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Lipid Profile and Oxidative Stability of Commercial Egg Products

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ABSTRACT

This work was conducted to investigate the quality of lipids in various commercial egg products. Lipids of egg yolk were extracted from fresh egg, Pidan (preserved egg), tea egg, simmered egg, iron egg (deeply simmered and dried egg) and salted egg by Folch reagent, a mixture of chloroform and methanol at 2:1 volume ratio. The extracts and commercial yolk oil were analyzed to determine lipid profile, fatty acid composition, thiobarbituric acid value (TBA value), acid value and peroxide value. Results showed that the quality of yolk lipids was quite different. The lipid content ranged from 27% for Pidan to 46% for iron egg. Phospholipid content was highest in fresh egg (about 351 mg/g oil), while lowest in Pidan (about 175 mg/g oil). Cholesterol content of fresh egg yolk oil was the highest, about 38 mg/g oil, while the Pidan yolk oil contained the least cholesterol (28.51 mg/g oil). On the other hand, fatty acid compositions of all yolk lipids were consistent. Oleic, palmitic and linoleic acids were the three most abundant fatty acids. Acid and TBA values of lipids in processed eggs were generally higher than those in fresh eggs. Commercial yolk oil products also showed significant differences in lipid profile and fatty acid composition. The ranges of triglyceride, cholesterol and phospholipid contents were 59-95%, 0.9-18% and 1.8-33%, respectively. The yolk oils with higher liquid characteristic showed lower content of phospholipid and higher triglyceride content. Meanwhile, the yolk oil products with higher phospholipid content contained more 18:0, 20:4 and 22:6 fatty acids and less 16:1 and 18:1 fatty acids. Peroxide value was not detectable in all egg yolk extracts and yolk oils.

Key words: yolk oils, phospholipids, cholesterol, fatty acid composition, acid value, TBA value

INTRODUCTION

As one group of the major agricultural products in Taiwan, eggs are the nutritious favorites providing complete proteins, lipids, iron, vitamin A, vitamin B_1, vitamin B_2 and niacin. Each egg consists of three components: the shell, the albumen and the yolk. According to Liu and Lee[(1)], a whole egg contains 11.0-11.1% shell, 59.7-60.6% albumen and 28.4-29.3% yolk. Yolk solids, 59.0-64.4% of total solids, are composed of 34.8-37.8% proteins and 62.4-65.2% lipids. Since most egg lipids are located in the yolk, they are susceptible to oxidation and are very important for quality control[(2)]. In addition, although yolk lipids generally include triglycerides, phospholipids, cholesterol and other trace compounds, different components of yolk lipids may result in adverse effects for the human health. For instance, lecithin was reported to benefit the reduction of cholesterol level in serum[(3)], whereas cholesterol elevated blood lipid level and increased the risk of cardiovascular diseases[(4)]. Therefore, it is important to understand lipid profile and the oxidative status of various egg products.

Eggs have been important for humans ever since history was recorded. Modern technology further contributed to the versatile uses of eggs. In addition to fresh eggs, other types of egg products in Taiwan include Pidan, salted egg, tea egg, simmered egg, iron egg and yolk oil. Except for the traditional duck egg products, Pidan and salted egg, tea egg, simmered egg and iron egg are usually processed hen eggs. Pidan is a special local product manufactured by laying or soaking whole eggs in 3-10% alkaline solutions or 5-20% saline solutions. Salted egg is made by soaking whole eggs in more than 30% concentrated saline solutions for over one month. Tea eggs are well-known simmered eggs cooked with black tea. Iron egg is another local specialty made by repeatedly simmering unshelled boiled eggs two hours a day for one week[(2)]. The harsh conditions such as the strong alkaline treatment and overheating used in egg processing may influence the quality of final products.

The objective of this work was to investigate the lipid quality of various commercial egg products by determining the lipid profiles and oxidative stabilities.

