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# Lipolysis and Oxidation of Lipids during Egg Storage at Different Temperatures

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**Abstract**

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The aim of this study was to investigate lipolysis and lipid oxidation of stored eggs at different temperatures (4 and 22°C) by evaluating the changes in physicochemical index, lipid profiles, enzymatic activity, and oxidative index. The results showed that the changes in physicochemical index were more significant at 22°C than at 4°C. Weight loss, moisture content, and pH of egg yolk increased significantly ($P < 0.05$), whereas the yolk index decreased during storage. However, there was no significant difference in lipid profiles between 4 and 22°C storage temperature. The lipid composition analysis demonstrated that lipid hydrolysis took place during egg storage and resulted in a marked decrease of PL and increase of FFA. It was also found that the content of phosphatidylcholine (PC), phosphatidylethanolamine (PE), and phosphatidylinositol (PI) decreased significantly during storage. The correlation analysis showed that the lipid degradation is significantly positively related to lipase activity ($P < 0.05$), and the marked changes of lipid fractions are results of both hydrolysis and oxidation. It can be concluded that the egg physicochemical index and lipase activity were greatly influenced by temperature during storage, but the yolk lipid stability was not significantly influenced by storage temperature.

**Keywords**: hen egg; lipid hydrolysis; lipid oxidation

Hen eggs are not only one of the most versatile foods with a high nutritional value for humans, but also are efficient ingredients in many food products, because of their nutritional, organoleptic, and functional properties (Anton 2013). In developed countries, eggs are mainly transported and stored under chilling conditions. However, there is no established cold-chain system for egg transportation and storage in China. Long-term storage, particularly under room temperature, may result in a variety of complex changes in eggs, such as quality loss (Akyurek & Okur 2009), lipid, and protein oxidation (Botsoglou *et al.* 2013; Ren *et al.* 2013), and changes in the fatty acid composition as well as protein conformation (Qiu *et al.* 2012).

Egg yolk lipids are more susceptible to oxidation due to a relatively high concentration of polyunsaturated fatty acids (PUFA) (Abreu *et al.* 2014). Lipid oxidation of egg yolk during processing has attracted much interest among researchers. Several studies have demonstrated that processing methods significantly affect the lipid oxidation of egg yolk, and the oxida-

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tion levels are higher in scrambled eggs compared with hard-boiled eggs (Cortinas *et al*. 2003). Mazalli and Bragagnolo (2009) also reported a decrease in PUFA content as a result of heat treatment of eggs. Compared with processing, the lipid oxidation of egg yolk during storage was moderate. To date, only a few studies have reported the oxidation of egg yolk lipids during storage. For instance, Ren *et al*. (2013) found that malondialdehyde (MDA) content in yolk samples increases significantly when stored at 4°C for 28 days, and storage also results in a reduction of long chain n-3 PUFA. Botsoglou *et al*. (2012) reported that storage (4°C, 60 days) decreases the proportion of PUFAs and increases that of MUFAs in egg yolks. However, these reports focused only on the change of fatty acids during egg storage, but the variation of lipid fractions and endogenous enzymatic activity were not reported. Thus, there has been scarce systematic information available on the lipolysis and oxidation of lipids during hen egg storage so far.

The objective of this study was to investigate lipolysis and lipid oxidation in hen egg yolks during storage below 4 and 22°C, and to analyse the relationships between the egg yolk lipolytic enzymatic activities and lipid oxidation.

## MATERIAL AND METHODS

***Sampling and sample preparation***. A total of 300 White Leghorn hen eggs, weighing from 58 g to 62 g, were produced by 46-wk-old hens of the same strain. The egg samples were obtained from the Poultry Research Centre Farm of Huazhong Agricultural University. Shortly thereafter, the unwashed eggs were selected and classified and randomly divided into two groups, stored at a constant temperature and in a humidity incubator at 22 ± 1 and 4 ± 1°C, respectively. The relative humidity was set at 50% for all treatments. Eggs were randomly selected for sampling every 10 days for 50 days.

***Physicochemical analysis***. The moisture content of egg yolk was determined according to ISO1442:1997(E) and presented as g/100 g of egg yolk. About 1 g of homogeneous yolk was weighed and put into a weighing aluminium specimen box. Both the yolk and the box were dried at 100 ± 5°C to a constant weight to calculate the moisture content of yolk.

Five grams of egg yolk and 5 ml of distilled water were mixed thoroughly using a Fluko FA25 homogeniser (Fluko, China) at 5000 rpm for 10 × 3 seconds. The mixture pH was measured using a pH meter (STARTER-3C; Ohaus, USA).

