laboratories.

Fourier transform infrared spectroscopy (FTIR) was widely used for authentication of certain food products such as coffee (4) and meat products (5). Van de Voort *et al.* (6) developed an FTIR method for evaluation of the oxidative state of oil for monitoring changes in edible oils undergoing thermal stress. An FTIR method was developed for the determination of the solid fat index (7), and a sampling method was developed for protein and fat analysis of cheese samples by FTIR spectroscopy (8). FTIR instruments have many advantages over the conventional dispersive instruments, with more energy throughput, excellent wavenumber reproducibility and accuracy, extensive and precise spectral manipulation capabilities, and advanced chemometric software to handle calibration development (9). Furthermore, FTIR reduces the use of large solvent quantities associated with wet chemical methods, making the method development using quantitative FTIR oil analysis desirable.

We recently reported a new method for quantitative determination of phospholipid in vegetable oils by FTIR using calibration equations generated by a known standard phospholipid mixture (10). High correlations ($R^2 \geq 0.968$) were observed between band areas and the phospholipid concentration. The optimal absorption band from 1200–970 cm^{-1} was found very use-

*To whom correspondence should be addressed.
E-mail: aproctor@comp.uark.edu

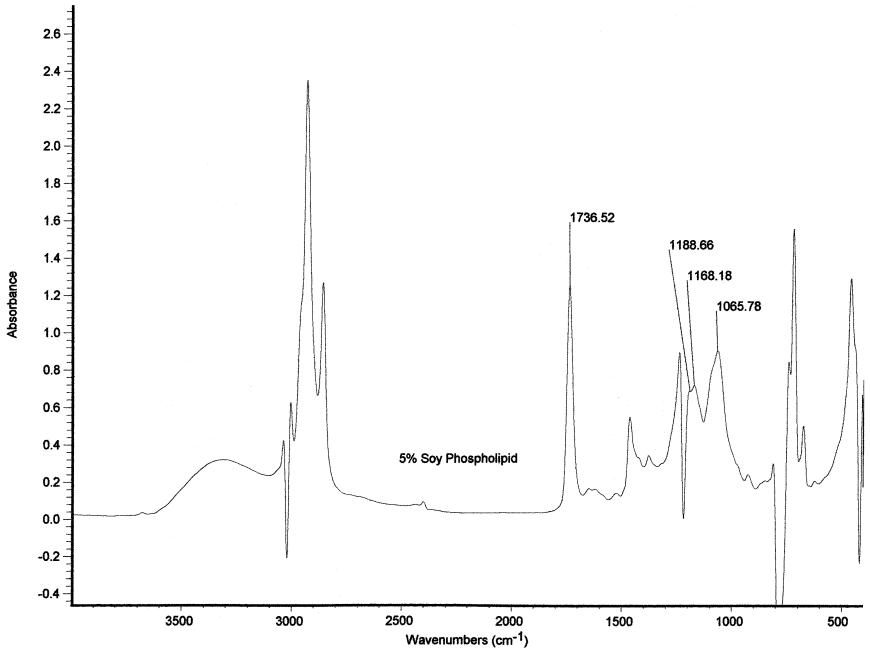

FIG. 1. A Fourier transform infrared (FTIR) spectrum of 5% soy phospholipid (PC) (Lecigran 5750; Riceland Foods Inc., Stuttgart, AR) in chloroform. 100 scans were co-added at a resolution of 4 cm^{-1}.

ful for the identification and quantitation of phospholipid content of oil. However, there is a need to develop a method that can accurately and rapidly provide data about the concentration of phospholipids present in vegetable oils, enabling a general FTIR approach for the food industry to be established to replace time-consuming and tedious protocols such as the Bartlett method.

The main objective of this study was to compare quantitative determination of phospholipid in soy lecithin by the FTIR method to the commonly used Bartlett phospholipid method of phosphorus determination.

EXPERIMENTAL PROCEDURES

Chemicals and reagents. All solvents used were purchased from Fisher Scientific (Fairlawn, NJ). Lecigran 5750 (97.3% phospholipids) was obtained from Riceland Foods Inc.