MATERIALS AND METHODS

I. *Materials*

Sixteen each of fresh hen eggs, Pidan and salted eggs were purchased from local convenient stores. Iron eggs were obtained from a Tamsui amusement park and local convenient stores. Tea eggs and yolk oil were purchased

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from convenient stores and local markets, respectively. All solvents and reagents were of analytical grade. Methanol, chloroform and *n*-hexane were purchased from Merck Co. Cholesterol, cholestane, thiobarbituric acid, potassium iodide, sodium thiosulfate, sodium hydroxide were obtained from Sigma-Aldrich (St. Louis, MO, USA).

II. *Methods*

(I) *Extraction of lipids from yolk*

For fresh egg, Pidan, salted egg, tea egg, simmered egg and iron egg, yolk was separated and then extracted according to Folch *et al.*[5]

(II) *Determination of the phospholipid content of yolk*

The contents of phospholipids in fresh egg, Pidan, salted egg, tea egg and iron egg were determined according to the method described by Murphy and Roley[6]. Briefly, 1 g of extracted lipids was mixed with 0.2 g of zinc oxide in a crucible and, after being burnt or carbonized, was incinerated to ash in a furnace at 600˚C for 5-6 hr. The cooled residue was then heated to boil with 40 mL of 4 times diluted HCl and 1 mL of concentrated nitric acid. The mixture was cooled down, adjusted to 250 mL, and filtered. One milliliter of the filtrate was mixed with 8 mL molybdate-vanadate solution in a 50-mL volumetric flask and adjusted to volume with distilled water. After 30 min, the absorbance was measured at 330 nm and the amount of phosphate was calculated based on a calibration curve. Phospholipid content was finally obtained by multiplying the phosphate content by 25 and represented by dioleylphosphatidyl choline.

(III) *Determination of the cholesterol content of yolk*[7]

Lipids extracted from yolk were separately saponified and cholestane was added as the internal standard. After extraction and adjustment of volume with *n*-hexane, the sample was subjected to gas chromatographic analysis. A Hewlett-Packard 5890 gas chromatograph equipped with a DB-1 fused silica capillary column (0.2 mm × 30 m, J&W Scientific) and a flame ionization detector (H_2 flow rate 30 mL/min, air flow rate 300 mL/min) was utilized. The column temperature was programmed at 280˚C initially and increased to 300˚C at 3˚C/min. The temperature of the injector and detector was 260˚C. Nitrogen was used as the carrier gas at flow rate 1 mL/min.

(IV) *Analysis of the lipid profile of yolk oil by thin layer chromatography*[8]

Lipid extracts were separately dissolved in EtOAc/EtOH mixture (1/1, v/v) at 3% concentration and 1 μL was applied to a TLC rod. A mixed solvent of lower polarity, *n*-hexane/ether/formic acid (70/30/1, v/v), was utilized for the first development so that neutral lipids and cholesterol were separated and determined by a flame ionization detector starting from the point about 20% full length above the origin. Since the lipids retained in the origin were not burned, the TLC bar was developed in chloroform/methanol/acetic acid/H_2O (75/45/1/1, v/v) again, and phospholipids were identified at this time.

(V) *Analysis of fatty acid composition by gas chromatography*

Lipids were derivatized to generate methyl esters of fatty acids according to the method described by Lee *et al.*[9] Briefly, 0.1 g of lipids was mixed with 3 mL of diethyl ether and 1 mL of 20% methanolic tetramethylammonium hydroxide (TMAH) solution. After 10 min, water was added to stop the reaction and methyl *n*-pentadecanoate was incorporated into the mixture as the internal standard. The organic layer was collected, dehydrated with anhydrous sodium sulfate, and subjected to gas chromatographic analysis. A Hewlett-Packard 5890 gas chromatograph equipped with a DB-Wax fused silica capillary column (0.25 mm × 30 m, J&W Scientific) and a flame ionization detector (H_2 flow rate 30 mL/min, air flow rate 300 mL/min) was utilized. The column temperature was programmed at 180˚C initially, increased to 230˚C at 2˚C/min, and held at 230˚C for 15 min. The temperature of injector and detector was 260˚C. Nitrogen was used as the carrier gas at flow rate of 1 mL/min.

