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## Phosphatidylcholine as a More Specific Basis for Egg Yolk Determination in Cosmetic Products

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### Introduction

With the availability of soy lecithin and semi-synthetic organic phosphoric acid esters (Emulsifier YN, Hostaphat and Crodafos esters\*) as food or cosmetic ingredients the reliability of egg yolk determinations based on lipoid-phosphor (3) became questionable. A much used alternative method based on cholesterol (4, 5), which is not so accurate because of great natural variations of cholesterol levels in egg yolk, has also become unreliable with introduction of technical cholesterol or cholesterol containing ingredients (e. g. woolfat) as cosmetic ingredients or in the presence of phytosterols containing food ingredients such as flours (6).

This work is an attempt to investigate a more specific determination of egg yolk, which could allow to detect the above-mentioned «adulterations». The proposed method is based on the most important phospholipid of egg yolk, namely phosphatidylcholine (abbreviated in this report as «PC»). By suitable solvents systems, PC is easily separated on a TLC plate from many ingredients of the products, such as neutral lipids and detergents and also from the other phospholipids of egg yolk or from other sources according to *Skipsky et al* (7). After TLC isolation the PC is quantified by GLC via its fatty acids methyl esters according to *Christie* (8); the fatty acid pattern is characteristic for the origin of the PC. By this way technical soy lecithin\*\*, which contain approx. 13% PC, is easily detected.

Quantification of the PC fraction from TLC was also done by colorimetry after extraction from the silica, destruction with a perchloric acid mixture and colour development with a molybdate reagent according to *Chen et al* (9). There was a fair agreement in the obtained data with those determined by gaschromatography.

\* Emulsifier YN (Cadbury Ltd, UK)  
Hostaphat emulsifiers (Hoechst, W. Germany)  
Crodafos emulsifiers (Croda GmbH, W. Germany).

\*\* From AKZO, Emmerich, W. Germany.

For determining the natural variations of PC contents of egg yolk, seven egg yolks of different origin were carefully separated and then determined for their PC contents by the proposed TLC-GLC and TLC-colorimetry methods. The observed variations of the PC contents are similar to the variations in lipid- $P_2O_5$  found by previous workers. Using the data obtained from these seven egg yolks we propose a multiplication factor of 17.4 for the % PC to obtain the % egg yolk. This factor is based on a PC content of 5.75%, which represents a mean value of egg yolks of diverse origins.

The proposed method has been applied to the analysis of egg yolk containing cosmetics, namely egg shampoos, for which the Dutch Cosmetic Act (10) requires a minimum of 1.6% egg yolk. As will be shown in this report, adulterations of soy lecithin or organic phosphoric ester emulsifiers do not influence the obtained data.

Finally some examples of the TLC isolation of PC from several food products are given. Such work is in progress in our laboratory and will be reported later.

### Experimental part

The product is extracted for total-lipids according to the method of *Bligh and Dyer* (11). By TLC on ordinary precoated silicaplates PC (phosphatidylcholine) is isolated using the solvent system of *Skipsky et al.* (7) and after preelution with a mixture of equal volumes of chloroform-methanol and acetone to remove neutral lipids and other interfering substances. The PC fraction is analysed by GLC according to *Christie* (8). For this purpose the PC is transesterified to fatty acid methyl esters that are analysed by GLC. From the obtained data the PC contents can be calculated and also the presence of soy lecithin can be deducted. By multiplication of the % PC by the factor 17.4 the egg yolk contents of the product is calculated.

The PC fraction can also be analysed by micro-phosphor colorimetric determination (9) after extraction from the silica, destruction with a perchloric acid mixture and colour development with molybdate. This phosphor determination, however, does not detect the presence of soy lecithin. The method is used in our work to check the obtained PC data from GLC.

### Reagents

For extraction: Chloroform, methanol

Anhydrous sodium sulphate

Buffer solution pH 8.4 (37.5 volumes of HCl 0.1 M + 62.5 volumes borax 0.05 M).

For TLC: Solvent mixture I: chloroform-methanol-acetone (equal volumes)

Solvent mixture II: chloroform-methanol-acetic acid-water (50+30+8+4, v/v)

Dichlorofluoresceine spray: 0.02% in methanol

Egg yolk reference solution: isolate fresh egg yolk and remove the white as much as possible. Dissolve in ethanol to obtain a solution of 1 ml = 20 mg egg yolk. Filter if necessary.

For GLC: Sodium methanolate 0.3 M

Internal standard solution: dissolve 94 mg methylheptadecanoate in 250 ml chloroform

Chloroform, pentane, acetic acid.

For micro-phosphor colorimetric determination:

Sulfuric acid conc., nitric acid conc. (65%), perchloric acid (60%)

a) Ascorbic acid 10% in water. Keep cool. Stable for several weeks

b) Ammonium molybdate 4 H<sub>2</sub>O: 2.5% in water

c) Sulphuric acid 6 N

Molybdate reagent: mix solutions (a)—(b)—(c) and water (1+1+1+2, v/v).