The eggs were weighed and then broken onto a platform, where the height and width of the egg yolk were measured using a vernier calliper. The yolk index and weight loss were determined as follows:

Yolk index = yolk height/yolk width

Weight loss (%) of whole eggs during storage = [(initial whole egg weight at day 0 – whole egg weight after storage)/initial whole egg weight at day 0] × 100

***Total lipid extraction and separation***. The yolk lipids were extracted with a mixture of chloroform and methanol (2 : 1 v/v) (Folch *et al*. 1957); after extraction, total lipid content was determined gravimetrically after the solvent removal under vacuum. Lipid extracts were kept at –20°C until further analysis. Lipid fractions were prepared using an $NH_2$-aminopropyl mini-column (100 mg; Varian, USA) according to the method of Jin (2010). Briefly, the lipid extracts (30–50 mg) were dissolved in 5 ml of $CHCl_3$/MeOH (2 : 1 v/v) and fractionated by passing through the mini-columns. Neutral lipids, free fatty acids, and phospholipids were eluted in a sequence with 5 ml $CHCl_3$/isoproponal (2 : 1 v/v), 5 ml diethyl ether/acetic acid (2%), and 5 ml MeOH/HCl (9 : 1 v/v).

***Identification of PL fractions***. The obtained phospholipid fractions were dried under nitrogen flow, diluted with hexane/isopropanol (3 : 2 v/v) to 0.3 ml and analysed by HPLC. The PL fractions were determined using the HPLC system (E-2695; Waters, USA) equipped with a PDA detector (at 205 nm) and an Agilent Zorbax Rx-SIL column (5 μm, 250 mm × 4.6 mm i.d.) operating at 25°C. Standards of phosphatidylcholine (PC), phosphatidylethanolamine (PE), and phosphatidylinositol (PI) were procured from Sigma (USA). An isocratic elution with hexane-isopropanol-0.1% glacial acetic acid (8 : 8 : 1 v/v) was performed at a flow rate of 0.8 ml/min. The identification of PC, PE, and PI was performed by comparing the retention time with standards.

***Lipase extraction and activities assay***. Crude lipases were extracted according to the method of Jin (2010) and collected for enzymatic assays.

Neutral lipase activity and acid lipase activity were evaluated according to the method of Vestergaard *et al*. (2000) using a fluorescence spectrometer (RF-5301 PC; Shimadzu Co., Japan) set at $\lambda_{ex}$ = 350 nm and $\lambda_{em}$ = 445 nm.

The phospholipase activity was determined using the same method as described for acid lipase, except

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that 150 mM sodium fluoride was added to the disodium phosphate/citric acid buffer with Triton X-100 and BSA (Hernández *et al*. 1999).

***Lipid oxidation analysis and lipoxygenase (LOX) activity assay***. Lipid oxidation was evaluated by assessing the thiobarbituric acid reactive substances (TBARS) as described by Siu and Draper (1978) and expressed as mg MDA/kg egg yolk. LOX was extracted as previously described by Jin *et al*. (2010) and collected for the LOX activity assay. The linoleic acid substrate solution was prepared as previously described by Gata *et al*. (1996). LOX activity was determined using a spectrophotometer (3-30K; SIGMA, USA) at 20°C by monitoring the increase in absorbance at 234 nm for 1 minute. One unit (U) of LOX activity was defined as the amount of enzyme resulting in an absorption increment of 0.001/min and per g protein.

***Statistical analysis***. Measurements for all of the groups were performed in triplicate, and the values were averaged and reported with their standard deviation (SD). One-way analysis of variance (ANOVA) and correlation analysis were performed using the SPSS Program (ver. 19.0). Principal component analysis (PCA) was performed using the Unscrambler software (ver. 9.7).

## RESULTS AND DISCUSSION

***Physicochemical changes in egg yolk during storage at different temperatures***. The physicochemical changes of hen eggs during storage at 4 and 22°C are presented in Table 1. Being consistent with previous studies (Wardy *et al*. 2013, 2014), the weights of all tested eggs decreased significantly during storage ($P < 0.05$), which was mainly caused by the loss of water through the porous shell (Wardy *et al*. 2014), and the higher the storage temperature, the faster the moisture migration. These changes indicated an intense effect of storage temperature on the weight loss of eggs during storage. Though the total weight of stored eggs decreased significantly, the moisture content of egg yolk increased markedly during storage (Table 1), indicating water diffusion between albumen and egg yolk through the yolk membrane.