(VI) *Determination of acid value, peroxide value and thiobarbituric acid value*

The acid value and peroxide value were determined according to the Chinese National Standard analytical methods for edible oil CNS N6082[10] and CNS N6085[11], respectively.

Aliquots of lipids were distilled to extract malondialdehyde, which was then subjected to the reaction with thiobarbituric acid. The absorbance was finally measured at 532 nm[12,13].

(VII) *Statistical Analysis*

The data were presented as mean ± SD (n = 16) and compared by analysis of variance (ANOVA) [14].

RESULTS AND DISCUSSION

I. *Lipid Content of Commercial Egg Products*

The lipid contents in the yolks of the selected egg products ranged from 26.65% in Pidan to 46.33% in iron eggs. The tea egg yolk and the simmered egg yolk possessed similar contents of lipids as the fresh egg yolk (Table 1). The fact that iron egg yolk and salted egg yolk contained higher lipid contents could be the results of the

significant dehydration during processing. For instance, the moisture content of salted egg yolk was determined to be 28.46 ± 1.24% (n = 3), which is much lower than the water content of the fresh egg yolk, about 48%[15]. High concentrations of sodium chloride in salted egg white may drive the flow of water from yolk to egg white and result in the decrease of moisture content and the increase of fat content in yolk. On the other hand, the lower lipid content of Pidan yolk may be due to the hydrolysis of the yolk lipids caused by saponification, which draws more water to the yolk.

Table 1. Lipid contents of commercial egg yolks obtained from commercial egg products*

Item	Lipid content (%)**
Fresh egg yolk	38.21 ± 2.01[b]
Tea egg yolk	37.09 ± 1.07[b]
Simmered egg yolk	39.31 ± 3.09[b]
Iron egg yolk	46.33 ± 3.38[a]
Salted egg yolk	45.21 ± 1.65[a]
Pidan yolk	26.65 ± 4.74[c]

*Lipid content was calculated on wet basis.

**Data are presented as mean ± SD. Values within the same column bearing different superscript letters are significantly different as determined by Duncan's multiple range test ($p < 0.05$).

Table 2. Phospholipid and cholesterol contents of the egg oils prepared from the commercial egg products (mg/g oil)

Oil	Phospholipids*	Cholesterol*
Fresh egg oil	350.50 ± 2.60[a]	38.15 ± 3.41[a]
Tea egg oil	338.80 ± 9.34[a]	34.14 ± 1.51[a]
Simmered egg oil	349.50 ± 4.89[a]	36.52 ± 1.44[a]
Iron egg oil	343.50 ± 5.90[a]	34.51 ± 2.14[a]
Salted egg oil	233.50 ± 3.16[b]	37.51 ± 2.14[a]
Pidan oil	175.10 ± 2.67[c]	28.51 ± 3.01[b]

*Data are presented as mean ± SD. Values within the same column not bearing the same superscript letter are significantly different as determined by Duncan's multiple range test ($p < 0.05$).

II. *Phospholipid and Cholesterol Contents of Commercial Egg Products*

As shown in Table 2, the phospholipid contents of iron egg oil, tea egg oil, simmered egg oil and fresh egg oil were about 338.8 to 350.5 mg/g oil. Pidan oil contained the lowest amount of phospholipids, 175.1 mg/g oil. For a fresh hen egg yolk with 7 grams of oil, it contains about 2450 mg of phospholipids. For a Pidan yolk with 5 grams of oil, it contains about 875 mg of phospholipids. These calculations indicate the amount of phospholipids that people can obtain from the consumption of these egg products. Although the lipid and moisture contents of fresh duck egg yolk were shown to be very close to those of fresh hen egg yolk[2], we found that the amount of extractable lipids in Pidan yolk was far less than that in fresh hen egg yolk. It is suspected that the harsh treatment of Pidan may induce interactions between lipids and proteins and result in unextractable products, but the mechanism needs further investigation.

