Nephelometric Determination of Phosphorus in Soybean and Corn Oil Processing

Roger D. Sinram
A.E. Staley Manufacturing Co., Research and Development, 63 Building, 2200 E. Eldorado St., Decatur, IL 62525

A procedure to measure phosphorus content of soybean and corn oil samples has been developed using nephelometry (turbidity). The method uses the relationship between phosphorus level due to phosphatides in vegetable oil and turbidity formed in phosphatide mixtures. The rapid 10-min determination of phosphorus in process samples is 30 times faster than colorimetric methods. Phosphorus vs turbidity data formed nearly linear relationships for crude, degummed, once-refined, bleached and deodorized soybean and corn oil process samples.

The major phosphatides in crude soybean and corn oils include phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol, phosphatidic acid, phosphatidylserine and phosphatidylglycerol (1). Phosphatide level in crude oil, calculated by multiplying phosphorus content (ppm) by 31.7, determines potential lecithin yield, as well as the amount of hydration water necessary for adequate degumming (2). Typical phosphatide levels in crude soybean oil are about 2.5% and in crude corn oil about 1.5%. These levels often vary regionally and seasonally, and require monitoring by the oil processor.

Through each step of the vegetable oil refining process, phosphorus levels are reduced as shown in Table 1. Deodorized oils must be refined to acceptable phosphorus levels (<5 ppm) to minimize derivatives that may lead to off-flavor precursors and oxidative instability (3). Studies by Ottissen and Jensen (4,5) and Beckman (6) suggested that phosphorus levels ranging from 4–8 ppm in bleached soybean oil may partially deactivate nickel catalyst and have an adverse effect on selectivity during vegetable oil hydrogenation. Additional studies by Sambuc et al. (7) have verified the importance of removing phosphorus to low levels in vegetable oil to give a stable product.

Monitoring phosphorus levels in vegetable oil refining has been difficult due to lengthy analytical time requirements. The standard colorimetric phosphorus analysis for vegetable oil (AOCS Official Method Ca 12-55) (8) is both lengthy and tedious, often requiring 4–5 hr for completion. Gente-Jauneaux and Prevot (9) have reported using flameless atomic absorption with lanthanum to determine phosphorus level in vegetable oils. Hartman et al. (10) have developed an alkali digestion phosphorus procedure for use with lipids and lipid-containing materials such as lecithin. Racicot and Handel (11) have described a chromatographic method to determine phospholipid content in crude and degummed soybean oil. The validity of some of these methods has been evaluated by Koifman et al. (12). As with the official AOCS method, all of these procedures are time-consuming and require expensive, sophisticated equipment and high levels of expertise not always available in oil refinery laboratories. The purpose of this investigation was to develop an alternative phosphorus measurement procedure using nephelometry, which would use simple equipment and methodology and provide rapid reliable results for oil processing quality control. This procedure was accepted as a recommended practice by the AOCS Uniform Methods Committee on November 25, 1985.

EXPERIMENTAL

Material required. A HACH Ratio Turbidimeter, Model 18900-00, was used with 2.5 × 9.6 cm sample cells (HACH Cat. No. 20849-00). The instrument was standardized using latex standard cells (HACH Cat. No. 20848-00). Spectral grade acetone was used. All corn and soybean oil samples (A.E. Staley Mfg. Co.) were filtered and found to be low in moisture and soap. Hydrogenated oil samples were first heated to 50 C in a microwave oven before analysis.

Phosphorus measurement procedure. The turbidimeter was allowed to warm up for at least 15 min. Sample size varied depending on the expected amount of phosphorus in each product and included 0.33 g for crude corn and soybean oils, 1.67 g for degummed and once-refined oils and 8.35 g for bleached and deodorized oils. The sample was weighed into a 50-ml volumetric flask, filling with acetone to the 50 ml mark. The flask was stoppered and shaken, and the phosphatide mixture was poured into a sample cell (ca. 30 ml at 25 C). The sample was capped, thoroughly shaken (by hand for about 10 sec), wiped clean and placed in the turbidimeter with proper consistent alignment. The correct instrument range (2, 20 or 200 NTU) was selected. After 5 min, the NTU reading was recorded. The NTU value for the acetone blank was subtracted from the reading. The phosphorus level was either estimated directly from a phosphorus-NTU correlation curve or calculated from a mathematical equation, unique for each oil type.

