Inorganic Phosphate in Crude Palm Oil: Quantitative Analysis and Correlations With Oil Quality Parameters

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ABSTRACT

A convenient method, not involving ashing or acid digestion, for the determination of inorganic phosphorus content in crude palm oil, is developed. Direct extraction from solvent-diluted palm oil by dilute acid allows the inorganic phosphates to be analyzed by the usual phosphomolybdenum blue complex. Using this method, phosphate analyses in palm oil samples were correlated with other oil quality parameters and the results discussed.

INTRODUCTION

Recent studies (1-2) have shown that for crude and refined palm oils the phosphorus content may be an essential specification of oil quality. In an earlier study (3) we have inferred that in commercial crude palm oil inorganic phosphate accounts for most of the total phosphorus (ca. 20 ppm) analyzed, and phospholipids are present only in lesser amounts (0.8-3.3 ppm as P). Althouth these quantities of phosphorus in crude palm oil are relatively small in comparison to other edible oils, e.g. crude soybean oil contains about 600 ppm of P, the phosphorus content has been implicated as a possible cause of lower oil quality encountered in some samples. This appears to contradict earlier views on the role of phosphorus or phospholipids in oil, since it has been documented previously (4-7) that phospholipids have antioxidant properties or can act synergistically as antioxidants. We believe that separate and even conflicting roles may be played by inorganic phosphates and phospholipids. Furthermore, methods of phosphorus determination commonly employed. i.e. IUPAC and AOCS methods (8-9), analyze only for the total phosphorus content but do not distinguish these two forms of phosphorus. The recently developed atomic absorption method using an electrothermal atomizer (10-11) may be a convenient alternative, but this also analyzes for the total phosphorus. In general, colorimetric methods are more likely to gain wider acceptance for routine quality control because of the simplicity of equipment and low cost. These methods are not without drawbacks, because the necessity of ashing and/or acid digestion makes them inconvenient. In this paper we have developed a method suitable for the routine determination of inorganic phosphates in palm oil samples, taking into account the relatively low concentration of P and obviating the necessity of ashing or acid digestion procedures. Using the method developed we have examined the inorganic phosphorus content of several palm oil samples; correlations of P with other quality parameters for palm oil were examined and the overall effects of phosphorus on oil quality discussed.

EXPERIMENTAL

Materials

Crude palm oil samples were obtained from a few palm oil mills in West Malaysia. Pure dipalmitoylphosphatidylcholine and phosphatidylethanolamine were purchased from Sigma (St. Louis, Missouri) and phosphatidic acid from General Biochemicals (Chagrin Falls, Ohio). The phospholipid purity was checked by thin-layer chromatography (TLC) before use. All other chemicals were of analytical or pure reagent grade and were used without further purification. Solvents were redistilled before use.

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). Atomic absorption was performed on an Instrumentation Laboratory model 151 aa/ae spectrometer, and microanalysis was carried out by Microanalytical Laboratory, National University of Singapore, Singapore. A stock standard phosphate solution containing 100 ppm P was prepared by dissolving 0.43868 g of dry potassium dihydrogen phosphate in 1.00 L of distilled water.

Method

Colorimetric analyses of inorganic phosphorus components were performed by first extracting the palm oil with dilute mineral acids; the orthophosphate content then was determined by a conventional phosphomolybdenum blue method (12). Typically a crude palm oil solution of 20% (w/v) in hexane was prepared. A 5 ml aliquot of this solution was transferred to a 50 ml flat bottom flask containing 5 ml of 2M HCl. Extraction was carried out by magnetic stirring and reflux for at least 20 min. After cooling, aqueous hanging droplets if present were taken down with a small amount of hexane from a dropper and the aqueous extracts transferred to a test-tube using a dropper. Four ml of the extract was pipetted into a 20 ml volumetric flask and neutralized by dropwise addition of 50% (w/v) potassium hydroxide (phenolphthalein indicator). The solution immediately was made acidic again by the addition of two drops of concentrated hydrochloric acid. Eight ml of 0.015% (w/v) aqueous hydrazine sulfate solution and 2 ml of 2.5% (w/v) sodium molybdate solution in 5M dilute sulfuric acid were added and the flask well shaken. The mixture was heated in boiling water for 10 min and after cooling made up to 20 ml. The spectrum and absorbance were recorded in a 10 mm cell against a distilled-water blank, and the absorbance was corrected for that of the reagent blank of the same dilution. Typical observed absorbances ranged from 0.3 to 0.7 for crude palm oil samples containing inorganic phosphate in the range 9-20 ppm of P and less than 0.01 absorbance unit for reagent blanks. Phosphorus levels were read from a calibration curve obtained from orthophosphate standards treated under the same conditions. Results obtained are shown in Table I. Under the conditions used the molar extinctions at 820 nm and 660 nm were 26,600 and 9,300 absorbance unit/g mole P respectively. Concentration of phosphorus in the test solution is read from the calibration curve or calculated using the following equation:

$$\text{ppm of P} = A/\epsilon \times W \times 10^6/1000$$

where A = absorbance at 820 nm, ϵ = molar extinction at 820 nm = 26,600, W = atomic weight of phosphorus = 30.9738. Concentration of phosphorus in the oil sample

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TABLE I

Extraction of Inorganic Phosphorus from Crude Palm Oil with 2M HCl

Sample	Extraction time (min)	Inorganic phosphorus (ppm)
A	20	9.06 ± 0.17[a]
B	20	13.66
B[b]	20	13.31
B[c]	20	13.71
C	10	8.43
C	20	8.54[d]
C	30	8.48
D	20	7.52
D[e]	15	7.50 ± 0.12[a]
E[e]	15	14.99
E[e]	15	14.90[f]
F	20	18.97
F	20	18.74[g]

[a]Replicate of 6.

[b]4M HCl used for extraction.

[c]2M H_2SO_4 used for extraction.

[d]Second extraction provided 0.003 absorbance unit (0.09 ppm P).

[e]Cold extraction by alternative method.

[f]Standard addition method, recovery = 101.4%, linear correlation coefficient, r = 0.999.

[g]Standard addition method, recovery = 104.6%, r = 0.998.

can then be calculated as follows:

$$\text{ppm of P in oil} = A/\epsilon \times W \times 10^6/1000 \times V/w \times 5/4 = 29.11\ A$$

where V = volume of the test solution = 20.0 ml, w = weight of oil sample used = 1.00 g, and the factor 5/4 is required, as only 4 ml out of the 5 ml acid extract were used in the present experiments.

Alternative Method of Extraction

1.00 g of crude palm oil was weighed into a 20 × 2 cm stoppered boiling tube, and 5 ml each of hexane and 2M hydrochloric acid were added. The tube was well shaken by a mechanical shaker for 15 min after which the contents were boiled briefly to break up any emulsion that may have formed. After cooling, 4 ml of the aqueous extract was pipetted into a 20 ml volumetric flask and heated in a boiling water bath for 15 min. On cooling the solution was neutralized by potassium hydroxide and treated as described above before colorimetric determination. This alternative method is suitable for laboratories where magnetic stir-refluxing apparatus is not normally available.

Optimization of Inorganic Phosphate Extraction

A study of the time required to achieve maximum extraction of inorganic phosphate was carried out using the stir-reflux method, and the results are as shown in Table I. The effects due to the use of different acids at various concentrations were studied. Efficiencies of single and double extractions also were compared.

Effect of Phospholipids

The effect of phospholipids present on the determination of inorganic phosphate was studied by adding phosphatidylcholine (179 μg), phosphatidylethanolamine (195 μg) and phosphatidic acid (254 μg) into the palm oil solutions before extraction. The results are shown in Table II. TLC using solvent systems as described previously (3) was used to check the purity of phospholipid standards. Possible interference by glycerol phosphate also was investigated.

TABLE II

Effect of Phospholipids on Inorganic Phosphorus Determination in Crude Palm Oil

Sample	Added phospholipid[a] (ppm) (as P in ppm)	Inorganic P (ppm)
A	PA, 254 (10.6)	9.21
A	0	9.24
D	PC, 179 (7.6)	7.52
D	PE, 195 (8.7)	7.55
D	0	7.52

[a]PA = phosphatidic acid; PC = phosphatidylcholine, and PE = phosphatidylethanolamine.