Prepare daily.

KH<sub>2</sub>PO<sub>4</sub> p. a.

### *Apparatus and laboratory aids*

Sepfunnels 100 ml.

Laboratory centrifuge.

Precoated silicaplates 0.25 mm, Schleicher and Schüll F1500 (art. 355100).

Test plates for resolution of phospholipids (in particular phosphatidylcholine) with egg yolk reference solution (compare with fig. 4—6).

NB. For lipid rich extracts (mayonnaise, salad dressing etc.) use silicaplates with «concentration zone», for instance: Merck 60F254 with concentration zone (art. 11798).

GLC column: glass, 10% DEGS on Chromosorb WAW DMCS, 1/4 inch x 150 cm. Column temperature 150 °C.

Heating block (aluminium) with thermostatic control (60 °—150 °—250 °C). Cylindrical holes (13 mm x 70 mm) approx. 20 holes. Dimensions (approx. 120 mm diameter, 90 mm high).

Test tubes for destruction 12 mm x 100 mm

Glass reaction vials 5 ml with screw cap.

### *Extraction*

(Total lipid extraction according to *Bligh and Dyer* (11).)

Weigh accurately in a sepfunnel *s* grams sample, which should contain 200—500 mg egg yolk\*.

* Egg shampoos with ca. 2% egg yolk:	<i>s</i> = 10 g
Mayonnaise with ca. 6% egg yolk:	4 g
Salad or frites dressing with ca. 2%:	10 g
Macaroni/egg noodle with ca. 5%:	5 g
Egg biscuit with ca. 1% egg yolk:	20 g
Advocate (licquor) with ca. 10% yolk:	2 g

Add 50 ml methanol and 50 ml chloroform. Mix.

Add 36 ml buffer solution of pH 8.4. Shake 30 s.

Leave mixture until phase separation. If necessary centrifuge in suitable flask and remove upper layer by suction. Use lower chloroform layer. Dry over anhydrous sodium sulphate. Decant chloroform fraction. Flush with 2 x 4 ml chloroform. Evaporate solvent from combined chloroform filtrates under reduced pressure. Dissolve residue in a mixture of chloroform-methanol (1+1, v/v) to volume of 10.0 ml.

### *Thinlayerchromatography*

Divide 20 x 20 cm silicaplates in 5 sections of 4 cm. Each section can be used for the TLC of 100  $\mu$ l of the total lipid extract. If a micro phosphor determination will be made, the middle section of the plate is used as a blank. In this report — as mentioned before — each extract has been analysed by «GLC PC-fatty acid» as well as by «colorimetric micro-phosphor» determination for comparison. So the 5 sections of a plate were used as follows: Section I and II for the GLC analysis, section III as a blank for the micro-phosphor analysis and sections IV and V for the micro-phosphor analysis.

Apply 100  $\mu$ l of the total lipid extract on the starting line over approx. 4 cm.

NB. Lipid rich extracts, for example from mayonnaise or frites/salad sauces, require the special TLC plates with a «concentration zone».

Elute first with solvent mixture I to remove neutral lipids to front. The polar lipids (including PC) remain practically on the start. Time approx. 3 h. Dry plates for 10 min at 80 °C. Cool to room temperature. Elute with solvent mixture II to separate PC from the other phospholipids. Time approx. 1 h. Dry plate at room temperature. Visualize with dichlorofluoresceine spray and viewing under 254 nm UV light. Mark zones with pencil. Spray plates with water. The silica turns to pink, the neutral lipids become yellow, while the phospholipids — including PC — remain white.

Localization of PC in the beginning of the work is aided by using the reference egg yolk standard in one of the sections. After some experience such an aid is not needed.

### *GLC fatty acids analysis of PC fraction*

Remove quantitatively marked PC fraction from TLC plate, and pulverize carefully. Heat with 1.5 ml sodium methanolate 0.3 M at 50 °C during 30 min with continuous stirring (magnetic). Cool. Neutralize with 1 drop acetic acid. Add 100  $\mu$ l of the internal standard solution and then 1.5 ml of water. Extract succesively 3 times with 5 ml portions of ether. Dry combined ether fractions with sodium sulphate. Remove ether by blowing with nitrogen. Dissolve residue in 200  $\mu$ l pentane. Inject 3  $\mu$ l for GLC. Calculate % PC from integrated peak areas by using following formula:

$$\% \text{PC} = \frac{V \cdot A \cdot F \cdot 100 \cdot 1.371}{I \cdot s \cdot 10^6}$$

- $V$  = total peak area methyl fatty acids  
 $I$  = peak area internal standard  
 $A$  = dilution factor: total ml extract / ml extract on TLC plate  
       In this report: 10/0,1 = 100  
 $s$  = grams sample  
 $F$  = 100  $\mu$ l internal standard = 37.6  $\mu$ g

NB. The factor 1.371 is the multiplication factor to convert methyl fatty acid amount to PC, as used by *Christie* (12). It is also to be calculated from the ratio of the masses of PC and the C 16:0 methyl ester: 778/568 = 1.370.