Standard calibration and blank. Specially prepared latex standards (1.8, 18 and 180 NTU) were used to calibrate the instrument, per manufacturer's directions. A blank of 30.0 ml of pure acetone was run prior to oil-acetone samples to assure that the acetone reading was within acceptable limits.

Reproducibility of instrument and nephelometric method. The reproducibility of the ratio turbidimeter and nephelometric method was examined. Samples of crude and once-refined soybean oil and of pure acetone were prepared. Each sample was run on the turbidimeter, reshaken and rerun a total of 10 times to determine instrument reproducibility. To indicate method reproducibility, samples of crude, degummed, once-refined and deodorized soybean oils were each analyzed nephelometrically a total of 10 times, using a new sample for each analysis. The same sample cell was used for each rerun.

RESULTS AND DISCUSSION

The resulting phosphorus vs NTU data for each oil type and degree of processing were plotted graphically as shown in Figure 1 for crude soybean oil. For corn oil,

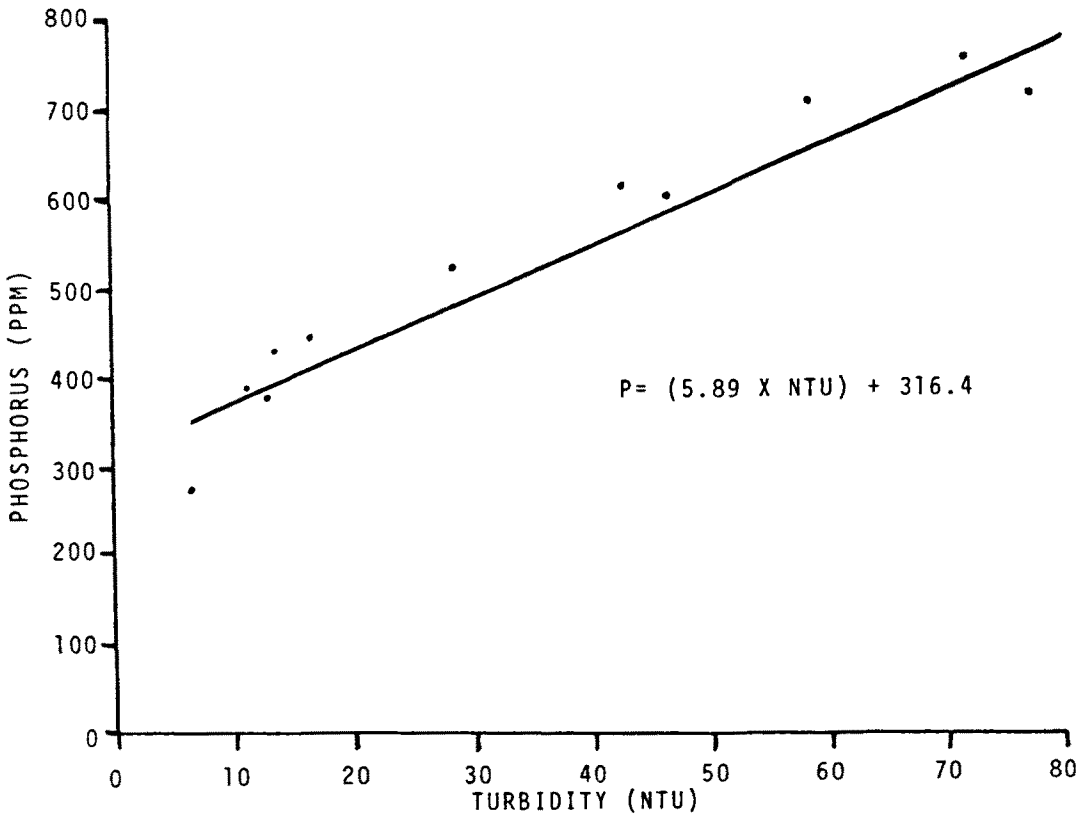

FIG. 1. Correlation graph of phosphorus vs turbidity for 0.33-g samples of crude soybean oil in 50.0 ml of acetone. Linear correlation coefficient: 0.941.