The lowest cholesterol content, 28.51 mg/g oil, was found in Pidan oil (Table 2). The other egg products contained similar amounts of cholesterol, ranging from 34.14 to 38.15 mg/g oil with no significant differences. Based on the data shown in Table 2, a fresh egg yolk with 7 g of oil and a Pidan yolk with 5 g of oil may contain 267 mg and 143 mg of cholesterol, respectively.

III. *Lipid Profile of Yolk Oil*

Table 3 shows lipid profile of commercial yolk oil products. Sixteen samples can be classified into 3 groups based on their total phospholipid contents. There were 8 out of 16 samples, i.e. 50%, containing more than 20% phospholipids, 5 samples, i.e. 31.3%, containing 13-18% phospholipids, and 3 samples, i.e. 18.7%, containing less than 5% phospholipids.

Table 3. Lipid compositions of commercial yolk oils (%)*

Sample no. (state of oil)	TG	Cholesterol	PE	PC	Others	Total
1 (solid)	67.97	4.23	2.49	19.07	6.24	27.80
2 (liquid)	94.31	2.61	ND	ND	3.08	3.08
3 (liquid)	94.84	3.32	ND	ND	1.84	1.84
4 (solid and black)	64.06	7.89	ND	ND	28.05	28.05
5 (solid and black)	60.09	6.86	ND	ND	33.05	33.05
6 (solid and black)	74.07	12.88	ND	ND	13.05	13.05
7 (solid and black)	69.25	17.61	ND	ND	13.14	13.14
8 (solid)	68.06	2.98	ND	13.87	15.09	28.96
9 (liquid)	71.74	0.90	ND	ND	27.36	27.36
10 (liquid)	58.68	16.26	ND	ND	25.06	25.06
11 (liquid)	77.99	5.26	ND	ND	16.75	16.75
12 (liquid)	74.89	2.72	ND	ND	22.39	22.39
13 (liquid)	76.05	2.58	ND	ND	21.37	21.37
14 (liquid)	93.46	2.25	ND	ND	4.29	4.27
15 (solid)	81.22	2.82	ND	7.89	8.07	15.96
16. home-made yolk oil (solid)**	75.11	7.11	ND	ND	17.78	17.78

* TG, triglycerides; PE, phosphatidylethanolamine; PC, phosphatidylcholine; ND, not detectable.

** Yolk oil was made by heating yolk on a hot plate at 260˚C for 24 hr.

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Meanwhile, yolk oil samples can be classified into 4 groups according to their cholesterol content (Table 3). The group of yolk oil with the highest cholesterol content, more than 10%, included 3 samples. The second group consisted of 4 samples with cholesterol contents of about 5 to 8%. Furthermore, there were 2 samples containing 3-5% cholesterol and 7 samples containing less than 3% cholesterol.

The manufacturing process seems to be crucial in obtaining commercial yolk oil products that contain high levels of phospholipids and low level of cholesterol and are effective in the reduction of blood lipids. According to Liu *et al.*[16], lipid profile could be significantly affected by the extraction method. They reported that an egg yolk oil with rich phsopholipids (90%) and very low cholestrol (0.9%) was obtained by firstly extracting the egg yolk with alcohol, and followed by extracting the alcohol extract with acetone to remove the cholestrol therein. The fact that only commercial yolk oils such as samples 6 and 7 in Table 3 contained low level of phospholipids and high level of cholesterol suggests the manufacturing process of commercial products needs modification to improve the quality.

