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# Isolation and Characterization of Egg Lecithin

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A modified method for the isolation of chromatographically and spectroscopically pure egg lecithin is described and the final product is characterized. Instead of the customary ethanol or chloroform-methanol, diethyl ether is used for extraction in this method. The chromatography is carried out on a silicic acid-silicate column with 6:1 and 3:1 (v/v) mixtures of chloroform and methanol, pure methanol, and pure chloroform as eluting solvents.

The special precautions taken to avoid degradation and oxidation of the product were deoxygenation of the solvents and addition of 4-methyl-2,6-*tert*-butylphenol (BHT) as antioxidant. Moreover, the chromatography was carried out at +4°C under nitrogen.

The values obtained in the characterization of the product are compared with those reported earlier by other authors. Similar values were obtained for the percentages of nitrogen, phosphorus, glycerol, and total fatty acids, but the fatty acid composition differs considerably. This stresses the importance of a well-defined method for the isolation of egg lecithin.

No satisfactory method for the isolation of egg lecithin was found in the literature when the author first undertook the study of its physical-chemical properties. The major features required of such a method are that it is fast and cheap and gives a homogeneous and undegraded product.

The classical method for the isolation of egg lecithin is the cadmium chloride procedure.[1] Isolation with solvents and column chromatographic methods are nowadays used. Before the extraction of the phospholipids, egg yolks are usually treated with acetone, whereupon dehydration takes place at the same time as the neutral lipids are dissolved and the phospholipids are precipitated.[2–5] Lyophilization before extraction with acetone has also been used.[3] Ethanol [2,4,5] and chloroform-methanol mixtures [3] have been used to extract the crude phospholipids.

Silicic acid [4] and alumina,[2,5] alone and in combination, have been used in the column chromatographic separation of the phospholipids isolated by solvent extraction. It has been reported that the silicic acid-silicate column is the only one that separates lecithin and sphingomyelin quantitatively.[6] Ethanol [2] and various methanol-chloroform mixtures have been used as

eluents.[3–5] Degradation of lecithin in alumina columns has been reported by Renkonen.[7] In contrast, no degradation has been detected in silicic acid columns.[8]

The results of earlier characterizations of egg lecithin differ considerably.[2,5,9] This shows that different methods may give products of variable composition. Because the physical-chemical properties of lecithins vary with the fatty acid composition, it is of great importance that the isolated product is homogeneous and well-defined. IR spectra of egg lecithin have apparently not been reported earlier, but density values for egg lecithin have been presented by Elworthy.[10]

## EXPERIMENTAL

*Materials and methods.* The yolks used in the work were taken from fresh eggs and the treatment was begun immediately after the whites had been separated.

All solvents were guaranteed reagents from E. Merck AG. They were deoxygenated and 0.005 % 4-methyl-2,6-*tert*-butylphenol (BHT) was added as an antioxidant.

Silica Gel G, according to Stahl, E. Merck AG, was used in the TLC analyses. The silicic acid used in column chromatography was an analytical reagent (100 mesh) from Mallinckrodt that had been treated according to Rouser and co-workers.[6]

The column used was a Pharmacia column of type SR 25/45 which was connected to a pressure vessel containing the solvent.

The lecithin standard was chromatographically homogeneous L-α-lecithin from the California Biochemical Corporation.

In the chemical characterization of the lecithin, glycerol was estimated according to Renkonen,[11] nitrogen with a Coleman Model 29 Nitrogen Analyzer and phosphorus by the perchloric acid procedure.[12] The fatty acid composition was estimated by GLC of their methyl esters on a Perkin-Elmer F 11 Gas Chromatograph equipped with a column of Chromosorb W coated with EGGS-X. The temperature was programmed from 150 to 180°C at a rate of 4°C/min. Nitrogen was used as carrier gas. Total fatty acids were determined by the method of Tattrie.[9]

The IR spectrum was recorded with a Perkin-Elmer Model 700 Infrared Spectrophotometer equipped with a sodium chloride cell. The solvent was carbon tetrachloride. The UV spectrum was measured with a Perkin-Elmer Model 402 UV-visible Spectrophotometer using petroleum ether as solvent.