Recovery from Standard Addition

Additions of 0, 5, 10 and 20 μg of inorganic P as KH_2PO_4 solution (100 ppm) were made to 5 ml samples of 20% (w/v) palm oil in hexane solution and were thoroughly mixed by magnetic stirring. Extractions were carried out by stir-reflux with 2M hydrochloric acid as described above. Results obtained are shown in Table I.

Total Phosphorus Determination

Determination of the total phosphorus content including both inorganic and organic forms in oil was made using the AOCS method (9) with slight modifications. The time of the acid digestion of the ash residue was increased from 10 to 30 min, and no further dilution was made to the 50 ml flask used. Using this method and that described above for inorganic phosphorus, 24 samples of crude palm oil were studied to compare and correlate the phosphorus constituents with other quality parameters.

Isolation of Inorganic Phosphate Constituents

Dilution of 150 g of crude palm oil with ca. 27 ml of hexane or petroleum ether and centrifuging the solution at 2000 rpm for 25 min, at 40 C gave 0.1 g of a sticky residue (varied widely with samples) containing a relatively high content of phosphorus (ca. 1500 ppm) as determined by the ashing procedure of AOCS. Phospholipids can be extracted from the residue by chloroform and methanol. The remaining insoluble residue analyzed for 1.70% Ca, 0.30% P, 2.20% Mg, 1.64% Fe, 30.66% C, 4.42% H, and 1.77% N. The Fe, Ca, Mg and P accounted for 25% of the ash of the residue, while the carbon/hydrogen analyzed was presumably from cellulose, which is quite abundant in the meshings of the palm fruit. The residue containing phosphorus was studied by TLC and proton nuclear magnetic resonance (NMR) spectra and found not to contain phytic acid, glycerol phosphate or glucose phosphate. The behavior, however, was like calcium and iron phosphate or their condensed phosphate forms. These inorganic phosphates were dissolved in dilute acids and determined as phosphomolybdate as described above for inorganic phosphate.

RESULTS AND DISCUSSION

The quantitative extraction of inorganic materials such as iron and copper from oil and fat samples using dilute strong acid solutions has been reported previously (13,14). Dilution of oil samples with suitable organic solvents not only prevents excessive emulsion formation but also facilitates the extraction. In fact, solvent dilution of crude palm oil

helps to precipitate colloidal insoluble matter which can then be isolated by centrifugation. The precipitated residue contained significant amounts of inorganic phosphates and other inorganic materials. It also was found that the inorganic phosphates in the oil could readily be extracted out by dilute mineral acids after solvent dilution of the oil. Results obtained for the extraction of 20% solutions of crude palm oil in hexane, as presented in Table I, show that the extraction of inorganic phosphate was complete after 20 min of vigorous stirring at reflux temperature. A longer extraction time does not significantly increase the phosphate extracted; e.g., the phosphorus level found in a second consecutive extraction increased by only 1% of the result from a single extraction, indicating a high efficiency in the extraction partition. Hydrochloric acid of 2M strength was found to be adequate, while acids of higher molarities tested did not give better results. The alternative method of extraction was found to be equally efficient (Table 1), and this may be convenient for the analysis of a large number of samples (e.g., the electric shaker can handle 8 samples at a time).

Samples also were analyzed by standard addition using potassium dihydrogen phosphate, and the results are shown in Table I. It can be seen that quantitative recovery of the added phosphorus was achieved, and this agrees with the phosphorus content obtained by a calibration curve within experimental error (Table I).