GLC-conditions: Column: glass filled with 10% DEGS on Chromosorb WAW DMCS,  $\frac{1}{4}$  inch x 150 cm. Temperature 150 °C.

#### *Colorimetric micro-phosphor determination of the PC fraction*

Remove quantitatively marked PC fraction from TLC plate. Extract PC quantitatively with 3 times 2 ml of a warm mixture of chloroform-methanol (1+1, v/v) by means of a micro glass filter (ca. 2 ml). Promote extraction by pulverizing silica with glass rod. Avoid silica particles in the filtrate, which cause errors in the colorimetric determination.

Keep combined filtrates in the small test tubes for destruction. Put a carborundum stone in test tube to aid boiling. Heat test tube in heating block and set heat on 60 °C. Blow constantly nitrogen above solvent surface, as this will avoid splashes. To dry residue add 3 drops conc. sulfuric acid, 8 drops of nitric acid and 4 drops of perchloric acid. Increase heat to 150 °C until black residue is obtained. Then — while keeping hot — add 1 drop nitric acid. Repeat addition of nitric acid 6—8 min. Usually a destruction time of 45 min is sufficient. Then heat for 15 min at 250 °C. Cool to room temperature. Add 3 ml of water and 4 ml of molybdate reagent. Heat mixture at 55 °C (waterbath) during 1 h. Cool and measure absorbance at 820 nm against water. A blank portion of scraped silica (middle section of TLC plate: see thinlayerchromatography) at the height of PC should undergo the same manipulations as described above and is used as a correction of the colorimetric measurements.

A calibration curve must be made, to find the phosphor content of the sample. Standard solutions are made from the following stock solution: Dry  $\text{KH}_2\text{PO}_4$  at 105 °C until constant weight. Weigh 0.4393 g and dissolve in 1 l water. Pipet 50.0 ml and dilute further to 1 l with water. This solution will contain 5  $\mu$ g  $P$  per ml. Develop colour as described above with respectively 0—0.4—1.2—1.6—2.0 ml of the standard phosphate solution. These solutions contain respectively: 0—2—6—8—10  $\mu$ g  $P$ .

Calculate PC contents from obtained data with the following formula:

$$\% \text{ PC} = 25.4 \times \frac{P \cdot A}{s} \cdot 10^{-4}$$

in which P =  $\mu\text{g}$  phosphorous found in the PC fraction

A = dilution factor: total ml extract / ml extract on TLC plate

In this report:  $10/0.1 = 100$

s = grams sample.

### Results and Discussion

The proposed methods for PC were tested for repeatability on one egg yolk sample. The results shown in table 1 have proofed the reliability of the methods.

The fatty acids composition of ovo- and soy phosphatidylcholine were determined using the proposed TLC-GLC method. Table 2 compares the results with

*Table 1.* Repeatability of the proposed methods

One freshly isolated egg yolk sample has been determined several times for phosphatidylcholine by the proposed methods

Method 1: TLC-Colorimetry

Method 2: TLC-GLC

%PC by Method 1		Method 2	
	5.73		5.84
	6.03		5.74**
	5.74		5.615**
	5.92		5.315**
	6.07		5.75**
	5.96		5.485**
	(5.11*)		5.47**
	5.59		5.575**
	(5.07*)		5.84**
			5.925**
			5.64
			5.76
			5.595**
			5.73
Mean	5.86		5.66
s	= 0.18		s = 0.17
n	= 7		n = 14

\* Discarded data. Probably losses during destruction.

\*\* Mean of two determinations on 1 TLC plate.

those from previous workers. There is a fair agreement between the data, so that characterization of ovo- and soy-PC can be done by the obtained fatty acids pattern. Ovo-PC is characterized by the fatty acids pattern of:

C 16:0—35% C 18:0—15% C 18:1—34% C 18:2—16% C 18:3—0%

as soy-PC has:

C 16:0—13% C 18:0—3% C 18:1—8% C 18:2—69% C 18:3—5%

Table 2. Fatty acids composition of ovo- and soy phosphatidylcholine

Ovo phosphatidylcholine	C 16:0	C 18:0	C 18:1	C 18:2	C 18:3
Egg yolk no 1	36 <sup>0</sup> / <sub>0</sub>	13 <sup>0</sup> / <sub>0</sub>	35 <sup>0</sup> / <sub>0</sub>	16 <sup>0</sup> / <sub>0</sub>	—
Egg yolk no 2	36 <sup>0</sup> / <sub>0</sub>	14 <sup>0</sup> / <sub>0</sub>	35 <sup>0</sup> / <sub>0</sub>	15 <sup>0</sup> / <sub>0</sub>	—
Egg yolk no 3	30 <sup>0</sup> / <sub>0</sub>	20 <sup>0</sup> / <sub>0</sub>	35 <sup>0</sup> / <sub>0</sub>	15 <sup>0</sup> / <sub>0</sub>	—
Egg yolk no 4	34 <sup>0</sup> / <sub>0</sub>	15 <sup>0</sup> / <sub>0</sub>	34 <sup>0</sup> / <sub>0</sub>	17 <sup>0</sup> / <sub>0</sub>	—
Egg yolk no 5	31 <sup>0</sup> / <sub>0</sub>	16 <sup>0</sup> / <sub>0</sub>	37 <sup>0</sup> / <sub>0</sub>	16 <sup>0</sup> / <sub>0</sub>	—
Egg yolk no 6	34 <sup>0</sup> / <sub>0</sub>	16 <sup>0</sup> / <sub>0</sub>	34 <sup>0</sup> / <sub>0</sub>	16 <sup>0</sup> / <sub>0</sub>	—
Average	35 <sup>0</sup> / <sub>0</sub>	15 <sup>0</sup> / <sub>0</sub>	34 <sup>0</sup> / <sub>0</sub>	16 <sup>0</sup> / <sub>0</sub>	—
NB. Compare with following data of previous workers:					
Lundberg (1973) (15)	36 <sup>0</sup> / <sub>0</sub>	18 <sup>0</sup> / <sub>0</sub>	29 <sup>0</sup> / <sub>0</sub>	14 <sup>0</sup> / <sub>0</sub>	—
Hanahan (1951) (16)	32 <sup>0</sup> / <sub>0</sub>	16 <sup>0</sup> / <sub>0</sub>	30 <sup>0</sup> / <sub>0</sub>	17 <sup>0</sup> / <sub>0</sub>	—
Singleton (1961)	38 <sup>0</sup> / <sub>0</sub>	9 <sup>0</sup> / <sub>0</sub>	33 <sup>0</sup> / <sub>0</sub>	17 <sup>0</sup> / <sub>0</sub>	—
Tattrie (1959) (17)	36 <sup>0</sup> / <sub>0</sub>	15 <sup>0</sup> / <sub>0</sub>	37 <sup>0</sup> / <sub>0</sub>	12 <sup>0</sup> / <sub>0</sub>	—
Supelco (in Lipid Reporter, 1975)	34 <sup>0</sup> / <sub>0</sub>	13 <sup>0</sup> / <sub>0</sub>	35 <sup>0</sup> / <sub>0</sub>	17 <sup>0</sup> / <sub>0</sub>	—
Soy phosphatidylcholine***	C 16:0	C 18:0	C 18:1	C 18:2	C 18:3
Soy lecithin no 1*	14 <sup>0</sup> / <sub>0</sub>	4 <sup>0</sup> / <sub>0</sub>	6 <sup>0</sup> / <sub>0</sub>	69 <sup>0</sup> / <sub>0</sub>	7 <sup>0</sup> / <sub>0</sub>
Soy lecithin no 2**	12 <sup>0</sup> / <sub>0</sub>	3 <sup>0</sup> / <sub>0</sub>	10 <sup>0</sup> / <sub>0</sub>	68 <sup>0</sup> / <sub>0</sub>	7 <sup>0</sup> / <sub>0</sub>
Average	13 <sup>0</sup> / <sub>0</sub>	3 <sup>0</sup> / <sub>0</sub>	8 <sup>0</sup> / <sub>0</sub>	69 <sup>0</sup> / <sub>0</sub>	7 <sup>0</sup> / <sub>0</sub>
NB. Compare with following data from (Supelco Lipid Reporter, 1975)					
Vegetable lecithin (soy ?)	18 <sup>0</sup> / <sub>0</sub>	4 <sup>0</sup> / <sub>0</sub>	12 <sup>0</sup> / <sub>0</sub>	61 <sup>0</sup> / <sub>0</sub>	5 <sup>0</sup> / <sub>0</sub>

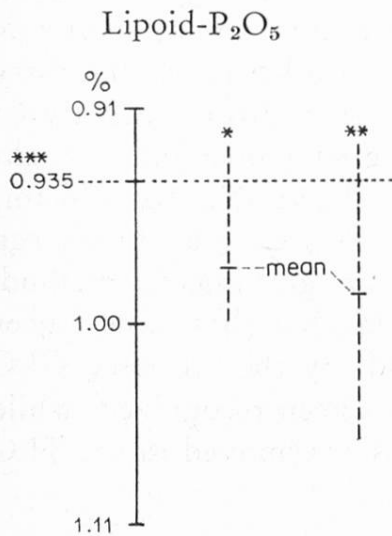
\* Commercial soy lecithin. Origin unknown.