TABLE 1

Linear Correlation Coefficients for Phosphorus vs Turbidity Data, Equations for Converting NTU to Phosphorus for Soybean and Corn Oils, and Recommended Nephelometric Sample Sizes

Oil type	Phosphorus (ppm) limits	Linear correlation coefficient	Equation for curve	Recommended sample size in 50 ml acetone (g)
Soybean				
Crude	316–700	0.941	P = (5.89 × NTU) + 316.4	0.33
Degummed	20–170	0.970	P = (5.32 × NTU) + 3.38	1.67
Once-refined	0–20	0.965	P = (8.26 × NTU) − 4.49	1.67
Bleached	0–5	0.926	P = (1.27 × NTU) − 0.225	8.35
Deodorized	0–3	0.957	P = (1.72 × NTU) − 0.528	8.35
Corn				
Crude	250–560	0.927	P = (5.62 × NTU) + 97.2	0.33
Degummed	16–136	0.999	P = (3.69 × NTU) − 2.77	1.67
Once-refined	0–16	0.975	P = (1.42 × NTU) − 2.21	1.67
Bleached	0–4	0.999	P = (2.60 × NTU) − 1.05	8.35
Deodorized	0–2	0.870	P = (0.99 × NTU) + 0.027	8.35

linear correlation coefficients ranged from 0.87 for deodorized oil to 0.999 for degummed and bleached oils. For crude and processed soybean oils, correlation coefficients were greater than 0.92. From each set of data, least square fit regression analyses were used to calculate the equation for the curve (Table 1). In the test for instrument reproducibility (Table 2), greater turbidity values were seen for once-refined oil and for the acetone blank as time progressed. The increased acetone turbidities correlated well with increasing sample temperatures. The crude sample, being in a high NTU range, was not affected by increasing temperature. As with instrument reproducibility, small standard deviations and coefficients of variation were seen (Table 3) for NTU method reproducibility. In addition, good linear correlation coefficients, averaging 0.953 for all the sets of phosphorus-NTU data, indicate that the procedure was quite accurate for all phosphorus ranges tested.

Although the nephelometric phosphorus method is quite simple, several factors can cause erroneous results.

TABLE 2

Instrument Reproducibility for Acetone Blank and Reshaken Samples of Crude and Once-refined Soybean Oils

Reshake trial no.	Temp (C)	Turbidity (NTU)		
		Acetone blank (0.0 g)[a]	Crude soybean oil (0.33 g)[a]	Once-refined soybean oil (1.67 g)[a]
1	23.5	0.44	41	0.68
2	23.5	0.45	42	0.68
3	24.0	0.45	40	0.70
4	24.5	0.49	40	0.68
5	25.0	0.48	39	0.74
6	26.0	0.48	39	0.64
7	27.0	0.50	40	0.83
8	28.0	0.50	40	0.82
9	28.0	0.50	40	0.86
10	29.0	0.50	41	0.85
Range	23.5–29.0	0.44–0.52	39–41	0.68–0.85
Mean	25.9	0.48	40.2	0.75
Standard deviation	1.94	0.03	0.92	0.08
Coefficient of variation	7.75	5.50	2.29	10.31

Linear correlation coefficient between acetone blank temperature and turbidity: 0.91.

[a]Oil sample wt in 50 ml of acetone.

TABLE 3

Nephelometric Method Reproducibility for Various Refining Stages of Soybean Oil, Using Different Samples of the Same Oil for Each Series of Trials

Trial no.	Turbidity			
	Crude soybean oil (0.33 g)[a]	Degummed soybean oil (1.67 g)[a]	Once-refined soybean oil (1.67 g)[a]	Deodorized soybean oil (8.35 g)[a]
1	43	3.0	0.55	0.70
2	42	3.2	0.50	0.78
3	44	3.2	0.47	0.69
4	39	3.1	0.48	0.71
5	38	3.5	0.55	0.73
6	43	3.0	0.56	0.86
7	36	3.6	0.47	0.72
8	45	3.7	0.58	0.78
9	40	3.7	0.57	0.80
10	42	3.0	0.47	0.80
Range, NTU	36–45	3.0–3.7	0.47–0.58	0.69–0.80
Range, P (ppm)	528–581	19–23	0	0
Mean	41.2	3.3	0.52	0.76
Standard deviation	2.85	0.29	0.04	0.06
Coefficient of variation	6.94	8.92	8.84	7.29

[a]Oil sample wt in 50 ml of acetone.