The effect of phospholipids in crude palm oil on the determination of inorganic phosphate also was examined. Phosphatidic acid, phosphatidylcholine and phosphatidylethanolamine, when added in about 4 to 5 times the average amounts normally encountered in crude palm oil, were found not to increase the value of inorganic phosphate extracted (Table II). This means that the decomposition of phospholipids during the course of the extraction was negligible. The effect of glycerol phosphate, if any were present, also was shown to be negligible. Under similar experimental conditions, it was found that glycerol phosphate contributed only 0.005 absorbance unit per ppm of glycerol phosphate solution. Phospholipids are known to form weak blue complexes with molybdate (15,16), but these are soluble in organic rather than aqueous media and therefore will not contribute significantly to the present analyses. In aqueous-acidic solutions presently used (pH < 0.3) only the orthophosphate-molybdenum blue complex was analyzed colorimetrically; this complex is likely of the form described by Crouch (17), i.e. 5 moles of Mo(VI) combined with 1 mole of phosphate, and since the stability of the complex is dependent on the acidity, care should be exercised to maintain the acidity if dilution needs to be carried out, e.g. from samples containing very high levels of phosphate.

The possible interference by other impurities to the phosphomolybdenum blue color also was examined. Under the experimental conditions, silicate was coextracted at up to 30 ppm but did not interfere with the determination of orthophosphate at 10 ppm level. Other possible interfering agents, arsenic and germanium, may be ignored as they are present, if at all, at only trace levels and therefore will not have any significant effect on the determination.

Using the procedure for the analysis of inorganic phosphate as described, we have analyzed 24 samples of crude palm oil. Total phosphorus content was determined by the AOCS ashing method, and the difference between the two methods generally compares well with the phospholipid content as described in the accompanying paper (15). Linear correlations with other commonly used oil quality parameters were attempted, and the results are summarized in Table III. A strong linear correlation was observed between inorganic phosphate and free fatty acid, and this is illustrated in Fig. 1. Free fatty acid content does not correlate very well with phospholipid values (15), but the apparent linear correlation of total phosphorus with free fatty acid (Table III) is due to the good correlation with inorganic phosphate, which is in a large excess (ca. 8 times) over phospholipid phosphorus. A significant correlation of inorganic phosphate with iron may be due to the fact that both constituents already correlate with free fatty acid; otherwise it would mean that iron is present in the phosphate or mixed phosphate forms. Weak but significant correlations between inorganic phosphate with the extinction values (at 233 and 269 nm) may similarly be caused by the good correlations of inorganic phosphate with free fatty acid and iron contents. The two extinction values at best approximately reflect the content of oxidation products, the former with hydroperoxide/peroxide content and the latter with conjugated degradation products arising from further decomposition of peroxides (18). An increase of oxidized products as reflected by the extinction values may therefore be expected from increasing iron content in the oil. Noteworthy is the result that poor oil bleachability (residue red) does not appear to be due to inorganic phosphate, which means that free fatty acid alone or with prooxidant metals may be the primary cause of this and perhaps most other problems related to crude palm oil deterioration. However, phosphate or rather phosphoric acid can be problematic as it will react with phospholipids during "degumming" (15) at the refinery, and the products formed have to be removed by Tonsil or bleaching earths. The origin of inorganic phosphate in crude palm oil was not determined, but because the palm fruit oil contains 40-45 ppm phosphorus in phospholipid form and phospholipase D activity was detected (3), it is likely that inorganic phosphate arises mainly from the phospholipids, presumably by phospholipase action and/or further chemical degradation. This behavior may be confined to palm oil as damaged fruits invariably suffer biochemical degradation in transit to the mill.

As far as we know the proposed method is the only method that analyzes selectively for inorganic phosphates in palm oil. This provides for a convenient and inexpensive method for the quantitative speciation of inorganic phosphorus in palm oil. As inorganic phosphate rather than phospholipids represents the major phosphorus component

TABLE III

Linear Correlation of Inorganic and Total Phosphorus Content with Other Palm Oil Parameters

Parameter[a]	Linear correlation coefficient, r[b]	
	Total phosphorus	Inorganic phosphorus
FFA	0.71	0.72
PV	0.10	0.23
AV	0.00	0.21
$\epsilon^{1\%}$ @ 233 nm	0.29	0.40
$\epsilon^{1\%}$ @ 269 nm	0.53	0.44
Fe	0.50	0.63
Cu	0.03	0.14
Residue Red	0.06	0.00

[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.