\*\* From Akzo, Emmerich, W. Germany.

\*\*\* Soy lecithin contains two other important phospholipids: Phosphatidylethanolamine and phosphatidylinositol. (See fig. 4 and 6.)

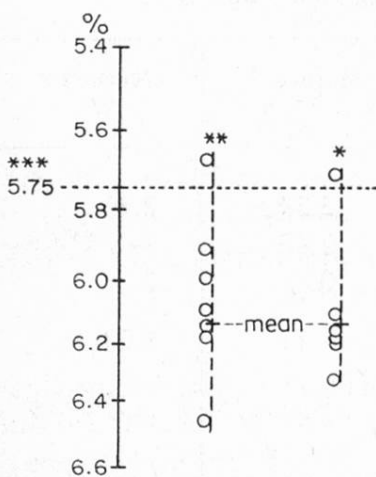


A crucial criterium for the validity to use PC as a basis for egg yolk determinations is the natural variations of the PC contents. Not sufficient data could be found in literature. So seven eggs from different origin were carefully selected, and the yolks separated for the work. The PC has been determined by both methods and the results are shown in figure 1, in which a comparison is made with the natural variations of lipoid  $P_2O_5$  (the basis for many official methods of egg yolk determinations). It seems that these seven egg yolks have variations of PC contents, which are of the same order of those lipoid- $P_2O_5$  as found from literature sources. We can therefore conclude that PC can be used as a basis for



Sources: \* Food Inspection Lab. of Amsterdam (1963) (13), from 8 egg yolks of different origin.  
 \*\* Data from Walter (1951) (14), 78 egg yolks of different origin.  
 \*\*\* Dutch Food Law (Warenwet) (3) adopted % Lipoid- $P_2O_5$  0.935%.

Phosphatidylcholine  
(this report)



	%PC	
	*	**
Egg yolk (fresh) no 1	6.18	6.46
Egg yolk (fresh) no 2	6.41	6.10
Egg yolk (fresh) no 3	6.12	5.98
Egg yolk (fresh) no 4	6.19	6.14
Egg yolk (fresh) no 5	6.27	6.15
Egg yolk (fresh) no 6	6.24	5.92
Egg yolk (fresh) no 7	5.69	5.66

Average % PC 6.06 6.07

Commercial egg yolk (bulk material) 4.48 4.56

\* TLC-colorimetry  
 \*\* TLC-GLC  
 \*\*\* Proposed % phosphatidylcholine for egg yolk analysis: 5.75% PC

Fig. 1. Variability of lipoid- $P_2O_5$  and phosphatidylcholine in egg yolks of different origin

egg yolk determinations. We are, however, fully aware that such a statement must be confirmed by many more determinations in the future.

In figure 1 a commercial sample of egg yolk (in bulk) shows a contents of 4.5% PC. This is not unexpected, as bulk egg yolk material always contain residual egg white.

It was necessary to define a multiplication factor for PC to obtain the egg yolk percentage. The factor of 17.4 is based on a PC content of 5.75%. The choice of a lower PC content rather than the mean value has been made from the same practical reasons as has been done by previous workers in the past for lipid  $P_2O_5$ . This is clearly seen in figure 1.

The proposed methods were then tested to analyse «model» egg shampoos, which were prepared in our laboratory by additions to a commercial shampoo, consisting of the conventional ingredients, such as fatty acids ether sulphates, fatty acid diethanolamide and ethyleneglycolmonostearate. The additions are stated in table 3. Shampoo 1 can be considered as a «true» egg shampoo. No 2 might be a falsification using soy lecithin as the phospholipid additive. No 3 is a shampoo that does not contain any phospholipid, but nevertheless gave a «false» egg yolk content of 10% (!) as determined by the lipid- $P_2O_5$  gravimetric method. No 4 and 5 are egg shampoos with a low egg yolk content, but that have higher egg yolk ciphers if determined by the lipid- $P_2O_5$  method. By the proposed GLC method in all these cases adulteration with soy-lecithin has been recognized, while adulterations with organic phosphoric acid esters were easily removed in the TLC isolation step.

Table 3. Analysis of modelshampoos

Addition to a sample of a commercial shampoo	% Egg yolk found by		
	Method 1 *	Method 2 **	Method 3 ***
1. Freshly isolated egg yolk 2.27%	2.35	2.34	2.35
2. Soy lecithin 1.16%*****	4.64	2.40	— ****
3. Trioleylphosphate 2.01%*****	10.30	0	0
4. Mixture of egg yolk 1.14% + trioleylphosphate 1.0%	6.40	1.11	1.04
5. Mixture of egg yolk 1.14% + soy lecithin 0.58%	3.45	2.01	— ****

\* Via lipid- $P_2O_5$ , gravimetric (Dutch official method for mayonnaise).

