14. Tanabe, K., "Solid Acids and Bases, Their Catalytic Properties," Academic Press, New York, 1970, pp. 2, 5-7, 35-37, 54-55, 107.
15. Rindt, J.R., "Heterogeneous Catalysis of Solvent Refined Lignite to Obtain Chemical Feed Stocks," Master's thesis, Univ. of North Dakota, Grand Forks, ND, 1979, pp. 52-53.
16. Wu, W.R.K., and H.H. Storch, "Hydrogenation of Coal and Tar," Bureau of Mines, Washington, DC, 1968, p. 63.
17. Dykstra, G.J., and S.C. Sorenson, Veg. Oil Diesel Fuel Semin. III, Peoria, IL, 1983, pp. 38-44.

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♣Total Phospholipids in Crude Palm Oil: Quantitative Analysis and Correlations with Oil Quality Parameters.

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ABSTRACT

Phospholipids, selectively extracted from crude palm oil and free of colored carotenoids, can be quantitatively determined by the phospholipid-molybdenum blue complex in hexane. A study of the phospholipid in crude palm oil in relationship to other components affecting oil quality shows that they have a beneficial effect while any detrimental effect may be of an indirect nature.

INTRODUCTION

Phosphorus constituents in edible oils generally are considered as undesirable impurities causing refining problems and oil losses (1,2). In commercial crude palm oil, phospholipids and total phosphorus are reported present at 0.8-3.3 ppm (as P) and ca. 20 ppm (as P) respectively (3). Although the two sources of phosphorus (inorganic phosphates and phospholipids) are chemically distinct, usually only the total phosphorus content is determined and then converted to equivalent phospholipid value. This is because simple, reliable methods of phospholipid quantitation are not generally available at present for routine quality control analyses. Furthermore, phospholipids seldom are differentiated from other phosphorus compounds. Recently we have described a thin layer chromatographic (TLC) procedure for palm oil phospholipids (3). More recently a Iatroscan-Chromarod method also has been reported (4) for some oils. For other edible oils colorimetric methods (5-7) based on molybdenum blue complexes have been tried with varying degrees of success, while a gravimetric method is possible when large amounts of phospholipids are present as in rapeseed oil (8). In this paper we describe a simple analytical method for the determination of total phospholipids in crude palm oil. Correlations of phospholipid values with other quality parameters of palm oil are studied, and the effect of phospholipids on palm oil quality is discussed.

EXPERIMENTAL

Materials

Crude palm oil samples were obtained from a few palm oil mills in West Malaysia. Dipalmitoylphosphatidylcholine and dipalmitoylphosphatidylethanolamine were purchased from Sigma (St. Louis, Missouri), whereas phosphatidic acid was from General Biochemicals (Chagrin Falls, Ohio). All other chemicals were of analytical or reagent grades and were used without further purification. Solvents were redistilled before use. Modified Zinzadze's reagent was as described previously (3).

Visible absorption spectra were recorded on a Beckman DU-7 spectrometer in a 10 mm cell. Mechanical vessel shaking was carried out by an electric flask shaker (Griffin and George, Great Britain).

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Procedure

Crude palm oil (10.0 g) was stirred magnetically and refluxed with methanol-acetic acid (95:5, v/v, 20 ml) for 20 min. After cooling, the methanolic layer was separated and the oil was washed once with methanol (10 ml). The combined methanolic portion was rotary-evaporated to dryness, and chloroform (3 ml) was added immediately to redissolve the extract. The extract was then purified through a short column of acid-treated Florisil (2.5 g, 10 cm × 0.8 cm) by eluting with chloroform (10 ml), acetone (10 ml) and finally with methanol-acetic acid (98:2, v/v, 15 ml). The methanolic eluant was collected and rotary-evaporated to dryness in a 20 cm × 2 cm boiling tube. The purified phospholipids were determined by a procedure similar to those reported (6,7,9), but with modifications. Modified Zinzadze's reagent (4 ml) was added and the contents were well-shaken for 30 min. The blue complexes formed were extracted by shaking with hexane (5 ml) for 1 min. A visible spectrum was recorded and absorbance was read at 711 nm, λ_{max} for crude palm oil phospholipids. A sample reagent blank was found to be negligible. Hence, hexane can be used as blank.

The complex formation and extraction procedure was tested using both synthetic phosphatidylcholine and purified crude palm oil phospholipids for different time periods. Recovery of phospholipids by extraction was studied using a standard addition technique by spiking 5.0 g aliquots of crude palm oil sample with 100, 200 and 500 μg of synthetic dipalmitoylphosphatidylcholine in methanol. The phospholipids were then extracted and purified as described above.

