The Fatty Acid Composition of Egg Yolk Lipids from the Sea-turtle

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Introduction

In earlier summer, sea-turtles lay a lot of eggs on the sandy coast of South Kyushu-province and Yaku-island in Japan. Generally one sea-turtle lays more than 100 eggs in a night. Avian eggs have hitherto been utilized as one of the preferred foods for human consumption. In anticipation of the effect for the sickness of the stomach and bowels, the sea-turtle's egg yolk has been eaten by some villagers in Kagoshima prefecture in a cooked or a salted state. The eatable part of the egg is to be the yolk, because of the heat-uncoagulation of the egg white and the remarkable samllness of protein amount (0.8%) in the egg white, as shown in the previous report[4]. Nowadays, sea-turtle's eggs have been occasionally marketing at some stores. Previously, the authors showed that the protein and its essential amino acids contents of the sea-turtle's egg yolk were larger than those of chicken's yolk[5]. This paper describes the fatty acid composition of the lipids of sea-turtle's egg yolk and the phosphorus content in the yolk compared with those of chicken's yolk.

Materials and methods

Materials—The eggs of sea-turtle (*Caretta caretta olivacea*) were collected within a few minutes after being laid on the sand of the Kurio-coast of Yaku-island, Kagoshima prefecture, and brought to our laboratory after several days. Chicken eggs laid by the hen fed on the commercial formula feed were also used as materials. Eggs were weighed, broken with scissors, and the yolks were separated from their respective whites. Traces of egg white which adhered to the yolk were carefully removed with the aid of injector, adding a small amount of water. The yolk content was, then, poured into a tared glass dish and weighed. The yolk was blended with a homogenizer at a slow speed, lyophilized and used as the analytical sample.

Extraction and analysis of yolk lipids—Yolk lipids were extracted with ethyl ether, using the Soxhlet extracting apparatus and with chloroform-methanol mixture (*v/v*, 2:1) at room temperature according to the method of Folch et al.[3], separately. The preparation of fatty acid methyl esters in the lipids by interesterification was achieved in accordance with the procedure described by Takahashi et al.[11], at the same time referring to the method of Stoffel et al.[10] Eight *mg* of lipids was dissolved in 8 *ml* of su-

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perdry methanol saturated with hydrogen chloride and 1 *ml* of benzene, and the mixture was refluxed in a boiling water bath for 2 hours, with frequent shaking at the start to dissolve the lipids. After cooling to room temperature, 3.8 *ml* of water was added, and the methyl esters were extracted three times with 20 *ml* of petroleum ether.

The pooled extracts were concentrated to a small volume under reduced pressure and diluted to 25 *ml* with petroleum ether. The solution was then simultaneously neutralized and dehydrated with anhydride sodium sulfate and sodium carbonate (*w/w*, 4:1), and the supernatant was employed as the sample for gas-liquid chromatography. The analysis of fatty acid methyl esters was carried out by gas-liquid chromatography, in a Shimazu GC 4B gas chromatograph equipped with a hydrogen flame ionization detector. The employing conditions were as follows: Stainless column, $3mm \times 3m$; packings, DEGS-H_3PO_4, 5～1% on chromosorb W 60～80 mesh; column temperature, 180°*C*; injection temperature, 200°*C*; carrier gas, nitrogen with a flow rate 20 *ml/min*. Chromatographic peaks were identified by comparing their retention times against those of known standards. Quantitation of the various peaks was accomplished by triangulation.

Determination of phosphorus contents of egg yolk and yolk lipids--Phosphorus contents were determined according to the procedure of Lucena-Conde et al.[6] Ten *g* of lyophilized yolk powder and 1.5 *g* of the extracted lipids were separately burnt and ignited at 500°*C*, in the electric oven. Ten *ml* of hydrochloric acid (18%) was added on the ash and the solution was evaporated to dryness on a boiling water bath. Five *ml* of 9% HCl and 10 *ml* of water were added on it, then it was filtered and diluted to an appropriate volume. An aliquot of the dilution in a 25 *ml* volumetric flask was treated with 0.75 *ml* of the Lucena-Conde reagent, and diluted with 15 *ml* of water. After warming on the water bath to 95°*C* for 10 *min*, the reaction mixture was allowed to cool, then diluted, and the absorbance at 840 *nm* was measured spectrophotometrically.

Results and discussion

The lipid content of the fresh yolk from eggs of various strains of hens is between 32 and 36%.[7] The mean content on the basis of dry yolk is 65%. Some general components of the sea-turtle's egg yolk were as shown in Table 1. In comparison with the chicken egg yolk, in the turtle's yolk, the lipid amount extracted with ethyl ether are remarkably small, though both protein content and soluble nitrogen-free matter content are very large. These results were obtained by the previous investigation.[5] In the present experiment, the lipids content of turtle's yolk extracted with ethyl ether, using

Table 1. General components of sea-turtle's egg yolk compared with those of chicken yolk

	Wt. of an egg yolk (*g*)	Crude protein (%)	Lipid: (ether-extract) (%)	Ash (%)	Soluble N-free matter (%)
Turtle	13～14	54.0	29.1	4.0	12.9
Chicken	17～20	33.4	56.8	3.6	6.2

The contents of component are values on the basis of dry matter.

