Lipids in Egg White

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ABSTRACT Fresh egg white contained about 0.02% lipids, and 13–15% phospholipids for the lipids. They were made up of triglyceride, diglyceride, free fatty acid, cholesterol ester and cholesterol in acetone-soluble materials, and phosphatidylcholine (PC), lysophosphatidylcholine (LPC), phosphatidylethanolamine (PE), sphingomyelin (SPM) and unknown material with choline base in acetone-insoluble materials. Triglyceride was present in larger amounts. The amounts of PC, SPM, LPC and PE were 43, 32, 24% and trace, respectively. It was suggested that more than half of lipids were present in bound form with protein. Lipid contents in egg white slightly increased during storage at 30 ± 1°C. and 12 ± 1°C., but little increased at 4 ± 2°C. It was shown that no phospholipid but triglyceride and cholesterol ester might pass from egg yolk into albumen during storage.

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INTRODUCTION

SMITH (1951) reported that yolk lipids would pass from yolk into egg white through intact membranes, and resulted in poorer egg white performance in whip and angel food cakes tests, having bad effects upon the egg white thinning. On the basis of these data, it seemed that lipids in egg white, although their amounts were only small, had highly significant effects on the character of egg white. But, to date, little has been known about the nature of the lipids in egg white. This work was undertaken in order to make the components of lipids in egg white clear, and to determine the changes of lipid contents in the egg white of stored eggs.

MATERIALS AND METHODS

Materials. Infertile eggs of White Leghorn hens were obtained from the laying house in Nagoya University.

Extraction, Separation and Analyses of Lipids in Egg White. 1. Extraction and Analyses of Lipids. Twelve to fifteen eggs were used within 24 hr. after laying or after storagef or the determination of lipid contents, and all analyses were made in duplicate.

Egg white was carefully separated from yolk by breaking eggs one by one using a metal filter plate, and acidified to pH 2–3 with 1 N HCl to liberate fatty acids. Lipids in the acidified egg white were extracted with ethanol-ethyl ether (3:1) and chloroform-methanol (2:1) (Entenman, 1961). In the first extraction, the egg white was homogenized with 2 volumes of cold ethanol-ethyl ether, followed by standing at 5°C. overnight and then filtering. The residue was re-extracted twice with 1 volume of chloroform-methanol. Solvents in the combined extracted solution were removed under the reduced pressure almost to dryness. The residual extract was re-dissolved in chloroform-methanol, washed with 0.2 volumes of distilled water, dried over anhydrous Na_2SO_4, filtered through filter paper (Toyo Roshi No. 2), and evaporated under the reduced pressure almost to dryness. The dehydrated lipid extract was analyzed for total lipid weight by drying in desiccator at 10°C. to constant weight and for its phosphorus content by the method of Allen (1940). This lipid extract contained the nonphospholipid-phosphorus. So amounts of phospholipid-

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phosphorus were determined on phospholipids (Abramson and Blecher, 1964) which were separated by silica gel thin layer chromatography using petroleum ether, ethyl ether and acetic acid (80:30:1) (Noda and Ikegami, 1966) as the solvent system. The approximation of quantity of phospholipid was calculated on multiplying the phosphorus content by 25.

2. Extraction and Analyses of Free and Bound Lipids. For the separation of free and bound lipids, free lipids were extracted twice with 1 volume of ethyl ether from the lyophilized egg white, and the residual bound lipids were extracted with chloroform-methanol. Amounts of lipids in each solvent were analyzed as described above.

3. Separation of Acetone-Soluble and Acetone-Insoluble Materials. The weighed lipids were treated with acetone containing magnesium chloride to separate the acetone-soluble materials from the acetone-insoluble materials. The acetone-soluble materials were made up mostly of fats, free fatty acids and unsaponifiable matter. The acetone-insoluble materials mainly comprised the phospholipids.

The acetone-soluble materials were separated by silica gel thin layer chromatography. The solvent system used was petroleum ether, ethyl ether and acetic acid (Noda and Ikegami, 1966) described above. The acetone-insoluble materials were also separated by silica gel thin layer chromatography, of which the solvent system was chloroform, methanol and water (65:25:4) (Kaufmann *et al.*, 1966).

4. Semiquantitative Analysis of Phospholipids. Semiquantitative analysis of phospholipids was done according to Abramson and Blecher (1964), using the same solvent system as in the separation of acetone-insoluble materials. Each phospholipid was removed from the silica gel chromatograms, and its content was determined as described above.