TABLE 1
FTIR Band Assignment and Regression Equations for Standard Phospholipid (PL) Mixture

Bands	Vibrational mode	Equations[a]	R^2
1	C=O 1765–1720 cm^{-1}	$y = -0.03 + 0.30$ (PL)	0.998
2	PO_2 1200–1145 cm^{-1}	$y = -0.11 + 0.48$ (PL)	0.988
3	P-O-C 1145–970 cm^{-1}	$y = 0.16 + 0.14$ (PL)	0.976
4	P-O-C + PO_2 1200–970 cm^{-1}	$y = 0.05 + 0.31$ (PL)	0.980

[a]$y = a + bx$; where x = PL is the percentage of phospholipid in the standard solution (1, 2, 5, 7, 10, 12, 15, 18, 20, and 30%) consisting of 39% phosphatidylcholine, 22% phosphatidylinositol, and 27% phosphatidylethanolamine. FTIR, Fourier transform infrared spectrometry. R^2, correlation of determination.

TABLE 2
One-Way Analysis of Variance for the FTIR and Bartlett Methods[a]

Actual	Number	FTIR			Bartlett		
PL (%)	of runs	Means	SD	CV	Means	SD	CV
0.97	10	0.92	0.03	3.59	0.80	0.07	8.95
2.42	10	2.32	0.22	9.45	1.15	0.25	21.29
4.85	10	4.49	0.24	5.32	2.25	0.82	36.55
9.70	10	8.29	0.33	3.95	8.23	1.80	21.82
14.55	10	13.02	0.50	3.81	13.19	6.43	48.73
19.4	10	20.08	1.09	5.43	22.47	6.62	29.44

[a]SD, standard deviation; CV, coefficient of variance. See Table 1 for other abbreviations.

TABLE 3
Mean Comparisons for Percentage Phospholipid—FTIR Method

Student's *t*-test multiple pairwise comparison[a]

FTIR PL (%)	19.4	14.55	9.7	4.85	2.42	0.97
19.4	–0.47	6.59	11.32	15.12	17.29	18.69
14.55	6.57	–0.47	4.26	8.06	10.23	11.63
9.7	11.32	4.26	–0.47	3.32	5.50	6.90
4.85	15.12	8.06	3.32	–0.47	1.70	3.10
2.42	17.29	10.23	5.50	1.70	–0.47	0.93
0.97	18.69	11.63	6.90	3.10	0.93	–0.47

Tukey-Kramer HSD-multiple pairwise comparison

FTIR PL (%)	19.4	14.55	9.7	4.85	2.42	0.97
19.4	–0.69	6.36	11.10	14.89	17.07	18.46
14.55	6.36	–0.69	4.04	7.84	10.01	11.41
9.7	11.10	4.04	–0.69	3.10	5.28	6.68
4.85	14.89	7.84	3.10	–0.69	1.48	2.88
2.42	17.07	10.01	5.28	1.48	–0.69	0.71
0.97	18.46	11.41	6.68	2.88	0.71	–0.69

[a]Positive values show means that are significantly different at $\alpha = 0.05$. See Table 1 for abbreviations.

(Stuttgart, AR). Phosphatidylcholine (PC), phosphatidylinositol (PI), and phosphatidylethanolamine (PE) (all of >97% purity) were purchased from Sigma Chemical Company (St. Louis, MO).

Calibration standards. Calibration curves were generated using 1, 2, 5, 7, 10, 12, 15, 18, 20, and 30% phospholipid standard (39% PC, 22% PI, and 27% PE) in chloroform. Van de Voort *et al.* (7) found that there was a tendency for calibration to drift over time, due mainly to changes in instrument performance. Therefore, the standards were run in between every 10 sample runs to make sure that the instrument was properly calibrated. Calibration equations generated from the curve were used in the determination of percentage phospholipid by FTIR, according to the method developed by the authors using band areas 1200–970 cm^{-1} (10).

Phospholipid determination by FTIR. An Impact 410 FTIR instrument (Nicolet, Madison, WI) was used for analysis. Sodium chloride stainless steel precision cells were used with a path length of 0.1 mm. A nominal resolution of 4 cm^{-1} and scan number of 100 were used to generate the spectra at automatic gain to maximize the detector signal-to-noise ratio.

Lecigran 5750 concentrations at 1, 2.5, 5, 10, 15, and 20% phospholipid concentrations dissolved in chloroform were prepared. The phospholipid bands analyzed were those between 1200 and 970 cm^{-1}. Four bands were used to determine the percentage phospholipid (10), which was between 1765 and 1720 cm^{-1} (band 1) due to the C=O vibration; between 1200 and 1145 cm^{-1} (band 2) due to the PO_2 vibration; between 1145 and 970 cm^{-1} (band 3) due to P-O-C ν_{sym} vibration; and between 1200 and 970 cm^{-1} (band 4) due to both P-O-C and PO_2 vibrations. Bands 1 and 4 were used for the calculation of total percentage phospholipid. Ten replicates per sample were run, and the results were correlated with the actual phospholipid content provided by the lecithin supplier (11).