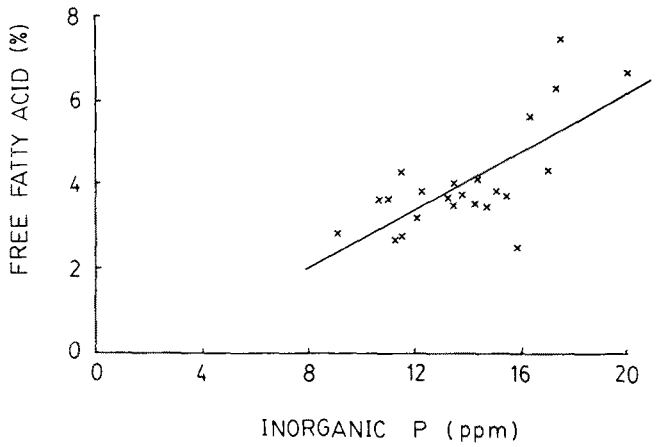

FIG. 1. FFA/inorganic phosphorus correlation.

in crude palm oil and probably also in refined palm oil which has been treated with phosphoric acid, routine quality control using this method would be preferable as ashing and/or acid digestion are not necessary.

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☘A Convenient Preparaton of γ-Lactones and Dialkyltetrahydrofurans From the Reaction of Fatty Acids with Epoxides Using Lithium Naphthalenide

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ABSTRACT

Fatty acids reacted with epoxides using lithium naphthalenide in the presence of diethylamine to give corresponding 4-hydroxy acids. These 4-hydroxy acids easily tended to cyclize into their corresponding γ-lactones by refluxing in benzene. Reduction of these γ-lactones with lithium aluminum hydride followed by intramolecular dehydration with potassium bisulfate afforded corresponding dialkyl tetrahydrofuran derivatives in high yields. For example, 4-methyl-2-(8-nonenyl) γ-butyrolactone (III) was obtained from 10-undecylenic acid and propylene oxide. 2-Methyl-4-(8-nonenyl) tetrahydrofuran (IV) was produced from (III). 2-Methyl-4-(8-nonenyl) and 2-ethyl-4-(8-nonenyl) tetrahydrofurans are woody smelling and may be used as perfumery materials.

INTRODUCTION

As perfumery and flavor materials, various alcohols, aldehydes, ketones, ethers, esters and lactones are available (1). Syntheses of compounds containing those functional groups are widely investigated, and extensive preparation methods for lactones are well known (2-9).

Preparation methods for γ-lactones from lower fatty acids and epoxides also are reported (10-13). However, reactions of higher fatty acids and epoxides are not well investigated. Recently, we reported that lithium naphthalenide is an excellent reagent for various synthetic organic reactions (14-16). In connection with these studies of lithium naphthalenide, we now report the reaction of higher fatty acids and epoxides using lithium naphthalenic in the presence of diethylamine. A variety of new γ-butyr lactones were obtained, and the conversion of these butyrolactones to tetrahydrofuran derivatives was pe formed by the reduction with lithium aluminum hydric followed by intramolecular dehydration with potassiu bisulfate.

EXPERIMENTAL

Reaction of 10-Undecylenic Acid (I) with Propylene Oxide (II)

To 0.1 mol (12.8 g) of naphthalene in 150 ml of tetr hydrofuran, 0.2 mol (1.4 g) of metallic lithium cutting was added, and the mixture was agitated at room temper ture in an atmosphere of dry nitrogen. After 1 hr, 0.2 m (14.6 g) of diethylamine was added. After agitation fo 1 hr, 0.1 mol (18.4 g) of 10-undecylenic acid, I, in tetr hydrofuran (100 ml) was slowly added. After 2 hr, 0.2 m (11.6 g) of propylene oxide II was added to the reactio mixture, which was left overnight. The mixture was re fluxed for an additional 8 hr. The acidic materials wer separated as reported previously (11) to give a mixture o unreacted I and the 4-hydroxy acid. The acidic materia mixture was dissolved in 300 ml of benzene, refluxed fo 8 hr and cooled to room temperature. The benzene solutio