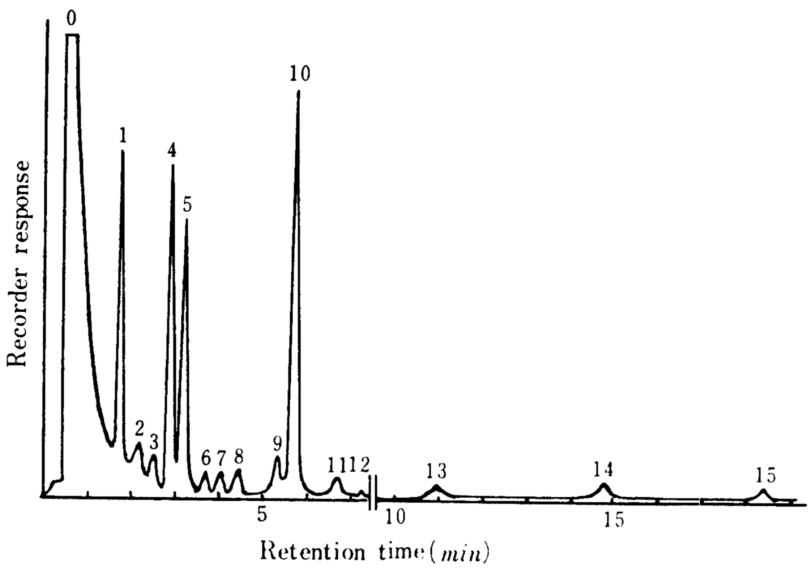

Fig. 1 Gas chromatogram of fatty acid methyl esters from the turtle's yolk lipid extracted with $CHCl_3$-MeOH.

Employing conditions: stainless column, 3*mm* × 3*m*; packings, DEGS-H_3PO_4, 5 ∼ 1% on chromosorb W 60–80 *mesh*; column temp., 180° *C*; injection temp., 200° *C*; carrier gas, N_2 with a flow rate 20 *ml/min*; detector, H_2F.I.D.

Marks: 0, solvent (petroleum ether)
1, myristic; 2, myristoleic; 3, pentadecanoic;
4, palmitic; 5, palmitoleic; 6, unknown;
7, heptadecanoic; 8, heptadecenoic; 9, stearic;
10, oleic; 11, linoleic; 12, linolenic;
13, cis-5-eicosenoic; 14, heneicosanoic;
15, arachidonic A.

the Soxhlet extracting apparatus was 31.9%, and the lipids content extracted with chloroform-methanol (*v/v*, 2:1), 40.2%. It was, therefore, confirmed that chloroform-methanol are more effective than ethyl ether on the extraction of the lipids from the sea-turtle's egg yolk.

The pattern of fatty acid methyl esters of the turtle's yolk lipids obtained by gas-liquid chromatography was as shown in Fig. 1. In the chromatographic figure, fifteen peaks well-separated can be observed. By comparing their retention times with those of known standards, the fatty acids could be respectively identified with myristic (14:0), myristoleic (14:1), pentadecanoic (15:0), palmitic (16:0), palmitoleic (16:1), unknown, heptadecanoic (17:0), heptadecenoic (17:1), stearic (18:0), oleic (18:1), linoleic (18:2), linolenic (18:3), cis-5-eicosenoic (20:1), heneicosanoic (21:0) and arachidonic (20:4) acids, in the order of the appeared peaks. Various fatty acids amounts were calculated from the equation curve ($y = 11.6 \times 10^{-2}x$) for palmitic acid treated under the same experimental condition. In the equation, y is the area (cm^2), and x, the concentration ($10^{-2}r$).

Table 2 shows the fatty acid composition of total lipid of sea-turtle's yolk compared with that of chicken yolk. The analytical data of Ootake et al.[8] was represented in that table, too. The fatty acid composition of the lipids extracted with chloroform-methanol from the turtle's yolk was appreciated to be quite similar to that of ether extract, though there was a difference between the amounts of both lipids, as described precedingly.

Table 2. Fatty acid composition of total lipids of sea-turtle's egg yolk compared with that of chicken yolk

Fatty acid	Turtle lipid ($CHCl_3$-MeOH extract)	Turtle lipid (ether extract)	Chicken lipid ($CHCl_3$-MeOH extract)	Chicken lipid* ($CHCl_3$-MeOH extract)
14:0	9.3	9.0	0.4	0.3
14:1	0.8	0.8	0.4	trace
15:0	0.8	0.7	trace	trace
16:0	16.5	16.9	26.3	27.8
16:1	15.3	14.9	3.3	3.3
Unknown	0.4	0.5	—	—
17:0	0.7	0.7	trace	0.3
17:1	1.1	1.0	trace	0.2
18:0	4.4	4.7	10.7	8.6
18:1	43.6	43.1	48.3	46.2
18:2	1.9	2.1	10.0	10.6
18:3	trace	trace	—	0.4
20:1	1.5	1.5	—	0.2
21:0	2.1	2.2	0.5	—
20:4	1.7	1.8	—	1.5
20:6	—	—	—	0.8
Satd. acid (A)	33.8	34.2	37.9	36.7
Unsatd. acid (B)	65.9	65.2	62.1	63.3
B/A	1.9	1.9	1.6	1.7

Values are weight % on the basis of total fatty acids.
*The data were cited from the reference No. (8).