In the present study, the egg yolk pH increased from 6.10 in fresh eggs to 6.32 and 6.43 in stored eggs (50 days) at 4 and 22°C, respectively. This pH increase is attributed to the carbon dioxide loss from the breakdown of carbonic acid in egg yolks (Wardy *et al*. 2013). The yolk pH at 22°C was significantly higher than at 4°C, which indicated that high temperature may accelerate the loss of carbon dioxide.

In accordance with previous reports (Nongtaodum *et al*. 2013; Wardy *et al*. 2013), the yolk index of all tested eggs significantly decreased during storage (Table 1), by 62.73% at 22°C vs. 7.33% at 4°C. The decrease in the yolk index is closely related to the progressive weakening of the vitelline membrane, reduction in total solids, and progressive transition of egg yolk rheological properties (Torrico *et al*. 2014). Our results showed that room temperature (22°C) storage can accelerate the deterioration of eggs quality, and refrigeration temperature (4°C) is suitable for egg storage.

***Changes in lipid composition and PL classes during storage***. In fresh hen egg yolk, the total lipid (TL) content was 59.70 g/100 g of the yolk dry matter, including 53.21% neutral lipids (NL), 45.55% phospholipids (PL), and 1.24% free fatty acids (FFAs), which is similar to previous studies by Li-Chan *et al*. (1995).

There was a significant ($P < 0.05$) decrease of TL content in all egg yolks of the two test groups (Table 2). At the end of storage, the TL content decreased

Table 1. Changes in the physicochemical indices during egg storage (mean ± SD)

| Variables | T (°C) | 0 day | 10 days | 20 days | 30 days | 40 days | 50 days |
|---|---|---|---|---|---|---|---|
| Weight loss (%) | 4 | $0^{f}$ | $0.62 \pm 0.02^{eB}$ | $1.28 \pm 0.02^{dB}$ | $2.32 \pm 0.07^{cB}$ | $2.71 \pm 0.05^{bB}$ | $2.82 \pm 0.01^{aB}$ |
| | 22 | $0^{f}$ | $0.95 \pm 0.02^{eA}$ | $2.53 \pm 0.09^{dA}$ | $3.88 \pm 0.07^{cA}$ | $4.96 \pm 0.04^{bA}$ | $5.63 \pm 0.03^{aA}$ |
| Moisture content (%) | 4 | $46.96 \pm 0.30^{d}$ | $47.64 \pm 0.06^{cB}$ | $48.36 \pm 0.14^{bB}$ | $48.61 \pm 0.06^{abB}$ | $48.71 \pm 0.08^{abB}$ | $48.92 \pm 0.31^{aB}$ |
| | 22 | $46.96 \pm 0.30^{d}$ | $49.32 \pm 0.08^{cA}$ | $50.83 \pm 0.03^{bA}$ | $50.58 \pm 0.05^{bA}$ | $51.52 \pm 0.09^{aA}$ | $51.29 \pm 0.25^{aA}$ |
| pH value | 4 | $6.10 \pm 0.01^{e}$ | $6.14 \pm 0.01^{dA}$ | $6.20 \pm 0.01^{cA}$ | $6.27 \pm 0.01^{bB}$ | $6.31 \pm 0.01^{aB}$ | $6.32 \pm 0.01^{aB}$ |
| | 22 | $6.10 \pm 0.01^{e}$ | $6.12 \pm 0.01^{dA}$ | $6.21 \pm 0.01^{cA}$ | $6.30 \pm 0.01^{bA}$ | $6.40 \pm 0.01^{aA}$ | $6.43 \pm 0.01^{aA}$ |
| Yolk index | 4 | $0.49 \pm 0.01^{a}$ | $0.49 \pm 0^{abA}$ | $0.48 \pm 0.01^{abA}$ | $0.47 \pm 0.02^{abA}$ | $0.47 \pm 0^{cA}$ | $0.46 \pm 0.02^{dA}$ |
| | 22 | $0.49 \pm 0.01^{a}$ | $0.34 \pm 0.02^{bB}$ | $0.31 \pm 0.01^{cB}$ | $0.28 \pm 0.01^{dB}$ | $0.24 \pm 0^{eB}$ | $0.18 \pm 0.01^{fB}$ |

$^{a–f}$mean values in the same row with different superscripts differ significantly ($P < 0.05$); $^{A–B}$values of two experimental groups with different letters differ significantly ($P < 0.05$)