The determinations of inorganic phosphates and total phosphorus are described in detail in the accompanying paper (10).

Phosphoric Acid Degumming of Crude Palm Oil

A sample of crude palm oil (100 g) was treated with 85% phosphoric acid (0.1 ml) at 90 C under argon for 20 min with magnetic stirring. Tonsil Optimum FF bleaching earth (2 g) was then added and further stirred at 90 C for 30 min. The hot oil was filtered through a sintered glass funnel (fine porosity) under partial vacuum. Ten g of the degummed oil were then analyzed for phospholipid content using the procedure described.

RESULTS AND DISCUSSION

The low levels of phospholipids in palm oil preclude the use of gravimetric methods for their determination. The thin layer chromatographic method (3) previously used by us is suitable but suffers from the disadvantage that relatively skillful techniques are required in order to obtain quantitative and reproducible results. The Iatroscan chromato-

graphic method of analysis described for vegetable oils (4) also faces the same disadvantage in addition to non-specificity of detection and the requirement of specialized equipment. Since phospholipids are known to form molybdenum blue complexes directly (5), a colorimetric method appears to be attractive. In crude palm oil there are relatively large amounts of inorganic phosphate (but not phytic acid) in comparison to phospholipids (3,10). However, colorimetric methods based on molybdenum blue can be used to analyze inorganic phosphates and phospholipids selectively due to differences in chemical properties; these complexes can be formed under different conditions. The phospholipid-molybdenum blue complex is soluble in non-polar solvents such as hexane and chloroform, whereas the inorganic phosphorus-molybdenum blue is soluble in polar solvents such as aqueous systems and alcohols. Thus, these complexes do not interfere with each other. The method described by Raheja et al. (6) involved complex formation of phospholipids with chromogenic solution after TLC separation, whereas Totani et al. (7) reported that phospholipids are extractable from vegetable oils by glacial acetic acid. These two methods are not directly applicable to crude palm oil, mainly due to the low levels of phospholipids and the interference caused by carotenoids present in palm oil.

We have found conditions to selectively extract phospholipids from crude palm oil by partitioning the oil matrix with acidified methanol. The extracts, however, still contained impurities which could interfere with direct color development with molybdate. A short pipette column of acidic Florisil was therefore used for the rapid removal of non-phospholipid impurities. Carotenoids, for example, were removed together with other non-polar lipids by chloroform elution. Phospholipids were eluted by acidic methanol and recovered before color development with modified Zinzadze's reagent. The optimum time required for the colored complex formation was found to be 30 min (Fig. 1). The phospholipid-molybdenum blue complex was extracted into hexane and the visible spectrum recorded; a typical spectrum is shown in Figure 2 with the peak absorption wavelength at 711 nm. The spectra of molybdenum complexes of phosphatidylcholine (PC), and phosphatidic acid (PA), also displayed in Figure 2, show only small differences. A reconstituted phospholipid mixture containing the major phospholipids in typical crude palm oil samples (i.e. 50%, 20% and 30% of PC, PE and PA respectively) gave a molybdenum blue complex with a spectrum essentially the same as that shown in Figure 2, i.e. having λ_{max} at 711 nm. Not much is known of the nature of the phospholipid-molybdenum complex, but the solubility characteristics indicate that the fatty acid chains are intact and this was verified from the proton nuclear magnetic resonance spectrum in deuterochloroform. It is possible that the complex may be similar to the orthophosphate-molybdenum blue complex (10) with as many as 5 molybdenum atoms to each atom of phosphorus (5).

Calibration curves for 3 phospholipid standards are presented in Figure 4, and the equations for the curves are as follows:

$$PA: Y = 7.52 \times 10^{-4} X - 0.011 \qquad r = 0.998$$

$$PC: Y = 7.77 \times 10^{-4} X - 0.000 \qquad r = 0.997$$

$$PE: Y = 7.68 \times 10^{-4} X - 0.007 \qquad r = 0.985$$

where r is the linear correlation coefficient. The absorption maxima and extinction coefficients are summarized in Table I, where a comparison of the molybdenum blue complex of orthophosphate is also given. Based on the

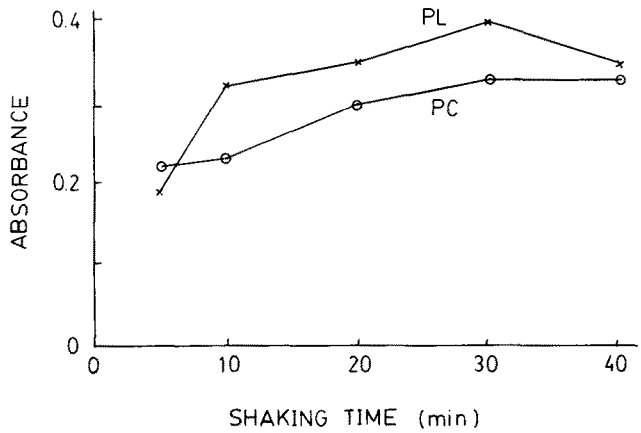

FIG. 1. Development of phospholipid-molybdenum blue complexes with time. (PL): Purified palm oil phospholipids. (PC): Phosphatidylcholine.