Phospholipid quantitation by Bartlett. The phospholipid phosphorus content of samples at various concentrations were determined by the method of Bartlett (3). Ten replicate samples were run, and the results were correlated to those determined by the acetone-insoluble method (11) provided by the supplier.

Statistical analysis. The JMP® statistical and data analysis program (SAS Institute Inc., Belmont, CA) was used for data analysis. The one-way analysis of variance was used for multiple comparisons of the means for all the phospholipid concentrations using the two methods. The coefficients of determination (R^2) and variances were used to compare the two methods in phospholipid determination.

RESULTS AND DISCUSSION

Phospholipid determination by FTIR. Figure 1 shows a typical FTIR spectrum of a phospholipid sample, as well as the bands used for determination of phospholipid concentration Table 1 shows the regression equations for bands 1 through 4 used to calculate the percentage phospholipid by FTIR. High coefficients of determination ($R^2 \geq 0.976$) were obtained for bands 1 to 4.

Table 2 shows the results of the one-way analysis of variance. The standard deviation from the actual means for the percentage phospholipid for all the sample concentrations was found to lie between 0.03 and 1.09. The coefficient of variance (CV) was found to lie between 3.59 and 9.45% (Table 2). This low CV was indicative of the high precision of the FTIR method in the determination of phospholipid content in soy lecithin. A comparison of the means for percent phospholipid concentration using the Student's *t*-test for the balanced data (10 replicates each for six different concentrations) was found to be significantly different at $P < 0.05$ (Table 3, Fig. 2). The same was observed for the comparison for all pairs using the Tukey-Kramer HSD test. The non-overlapping comparison circles for both the Student's test and the *t* test show that the values are significantly different from each other (Fig. 2). This shows that the FTIR method can accurately distinguish between increasing concentrations of phospholipids.

Phospholipid determination by Bartlett. The coefficient of determination (R^2) was 0.821 for the Bartlett method. Generally, at phospholipid concentrations greater than 2.5%, there was an increased deviation from the actual percentage phospholipid values for the Bartlett method. The higher the concentration, the further the results were from the actual percentage phospholipid concentration expected (Fig. 3). Table 2 shows that the Bartlett method gave standard deviations from the mean ranging from 0.07 to 6.62 for the different phospholipid concentration groups. The CV was used to compare the Bartlett method routinely used for phospholipid content determination to the FTIR method. The CV was found to be low at a concentration of 0.97% PL (8.95%), but at concentrations >1 the CV was