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Table 2. Changes in lipid composition during egg storage (mean ± SD; data were mean values of three replicates)

| Variables | T (°C) | 0 day | 10 days | 20 days | 30 days | 40 days | 50 days |
|---|---|---|---|---|---|---|---|
| Total lipids (TL)[1] | 4 | 59.70 ± 1.10$^{a}$ | 58.46 ± 1.01$^{aA}$ | 56.56 ± 0.56$^{cA}$ | 54.08 ± 0.54$^{dA}$ | 52.86 ± 1.08$^{cdA}$ | 52.14 ± 0.67$^{dA}$ |
| | 22 | 59.70 ± 1.10$^{a}$ | 58.28 ± 0.65$^{b\ A}$ | 56.18 ± 0.58$^{c\ A}$ | 53.81 ± 0.41$^{dA}$ | 52.58 ± 0.24$^{eA}$ | 51.29 ± 0.39$^{fA}$ |
| Neutral lipids (NL)[2] | 4 | 53.21 ± 1.66$^{b}$ | 55.30 ± 3.38$^{abA}$ | 56.82 ± 0.56$^{aA}$ | 56.93 ± 0.79$^{aA}$ | 57.23 ± 1.70$^{aA}$ | 56.35 ± 0.34$^{aA}$ |
| | 22 | 53.21 ± 1.66$^{c}$ | 55.23 ± 1.04$^{bcA}$ | 56.75 ± 0.78$^{abA}$ | 57.85 ± 2.20$^{aA}$ | 58.78 ± 0.73$^{aA}$ | 57.62 ± 0.23$^{bA}$ |
| Free fatty acids (FFA)[2] | 4 | 1.24 ± 0.28$^{c}$ | 1.44 ± 0.48$^{bcA}$ | 1.10 ± 0.17$^{cA}$ | 1.48 ± 0.07$^{bcB}$ | 1.79 ± 0.17$^{bA}$ | 3.26 ± 0.31$^{aB}$ |
| | 22 | 1.24 ± 0.28$^{c}$ | 1.68 ± 0.29$^{bcA}$ | 1.77 ± 0.52$^{bcA}$ | 1.83 ± 0.12$^{bA}$ | 2.08 ± 0.014$^{bA}$ | 4.12 ± 0.20$^{aA}$ |
| Phospholipids (PL)[2] | 4 | 45.55 ± 0.85$^{a}$ | 43.26 ± 3.38$^{abA}$ | 42.08 ± 0.54$^{bA}$ | 41.59 ± 0.73$^{bA}$ | 40.98 ± 1.65$^{bA}$ | 40.22 ± 0.39$^{bA}$ |
| | 22 | 45.55 ± 0.85$^{a}$ | 43.09 ± 0.80$^{bA}$ | 41.48 ± 0.68$^{bcA}$ | 40.32 ± 2.28$^{cdA}$ | 39.14 ± 0.63$^{dA}$ | 38.26 ± 0.11$^{dB}$ |

$^{a–f}$mean values in the same row with different letters differ significantly ($P < 0.05$); $^{A–B}$values of two experimental groups with different letters differ significantly ($P < 0.05$); [1]expressed as g/100 g dry egg yolk; [2]expressed as g/100 g lipid

to 52.14 g/100 g and 51.29 g/100 g of the yolk dry matter for 4 and 22°C storage groups, respectively. The significant decrease of the TL content was closely related with the variation of lipid fractions. During egg storage, the lipid components (especially the phospholipids) can be hydrolysed by the endogenous enzyme of egg yolk and give off free fatty acids which are the main substrates of lipid peroxidation. Lipid peroxidation can generate hydroperoxides and secondary oxidation products and finally decrease the lipid contents. Comparing the two experimental groups, it was found that the lipid content was not significantly affected by storage temperature. This has not been reported elsewhere in the literature to the best of our knowledge. Neutral lipids gradually increased during the whole storage period. At the end of storage, the NL content in total egg yolk lipids was 56.35 and 57.62% for the 4 and 22°C storage groups, respectively. In contrast to the NL, both the PL and FFA exhibited more significant changes during storage. The PL significantly ($P < 0.05$) decreased during storage by 11.7 and 16% at the end of 4 and 22°C storage, while the FFA content significantly increased from 1.24 to 3.26 and 4.12 g/100 g of lipid, respectively. The variation of lipid fractions at 4°C storage was not significantly different from that at 22°C. The increase in FFA content was demonstrated to be primarily due to the hydrolysis of PL by phospholipases in yolk lipids (Braddock & Dugan 1972). A high content of PUFA in the Sn-2 of the glycerin skeleton and the emulsification of phospholipids make them more vulnerable to be attacked from free radicals.