Appropriate sample size, depending upon phosphorus content, should be measured to the nearest 0.01 g. The effect of sample size in 1-g increments on turbidity is shown in Figure 2 for deodorized soybean oil. The interior and exterior of the sample cell and cap must be cleaned only with low-NTU acetone between analyses. The sample cell, its exterior wiped free of debris, must be properly aligned in the instrument and remain for exactly 5 min to allow dissipation of air bubbles and equilibrium of phosphatide micelles. If after 5 min the instrument reading is still fluctuating, repeat the analysis. The turbidimeter should be calibrated before use with latex standards or oils of known phosphorus levels. An acetone blank must be run whenever a new lot of acetone is used.

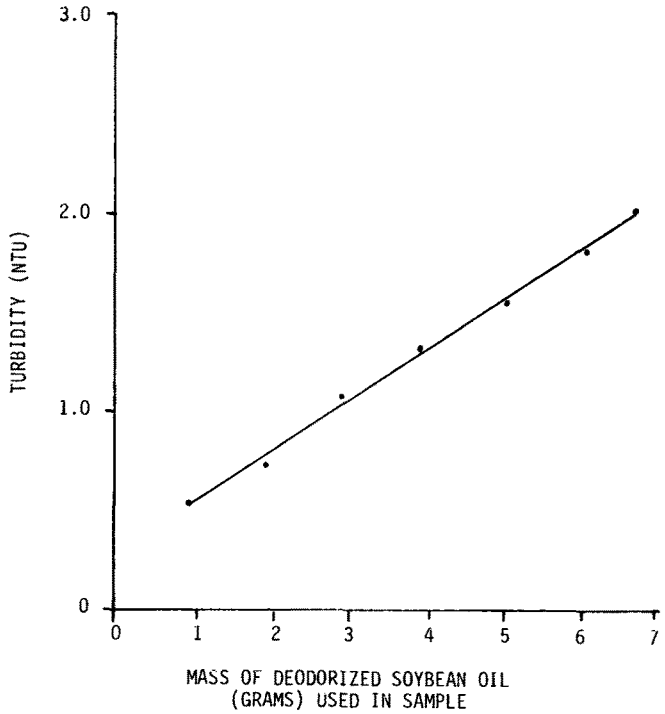

FIG. 2. Effect of sample size on turbidity of deodorized soybean oil samples in acetone. Linear correlation coefficient: 0.988; acetone blank: 0.28 NTU.

An acceptable acetone should have a reading less than 0.5 NTU. Oil impurities, such as filter aid, dirt and metal fragments, high moisture levels (>.5%) and soap (>500 ppm) may give erroneous high NTU values. Impurities can be removed by passing the heated (to 50 C) sample through Whatman No. 2 fluted filter paper.

This procedure enabled the quantitative determination of phosphorus using turbidity of oil-acetone mixtures. Initially designed for measuring phosphorus in degummed and once-refined soybean oil process samples, the procedure was later modified to be effective for oils of different degrees of refining, ranging from crude through deodorized soybean and corn oils. Initially for each of approximately 100 oil samples, both phosphorus determinations and turbidity readings were obtained to develop correlation curves.

This procedure may be applicable to other vegetable oils or animal fats for which phosphorus level is desired. For oils other than corn or soybean, optimum sample sizes would need to be established and correlation data of phosphorus (using the official method) and turbidity would need to be generated, as was done in this investigation. Since deviations in oil quality, such as clarity and moisture, are very likely to occur among processors, it is recommended that soybean and corn oil refiners establish phosphorus-turbidity correlation data for their particular oil products.

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