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Effect of storage on n-3 PUFA-enriched eggs
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1. Introduction
Humans have a long history of egg consumption. Due to their high protein content, low fat content, low calorie content, high absorption rate and high conversion rate, eggs have become an attractive source of low-cost and high-quality animal protein that is widely accepted by consumers around the world. In the last decade, egg consumption by Chinese consumer has exceeded 24 million tons per year and it is on the rise, indicating that eggs are important staple food in most households (Wang, Chen, & Liu, 2015). In addition to providing essential nutrients such as folate, selenium, iron, and vitamins A and B-12 (Herron & Fernandez, 2004), eggs can also easily be enriched with omega-3 polyunsaturated fatty acids (n-3 PUFA).

The level of n-3 PUFAs in eggs can be increased by feeding the hens diets rich in these fatty acids (Ferrier et al., 1995; Fraeye et al., 2012). Studies have shown that n-3 PUFAs have several health benefits, such as reducing the incidence of cardiovascular diseases, decreasing blood pressure and promoting the development of visual and cognitive abilities in infants and young children (Juturu, 2008; Lemahieu et al., 2015).

n-3 PUFA enriched eggs are as highly perishable as conventional eggs, and their freshness and quality begin to decline immediately after production at the poultry farm (Li, Zhang, Jia, & Liu, 2017). Moreover, n-3 PUFAs increase the degree of unsaturation in egg yolk but may enhance the sensitivity to lipid oxidation (Galobart, Barroeta, Baucells, & Guardiola, 2001). Oxidized fatty acids and their denatured products are harmful to the human body and may have adverse effects after absorption (Kubow, 1993). The lipids in eggs can be oxidized during storage, especially at high temperatures (Mohiti-Asli, Shariatmadari, Lotfollahian, & Mazuji, 2008). Therefore, the European Union recommends that the eggs were kept under refrigerator at home ((EC) No 589/2008 and (EC) No 1234/2007), and the United States Department of Agriculture requires eggs to be stored at 7.2°C or lower and at a relative humidity of 40 to 70% for during extended storage (Li et al., 2017). Most available data on egg quality are focused on normal eggs, whereas data on the effects of storage conditions on the quality of n-3 PUFA enriched eggs are scarce.

The objective of this study was to evaluate the effects of storage conditions on n-3 PUFA enriched and normal eggs.
In this comparative assessment, the fatty acid content and composition, lipid oxidation and the contents of free amino acids were analyzed.

2. Materials and methods

2.1. Materials

Both types (normal and n-3 PUFA enriched) of fresh shell eggs were supplied by Sundaily farm located in Sichuan, China. The eggs were stored at 4°C and 25°C for 24 days and analyzed every 6 days. The egg samples were immediately frozen at −80°C and then freeze dried until further analysis.

2.2. Analysis of the fatty acid composition

The fatty acids in the eggs were analyzed according to the National Standards for Determination of Fatty Acids in Foods (GB 5009.168–2016). The lipid fraction of the egg was extracted, after the addition of an internal standard (C36H68O6, CAS: 13552-80-2), using ether/petroleum ether (1:1). The extraction was performed three times, and the mixture of ether and petroleum ether was removed by rotary evaporation. The lipid fraction was then methylated according to the National Standards for Determination of Fatty Acids in Foods (GB 5009.168–2016). The fatty acid methyl esters were analyzed by a standard gas chromatography method (Model 7890A, Agilent Technologies, Palo Alto, CA, USA) using an SP-2560 column (100 m × 0.25 mm ID; Supelco Inc., Bellefonte, PA, USA) with a flame ionization detector. The initial column oven temperature was 100°C, and the temperature was increased at 10°C min-1 to 180°C; held at that temperature for 6 min, increased at 1°C min-1 to 200°C, held there for 20 min, and finally increased to 230°C at 4°C min-1. The injector and detector temperatures were maintained at 270°C and 280°C, respectively.