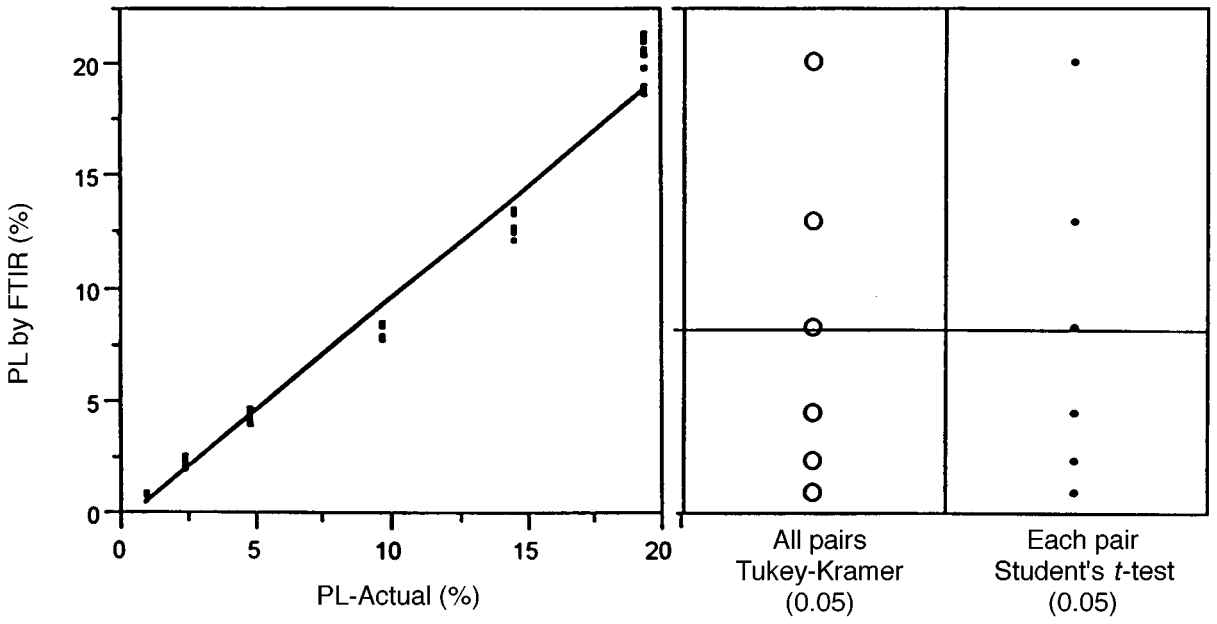

FIG. 2. A graphical representation of the regression analysis, the Tukey-Kramer test, and Student's *t*-test between the percentage FTIR and the percentage actual phospholipid concentrations for 10 replications each of six different concentrations at $\alpha = 0.05$ level of significance. See Figure 1 for abbreviations.

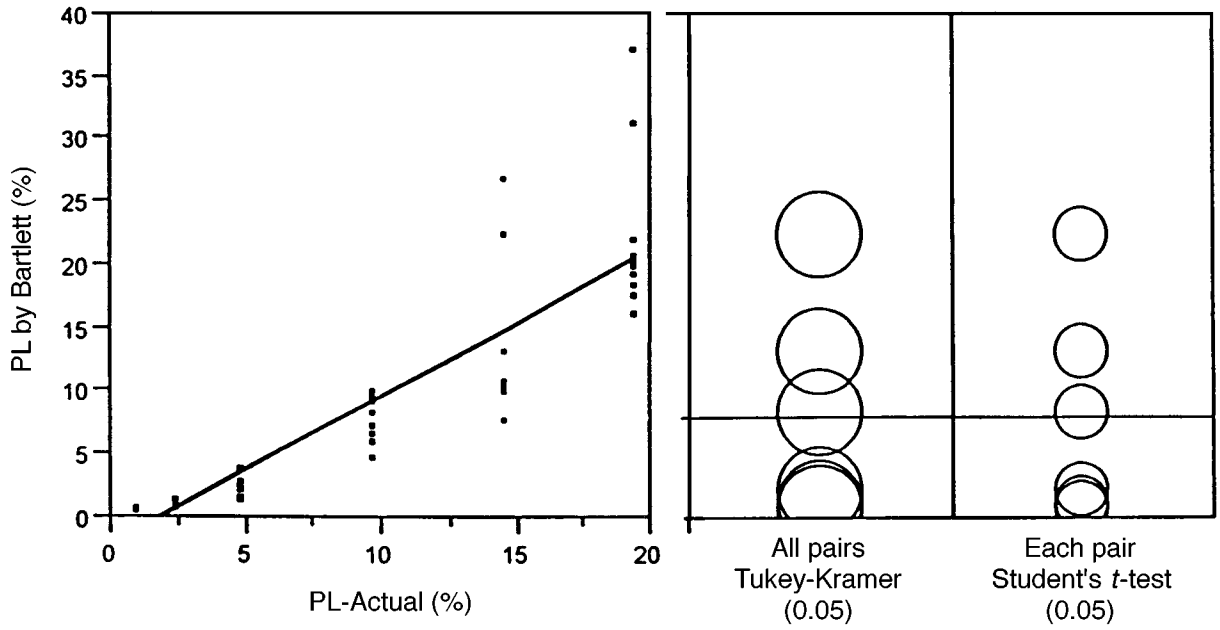

FIG. 3. A graphical representation of the regression analysis, the Tukey-Kramer test, and Student's *t*-test between the percentage Bartlett and percentage actual phospholipid concentrations for 10 replications each of six different concentrations at $\alpha = 0.05$ level of significance. See Figure 2 for abbreviation.

very high (21.29 to 48.73%), showing the lower accuracy and precision of this method compared to the FTIR method, which gave CV values between 3.59 and 9.45% for the same concentration range. A comparison of mean percent phospholipid concentration using the Student's *t*-test for the balanced data (10 replicates each for six different concentrations) was not significantly different at the $\alpha = 0.05$ level of significance at phospholipid concentration <5% (Table 4). The same was observed in the comparison for all pairs using the Tukey-Kramer HSD test, where the comparison