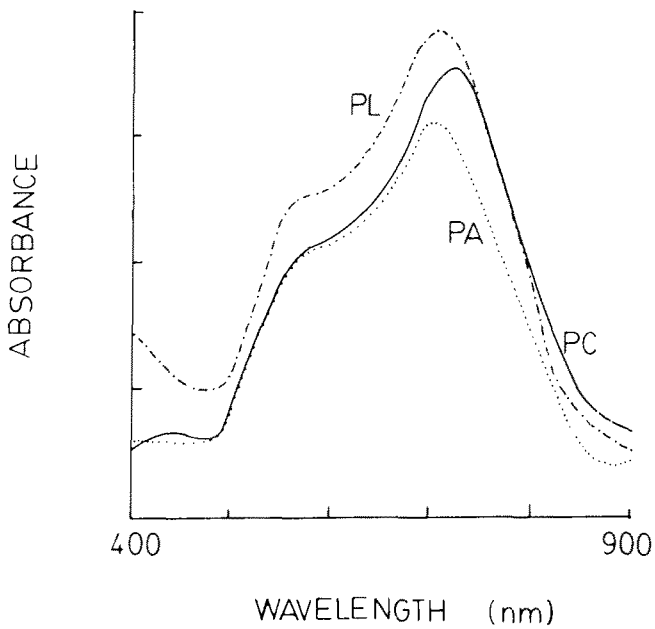

FIG. 2. Spectra of phospholipid-molybdenum blue complex. (PL): Palm oil phospholipids. (PC): Phosphatidylcholine. (PA): Phosphatidic acid. Phosphatidylethanolamine has a similar spectrum as PC above 500 nm.

TABLE I

Absorption Maxima and Extinction Coefficients of Phospholipids and Inorganic Phosphate as Molybdenum Blue Complexes

Compound[a]	Molecular weight	Maximum absorbance wavelength (nm)	Molar extinction coefficient[b]
DPPC	734.1	725	3,100
DPPE	692.0	725	3,000
PA	744	700	2,900
KH_2PO_4	136.1	820	26,600

[a]DPPC = dipalmitoylphosphatidylcholine. DPPE = dipalmitoylphosphatidylethanolamine. PA = phosphatidic acid, assumed as dioleyl, monosodium salt.

[b]All measured in hexane except for KH_2PO_4, which was in aqueous medium.

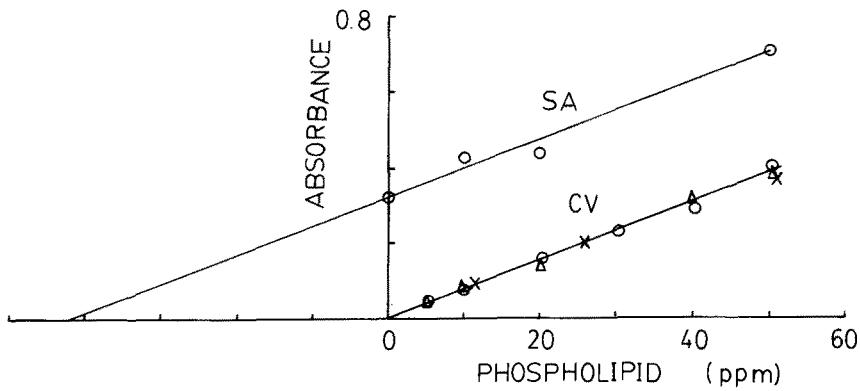

FIG. 3. Standard-addition and calibration curves of phospholipid-molybdenum blue complexes. (SA): Standard addition using phosphatidylcholine, based on 5.0 g oil samples. (CV): Calibration curve based on 10.0 g oil samples. (x——x): Phosphatidic acid (○——○): Phosphatidylcholine. (△——△): Phosphatidylethanolamine.