5. Gas Chromatographic Analyses. Gas chromatographic data of fatty acid methyl esters were obtained with Hitachi K-53 gas chromatography equipped with flame ionization detector. After saponification of the weighed lipids with 0.5 N alcoholic KOH, the separated fatty acids were converted to methyl esters with diazomethane.

Storage of Eggs and Freshness Tests. Fresh eggs within 24 hr. after laying were stored in the rooms, where they were well-ventilated at 4 ± 2, 12 ± 1 and 30 ± 1°C., respectively, for 4, 7, 15 and 30 days. Freshness of the eggs was estimated by log of their yolk index (Feeney *et al.*, 1956) and pH of albumen measured by glass electrode.

RESULTS AND DISCUSSION

A. Lipids in Egg White Obtained from Fresh Egg. 1. Lipid Contents and Analysis of Fatty Acid. In the weighed lipids, Beveridge-Johnson test for phosphorus (Beveridge and Johnson, 1949), Lieberman-Burchard test for cholesterols (Idler and Baumann, 1953) and ninhydrin test for protein or amino acids were all positive, and Molisch test for carbohydrate (Devor, 1948) was negative. And the infrared spectrum of the lipids was almost the same as that of glycerides. These results indicated the presence of glyceride, cholesterol and phospholipid. In the quantitative analyses, fresh egg white contained 0.020% (by weight) lipids, and 13–15% phospholipid for the weighed lipids. Smith (1951) reported that amounts of lipid in fresh egg white were about 0.015% by the monomolecular test. Though there might be the differences of egg and quantitative method, almost iden-

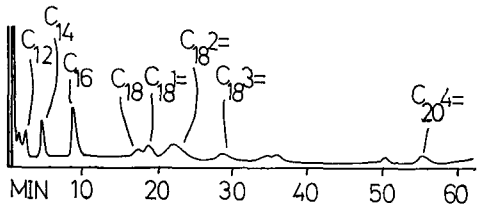

FIG. 1. Typical gas chromatogram of methyl esters of fatty acids obtained from egg white lipids.
Hitachi K-53, Flame ionization detector,
Column: EGS 25% on Shimalite
60–80 mesh, 4 mm. × 1 m.
Column temp.: 180°C.
N_2: 0.3 kg./cm.^{2}

tical value was obtained in solvent extract in this report.

The typical gas chromatogram of methyl esters of fatty acids is shown in Fig. 1.

Methyl esters of lauric acid (C_{12}), myristic acid (C_{14}), palmitic acid (C_{16}), stearic acid (C_{18}), oleic acid ($C_{18}1=$), linoleic acid ($C_{18}2=$), linolenic acid ($C_{18}3=$) and arachidonic acid ($C_{20}4=$) were found in the gas chromatogram, by the comparison of their retention times with known methyl esters. The methyl ester found in higher concentration was $C_{18}2=$.

2. Acetone-Soluble Materials. Separation of acetone-soluble materials is shown in Fig. 2. It was found that cholesterol ester, triglyceride, free fatty acid, diglyceride, and cholesterol were present in fresh egg white. Triglyceride was present in larger amounts, and the others in small amounts. More minor constituents were not investigated. For the detection of these lipids fifty percent H_2SO_4 spray and I_2 vapors were used.

3. Acetone-Insoluble Materials. Separation of acetone-insoluble materials is shown in Fig. 3. As the various standard materials of phospholipids, phosphatidylcholine (PC), lysophosphatidylcholine (LPC), phosphatidylethanolamine (PE), sphingomyelin (SPM) extracted from egg yolk (Rhodes and Lea, 1957) were used. Spot name b, c, d, and e in Fig. 3 were positive for Dragendorff's reagent, and spot name a was positive for ninhydrin. By comparing with standard phospholipids on the thin layer chromatogram, PC, LPC, PE and SPM were found in lipids of egg white. Spot name c, un-