TABLE 4
Mean Comparisons for Percentage Phospholipid—Bartlett Method

Student's *t*-test multiple pairwise comparison[a]

Bartlett PL (%)	19.4	14.55	9.7	4.85	2.42	0.97
19.4	–3.45	5.83	10.78	16.77	17.86	18.22
14.55	5.83	–3.45	1.50	7.49	8.58	8.94
9.7	10.78	1.50	–3.45	2.52	3.62	3.98
4.85	16.77	7.49	2.53	–3.45	–2.36	–2.01
2.42	17.86	8.58	3.62	–2.36	–3.45	–3.10
0.97	18.22	8.94	3.98	–2.01	–3.10	–3.45

Tukey-Kramer HSD-multiple pairwise comparison

Bartlett PL (%)	19.4	14.55	9.7	4.85	2.42	0.97
19.4	–5.09	4.19	9.15	15.13	16.23	16.58
14.55	4.19	–5.09	–0.13	5.85	6.95	7.30
9.7	9.15	–0.13	–5.09	0.90	1.99	2.34
4.85	15.13	5.85	0.90	–5.09	–4.00	–3.64
2.42	16.23	6.95	1.99	–4.00	–5.09	–4.74
0.97	16.58	7.30	2.34	–3.64	–4.74	–5.09

[a]Positive values show means that are significantly different at the $\alpha = 0.05$ level of significance. See Table 1 for abbreviation.

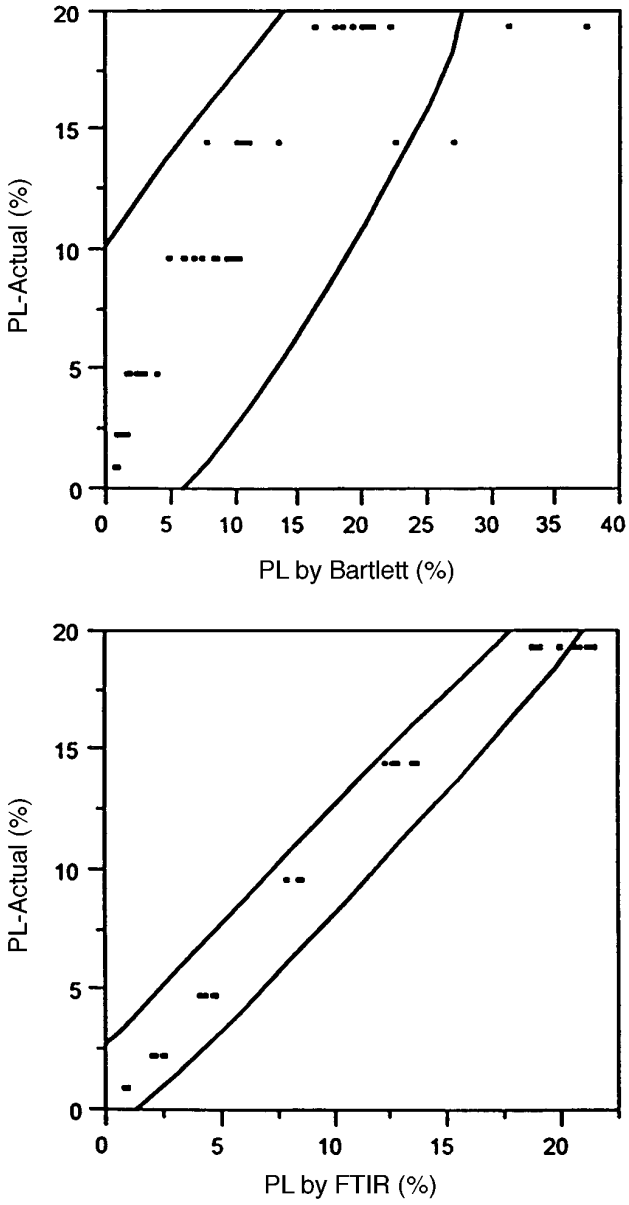

FIG. 4. Scatter plot matrices for percentage phospholipid by the Bartlett and FTIR methods compared to the actual percentage phospholipid, respectively. See Figure 2 for abbreviation.

circles overlapped at the 1–15% phospholipid concentration range. The overlapping comparison circles for both the Student's *t*-test and the Tukey-Kramer HSD test show that the values at the given phospholipid concentrations are not significantly different from each other.