The thin-layer plates were developed as described by Skipsky and co-workers.[13]

The density was measured by the displacement method.[10]

The lyophilization was carried out in a Christ Model L 2 Freeze Dryer.

*Extraction of crude phospholipids.* The yolks (about 60 g) of twelve eggs were lyophilized overnight. The resulting cake was homogenized by mixing with 150 ml of diethyl ether in a Waring blendor. The mixture was filtered and the filtrate evaporated almost to dryness in a rotary evaporator. The crude phospholipids were precipitated by adding 150 ml of cold acetone. The precipitation was repeated by dissolving the phospholipids in 10 ml of cold diethyl ether, filtering to remove any undissolved matter and adding 100 ml of cold acetone. The solvent was then removed and the crude phospholipids were stored under nitrogen at −20°C until further treated. The average yield of crude phospholipids was 4 % based on the weight of the fresh yolks.

*Column chromatographic separation.* The Pharmacia type SR 25/45 column that was used was filled with 100 g of activated silicic acid−silicate. The crude phospholipids were applied in 5 ml of chloroform. The load was 1 mg P/g $SiO_2$. The flow rate was held at 2 ml/min with compressed nitrogen, and 10 ml fractions were collected on a fraction collector. The chromatography was carried out at +4°C in a thermostated room. The eluting solvents were (1) 200 ml of 6:1 chloroform:methanol, (2) 150 ml of 3:1 chloroform: methanol, (3) 150 ml of pure methanol, and (4) 200 ml of pure chloroform. After such an elution sequence, the column was ready to be used again. The cephalin was eluted by solvent (1), the lecithin by solvent (2), and sphingomyelin and lysolecithin by solvent (3).

After the elution (6 h), the contents of the tubes were analysed by TLC. The tubes containing pure lecithin were evaporated to dryness under nitrogen in a rotary evaporator. The product was stored in acetone at −20°C or, if it was used at once, was lyophilized and dried to constant weight over phosphorus pentoxide under nitrogen in a vacuum desiccator. The average yield of pure lecithin from 60 g of fresh yolks was 1.6 g.

*Characterization of the product.* The purity of the final product was tested by TLC. Only one spot was detected on charring with sulphuric acid. Because this method is sensitive to 0.1 μg of lipids, the product was regarded as chromatographically pure (see Fig. 1).

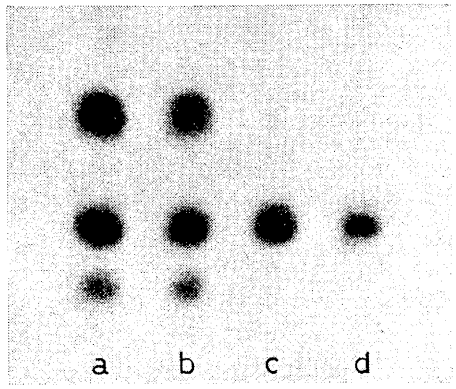


*Fig. 1.* Thin-layer chromatograms of (a) total phospholipids in egg yolk, (b) phospholipids applied to the column, (c) final product, (d) synthetic L-α-lecithin.

As further evidence of the purity of the product, the IR and UV spectra were recorded. The spectra showed that the product was spectroscopically pure and undegraded (see Figs. 2 and 3).

The results of the chemical analyses of the pure product can be compared with those of Hanahan *et al.*,[2] Singleton *et al.*,[5] and Tattrie [9] in Table 1.

The fatty acid composition, expressed as percentages of the total fatty acids in the homogeneous egg lecithin, determined by GLC analysis in this work and those reported by Hanahan *et al.*,[2] Singleton *et al.*,[5] and Tattrie,[9] is as shown in Table 2.