\*\* Proposed TLC-colorimetry method via phosphatidylcholine.

\*\*\* Proposed TLC-GLC method via phosphatidylcholine.

\*\*\*\* Presence of soy lecithin detected.

\*\*\*\*\* Hostaphat KO 300 N (Hoechst).

\*\*\*\*\* From Akzo, Emmerich, W. Germany.

We have also analysed eight commercial samples of egg shampoos from the Dutch market (table 4). Most of them do contain the minimum of 1.6% egg yolk that has been defined by law (10). Four samples (no 2—3—4—5) showed much lower contents by the proposed methods than by the gravimetric lipid- $P_2O_5$  method. No adulteration of soy lecithin has been detected from the GLC data. Discrepancies can be attributed to other organic phosphor compounds. No search has been made for the actual cause.

Figures 2 and 3 have been added for the sake of clarity, which are respectively obtained from the determination of PC and its fatty acids composition of one of the egg yolks mentioned in table 2 and of a commercial egg shampoo (no 5

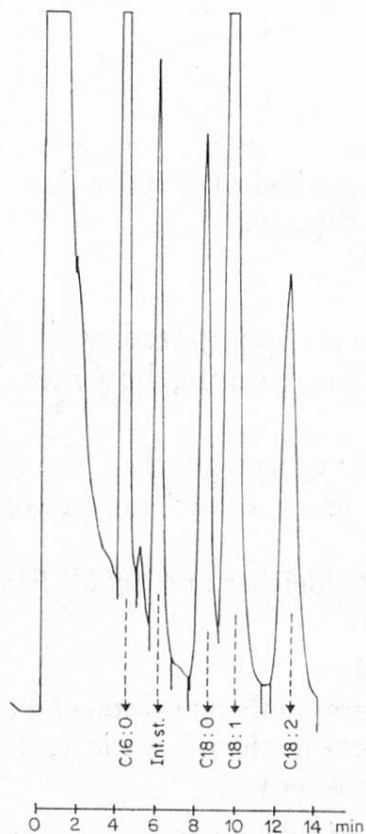


Fig. 2. GLC of methylesters of ovo phosphatidylcholine fatty acids TLC-GLC determination of PC in a fresh egg yolk sample

Column: Glass, 10% DEGS on Chromosorb WAW DMCS, 1/4 inch X 150 cm. Temperature 150 °C  
 Calculated from integrated data: 5.73% phosphatidylcholine  
 With fatty acids composition:  
 34.9% C 16:0  
 15.0% C 18:0  
 33.1% C 18:1  
 16.9% C 18:2

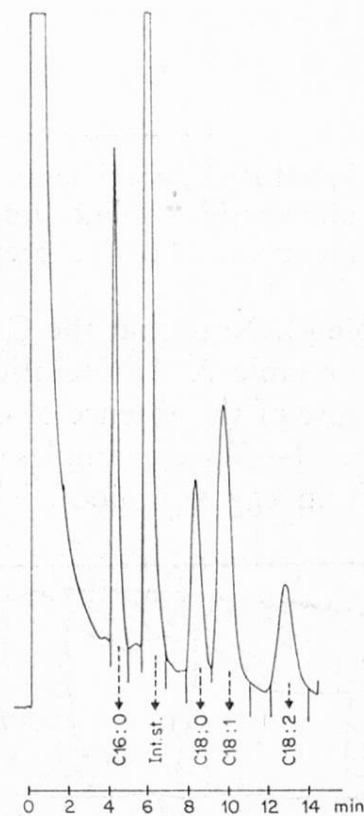


Fig. 3. GLC of methylesters of phosphatidylcholine fatty acids, isolated from a commercial egg shampoo TLC-GLC determination of PC in an egg shampoo

Column: See text  
 Calculated from integrated peak areas: 0.0747% PC, corresponding with  $0.0747 \times 17.4 = 1.30\%$  egg yolk. The fatty acids composition is:  
 28.1% C 16:0  
 20.2% C 18:0  
 35.2% C 18:1  
 16.4% C 18:2  
 indicating that no soy lecithin is present

Table 4. Analysis of commercial egg shampoos (1978, The Netherlands)  
% Egg yolk according to 3 methods

Sample no	Method 1 *	Method 2 **	Method 3 ***
1	2.58	2.05	2.05
2	1.74	1.16	1.02
3	1.54	1.12	1.10
4	1.60	0.99	0.96
5	1.32	0.96	0.97
6	1.74	1.74	1.68
7	1.62	1.63	1.56
8	1.89	1.63	1.68

