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Oxidative Stability of Silky Fowl Eggs. Comparison with Hen Eggs

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Oxidative stability of original silky fowl's eggs was investigated. The silky fowl's whole eggs indicated significant oxidative stability compared to hen's eggs in storage for 14 days. The hen eggs showed an increased amount of hydroperoxides on 6 days of storage at room temperature. In contrast, the silky fowl eggs showed restricted generation of hydroperoxides until 8 days and then a gradual increase. Though pigment extracted with chloroform/methanol (2:1) solvent from hen's whole egg turned brown for 14 days, the pigment extracted from silky fowl's whole egg slowly turned brown. Unsaturated fatty acids in silky fowl eggs were 62.5% among total fatty acids, while the unsaturated fatty acids of hen's eggs were 53.9%. It is speculated that the silky fowl eggs compared with that of hen eggs.

KEYWORDS: Oxidative stability; silky fowl; hen; egg; fatty acid

INTRODUCTION

Original silky fowl is mild and short, with a small and long head but a short neck. It can be easily distinguished from other chickens. The eggs of the original silky fowl are well-known in the Orient and for thousands of years have been credited with famously medicinal and health-promoting values. However, a modern scientific approach has only recently been applied to determine its medicinal chemical and biochemical components (1, 2). Recently, we reported that the silky fowl's eggs are considered to be a chemical storehouse and an excellent source of sialic acid (3), which is an important component for the protection of life such as receptor for rotavirus and influenza virus, *Vibrio cholerae*, and binding pathogenic *Escherichia coli* (4–7). We wanted to clarify a value and importance of the silky fowl's eggs. The object of the current study was to investigate oxidative stability of silky fowl eggs compared to hen eggs.

MATERIALS AND METHODS

Materials. Eggs of silky fowl and hen, White Leghorn origin, were a kind gift from Tokai Biken Co., Ltd., Gifu, Japan. Each fresh egg fraction was obtained from the eggs collected within a day after laying by a silky fowl and hen and immediately used for these experiments. Exactly the same feed and breeding conditions were given to the silky fowls and hens.

Measurement of Oxidative Stability Whole Eggs. Whole eggs obtained from 20 unfertilized eggs were homogenized. The mixture

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was exposed to air and kept at 25 °C for 14 days, and samples were taken for evaluation of stability every 2 days. Oxidative stability was evaluated by ferric thiocyanate (8) and 1,3-diethyl-2-thiobarbituric acid (DETBA) methods (9). The ferric thiocyanate analysis method was performed as follows. To the egg sample (50 μ L), 75% ethanol (2.35 mL), 30% ammonium thiocyanate (50 µL), and 20 mM ferrous chloride solution in 3.5% HCl (50 μ L) was added. After 3 min, the absorbance of the solution was measured at 500 nm in a 1 cm cuvette with a UV spectrophotometer (U-2000, HITACHI Co. Ltd., Tokyo, Japan). The DETBA method was carried out as follows. The egg (20 g) was extracted with methanol (100 mL). To the methanolic extract (100 μ L) from whole eggs, 200 μ L of 20 mM butylated hydroxytoluene (BHT, Sigma), 200 µL of 8% sodium dodecyl sulfate (SDS, Merck, Darmstadt, Germany), 400 µL of deionized water, and 3.2 mL of 12.5 mM DETBA (Aldrich Chemical Co., Milwaukee, WI) in sodium phosphate buffer (pH 3.0) were sequentially added. The mixture was stirred thoroughly, heated at 95 °C for 15 min, and then cooled on an ice bath. To the mixture, 4 mL of ethyl acetate was added, and the mixture was mixed and centrifuged at 2000 rpm at 20 °C for 15 min. The organic layer was recovered and measured by a HITACHI 650-40 spectrofluorometer with fluorescence excitation at 515 nm and emission at 555 nm. Browning index of the eggs was evaluated by estimating the color density of egg extract using a colorimeter at 420 nm (Nippon Denshoku Co. Ltd., Tokyo, Japan). The egg (20 g) was extracted with chloroform/ methanol (2:1) solvent (100 mL). The color density of the organic layer was estimated by the colorimeter.

Analysis of Fatty Acid of Egg. The fatty acids of the homogenized silky fowl and hen eggs were converted into methyl esters and analyzed with gas chromatography (GC) by using an Auto-System gas chromatograph, Perkin-Elmer, equipped with a split—splitless capillary injector and a flame ionization detector (FID) as described previously (10). The sample was separated by a 30 m \times 0.25 mm i.d. fused silica column (Rtx-2330). The FID temperature was set at 250 °C. The fatty acid compositions were analyzed in duplicate.

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Figure 1. Amount of hydroperoxides in whole silky fowl's eggs (\bigcirc) and hen's eggs (\blacksquare). Each value represents the mean \pm standard error in triplicate.