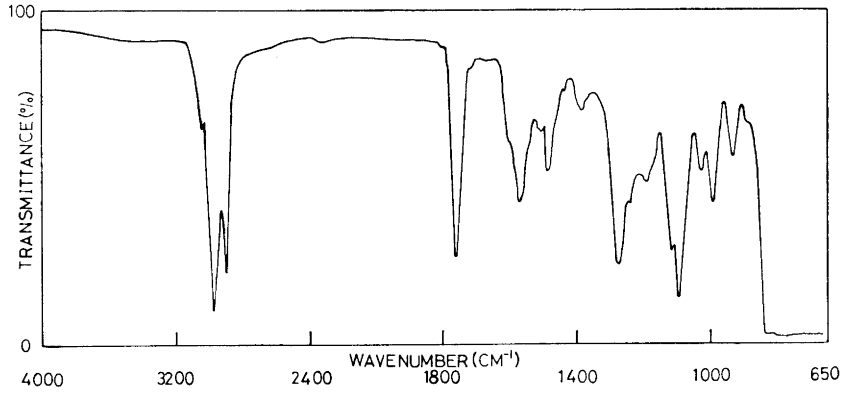


*Fig. 2.* IR spectrum of pure egg lecithin in carbon tetrachloride. The absence of the sphingomyelin absorption band at 1640 $cm^{-1}$ is to be noted.

*Table 1.* Analytical data of the isolated egg lecithin compared with those found by other authors.

| | This work | Ref. 2. | Ref. 5. | Ref. 9. |
|---|---|---|---|---|
| N, % | 1.82 | 1.79 | – | 1.82 |
| P, % | 4.02 | 3.90 | 3.97 | 3.94 |
| Glycerol, % | 12.00 | – | – | – |
| Fatty acids, % | 71.2 | 70.0 | 69.5 | 70.3 |

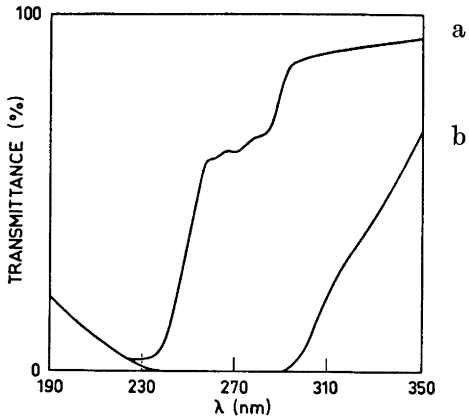


*Fig. 3.* UV spectra of pure egg lecithin in petroleum ether. Curve (a) is the spectrum of lecithin stored one week in acetone at – 20°C and curve (b) that of lecithin stored one week in air at + 4°C.

*Table 2.* Fatty acid composition of the isolated egg lecithin compared with that found by other authors.

| Fatty acid | This work | Ref. 2. | Ref. 5. | Ref. 9. |
|---|---|---|---|---|
| Palmitic | 35.9 | 32.0 | 37.7 | 35.7 |
| Palmitoleic | 0.9 | 1.0 | 3.1 | Trace |
| Stearic | 18.4 | 16.0 | 9.2 | 14.9 |
| Oleic | 28.7 | 30.0 | 32.9 | 37.0 |
| Linoleic | 13.9 | 17.0 | 17.0 | 12.4 |
| Arachidonic | 2.2 | – | – | – |

The average molecular weight of the fatty acids calculated from these values is 273.4. According to this average value the molecular weight of the egg lecithin was 768. The measured density of the lecithin was 1.018 g/cm³.

## DISCUSSION

Much time is saved in evaporating the solvent by using diethyl ether instead of ethanol or a chloroform:methanol mixture for the extraction. At the same time most of the sphingomyelin, which is insoluble in diethyl ether,

is separated. Most of the cephalin could have been separated by extraction with ethanol, but this is of lesser importance because it is much easier to separate cephalin than sphingomyelin from lecithin by chromatography. Because the evaporation of ethanol is very lengthy, this solvent was not used. One important condition that must be fulfilled when diethyl ether is used for extraction, however, is that the yolk has been completely dried. Besides lyophilization, dehydration with acetone was tried; but this proved less effective.

The chromatography was performed at about +4°C in order to avoid oxidation and degradation during column chromatography as far as possible. This required the testing of a particular elution sequence in chromatography at this temperature. Water was avoided so that the column would remain active. With the elution schemes described the column retained its activity for up to one year.

The data obtained in the analysis of the pure egg lecithin agree fairly well with those reported in earlier investigations. However, divergent fatty acid compositions are obtained when different isolation methods are used. This shows the importance of a well-defined method for the separation of egg lecithin because its molecular weight and physical-chemical qualities vary with the fatty acid composition.

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