Changes in PL classes in egg yolk during storage were further investigated (Table 3). In fresh egg yolk, the PL covered 45.55% of total lipids and PL fractions (PC, PE, and PI) accounted for 28.13, 9.49, and 4.43% of total lipids, respectively. The results proved that PL fractions in stored eggs were significantly affected by storage time, but the higher temperature (22°C) did not significantly enhance the rate of PL hydrolysis. The variation of egg yolk PL in the process of storage has never been reported. As the primary fraction of PL, PC content was decreased by 15.96 and 18.49% after 50 days of storage. Furthermore, the PE content decreased by 16.75 and 23.92% at 4 and 22°C, respectively. Phosphatidylethanolamine was more susceptible

Table 3. Changes in phospholipid classes during egg storage (mean ± SD; data were mean values of three replicates)

| Variables | T (°C) | 0 day | 10 days | 20 days | 30 days | 40 days | 50 days |
|---|---|---|---|---|---|---|---|
| PC[1] | 4 | 28.13 ± 1.16$^{a}$ | 27.86 ± 0.09$^{aA}$ | 26.60 ± 0.54$^{abA}$ | 25.52 ± 2.31$^{bcA}$ | 24.71 ± 1.25$^{cdA}$ | 23.64 ± 1.51$^{dA}$ |
| | 22 | 28.13 ± 1.16$^{a}$ | 26.71 ± 0.09$^{abA}$ | 25.95 ± 0.36$^{bA}$ | 24.94 ± 0.29$^{cA}$ | 23.41 ± 2.07$^{cA}$ | 22.93 ± 1.34$^{cA}$ |
| PE[1] | 4 | 9.49 ± 0.23$^{a}$ | 9.27 ± 0.99$^{aA}$ | 8.90 ± 0.03$^{abA}$ | 8.57 ± 0.43$^{abA}$ | 8.44 ± 0.95$^{abA}$ | 7.90 ± 0.60$^{bA}$ |
| | 22 | 9.49 ± 0.23$^{a}$ | 8.73 ± 0.08$^{abA}$ | 8.52 ± 0.81$^{abA}$ | 8.21 ± 0.69$^{bcA}$ | 7.88 ± 0.81$^{bcA}$ | 7.22 ± 0.11$^{cA}$ |
| PI[1] | 4 | 4.43 ± 0.45$^{a}$ | 3.47 ± 0.45$^{bA}$ | 2.62 ± 0.75$^{cA}$ | 2.45 ± 0.23$^{cA}$ | 2.29 ± 0.12$^{cA}$ | 2.26 ± 0.01$^{cA}$ |
| | 22 | 4.43 ± 0.45$^{a}$ | 3.11 ± 0.14$^{bA}$ | 2.59 ± 0.19$^{cA}$ | 2.43 ± 0.08$^{cA}$ | 2.31 ± 0.40$^{cA}$ | 2.18 ± 0.02$^{cB}$ |

PC – phosphatidylcholine; PE – phosphatidylethanolamine; PI – phosphatidylinositol; [1]expressed as g/100 g lipid; $^{a–f}$mean values in the same row with different letters differ significantly ($P < 0.05$); $^{A–B}$values of two experimental groups with different letters differ significantly ($P < 0.05$)

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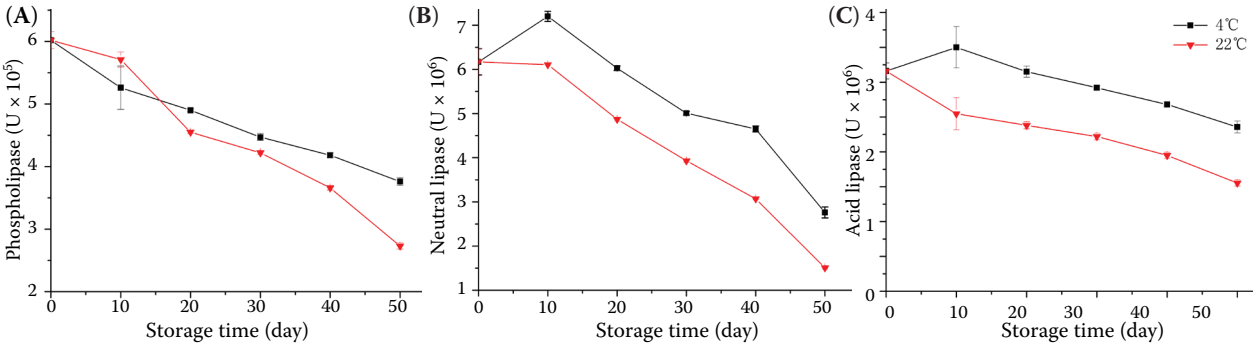


Figure 1. Variation in the activities of lipase during egg storage at different temperatures: (**A**) phospholipase, (**B**) neutral lipase, and (**C**) acid lipase

to oxidation, which is most likely due to its high content of polyunsaturated fatty acids and plasmalogen (Hernández *et al.* 1999). In addition, a significant reduction of PI content was found in the first twenty days of storage. However, the results did not exhibit a significant difference in PL classes between different storage temperatures. Perhaps, the decrease in PL fractions observed in this study was partly attributed to enzymatic hydrolysis (Keller & Kinsella 1973).