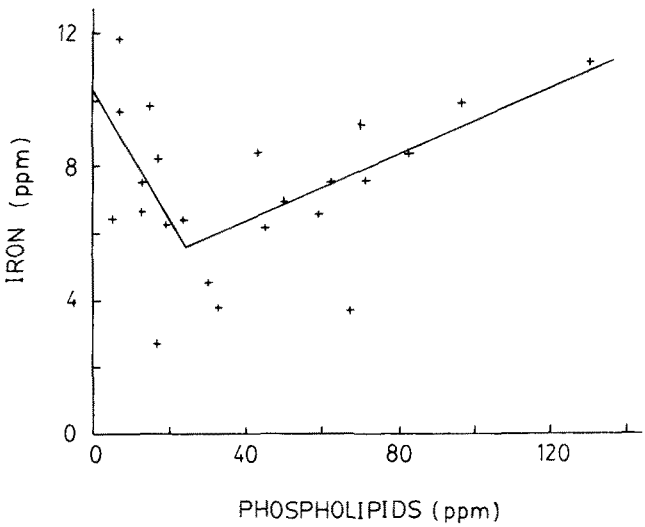

FIG. 4. Iron/phospholipid correlation.

TABLE II

Phospholipid Determination in Crude Palm Oil

Sample	Absorbance	Phospholipid (ppm)	As phosphorus[a] (ppm)
A	0.069	9	0.37
B	0.585 ± 0.031[b]	75 ± 4	3.1 ± 0.2
C	0.690 ± 0.038[c]	89 ± 5	3.7 ± 0.2

[a]Factor 24 was used for conversion of phospholipids to phosphorus; values encountered in this study range from 0.2 to 5.4 ppm.
[b]Replicate of 7.
[c]Replicate of 10.

phospholipid composition of the major components in crude palm oil, an average molar extinction coefficient of 3,000 may be used at 711 nm. The extinction coefficient of the complex is lower than that of the orthophosphate complex, but phospholipids at levels as low as 1 ppm can be determined readily. Table II shows typical results of determination of phospholipid content in crude palm oil samples and the equivalent phosphorus levels.

Recovery of phospholipids by the extraction method from crude palm oil is practically quantitative as shown by the same slope obtained for the calibration curve of PC and that of the standard addition method (Fig. 3). The phospholipid content obtained by the standard addition method (84 ppm as PC) is in good agreement with that obtained from the calibration curve (83 ppm).

TABLE III

Comparison of Phospholipid-phosphorus Analyzed by Different Methods

Sample	Method	Phospholipid-phosphorus (ppm)
A	AOCS[a]	0.42
	Colorimetry[b]	0.38
C	AOCS[a]	3.80
	Colorimetry[b]	3.71
	TLC[c]	3.3

[a]Modified AOCS method was used after extraction of phospholipids.
[b]Present method as described.
[c]Semi-quantitative method by thin layer chromatography as described in ref. 3.

Table III shows the results of palm oil phospholipid determinations using 3 methods: a semiquantitative estimate using TLC (3), a phosphorus assay of the ashings of purified phospholipids using modified AOCS method (10) and the colorimetric method described above. Good agreement between the values of the colorimetric and modified AOCS methods was observed. The value obtained by TLC was slightly lower, reflecting the lower precision of this method which is more suitable for rapid comparisons of phospholipid levels where high precision is not required.

The recovery of phosphorus from both the phospholipids and inorganic phosphates was found to be almost quantitative, as indicated by the good agreement between the sum of these two types of phosphorus determined independently and the total phosphorus determined by a modified AOCS method (Table IV).

For oil treated or "degummed" with phosphoric acid a new complication can arise as phospholipids react with phosphoric acid to form modified phospholipids containing possibly two or more condensed phosphate groups. TLC verified that modified phospholipids having low R_f values have been formed by phosphoric acid degumming. Phosphoric acid was found not to be effective for removal of palm oil phospholipids. For example, a sample containing 61 ppm of phospholipids was degummed by phosphoric acid, but the degummed oil still was found to contain 57 ppm of phospholipids. Therefore, phospholipid removal

TABLE IV

Phospholipid-phosphorus, Inorganic Phosphorus and Total Phosphorus in Crude Palm Oil

Sample	PLP[a] (ppm)	IP[b] (ppm)	TP[c] (ppm)	(PLP + IP)/TP × 100 (%)
A	0.38	8.88	9.88	93.7
B	3.17	8.03	11.54	97.0
C	3.71	6.60	10.89	94.7

[a]PLP = phospholipid-phosphorus, determined by present method as described. Factor 24 was used for conversion of phospholipid to phosphorus.

[b]IP = inorganic phosphorus, analyzed as in ref. 10.