Table 4. Fatty acid compositions of the egg oils prepared from commercial egg products (%)

Oil	Fatty acid							
	16:0	16:1	18:0	18:1	18:2	20:4	22:6	Others
Fresh egg oil	24.86	5.59	7.94	42.39	14.85	1.83	1.47	1.07
Tea egg oil	24.16	3.64	4.92	49.12	14.97	1.10	1.16	0.93
Simmered egg oil	26.65	3.73	8.62	46.84	10.99	1.16	1.98	0.11
Iron egg oil	28.31	4.37	7.49	45.86	10.33	1.46	2.17	0.11
Salted egg oil	27.86	2. 11	6.93	44.06	14.93	1.36	1.79	0.12
Pidan oil	25.65	2.36	6.66	45.20	15.41	2.73	1.09	0.30

Table 5. Fatty acid compositions of yolk oils (%)

Sample no.	Fatty acid											
(state of oil)	14:0	16:0	16:1	18:0	18:1	18:2	18:3	20:4	20:5	22:5	22:6	Others
1 (solid)	0.38	22.44	1.92	8.43	32.19	20.50	0.29	2.23	ND*	0.61	0.46	4.55
2 (liquid)	0.20	23.57	3.79	6.23	46.25	16.84	0.10	0.31	ND	ND	ND	2.71
3 (liquid)	0.48	24.88	3.62	7.24	46.52	13.14	0.40	0.44	ND	ND	0.20	3.08
4 (solid and black)	0.28	25.17	2.00	10.47	41.35	12.22	ND	0.82	ND	ND	ND	7.69
5 (solid and black)	0.34	24.98	1.99	9.68	42.52	11.18	ND	0.49	ND	ND	ND	8.82
6 (solid and black)	0.29	21.18	1.93	7.87	34.53	27.79	2.52	0.68	ND	ND	ND	3.21
7 (solid and black)	0.23	27.54	1.98	11.56	36.76	14.42	0.21	2.30	ND	ND	2.44	2.56
8 (solid)	0.21	23.61	1.96	9.47	30.84	24.77	0.15	2.99	ND	0.98	ND	5.02
9 (liquid)	0.25	19.15	1.13	3.70	46.09	26.58	0.85	0.52	ND	ND	ND	1.73
10 (liquid)	0.38	27.96	3.22	11.09	37.97	12.12	0.11	2.34	ND	0.36	1.22	3.23
11 (liquid)	0.22	20.13	1.48	3.95	46.19	23.58	0.88	0.50	ND	ND	ND	3.07
12 (liquid)	0.21	25.05	3.97	6.07	47.14	13.38	0.53	0.21	ND	ND	ND	3.44
13 (liquid)	0.56	25.24	4.32	6.27	45.54	14.24	0.50	0.45	ND	ND	ND	2.88
14 (liquid)	0.25	26.05	4.07	6.83	44.60	14.33	0.42	0.40	ND	ND	ND	3.05
15 (solid)	0.27	21.05	1.72	6.80	34.12	26.35	2.99	0.59	0.17	ND	1.49	4.45
16. home-made yolk oil (solid)**	0.48	42.19	1.88	12.09	24.29	14.44	ND	2.84	ND	ND	0.59	1.22

* ND, not detectable.

** Yolk oil was made by heating yolk on a hot plate at 260˚C for 24 hr.

Table 6. Acid value (AV) and TBA value of the egg oils prepared from commercial egg products

Egg oil	Acid value (mg KOH/g oil)	TBA value (mg MDA/kg oil)
Fresh egg oil	0.30 ± 0.53^{e}	0.17 ± 0.05^{cde}
Tea egg oil	6.74 ± 1.16^{c}	2.73 ± 0.77^{a}
Simmered egg oil	6.13 ± 2.40^{c}	0.61 ± 0.01^{de}
Iron egg oil	11.54 ± 2.10^{a}	1.61 ± 0.53^{bc}
Salted egg oil	4.15 ± 0.86^{d}	2.24 ± 0.78^{ab}
Pidan oil	9.23 ± 1.51^{b}	1.18 ± 1.04^{cd}

*Data are presented as mean ± SD. Values within the same column not bearing the same superscript are significantly different as determined by Duncan's multiple range test ($p < 0.05$).

