Extraction of Phospholipids from Plant Oils and Colorimetric Determination of Total Phosphorus

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ABSTRACT

A rapid extraction procedure to isolate phospholipids from plant oils is described. Glacial acetic acid is used as solvent and the extracted phospholipids are quantified by means of a colorimetric method.

INTRODUCTION

Existing methods for the determination of total phosphorus in lipids usually involve digestion (1-3), saponification (4), or chromatographic separation (5-7) followed by colorimetry. In this study, a rapid extraction method was developed to concentrate the phospholipid fraction of plant oils sufficiently for colorimetric determination.

The colorimetric reagent previously used by Vaskovsky and Kostetsky (8) and Raheja et al. (9) has been modified by us.

EXPERIMENTAL PROCEDURES

All chemicals were analytical reagent grade except where otherwise stated. L-α-Phosphatidylcholine (PC, egg yolk) was purchased from Sigma Chemical Co., St. Louis, MO. Lecithin (soybean) was obtained from Tokyo Kasei, Tokyo, and glycerol trioleate from BDH Chemicals, Poole, England. Soy oil samples were obtained from S.A. Oil Mills, Randfontein, whereas the sunflower seed oils were obtained from Nola Industries, Randfontein, Republic of South Africa. A Gilford 2400-S spectrophotometer was used for absorbance readings.

EXTRACTION OF PHOSPHOLIPIDS

Phospholipids were extracted from the plant oil with glacial acetic acid. One g of oil and 1 mL acetic acid were placed into a 10-mL glass test tube equipped with a ground-glass stopper. The tube was vigorously shaken for 1 min and afterwards centrifuged for about 1 min at ca. 1,200 × g. The ratio of oil to acetic acid can be changed, depending on the phospholipid content of the plant oil. In the crude plant oil, a ratio of oil to acetic acid of 1:2 can be used.

Aliquots of the acetic acid layer, containing between 1 and 12 μg P, are removed with a microsyringe for the colorimetric analysis. If the volume of the aliquot exceeds 50 μL, as in the case of refined oils, the appropriate volume is concentrated to below 50 μL by evaporation (30 C, reduced pressure); or by freeze-drying. This is necessary to prevent interference in the color development by excess acid.

CHROMOGENIC REAGENT

The preparation of the chromogenic reagent is done according to the description of Vaskovsky and Kostetsky (8) and Raheja et al. (9) except that tin (II) chloride is used instead of mercury.

Ammonium molybdate (1.6 g) is dissolved in 12 mL of hot water to give solution I. A few drops of concentrated hydrochloric acid and 0.1 g of tin (II) chloride are shaken with 8.0 mL of solution I for 30 min to give solution II.

Concentrated sulfuric acid (20 mL) is added carefully to the remainder of solution I and the resultant solution is added to solution II to give solution III.

Methanol (4.5 mL) and 2.0 mL water are added to 2.5 mL of solution III to produce the chromogenic solution, which is stable for at least 3 months when stored at 5 C.

COLORIMETRIC DETERMINATION

An aliquot of the acetic acid extract is added to 0.4 mL chloroform and 0.1 mL chromogenic reagent in a stoppered test tube. The tube is immersed into boiling water for ca. 1 min, then left to cool to room temperature. Chloroform (5 mL) is added to each test tube and the bottom layer (chloroform layer) removed for spectrophotometric analysis. It was found that the color was stable for at least 90 min. The phospholipid content was calculated using the formula:

$$\text{Total phospholipids (\%)} = \frac{b \times c \times 25}{a \times d} = 100,$$

where a = acetic acid extract sampled (μL); b = total phosphorus (μg) derived from standard curve; c = volume acetic acid (μL) used for extractions; and d = weight of oil (μg).

The absorption maximum for the prussian blue-phospholipid complex was found to be 735 nm (Fig. 1).

RESULTS AND DISCUSSION

A linear calibration curve for PC solutions in *n*-hexane, as measured by our chromogenic reagent (λmax. = 735 nm), was obtained. The least-squares method was applied to calculate the line of best fit for the data ($y = 0.031657x + 0.0080666$, correlation coefficient $\gamma = 0.99975$; for $0 < x \leq 12$). The recovery of PC added to glycerol trioleate was 99.5% with a coefficient of variation of 3.2% for 6 replicates. Recovery studies were also conducted at various extraction temperatures in the range 20-40 C and it was

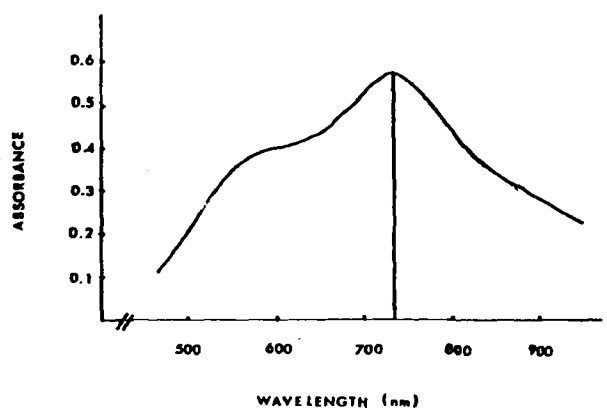

FIG. 1. Spectral curve of prussian blue-phospholipid complex.

found that the temperature change did not affect the recovery significantly.