[c]TP = total phosphorus, analyzed as in ref. 10.

was insignificant. In view of this fact it is doubtful that phosphoric acid "degumming" is essential since wet milling already ensures a low phospholipid content. In fact, in crude palm oil the level of phospholipid or even the total phosphorus content is lower than most of the phosphoric acid-degummed vegetable oils such as soybean, rapeseed, sunflower, peanut and corn oils.

We also have determined the total phospholipid content in 24 crude palm oil samples in order to study its correlation with other quality parameters such as Cu and Fe contents, extinction 233 and 269 nm values, and peroxide values. Results are summarized in Table V. Correlations usually are non-linear, and that for Fe is shown in Figure 4 where an interesting observation can be seen. Low concentrations of phospholipids (<20 ppm or 0.8 ppm as P) favor high concentrations of prooxidants Cu and Fe with an optimum concentration of ca. 1.3 ppm (as P) when the prooxidant concentrations are minimal. Similar profiles also are observed for correlations with extinction-233 nm and peroxide values. Qualitatively this is indicative of the fact that phospholipids do act as antioxidants or can act synergistically as antioxidants (12-15). The direct sequestering of soluble prooxidant ions or their hydrated forms by phospholipids to form inactive species is an attractive possibility. Results, however, show that higher levels of phospholipids also become undesirable as their relatively higher ease of oxidative breakdown [presumably due to the higher unsaturation (3)] causes an increase in "secondary" oxidation. This is evident in that a significant correlation is observed with the extinction 269 nm value (Table V), normally associated with secondary oxidation. It may be pointed out, however, that interpretation of the effect of relatively higher levels of phospholipids (>1.3 ppm as P) in palm oil may be obscured by the correspondingly high free fatty acid (FFA) values, there being a weak but significant linear correlation of FFA with phospholipid (see Table V). The observed result of high phospholipid levels may therefore be caused by increased levels of prooxidant metals which normally accompany increasing amounts of FFA.

TABLE V

Correlation of Phospholipid with Other Palm Oil Parameters

Parameter[a]	Linear correlation coefficient, r[b]
FFA	0.47
PV	0.22
AV	0.39
$\epsilon^{1\%}$ @ 233 nm	0.28
$\epsilon^{1\%}$ @ 269 nm	0.56
Fe	0.22
Cu	0.17
Residue red	0.02

[a]FFA = free fatty acid content (%); PV = peroxide value (m equiv/kg); AV = anisidine value; $\epsilon^{1\%}$ @ 233 nm and $\epsilon^{1\%}$ @ 269 nm are absorbances of 1% (w/v) oil solution in isooctane at 233 nm and 269 nm respectively; FE = iron content (ppm); Cu = copper content (ppm), and residue red = the Lovibond tintometer reading (5.25") of the oil after bleaching (in red unit).

[b]r = 0.47 significant at 99% confidence level. r = 0.34 significant at 95% confidence level.

The results of phospholipid research presented above and for a study of inorganic phosphate presented in a separate publication (10) indicate that, in palm oil, phospholipids are not directly detrimental to oil quality. Further studies need to be carried out in order to determine whether this is a general phenomenon in edible oils.

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REFERENCES

1. Evans, C.D., P.M. Cooney, C.R. Scholfield and H.J. Dutton, JAOCS 31:295 (1954).
2. Lezajic, J., I. Mezei, N. Dordevic and F. Smit, Bilt. Bil. Ulja 1:3 (1973).
3. Goh, S.H., H.T. Khor and P.T. Gee, JAOCS 59:296 (1982).
4. Du Plessis, L.M., and H.E. Pretorius, Ibid. 60:1261 (1983).
5. Galanos, D.S., Lipids 5:573 (1970).
6. Reheja, R.K., C. Kaur, A. Singh and I.S. Bhatice, J. Lipid Res. 14:695 (1973).
7. Totani, Y., H.E. Pretorius and L.M. du Plessi, JAOCS 59:162 (1982).
8. Standard Methods for the Analysis of Oils, Fats and Derivatives, 6th Edition, 1979, Part 1, IUPAC, Pergeman Press, England, Method 2.422.
9. Sandhu, R.S., Clin. Chem. 22:1973 (1976).
10. Goh, S.H., S.L. Tong and P.T. Gee, JAOCS 61:1601 (1984).
11. Crouch, S.R., and H.V. Malmstadt, Anal. Chem. 39:1084 (1967).
12. Working, E.B., Oil Soap (Chicago) 13:261 (1936).
13. Wisenhahn, G.A., Ibid. 14:119 (1937).
14 Eichberg, J., Ibid. 16:51 (1939).
15. Hudson, B.J.F., and S.E.O. Mahgoub, J. Sci. Food Agric. 32:208 (1981).

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