***Changes in lipolytic enzymatic activities during storage***. As far as we know, systematic research on the endogenous enzyme of egg yolk has been scarce. We found that the fresh eggs showed the highest neutral lipase activity ($6.17 \times 10^6$ U/g protein) and the lowest acid lipase activity ($3.16 \times 10^5$ U/g protein) (Figure 1). The activity of lipases continued to decrease throughout the storage period, and after 50 days storage at 22°C, the activities of neutral lipase, acid lipase, and phospholipase decreased by 75.6, 50.9, and 54.7%, respectively. A similar trend of the change was observed in lipase activities at 4°C with a relatively smaller range of the change. It suggested that the egg yolk endogenous enzyme played an important role in lipid degradation and rapid decline of enzymatic activities at 22°C compared with 4°C.

As a kind of water-soluble globulin, lipase combines the substrates through a hydrophobic area on the surface and hydrolyses these substrates (Reis *et al.* 2008). Hence, the major factor that affects the catalytic effect of lipase is whether there is a complete oil-water interface between substrates and the enzyme (Jin *et al.* 2011). Phospholipids are amphiphilic lipids and can be easily combined with lipase. Phospholipase showed an immediate activity among the three lipases, while playing the most important role in the lipolysis of egg yolk due to its remarkable hydrolysis of phospholipids in hen eggs during storage (Arthur & Choy 1987).

***Lipid oxidation and LOX activity changes during storage***. The evolution of TBARS and LOX activity during hen egg storage is shown in Table 4. In the present study, the TBARS value in fresh yolk at day 0 was 0.05 mg MDA/kg, and the value gradually increased during 50 days storage. With an extension of storage, lipid oxidation preceded an accumulation of secondary oxidation products as demonstrated by the increase in TBARS values. At the end of storage, the TBARS values reached 0.39 and 0.46 mg MDA/kg for 4 and 22°C storage groups, respectively. One of the reasons for the higher MDA value is that the high temperature could provide activation energy ($E_a$) for lipid oxidation (Jin *et al.* 2011).

In the present study, the LOX activity was determined to be 55.49 U/g in fresh egg yolk. During storage at 4 and 22°C of the first forty days, all of

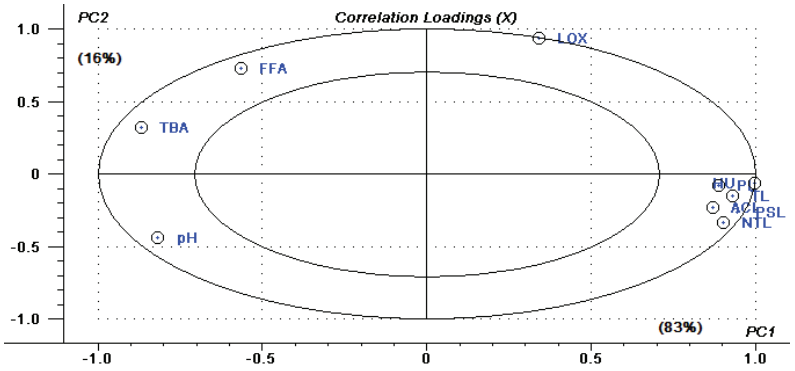


Figure 2. The correlation loading plot for the first two principal components; ellipses represent $r^2$ = 0.5 (50%) and 1.0 (100%)

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Table 4. Changes in the lipid oxidation indices and LOX activities during egg storage

| Variables | T(°C) | 0 day | 10 days | 20 days | 30 days | 40 days | 50 days |
|---|---|---|---|---|---|---|---|
| TBARS[1] | 4 | $0.050 \pm 0^{c}$ | $0.09 \pm 0.01^{bc3A}$ | $0.13 \pm 0.06^{bA}$ | $0.14 \pm 0.02^{bA}$ | $0.15 \pm 0.05^{bB}$ | $0.39 \pm 0.048^{aA}$ |
| | 22 | $0.050 \pm 0^{d}$ | $0.09 \pm 0.01^{dA}$ | $0.15 \pm 0.05^{cA}$ | $0.26 \pm 0.03^{bB}$ | $0.29 \pm 0.05^{bA}$ | $0.46 \pm 0.03^{aA}$ |
| LOX activity[2] | 4 | $55.49 \pm 4.3^{b}$ | $53.98 \pm 2.89^{bA}$ | $47.10 \pm 1.41^{cA}$ | $39.62 \pm 1.09^{dA}$ | $35.46 \pm 2.91^{dA}$ | $62.59 \pm 1.53^{aA}$ |
| | 22 | $55.49 \pm 4.3^{ab}$ | $51.76 \pm 3.50^{bcA}$ | $44.75 \pm 2.02^{cA}$ | $35.39 \pm 3.63^{dA}$ | $34.90 \pm 3.02^{dA}$ | $61.89 \pm 7.75^{aA}$ |