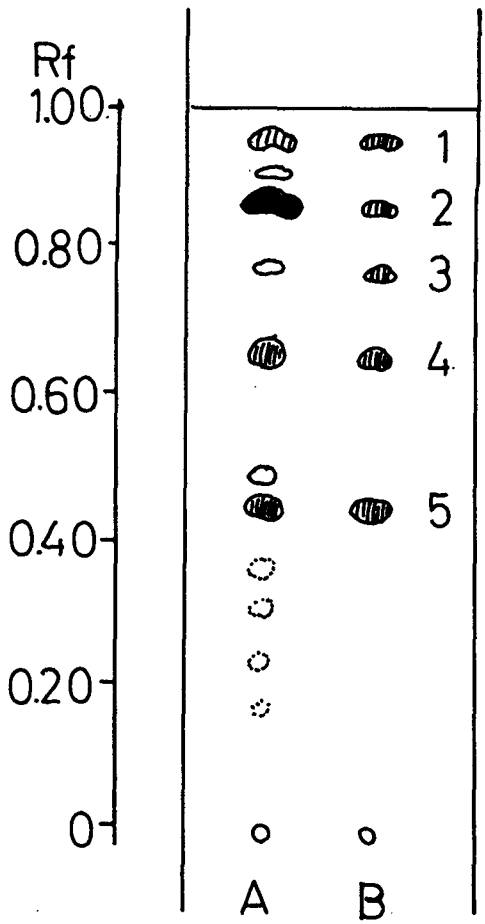

FIG. 2. Separation of acetone-soluble materials obtained from egg white lipids.
A: Acetone-soluble materials obtained from egg white lipids
B: Standard materials
1: cholesterol ester, 2: triglyceride, 3: free fatty acid, 4: diglyceride, 5: cholesterol
Solvent system: petroleum ether-ethyl ether-acetic acid (80:30:1)

identified material, should have the choline base in the molecule because of positive for Dragendorff's reagent.

Payne (1964) reported that sphingomyelin showed two spots on the thin layer chromatogram, depending on the difference in fatty acid composition. Spot name c might be sphingomyelin having the different fatty acid composition from sphingomyelin of spot name d. Momma *et al.* (1970) reported the presence of two unidentified polar lipids and lysophosphatidylethanolamine in polar lipid fraction of egg yolk by two-dimensional thin layer chromatography. It seems unlikely that spot name c corresponds to one of these compounds because their developed positions are apart from that of sphingomyelin. Phosphatidylserine, reported by Rhodes and Lea (1957), was not detected in this experiment.

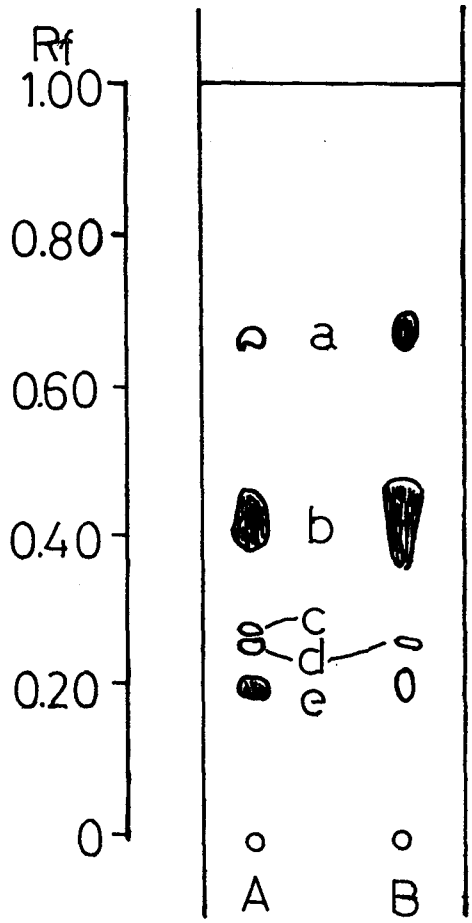

FIG. 3. Separation of acetone-insoluble materials obtained from egg white lipids.
A: Egg white lipids
c: unidentified material
B: Standard materials from egg yolk
a: phosphatidylethanolamine,
b: phosphatidylcholine,
d: sphingomyelin,
e: lysophosphatidylcholine
Solvent system: chloroform-methanol-water (65:25:4)

Each material on thin layer chromatogram was extracted with chloroform-methanol mixture, and their amounts of phosphorus were determined as described above. The constitution of phospholipid was 43, 32, 24% and trace of PC, SPM, LPC and PE, respectively, from their amounts of phosphorus. It was previously reported that egg yolk contained 33.2% phospholipids for the total lipids, and the main phospholipids were PC and PE, those contents being 24.9 and 8.3%, respectively (Marison and Woodroof, 1968). The data in this report indicated that the composition of phospholipids in egg white was different from those in egg yolk.