Table 7. Acid value of commercial yolk oils and solvent-extracted oils

Sample no. (state of oil)	Acid value (mg KOH/g oil)
4 (solid and black)	44.80
5 (solid and black)	28.00
9 (liquid)	0.93
10 (liquid)	1.40
11 (liquid)	2.24
12 (liquid)	3.70
16 home-made yolk oil A*	1.20
17 home-made yolk oil B*	6.41
20 hexane-extracted oil	5.14
21 alcohol-extracted oil	14.83

* Yolk oil was made by heating yolk on a hot plate at 260˚C for 24 hr.

IV. *Fatty Acid Compositions of Egg Products and Yolk Oil*

The fatty acid compositions of the oils prepared from commercial egg products were consistent (Table 4). Among the fatty acids, the first major fatty acid was oleic acid (C18:1), with a content of about 44-49%, followed by palmitic acid (C16:0) of about 24-28%, and then linoleic acid (18:2) of about 10-15%.

On the other hand, the fatty acid compositions of yolk oil samples were quite different as shown in Table 5, probably due to differences in manufacturing procedures. Nevertheless, the first three most abundant fatty acids were still oleic, palmitic and linoleic acids. In general, the yolk oil products such as samples 1, 4, 5 and 8 with higher phospholipid content contained more 18:0, 20:4 and 22:6 fatty acids and less 16:1 and 18:1 fatty acids. The data in Table 5 indicated that fatty acid composition of yolk oil has no relationship to its appearance.

V. *Acid Value, Peroxide Value and TBA Value of Egg Products and Yolk Oil*

The acid values of the egg oils prepared from all processed eggs were significantly higher than that of fresh egg oil (Table 6). Among all samples, iron egg oil had the highest acid value, 11.54 mg KOH/g oil, and Pidan oil had the second highest acid value, 9.23 mg KOH/g oil. The dramatic increase of free fatty acids was probably due to the repetitive heating of iron eggs and the harsh alkaline treatment of Pidan. Lipid hydroperoxides were neither detectable in yolk of all egg products nor yolk oil samples (data not shown). For the egg yolks of various egg products, the formation of lipid hydroperoxides may be prevented because yolk is embedded in the core of an egg and scarcely in contact with air. As for yolk oils, further studies are required to understand whether lipid hydroperoxides never formed or were completely decomposed.

TBA values of the egg oils prepared from egg products were 0.17-2.73 mg malondialdehyde/kg oil (Table 6). The highest TBA value was found in tea egg and probably due to the continuous heating for 12 to 48 hr, which was actually longer than the total heating time of iron egg since the latter was discontinuously heated for 1-2 hr within one week. Additionally, the seasonings may accelerate lipid oxidation in tea eggs during prolonged heating.

Table 7 shows that the acid values of commercial yolk oils with solid and black appearance were generally higher than those of liquid oil. The highest acid value, 44.80 mg KOH/g oil, was found in sample 4, while the lowest acid value, 0.93 mg KOH/g oil, in sample 9.

CONCLUSIONS

Lipid contents of commercial egg products significantly varied with the processing method. The lipid content of iron egg yolk was highest among all samples, about 46% of yolk weight, while that of Pidan yolk was lowest, about 27%. Results showed that Pidan oil contained the least amount of phospholipids, about 175 mg/g oil, and the least amount of cholesterol, about 28.5 mg/g oil. Fatty acid compositions of egg products and yolk oils showed that the three most abundant fatty acids were oleic, palmitic and linoleic acids. For measurement of oxidative stability, the highest level of TBA-reactive substances was found in tea egg oil and the maximum amount of free fatty acids in iron egg oil. As for yolk oil, we found the more solid the higher level of phospholipids. Meanwhile, the darker yolk oil contained more free fatty acids.

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