[1]TBARS expressed as mg MDA/kg egg yolk; [2]expressed as U/g protein; [a–f]mean values in the same row with different letters differ significantly ($P < 0.05$); [A–B]values of two experimental groups with different letters differ significantly ($P < 0.05$)

the LOX activities showed significant decreases ($P < 0.05$). Although high temperatures could enhance LOX activity (Jin *et al*. 2011), eggs stored at 22°C showed no significant difference in the LOX activity decrease compared to eggs stored at 4°C. Previous studies proved that hydroperoxides from lipid oxidation might induce the oxidation of thiol groups resulting in the inactivation of LOX (Fu *et al*. 2009). This might be the main reason for the marked decrease in LOX activity in eggs which have a relatively higher content of thiol compounds, such as sulfoprotein and sulphure-containing amino acids.

***Principal component and correlation analysis***. The principal component analysis showed the obvious separation of the stored egg samples from the PC1-PC2 scatter point plot. The graphical presentation of correlation loading (Figure 2) shows the overall potential relations between hydrolysis and lipid oxidation of 22°C storage eggs. The most important observation is the presence of negative relationships of lipid fractions (TL and PL) with pH, TBA, and FFA, indicating that a decrease of TL and PL accompanies an increase in pH, TBA, and FFA, which is consistent with our results in Table 1. Moreover, on the basis of the distribution of TL, PL, and lipases in Figure 2, it can be concluded that the lipid content was closely correlated with the hydrolysed lipids. In addition, FFA covaried closely with TBA, indicating that an increase in FFA was closely related with lipid oxidation because FFA mainly originated from PUFA of PL. Finally, as storage continued, the sample distribution gradually moved to the left of the score plot, which corresponded with Figure 2, demonstrating a decrease in PL, TL, and lipase activity and an increase in TBA, FFA, and pH with the increase of storage time.

## CONCLUSIONS

Based on these studies, it can be concluded that both lipolysis and lipid oxidation occurred in eggs during storage. Egg quality decreased and lipids contents changed significantly with the extension of storage time. The largest changes in lipid fractions were found in the PL fractions which were the main source of FFAs in eggs during storage. Lipolysis and lipid oxidation were critically influenced by storage temperature, and no significant difference was presented under different storage temperature. The correlation analysis demonstrated that the activity of acid lipase, neutral lipase, and phospholipase was significantly positively correlated with PL content, which indicates that indigenous enzymes play an important role in lipid hydrolysis. Moreover, the negative correlation between LOX activity and TL content proved that lipoxygenase may promote lipid degradation. Beyond the discovery of these variations, their mechanisms, particularly the mechanism of enzymatic oxidation, need to be studied further.

## References

Abreu V.K.G., Pereira A.L.F., Freitas E.R., Trevisan M.T.S., Costa J.M.C. (2014): Effect of anacardic acid on oxidative and color stability of spray dried egg yolk. LWT-Food Science and Technology, 55: 466–471.

Akyurek H., Okur A.A. (2009): Effect of storage time, temperature and hen age on egg quality in free-range layer hens. Journal of Animal and Veterinary Advances, 10: 1953–1958.

Anton M. (2013): Egg yolk: structures, functionalities and processes. Journal of the Science of Food and Agriculture, 93: 2871–2880.

Arthur G., Choy C. (1987): Phospholipases. In: Kuby S.A. (ed.): A Study of Enzymes. Vol. 2. Mechanism of enzyme action. Boca Raton, CRC Press: 39–561.

Botsoglou E., Govaris A., Pexara A., Fletouris D. (2012): Effect of processing and storage on the fatty acid composition of n-3 or n-6 fatty acid-enriched eggs. International Journal of Food Science and Technology, 47: 2388–2396.

Botsoglou E., Govaris A., Ambrosiadis I., Fletouris D. (2013): Olive leaves (*Olea europaea* L.) versus α-tocopheryl ac-

doi: 10.17221/174/2016-CJFS

etate as dietary supplements for enhancing the oxidative stability of eggs enriched with very-long-chain n-3 fatty acids. Journal of the Science of Food and Agriculture, 93: 2053–2060.