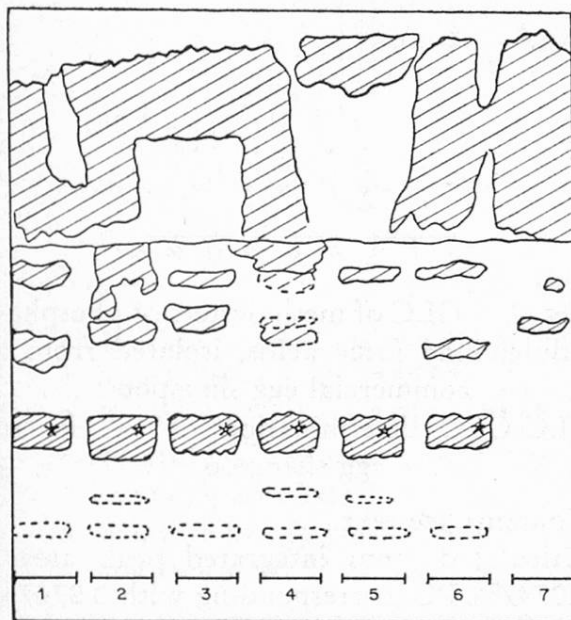
\* Via lipid  $P_2O_5$ , gravimetric method (Dutch official method of mayonnaise).

\*\* Via phosphatidylcholine, proposed method TLC-colorimetry.

\*\*\* Via phosphatidylcholine, proposed method TLC-GLC.

from table 4). Note that the C 16:0 is 28%, which is low compared with the 35% average of table 2. Adulteration of soy lecithin in this sample, however is unlikely because of the absence of C 18:3.

Figures 4—5 are examples of TLC plates that have been used in the isolation of PC from egg shampoos. The separation of PC from other compounds with



Silicaplates Schleicher and Schüll F1500  
(art. 355 100)

Twice eluted:

I Chloroform-methanol-aceton(1+1+1v/v)

II Chloroform-methanol-acetic acid-water  
(50+30+8+4 v/v)

\* = Phosphatidylcholine

1 = Shampoo with added 1.16% soy lecithin  
(50 mg) as extract

2 = Shampoo with added 2.27% egg yolk  
(50 mg) as extract

3 = Soy lecithin (reference)  
(0.76 mg) as extract

4 = Shampoo with 1.14% egg yolk + 1%  
triolelyphosphate (50 mg) as extract

5 = Egg yolk (reference) (1.6 mg) as extract

6 = Shampoo with 1.14% egg yolk  
+ 0.58% soy lecithin (50 mg) as extract

7 = Shampoo with 2% triolelyphosphate  
(50 mg) as extract

Fig. 4. Thinlayerchromatographic isolation of phosphatidylcholine  
from «model» egg shampoos

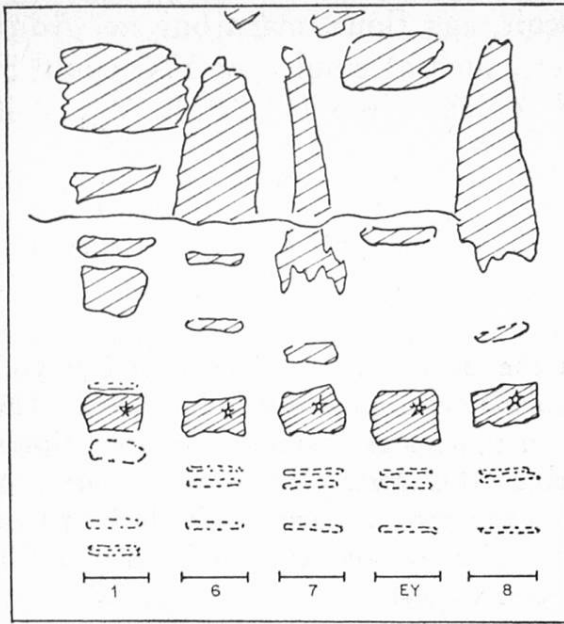


Fig. 5. Thinlayerchromatographic isolation of phosphatidylcholine from commercial egg shampoos

Silicaplates Schleicher and Schüll F1500  
(art. 355 100)

Twice eluted:

I Chloroform-methanol-aceton(1+1+1v/v)

II Chloroform-methanol-acetic acid-water  
(50+30+8+4 v/v)

Numbers 1-6-7-8 refer to commercial egg shampoos mentioned in table 4 (50 mg product as extract)

EY = egg yolk reference (1.6 mg)

\* = Phosphatidylcholine

Skipsky solvent mixture (II) is very good. Detergents and neutral lipids has been removed to the top by first solvent system.