Figure 2. Amount of malonaldehyde in whole silky fowl's eggs (\blacksquare) and hen's eggs (\blacksquare). Each value represents the mean \pm standard error in triplicate.

RESULTS AND DISCUSSION

Comparison of Oxidative Stability between Whole Silky Fowl Eggs and Hen Eggs. Comparison of the oxidative stability between whole silky fowl eggs and hen eggs was investigated by two methods, amount of hydroperoxides and amount of malonaldehydes in the eggs (Figures 1 and 2). After 4 days no hydroperoxides were observed in either type of egg at all. However, after 6 days, the amount of hydroperoxides in hen eggs increased drastically. In contrast, in the case of silky fowl eggs, after 8 days hydroperoxides were still nearly absent and then began to increase gradually (Figure 1). Apparently, silky fowl eggs showed greater stability toward oxidation than hen eggs. The generation of malonaldehydes in both eggs was also measured. At every sampling time, the amount of malonaldehydes in silky fowl eggs was lower than that in hen eggs. The time for malonaldehydes in the eggs to reach 0.1 mg (malonaldehydes/kg) was 8 and 11 days for hen and silky fowl eggs, respectively (Figure 2).

Observation of Browning of Whole Silky Fowl Eggs and Hen Eggs. Browning of whole silky fowl and hen eggs was observed using a colorimeter. Absorbance of extract of silky fowl eggs at 420 nm using a colorimeter was 0.134–0.187 for 14 days, while that of hen eggs was 0.157–0.307 (Figure 3). The silky fowl eggs suppressed formation of browning pigment in them. In contrast, the hen eggs turned brown by air oxidation during storage.

Analysis of Fatty Acid of Lipids in Whole Silky Fowl's Eggs and Hen's Eggs. The antioxidative (11, 12) and synergistic (13) activities of lipids have been reported to relate to the structural diversity of fatty acid compositions. To confirm



Figure 3. Observation of browning of whole silky fowl's eggs (\bigcirc) and hen's eggs (\blacksquare). Each value represents the mean ± standard error in triplicate.

Table 1. Fatty Acid Compositions of Whole Egg of Silky Fowl and Hen

fatty acids	silky fowl's eggs ^a	hen's eggs ^a
C14:0	0.4 ± 0.02	0.5 ± 0.06
C16:0	24.9 ± 2.0^{b}	33.1 ± 3.7
C16:1 (n – 9)	1.6 ± 0.01^{b}	2.7 ± 0.97
C16:1 (n – 7)	0.9 ± 0.01	0.4 ± 0.02
C17:0	0.1 ± 0.01	0.1 ± 0.01
C17:1	0.1 ± 0.02	0.1 ± 0.01
C18:0	11.8 ± 2.5	12.2 ± 2.4
C18:1 (n – 9)	35.3 ± 6.7^{b}	31.4 ± 4.7
C18:1 $(n - 7)$	1.2 ± 0.5	1.1 ± 0.3
C18:1 (n – 5)	trace	trace
C18:2	12.7 ± 2.8^{b}	14.4 ± 1.8
C18:3 (<i>n</i> – 6)	0.3 ± 0.02	0.2 ± 0.03
C18:3 (<i>n</i> – 3)	trace	0.1 ± 0.01
C20:0	0.3 ± 0.01	0.2 ± 0.02
C20:1	0.1 ± 0.02	0.1 ± 0.01
C20:3	0.2 ± 0.01	0.1 ± 0.02
C20:4 (<i>n</i> – 6)	4.9 ± 0.01^{c}	2.0 ± 0.07
C20:5	0.2 ± 0.01	0.1 ± 0.02
C22:5 (n – 3)	0.8 ± 0.01^{c}	0.2 ± 0.01
C24:1	trace	trace
C22:6 (n – 3)	4.2 ± 0.09 ^c	1.0 ± 0.04
saturates	37.5%	46.1%
unsaturates	62.5%	53.9%

^{*a*} Fatty acid concentration expressed as wt % of individual fatty acid methyl esters in the total fatty acid methyl esters. Values are the mean \pm standard deviation (n = 10). ^{*b*} Significantly different from hen's eggs (p < 0.05). ^{*c*} Significantly different from hen's eggs (p < 0.05).

the relationship between oxidative stability and the fatty acid compositions, we measured fatty acid compositions of lipids in whole silky fowl's eggs and hen's eggs. As shown in **Table 1**, the ratio of unsaturated fatty acids was 62.5% of total fatty acid in silky fowl eggs and the unsaturated fatty acid of hen's eggs was 53.9%. Our results suggested that the degree of unsaturation of fatty-acyl-chain-containing lipids was closely associated with their oxidative stability. Previously, it was reported that unsaturated fatty acids showed stronger antioxidative activity than saturated fatty acids (*14*). The present results are consistent with this previous report. It is speculated that the unsaturated fatty acid fatty acid chains of lipids contribute to the oxidative stability of silky fowl eggs.

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