Braddock R., Dugan L. (1972): Phospholipid changes in muscle from frozen stored Lake Michigan coho salmon. Journal of Food Science, 37: 426–429.

Cortinas L., Galobart J., Barroeta A.C., Baucells M.D., Grashorn M.A. (2003): Change in α-tocopherol contents, lipid oxidation and fatty acid profile in eggs enriched with linolenic acid or very long-chain ω-3 polyunsaturated fatty acids after different processing methods. Journal of the Science of Food and Agriculture, 83: 820–829.

Folch J., Lees M., Sloane-Stanley G. (1957): A simple method for the isolation and purification of total lipids from animal tissues. Journal of Biological Chemistry, 226: 497–509.

Fu X., Xu S., Wang Z. (2009): Kinetics of lipid oxidation and off-odor formation in silver carp mince: the effect of lipoxygenase and hemoglobin. Food Research International, 42: 85–90.

Gata J., Pinto M., Macias P. (1996): Lipoxygenase activity in pig muscle: purification and partial characterization. Journal of Agricultural and Food Chemistry, 44: 2573–2577.

Hernández P., Navarro J., Toldrá F. (1999): Lipolytic and oxidative changes in two Spanish pork loin products: dry-cured loin and pickled-cured loin. Meat Science, 51: 123–128.

Jin G.F., Zhang J., Yu X., Zhang Y., Lei Y., Wang J. (2010): Lipolysis and lipid oxidation in bacon during curing and drying-ripening. Food Chemistry, 123: 465–471.

Jin G.F., Zhang J., Yu X., Lei Y., Wang J. (2011): Crude lipoxygenase from pig muscle: partial characterization and interactions of temperature, NaCl and pH on its activity. Meat Science, 87: 257–263.

Keller J.D., Kinsella J.E (1973): Phospholipid changes and lipid oxidation during cooking and frozen storage of raw ground beef. Journal of Food Science, 38: 1200–1204.

Li-Chan E.C.Y., Powrie W.D., Nakai S. (1995): The chemistry of eggs and egg products. In: Stadelman W.J., Cotterill O.J. (eds): Egg Science and Technology. 4th Ed. New York, Haworth Press: 105–175.

Mazalli M.R., Bragagnolo N. (2009): Increase of cholesterol oxidation and decrease of PUFA as a result of thermal processing and storage in eggs enriched with n-3 fatty acids. Journal of Agricultural and Food Chemistry, 57: 5028–5034.

Nongtaodum S., Jangchud A., Jangchud K., Dhamvithee P., No H.K., Prinyawiwatkul W. (2013): Oil coating affects internal quality and sensory acceptance of selected attributes of raw eggs during storage. Journal of Food Science, 78: 329–335.

Qiu N., Ma M., Zhao L., Liu W., Li Y., Mine Y. (2012): Comparative proteomic analysis of egg white proteins under various storage temperatures. Journal of Agricultural and Food Chemistry, 60: 7746–7753.

Reis P., Miller R., Kragel J., Leser M., Fainerman V.B., Watzke H., Holmberg K .(2008): Lipases at interfaces: unique interfacial properties as globular proteins. Langmuir, 24: 6812–6819.

Ren Y., Perez T.I., Zuidhof M.J., Renema R.A., Wu J. (2013): Oxidative stability of omega-3 polyunsaturated fatty acids enriched eggs. Journal of Agricultural and Food Chemistry, 61: 11595–11602.

Siu G., Draper H. (1978): A survey of the malonaldehyde content of retail meats and fish. Journal of Food Science, 43: 1147–1149.

Torrico D.D., Wardy W., Carabante K.M., Pujols K.D., Xu Z., No H.K., Prinyawiwatkul W. (2014): Quality of eggs coated with oil-chitosan emulsion: Combined effects of emulsifier types, initial albumen quality, and storage. LWT-Food Science and Technology, 57: 35–41.

Vestergaard C.S., Schivazappa C., Virgili R. (2000): Lipolysis in dry-cured ham maturation. Meat Science, 55: 1–5.

Wardy W., Torrico D.D., Corredor J.A., No H.K., Zhang X., Xu Z., Prinyawiwatkul W. (2013): Soybean oil-chitosan emulsion affects internal quality and shelf-life of eggs stored at 25 and 4°C. International Journal of Food Science and Technology, 48: 1148–1156.

Wardy W., Martinez K.D.P., Xu Z.M., No H.K., Prinyawiwatkul W. (2014): Viscosity changes of chitosan solution affect physico-functional properties and consumer perception of coated eggs during storage. LWT-Food Science and Technology, 55: 67–73.

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