Figure 6 is an example of a TLC isolation of lipid rich food products such as mayonnaise and salad dressing. These lipid rich extracts, however, need special TLC plates with a concentration zone on the start. Otherwise not sufficient amounts of extracts could be placed on the start. The figure proofs that the proposed methods can be used to egg yolk analysis in food products, such as mayon-

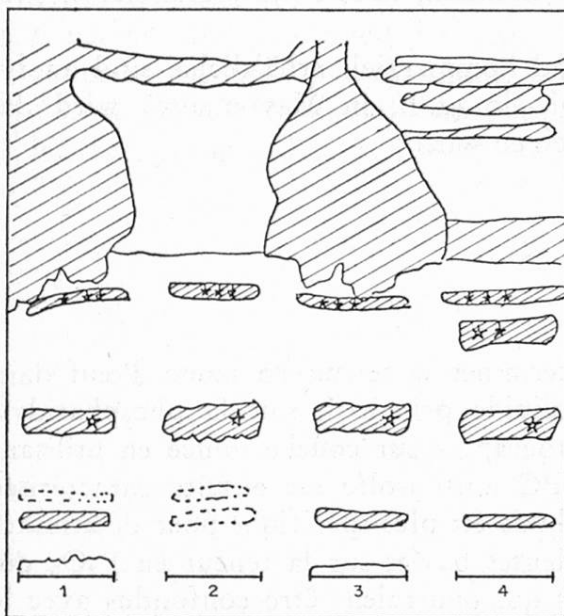


Fig. 6. Thinlayerchromatographic isolation of phosphatidylcholine from lipid rich food products (salad dressing, mayonnaise)

Merck silicaplates 60F 254 with concentration zone (art. 11798)

Twice eluted:

I Chloroform-methanol-aceton(1+1+1v/v)

II Chloroform-methanol-acetic acid-water  
(50+30+8+4 v/v)

\* = Phosphatidylcholine (PC)

\*\* = Phosphatidyl-inositol

\*\*\* = Phosphatidylethanolamine

1 = Salad dressing (80 mg)+

2 = Egg yolk reference (1.6 mg)+

3 = Mayonnaise (30 mg)+

4 = Soy lecithin reference (0.76)+

+ as extract

naise, salad dressing, advocate liquor, egg biscuit, egg flour, macaroni etc. Adulteration of edible organic phosphoric acid esters, such as emulsifier YN, could be used in mayonnaise and salad dressings and which will give a false egg yolk cipher if determined via lipid- $P_2O_5$ .

### *Summary*

This report is a first approach to investigate the possibility to determine egg yolk in cosmetic products via its main phospholipid, namely phosphatidylcholine (PC). This compound is isolated by TLC with a pre-elution on the plates to remove neutral lipids. The isolated PC is analysed further by quantitative GLC for its fatty acids. The proposed method is more specific for egg yolk than the two current methods based on lipid- $P_2O_5$  or on cholesterol. Ingredients that give rise to false ciphers by one of the current methods are easily removed or detected in this method.

Several model and commercial egg shampoos were analysed. Food products (mayonnaise etc.) analyses will be reported later.

### *Zusammenfassung*

Diese Arbeit beschreibt die Grundmethode, die erlaubt, in kosmetischen Produkten durch Analyse des hauptsächlichen Phospholipids — des Phosphatidylcholins (PC) — den Gehalt an Eigelb zu bestimmen. Diese Komponente wird, nach vorgehender Elution der neutralen Lipide, mittels Dünnschichtchromatographie isoliert. Das isolierte PC wird anschließend durch quantitative Analyse der Fettsäuren charakterisiert. Diese Methode ist, was die Bestimmung des Eigelbgehaltes anbelangt, spezifischer als frühere Methoden, basierend auf dem Gehalt an  $P_2O_5$  der Lipide oder dem Gehalt an Cholesterol. Ingredienzien, die mit dem Eigelb verwechselt werden könnten, haben bei dieser neuen Methode keinen Einfluß auf das Resultat.

Mehrere Kopfwaschmittel, Modellshampoos und kommerziell erhältliche, sind analysiert worden. Die Analyse von PC in Lebensmitteln (z. B. in Mayonnaise) wird das Thema einer Publikation sein, die demnächst erscheinen wird.

### *Résumé*

Ce travail décrit la méthode de base pour déterminer la teneur en jaune d'œuf dans les produits cosmétiques par analyse du phospholipide principal, soit la phosphatidylcholine (PC). Ce composant est isolé par chromatographie sur couche mince en utilisant une élution préliminaire des lipides neutres. La PC ainsi isolée est ensuite caractérisée par analyse quantitative des acides gras. Cette méthode est plus spécifique pour déterminer la teneur en jaune d'œuf que les méthodes précédentes basées sur la teneur en  $P_2O_5$  des lipides ou la teneur en cholestérol. Les ingrédients qui pourraient être confondus avec le jaune d'œuf n'interfèrent pas dans cette nouvelle méthode.

Plusieurs shampoings modèles et commerciaux ont été analysés. L'analyse de produits alimentaires (mayonnaise par exemple) fera l'objet d'une publication ultérieure.

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