In the fatty acids of turtle's yolk lipids, oleic, palmitic, palmitoleic, myristic, stearic, heneicosanoic, linoleic, arachidonic, cis-5-eicosenoic acids were found to be much in quantity, in that order. Furthermore, lesser amount of heptadecenoic, myristoleic, pentadecanoic, heptadecanoic and linolenic acids were appreciated. Of all fatty acids, the amount of oleic acid was exceedingly large, being similar to the predominance in the chicken yolk lipids. The comparison of the major fatty acids composition of turtle's yolk lipids with that of the chicken's shows that myristic, palmitoleic, heneicosanoic, and arachidonic acids are by far the more in the turtle than in the chicken, while oleic, palmitic, stearic and linoleic acids in the former are considerably less than in the latter. The ratio of the unsaturated acid to the saturated in the chicken yolk lipids amounted to 1.6, being near to the value (1.7) by Ootake et al.[8] and Privett et al.[9] The ratio in the turtle's yolk lipids was ascertained to be larger than that in the chicken's. Farr et al.[2] described that the analytical results of the fatty acids in the neutral and polar lipids of Bobwhite quail yolk are in general agreement with those reported already for the fatty acid content of chicken yolk. The fatty acid composition of yolk lipids from Japanese quail determined by authors was also similar to that of chicken yolk lipids, though the data were unpublished. Heneicosanoic and cis-5-eicosenoic acids, which were found in quite slight amount or not at all in the egg yolk from the birds belonging to the Phasianidae, such as chicken and quail, could be found in the sea-turtle's yolk.

It has been well-known that the constituents of the chicken yolk lipids, especially the fatty acids, are influenced by the hen's diet. Chen et al.[1] showed that the total amount of saturated fatty acid does not change even with a large alteration in dietary fatty acid composition, but that linoleic acid content of yolk increases with a concurrent decrease in oleic acid when the level of dietary polyunsaturated acid is raised.

In the turtle's yolk, 20: 5 and 20: 6 fatty acids could not been detected, though Privett et al.[9] found these polyunsaturated acids in the hen's yolk. Chicken eggs used by authors were the ones from the hen fed on the commercial formula feed. Various differences mentioned above between the fatty acid compositions of the sea-turtle and chicken yolks are presumed to be chiefly due to the differences in the diets of turtle and chicken.

In the previous report,[5] authors presumed that some differences may be contained in the constitutions of proteins of egg yolks from the turtle and from the domestic birds.

Table 3. Phosphorus amount in egg yolk and yolk lipid

	P in yolk (*mg* %)	P in yolk lipid (*mg* %)	Difference (*mg* %)
Turtle	1053	47	1006
Chicken	694	154	540

Table 3 shows the phosphorus amount in the turtle yolk and the yolk lipids. The phosphorus content of yolk was exceedingly larger in the turtle than in the chicken. However, the content in the turtle's yolk lipids was remarkably small: one third of that in the chicken's. This result suggests that the phospholipid content of turtle's yolk lipid is less than that of chicken's.

The differences between the phosphorus contents of the yolk and the yolk lipids, which were considered to be attributed to the phosphorus combined with proteins, were larger in the turtle than in the chicken. The turtle's yolk are, therefore, presumed to have a relatively large amount of phosphoprotein.

In the previous report,[5] authors recommended the turtle's yolk as the nutritionally preferable food on the protein intake. The turtle's yolk, however, was not considered to be necessarily better as a food than chicken's yolk, with respect to the supply of lipids and essential fatty acids.

Summary

1) Fifteen kinds of fatty acids could be detected in the egg yolk lipids from the sea-turtle by gas-liquid chromatography. Oleic, palmitic, palmitoleic, myristic, stearic, heneicosanoic, linoleic, arachidonic, cis-5-eicosenoic and heptadecenoic acids were found to be contained largely in the yolk lipids, in that order. Furthermore, lesser amount of myristoleic, pentadecanoic, heptadecanoic and unknown acids were appreciated.

2) A comparison of the composition of major fatty acid in the turtle's yolk lipids with that in the chicken's showed that myristic, palmitoleic, heneicosanoic and arachidonic acids are remarkably more in the turtle than in the chicken, while oleic, palmitic, stearic and linoleic acids in the former are considerably less than in the latter.

The ratio of the unsaturated acid to the saturated acid in the turtle's yolk lipids was ascertained to be larger than that in the chicken's.

3) The phosphorus content of yolk was exceedingly larger in the turtle than in the chicken. However, the content in the turtle's yolk lipids was remarkably small: one third of that in the chicken's one.

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