4. Free and Bound Lipids. Most of the lipids in lipoprotein, even though the substances containing the lipoprotein were lyophilized, could not be easily extracted with ethyl ether (Akabori and Ono, 1964). So, the lipids extracted with ethyl ether from the lyophilized egg white should be free. Acetone-soluble and -insoluble materials obtained from free and bound lipids were also separated on thin layer chromatograms, respectively. Their separations are shown in Fig. 4. And the amounts of free and bound lipids are shown in Table 1. This result shows that almost all of the phospholipids in egg white were obtained

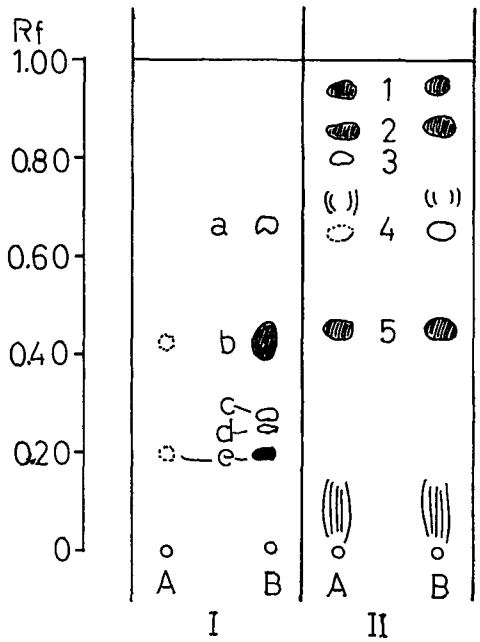

FIG. 4. Separation of acetone-soluble and -insoluble materials obtained from free and bound lipids in egg white.
I: Acetone-insoluble materials,
II: Acetone-soluble materials
A: Free lipids, B: Bound lipids
Solvent systems:
I: petroleum ether-ethyl ether-acetic acid (80:30:1)
II: chloroform-methanol-water (65:25:4)
The numbers, 1, 2, 3, 4 and 5 show the same as in Fig. 2.
The signs, a, b, c, d and e show the same as in Fig. 3.

from chloroform-methanol extract, although there were trace amounts of phospholipids in free lipids, and that phospholipids might be present as lipoprotein which could not be extracted with ethyl ether. The amount of phospholipids in bound lipids was, approximately, identical with those in the total weighed lipids described in A-1.

TABLE 1.—*Free and bound lipids in egg white*

	Free	Bound
Lipids (mg.) extracted from egg white (1 Kg.)	48 0.005%	104 0.010%
Phospholipid for total lipids	1.1%	13.0%

B. Lipids in Egg White Obtained from Stored Eggs. 1. Freshness of Egg. Freshness of egg was determined by pH of egg white and log of yolk index. The results are shown in Fig. 5. pH of egg white stored at 30 ± 1°C. attained to 9 within 4 days, but pH of the others was below 9 during the storage. Periods and temperatures of egg

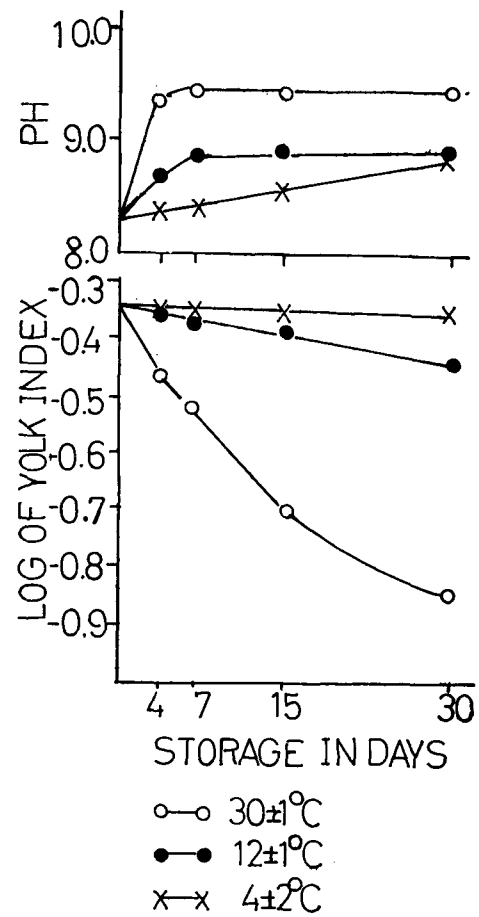

FIG. 5. pH of egg white and log of yolk index of stored egg.

storage had a significant effect on the measured factors. Rate of change with time for the factors differed significantly with temperature.