Comparative studies. Scatter plot matrices were used to further illustrate correlations between the actual percentage phospholipid and the percentage phospholipid for the Bartlett and FTIR methods (Fig. 4). The scatter plots for the Bartlett method were not diagonally oriented and the points were spread out, showing the large variation of this method from the actual percentage phospholipid. There was little variation in phospholipid concentration at 1–2.5% phospholipid content. However, at higher concentrations (>5%) a large variation was observed for the Bartlett method. This larger variation with increased concentration is evidenced by the CV values obtained (>20%) for the Bartlett test at concentrations greater than 5% phospholipid (Table 2). The CV values for the FTIR method at differing concentrations were much lower (3.59 and 9.45%) than for the Bartlett method (8.95 to 48.73%) for the same phospholipid concentration range. This better parameter estimate for the FTIR method compared to the Bartlett suggests that FTIR is a much better predictor of the percentage phospholipid at both low and high phospholipid concentrations. The matrices between the actual percentage phospholipid and percentage phospholipid by FTIR were elongated ellipses indicative of the strong pairwise linear association between the two (Fig. 4).

Tables 3 and 4 show the comparison of the means across the whole range of the 6% phospholipid using both the Student's *t*-test and the Tukey-Kramer test for both FTIR and Bartlett methods, respectively. Positive values show a pair of means that are significantly different at $\alpha = 0.05$ level of significance. There were significant differences between the percentage phospholipid groups run by FTIR, showing the ability of FTIR to distinguish between increasing concentrations of phospholipids. However, when the same was determined for the Bartlett percentage phospholipid, there were no significant differences between 0.97 and 4.85% phospholipid levels, signifying that the Bartlett method could not accurately distinguish between increasing percentage phospholipid contents at this concentration range.

REFERENCES

1. Przybylski, R., and N.A.M. Eskin, Phospholipid Composition of Canola Oils During the Early Stages of Processing as Measured by TLC with Flame-Ionization Detector, *J. Am. Oil Chem. Soc. 68*:241–245 (1991).
2. Davies, M.G., I.M. Zaward, and G.S.D. Weir, *Scientific and Technological Surveys No. 112*, Leatherhead Food Research Association, Leatherhead, Surrey, United Kingdom, 1979.
3. Bartlett, G., Phosphorus Assay in Column Chromatography, *J. Biol. Chem. 234*:446–468 (1959).
4. Downey, G., R. Briandet, R.H. Wilson, and E.K. Kemsley, Near- and Mid-Infrared Spectroscopies in Food Authentication: Coffee Varietal Identification, *J. Agri. Food Chem. 45*:4357–4361 (1997).
5. Ville, H., G. Maes, R. De Schrijver, G. Spincemaille, G. Rombouts, and R. Geers, Determination of Phospholipid Content of Intramuscular Fat by Fourier Transform Infrared Spectroscopy, *Meat Sci. 41*:283–291 (1995).
6. Van de Voort, F.R., A.A. Ismail, J. Sedman, and G. Emo, Monitoring the Oxidation of Edible Oils by Fourier Transform Infrared Spectroscopy, *J. Am. Oil Chem. Soc. 71*:243–253 (1994).
7. Van de Voort, F.R., K.P. Memon, and A.A Ismail, Determination of Solid Fat Index by Fourier Transform Infrared Spectroscopy, *Ibid. 73*:411–416 (1996).
8. Chen, M., and J. Irudayaraj, Sampling Technique for Cheese Analysis by FTIR Spectroscopy, *J. Food Sci. 63*:96–99 (1998).
9. Sedman, J., F.R. van de Voort, and A.A. Ismail, Application of Fourier Transform Infrared Spectroscopy, in *New Techniques and Applications in Lipid Analysis*, edited by R.E. McDonald and M.M. Mossoba, AOCS Press, Champaign, 1997, pp. 283–324.
10. Nzai, J.M., and A. Proctor, Determination of Phospholipids in Vegetable Oil by Fourier Transform Infrared Spectroscopy, *J. Am. Oil Chem. Soc. 75*:1281–1289 (1998).
11. *Official Methods and Recommended Practices of the American Oil Chemists' Society*, 4th edn., AOCS Press, Champaign, 1994, Method Ja4-46.

[Received July 6, 1998; accepted October 1, 1998]