Soybean and sunflower oils, at various stages of refinement, were analyzed by this method and the results are presented in Table I. Crude and degummed oils presented no problems during the analysis; however, care must be exercised with refined oils in the extraction and concentration stages to obtain reproducible results.

The main advantage of the proposed method is that ashing or digestion of the oil, prior to the colorimetric phosphorus analysis, is unnecessary. The proposed extraction procedure is simple and inexpensive.

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R. Bagnari made the phosphorus determinations.

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TABLE I

Phospholipid Content of Oils

Sample	% Phospholipids[a]	Coefficient of variation (%)
Soya oil		
Crude	1.17 ± 0.05[b]	4.3
Degummed	$(2.37 \pm 0.120) \times 10^{-2}$	5.1
Refined	$(1.49 \pm 0.01) \times 10^{-4}$	0.7
Sunflower seed oil		
Crude	$(2.48 \pm 0.15) \times 10^{-1}$	6.0
Degummed	$(1.24 \pm 0.07) \times 10^{-2}$	5.6
Neutralized	$(9.24 \pm 0.68) \times 10^{-4}$	7.4
Winterized	$(6.91 \pm 0.60) \times 10^{-4}$	8.7
Refined	$(5.83 \pm 0.42) \times 10^{-4}$	7.2
Glycerol trioleate PC solution	1.53 ± 0.07	4.6

[a]Mean of 10 replicates.
[b]Standard deviation.

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Oxidation of Unsaturated and Hydroxy Fatty Acids by Ruthenium Tetroxide and Ruthenium Oxyanions

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ABSTRACT

The reactions of ruthenium VIII tetroxide (RuO_4) and the ruthenium VII and VI oxyanions, perruthenate (RuO_4^-) and ruthenate ($RuO_4^=$) with hydroxy substituted and unsaturated fatty acids have been studied. At a 1:1 molar ratio, ruthenium tetroxide (RuO_4) and both oxyanions (RuO_4^- and $RuO_4^=$) oxidized 12-hydroxystearic acid to 12-ketostearic acid. With 9,10-dihydroxystearic acid, the type of oxidation products obtained depended on the amount of ruthenium oxidant used. At high ratios of oxidant to substrate, cleavage to pelargonic and azelaic acids occurred whereas at lower ratios, partial oxidation to diketo and acyloin derivatives predominated. The oxidation of oleic acid with excess ruthenium tetroxide (RuO_4) or perruthenate anion (RuO_4^-) gave the cleavage products pelargonic and azelaic acid through the intermediate formation of dihydroxy and diketo intermediates. Ruthenate anion ($RuO_4^=$) did not react with the double bond of oleic acid.

INTRODUCTION

Ruthenium tetroxide (RuO_4) is recognized as one of the more potent oxidizing agents of organic substrates (1-6). In practice, RuO_4 is conveniently generated from a catalytic amount of ruthenium dioxide and a large excess of an oxygen donor such as sodium meta-periodate or sodium hypochlorite (5); these oxidants also regenerate RuO_4 from any RuO_2 product formed during the oxidation of the organic substrate. The latter reoxidation procedure with RuO_2 has been successfully used for the oxidation of cyclobutanols to cyclobutanones (1), substituted benzene compounds to alicyclic carboxylic acids (1), alkynes to α-diketones or carboxylic acids (3), cycloalkanes to cycloalkanones or carboxylic acids (5), and cycloalkenes to aldehydes (7). In a previous study, we reported a procedure for the oxidative cleavage of monounsaturated fatty acids using ruthenium dioxide and sodium hypochlorite (8,9). The use of sodium hypochlorite as the cooxidant required that the reaction be done under basic conditions to prevent the formation of chlorine-containing compounds. However, ruthenium tetroxide is reported to be stable only below pH 7 (10). Under alkaline conditions (pH 7-12), ruthenium tetroxide is reduced to perruthenate anion (RuO_4^-) and at pH greater than 12, the perruthenate anion is further reduced to ruthenate anion ($RuO_4^=$) (10). When catalytic amounts of ruthenium dioxide together with large amounts of sodium hypochlorite are used for the oxidation of unsaturated fatty acids under alkaline conditions, it is unclear which ruthenium species are formed and which ones are oxidizing the unsaturated fatty acid. To better understand the reaction pathway of the oxidation and to identify the

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