2. Changes of Lipid Contents. Changes of lipid contents of egg white are shown in Fig. 6. It was reported that moisture was lost from the egg white through the shell pores and simultaneously migrated to the egg yolk during storage (Marison and Woodroof, 1968). So, each lipid obtained from the egg white of stored eggs was expressed as both percentages of whole egg white and egg white solid. Fig. 6 shows a slightly increased amount of lipids in egg white during storage at 30 ± 1 and 12 ± 1°C., but little increase even in 30 days of stored periods at 4 ± 2°C. Marison and Woodroof (1968)

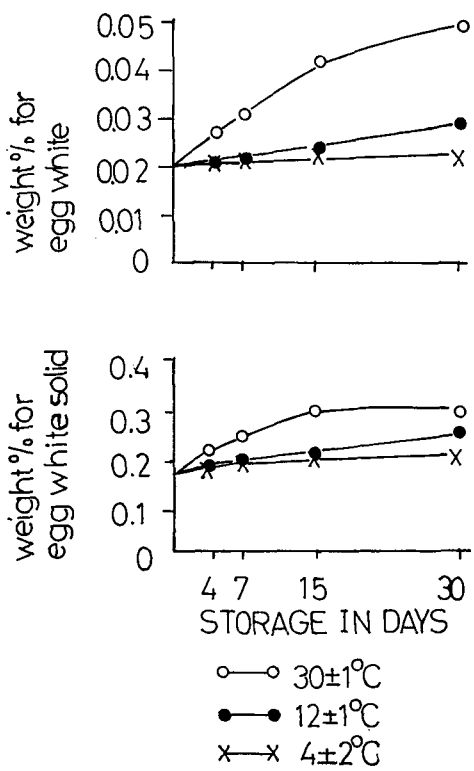

FIG. 6. Lipid contents in egg white of stored egg.

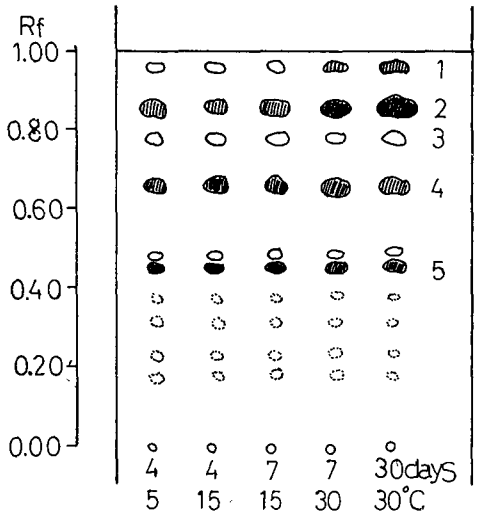

FIG. 7. Separation of acetone-soluble materials obtained from egg white lipids of stored egg.
Solvent system: the same as in Fig. 2.
The numbers, 1, 2, 3, 4 and 5 show the same as in Fig. 2.

reported that amounts of lipids in egg yolk slightly decreased in the stored egg at 12.8°C. for 21 days. It might be thought that these decreased part of yolk lipids passed through intact membranes from egg yolk to white. It was reported that egg yolk membrane weakened and became more elastic during storage (Fromm and Matrone, 1962; and Fromm, 1967). Lipid contents in egg white might increase with the deterioration of the egg yolk membrane, though not measured, during the storage.

The amount of phospholipid in each stored egg was 0.022–0.023% for the egg white solid, and no remarkable changes were found in phospholipid contents. The increased lipids in egg white might be the other lipids except phospholipid. On the basis of this data and the separated patterns in Fig. 7, it seemed to be reasonable to assume that no phospholipid but triglyceride and cholesterol ester were passed from egg yolk into white during storage.

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NEWS AND NOTES

(Continued from page 1563)

Richard Kathe was named General Manager of the Board. He has had wide experience in association work, most recently with the United Dairy Industry Association. The new headquarters will be at 205 W. Touhy Ave., Park Ridge, Illinois 60008. He will report directly to Dr. Gene Masters, General Manager, United Egg Producers, Atlanta, Georgia.

Dr. L. A. Wilhelm will continue as President of the organization.

E. D. Murphy, New York, New York, has been named Chairman of the Board. Vice Chairman is G. H. Biddle, Modesto, California; Treasurer—S. Casady, Jr., Des Moines, Iowa; and Secretary—J. S. Stroughan, Hilliard, Florida.

Janet Salstrom has joined the home economics staff. She is a graduate of Carthage College where she majored in foods and nutrition, with minors in chemistry and speech. She formerly worked for the American Dairy Association in its national headquarters.

ARMOUR NOTES

Armour and Company has purchased the turkey brooding and ranging facilities and the poultry feed mill operated by Graham Brothers, Inc. at Washington, Indiana.

Operations conducted by Graham Farms are not included in the agreement. The Graham Brothers management team of David and Ziba, Jr. will continue to manage the operation under Armour ownership and Armour expects to continue to work with local farmers as in the past.

Armour and Company has also acquired Wingate Feed and Farm Service, Inc., a turkey raising and feed milling operation at Wingate, North Car-

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