2.3. Extraction and analysis of the free amino acids

The free amino acids were analyzed using the method described by Gao, Clare, Rose, and Caldwell (2017). Approximately 100 mg of freeze-dried egg was extracted with 6 N hydrochloric acid at 110°C for 24 h. Then borate buffer (0.1 M, pH 8.8) was mixed with the sample solution. The solution was derivatized with diethyl ethoxymethylene-malonate at 55°C for 15 min. The mixture was cooled and centrifuged at 6000 g for 5 min at 4°C. The supernatant was held at that temperature for 4°C storage was higher than that in the eggs stored at 25°C. The high temperature makes the PUFAs more susceptible to oxidation by free radicals (Mazalli & Bragagnolo, 2011), and the temperature was increased at 10°C min-1 to 180°C; held at that temperature for 6 min, increased at 1°C min-1 to 200°C, held there for 20 min, and finally increased to 230°C at 4°C min-1. The injector and detector temperatures were maintained at 270°C and 280°C, respectively.

2.4. Assay of the total antioxidant capacity (T-AOC) and thiobarbituric acid reactive substances (TBARS)

The T-AOC and TBARS levels in eggs were measured by the corresponding assay kits. The kits for determining T-AOC were purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). The TBARS levels in eggs were measured by a QuantichromTM TBARS assay kit (DTBA-100) (Bioassay systems, Hayward, CA, USA).

2.5. Statistical analysis

All chemical analyses were conducted in triplicate and were reported as the mean value of triplicates. The data were analyzed using one-way analysis of variance (ANOVA) using Statistical Package for the Social Sciences v. 13.0 (SPSS, Chicago, IL, USA) followed by Duncan’s test. A P value < 0.05 was regarded as significant, and the data are presented as the mean ± standard deviation (SD).

3. Results and discussion

3.1. Effect of storage on the fatty acid composition

The fatty acid compositions of normal and n-3 PUFA-enriched eggs are shown in Table 1. In the normal eggs and n-3 PUFA-enriched eggs, the main fatty acids were palmitic acid (C16:0), oleic acid (C18:1), linoleic acid (C18:2ω-6) and stearic acid (C18:0). The n-3 PUFA-enriched eggs showed higher proportions of α Linolenic acid (C18:3ω-3, 6.44%), DHA (C22:6ω-3, 2.73%) and EPA (C20:5ω-3, 0.20%) than were found in the normal eggs, whereas the former showed a considerably higher proportion of total n-3 fatty acids (9.40%). These results corroborate previous studies with flaxseed supplementation in hen diets to obtain n-3 PUFA-enriched eggs (Lemahieu et al., 2015; Mattioli et al., 2016; Neijat, Ojekudo, & House, 2016).

In the n-3 PUFAs enriched eggs, storage for 18 days at 25°C increased the proportion of MUFAs from 41.44% to 44.27%, and then the level decreased to 41.33% (storage for 24 days), but the proportion of PUFAs decreased from 24.40% to 21.92% (storage for 18 days) and then increased to 25.86% (storage for 24 days). The contents of SFAs, especially α-linolenic acid, decreased in both groups of eggs during storage. These changes in the fatty acid composition of the eggs are due to lipolysis and lipid oxidation (Wang et al., 2017). During storage, the lipids can be hydrolyzed by the endogenous enzymes in the egg yolk, releasing free fatty acids, while lipid peroxidation in the eggs can produce hydroperoxides and secondary oxidation products, ultimately reducing the lipid content. The fatty acid compositions of the two experimental groups were not significantly affected by storage temperature (Figure 1). At the end of the storage period, the proportion of PUFAs in the eggs stored at 4°C storage was higher than that in the eggs stored at 25°C. The high temperature makes the PUFAs more susceptible to oxidation by free radicals (Mazalli & Bragagnolo, 2009). The selectivity of lipases for certain PUFAs may also be responsible for the increase (Yang, Ma, Qiao, Song, & Du, 2005).

3.2. Effect of storage on the free amino acid content

The compositions of free amino acids in normal and n-3 PUFA-enriched eggs are shown in Table 2. The total free amino acid contents in n-3 PUFA-enriched eggs (4424.93 µg/g) was higher than that in normal eggs (4160.40 µg/g). Essential amino acids accounted for 42.28% and 43.15% of the total amino acids in normal eggs and n-3 PUFA-enriched eggs, respectively. These results corroborate previous studies by Nimalaratne, Lopes-Lutz, Schieber, and Caldwell (2011). The contents of arginine (Arg) and alanine (Ala) in n-3 PUFA-enriched eggs were significantly lower than in
### Table 1. Changes in the fatty acid profile and total lipids during egg storage at 25°C. Means in the same row with different superscripts are significantly different \((P < 0.05)\).

| Fatty acids | Normal       | n-3 PUFAs enriched |
|-------------|--------------|--------------------|
|             | 0 d          | 6 d                | 12 d               | 18 d               | 24 d               |
|             | 0 d          | 6 d                | 12 d               | 18 d               | 24 d               |
|             | 0 d          | 6 d                | 12 d               | 18 d               | 24 d               |
|             | 0 d          | 6 d                | 12 d               | 18 d               | 24 d               |
|             |             |                   |                   |                   |                   |
| C 16:0      | 26.75 ± 1.01^a | 26.64 ± 0.63^a   | 27.07 ± 0.65^a    | 25.67 ± 1.02^ab   | 25.83 ± 0.09^ab   |
| C 16:1      | 273 ± 0.53^a  | 232 ± 0.02^a      | 265 ± 0.32^a      | 23.66 ± 0.61^a    | 27.54 ± 0.42^a    |
| C 18:0      | 939 ± 0.48^a  | 1008 ± 0.97^a     | 913 ± 0.57^a      | 971 ± 1.71^a      | 924 ± 0.59^a      |
| C 18:1      | 42.07 ± 0.32^abc | 42.46 ± 0.68^abc | 39.73 ± 0.21^abc | 43.12 ± 2.90^a    | 42.83 ± 1.50^ab   |
| C 18:2       | 1419 ± 1.41^a | 1402 ± 0.81^a     | 1604 ± 0.54^a     | 1418 ± 3.03^a     | 1451 ± 1.84^a     |
| C 18:3       | 0.10 ± 0.01^b | 0.08 ± 0.01^b     | 0.12 ± 0.00^c     | 0.08 ± 0.02^cd    | 0.08 ± 0.01^c     |
| C 19:0       | 0.34 ± 0.06^b | 0.30 ± 0.05^bc    | 0.66 ± 0.24^d     | 0.36 ± 0.16^b     | 0.37 ± 0.08^b     |
| C 20:1       | 0.20 ± 0.00^abc | 0.20 ± 0.00^abc  | 0.22 ± 0.01^e     | 0.22 ± 0.02^ab    | 0.24 ± 0.01^a     |
| C 20:2       | 0.10 ± 0.05^a  | 0.12 ± 0.02^a     | 0.15 ± 0.00^a     | 0.13 ± 0.01^a     | 0.14 ± 0.01^a     |
| C 20:3       | 0.14 ± 0.03^a  | 0.12 ± 0.01^a     | 0.17 ± 0.00^a     | 0.12 ± 0.04^a     | 0.15 ± 0.00^a     |
| C 20:3ω-6    | 0.00 ± 0.00^b  | 0.00 ± 0.00^b     | 0.00 ± 0.00^b     | 0.00 ± 0.00^b     | 0.00 ± 0.00^b     |
| C 20:4ω-6    | 225 ± 0.02^a   | 223 ± 0.02^a      | 209 ± 0.13^a      | 22.5 ± 0.18^a     | 203 ± 0.11^a      |
| C 20:5ω-3    | 0.02 ± 0.00^b  | 0.01 ± 0.00^b     | 0.01 ± 0.01^b     | 0.02 ± 0.01^b     | 0.00 ± 0.00^b     |
| C 22:6ω-3    | 0.94 ± 0.05^a  | 0.92 ± 0.04^a     | 0.82 ± 0.07^a     | 1.00 ± 0.04^a     | 0.97 ± 0.18^a     |
| SFA         | 3613.15 ± 1.40^abc | 3653.16 ± 0.69^abc | 36.20 ± 0.02^bc | 35.38 ± 0.69^abc | 35.07 ± 0.69^bcx  |
| MUFA        | 4500 ± 0.85^abc | 4497 ± 0.66^abc   | 42.59 ± 0.55^abcx | 45.70 ± 2.27^a | 45.82 ± 1.08^abc  |
| PUFAs       | 1807 ± 1.26^a  | 1779 ± 0.95^a     | 2008 ± 0.50^abc   | 18.14 ± 3.02^a    | 18.26 ± 1.64^a    |
| n-3         | 130 ± 0.01^b   | 123 ± 0.10^b      | 150 ± 0.17^b      | 1.38 ± 0.11^b     | 1.35 ± 0.10^b     |
| n-6         | 1668 ± 1.23^a  | 1644 ± 0.81^a     | 1843 ± 0.67^a     | 16.64 ± 2.90^a    | 16.77 ± 1.72^a    |
| n-6/n-3     | 1287 ± 0.86^a  | 1341 ± 0.42^a     | 1238 ± 1.84^a     | 12.00 ± 1.18^a    | 12.52 ± 2.16^a    |

\(^a\) Significant difference compared to 0 d. 
\(^b\) Significant difference compared to 6 d. 
\(^c\) Significant difference compared to 12 d. 
\(^d\) Significant difference compared to 18 d. 
\(^e\) Significant difference compared to 24 d.
normal eggs. Tyrosine (Tyr) was the most abundant amino acid (488.35 μg/g and 477.41 μg/g) in all samples, followed by glutamic acid (Glu) (471.61 μg/g and 431.86 μg/g) and leucine (Leu) (392.66 μg/g and 379.04 μg/g). Tyr and tryptophan (Trp) are the two major contributors to the antioxidant properties of egg yolk (Nimalaratne et al., 2011). Some free amino acids, such as Glu, Ala, and glycine (Gly), are responsible for flavor and taste (Yamanaka & Shimada, 1996).

During storage, the total amino acid contents in the egg samples significantly decreased, especially during storage at 25°C. Storage at room temperature caused reductions in the free amino acid contents of most honeys (Iglesias et al., 2006). The storage process tends to have a greater effect on the total amino acid content of n-3 PUFA-enriched eggs than normal eggs. These may be related to their high levels of polyunsaturated fatty acids. As the report of Nimalaratne, Schieber, and Wu (2016), the Tyr content was significantly reduced in n-3 PUFA-enriched eggs. However, the contents of most amino acids were not affected by storage temperature. Histidine (His) is an essential amino acid to infants and young children, and its levels decreased significantly during storage at 25°C.

### 3.3. Effect of storage on T-AOC and TBARS

The T-AOC and TBARS levels found in this study are shown in Figures 1 and 2. The T-AOC activity in normal eggs was higher than that of n-3 PUFA-enriched eggs. Similar to the free amino acid content in eggs (Table 2), the T-AOC activity tended to decrease during storage. Nimalaratne et al. (2016) found that the free amino acids were one of the main contributors to the antioxidant activity of eggs. The contents of n-3 PUFA and free fatty acids in n-3 PUFA-enriched eggs decreased during storage, which may result in a reduction in total antioxidant activity. The T-AOC of eggs stored at 4°C was higher than those stored at 25°C regardless of the type of storage.
of egg, which may be because the constituent responsible for the oxidative activity, such as free amino acids, were stable during low temperature storage.

Lipid oxidation occurs during egg storage (Meynier et al., 2014). The TBARS values increased in both normal and n-3 PUFA-enriched eggs stored at 25°C, while no changes in the TBARS values were observed during 12 d of storage at 4°C (Figure 3). These results were consistent with previous studies (Mohiti-Asli et al., 2008) showing that low temperature storage has less of an effect on egg lipid oxidation. During the storage of eggs, a series of physical and biochemical changes, including albumen liquefaction, pH increase, egg yolk flattening and increased in the air cell, can occur (Ragni, Al-Shami, Mikhaylenko, & Tang, 2007). These changes may cause an increase in the sensitivity of the free amino acids and fatty acids to fat oxidation, which may decrease the antioxidative capacity.

4. Conclusion

The results of the present study show that the fatty acid, free amino acids and antioxidant activities of n-3 PUFA enriched eggs and normal eggs changed during storage. In particular, n-3 PUFA-enriched eggs were more susceptible to lipid oxidation due to their higher PUFA proportions. The proportion of total free amino acids significantly decreased during storage processing, but the changes observed during storage at 25°C and 4°C were not significantly different. Twenty four days of storage at 25°C significantly reduced the total antioxidant capacity (T-AOC), and increased the thiobarbituric acid reactive substances (TBARS) values. Our results showed that the fatty acids and antioxidant activity in n-3 PUFA-enriched eggs are stable during storage at 4°C for 24 d.

Disclosure statement

No potential conflict of interest was reported by the authors.

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