RESEARCH NOTE

Research Note: Comparison of the texture, structure, and composition of eggs from local Chinese chickens and a highly selected line of egg-type chickens and analysis of the effects of lipids on texture

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ABSTRACT Egg yolk texture affects consumer egg preference. The sensory characteristics of eggs are affected by not only the cooking method but also the maternal breed. In this study, we investigated the texture, structure, and nutritional differences between the cooked yolks of eggs obtained from Hetian Dahei (**HTDH**) and Rhode Island Red (**RIR**) chickens. The springiness, cohesiveness, gumminess, chewiness, and resilience of HTDH egg yolks were lower, and the hardness was higher than those of RIR egg yolks. Moreover, scanning electron microscopy revealed that HTDH egg yolk particles were smaller and that HTDH egg yolks had a denser protein network than those of RIR egg yolks. Lipid and protein levels were higher, whereas water contents were lower in uncooked HTDH egg yolks than in uncooked RIR egg yolks. Liquid chromatography tandem mass spectrometry further revealed that lower cohesiveness was associated with higher levels and greater variety of lipids in egg yolks. Moreover, increased phospholipid levels reduced egg yolk cohesiveness. Thus, the eggs of local Chinese chicken breeds were superior to those of a highly selected broiler chicken breed in terms of texture, structure, and nutritional composition, which may influence egg variety selection.

Key words: egg yolk, texture, microstructure, lipidomics

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INTRODUCTION

Eggs are a nutrient-rich food commonly consumed after boiling, a relatively simple processing method. Compared with other cooking methods, boiling produces eggs with the highest nutrient content. However, boiled egg yolks are often criticized as having a poor texture. Hence, because egg yolk texture affects consumer egg preference (Franco et al., 2020), many studies have focused on assessing the sensory characteristics of egg yolks.

The sensory characteristics of eggs are affected by not only the cooking method but also the maternal breed. For example, the hardness, gumminess, and chewiness of cooked egg yolk from Mos chickens are higher than those from ISA Brown chickens (Franco et al., 2020). In China, consumers prefer to eat eggs from local Chinese breeds (Mi et al., 2019); however, it is unclear whether local Chinese breeds are actually superior to highly selected lines of broiler chicken breeds in terms of egg yolk sensory characteristics and nutrients. It is therefore important to characterize the texture, structure, and nutritional components of cooked yolks from eggs obtained from various chicken breeds.

During cooking, proteins and lipids in the yolk participate in yolk gel formation. After heating, the secondary yolk structure is destroyed, and internal functional groups are crosslinked by hydrophobic interactions, forming a network structure that results in a gel. The phospholipids in the egg yolk participate in gel formation through a calcium phosphate bridge (Strixner et al., 2013). However, the correlation between lipids and egg yolk texture and gel formation is unclear. In recent years, the application of lipidomics has increased exponentially, enabling systematic analysis of overall lipid contents (Primacella et al., 2018).

Here, we explored differences between Hetian Dahei (**HTDH**) and Rhode Island Red (**RIR**) egg yolks in terms of texture, structure, and composition and assessed the factors affecting texture. The correlations between lipids and texture were evaluated using lipidomic analysis. We aimed to reveal the differences in the textures and structures of different egg yolks in order to improve egg yolk quality during food processing.

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MATERIALS AND METHODS

All experimental procedures were approved by the Animal Management Committee (in charge of animal welfare issue) of the Institute of Animal Science, Chinese Academy of Agricultural Sciences (IAS-CAAS, Beijing, China) and performed in accordance with the guidelines.

Sample Collection

RIR and HTDH hens were collected from Hebei Rongde Breeding Company, and eggs laid within 24 h were randomly selected during sampling. In total, 430 HTDH eggs and 412 RIR eggs were used in this study. All the egg-laying hens were raised in separate cages under identical conditions and fed the same feed throughout the experiment. Birds had free access to water and a corn-soybean meal-based diet containing 628 g/kg corn, 216 g/kg soybean meal, 16 g/kg soybean oil, and 110 g/kg limestone.

Texture Profile Analysis of Boiled-Egg Yolks

All eggs were pretreated as follows. One minute after boiling water, the eggs were added and boiled for 5 min, and the heat was turned off. After 3 min, the eggs were removed from the hot water, and the entire yolk was separated from the egg albumin by hand and prepared for analysis at room temperature (26°C). A TA-XT2i texture profile analyzer (**TPA**; Texture Technologies, Scarsdale, NY) with a P50 probe was used to evaluate the texture characteristics of the cooked egg yolks.

Microstructure Determination of Boiled-Egg Yolks

Following a previously described method, the microstructures of 8 egg yolks for each chicken breed were observed using scanning electron microscopy (**SEM**; Hitachi SU8010; Hitachi Ltd., Tokyo, Japan). Samples were fixed with 2.5% glutaraldehyde solution and 0.2 M phosphate-buffered saline (pH 7.0), dehydrated with 1% osmic acid solution and an ethanol gradient, dried in a critical point dryer (LEICAEM CPD300; Hitachi Ltd.), and then coated on an MC1000 ion sputtering instrument (Hitachi Co., Ltd.). The treated samples were observed using SEM.

Determination of the Nutritional Components of Uncooked Egg Yolks

Moisture was determined by drying the sample to a constant weight in an oven at 105°C. The protein levels were determined using Kjeldahl's method (Folch et al., 1957). Fat was obtained by distillation and extraction, and the fat levels were determined using the gravimetric method (Ersoy and Oezeren, 2009).

Lipidomic Analysis Using Ultra-High-Performance Liquid Chromatography High-Resolution Tandem Mass Spectrometry

The samples were chromatographically separated using ultra-high-performance liquid chromatography (**UHPLC**) system (Agilent Technologies, Santa Clara, CA) equipped with a Waters CSH C18 chromatographic column (2.1 mm × 100 mm, 1.7 μm) at a column temperature of 55°C. The mobile phase flow rate was 0.3 mL/min. The injection volume was 2 μL. The mobile phase comprised (A) 10 mM ammonium formate and 0.1% formic acid aqueous solution and (B) isopropanol/acetonitrile (9:1, v:v) containing 10 mM ammonium formate and 0.1% formic acid.

The eluents were analyzed on a ThermoFisher Q Exactive Hybrid Quadrupole-Orbitrap Mass Spectrometry system (Agilent Technologies) in the heated electrospray ionization positive (**HESI+**) and negative (**HESI-**) modes. The spray voltage was set to 3.5 kV for HESI+ and HESI-. Both capillary and auxiliary (**Aux**) gas temperatures were 350°C. The sheath gas flow rate was 40 (Arb). The Aux gas flow rate was 10 (Arb). The S-Lens RF level was 50 (Arb).

Multivariate statistical analysis was performed using SIMCA software (v.14.1; Sartorius, Göttingen, Germany). For univariate statistical analysis, the normalized data were analyzed using the R platform. Structural identification of lipids was performed using LipidMatch (v.3.0.0).

Statistical Analysis

Standard scores were used to eliminate values other than ± 3. Statistical analyses were performed using SPSS software (v.25.0; IBM, Chicago, IL). Differences were considered statistically significant when the P values were less than 0.05. Based on the R platform (v. 3.5.1), Pearson correlation coefficients and heatmaps were analyzed.

RESULTS AND DISCUSSION

Comparison of the Texture Profiles of the Cooked Egg Yolks

The results of the TPA of the different eggs are shown in Figures 1A and 1B. The springiness, cohesiveness, gumminess, chewiness, and resilience of HTDH egg yolks were lower than those of RIR egg yolks, supporting the intense performance selection during production. By contrast, HTDH, a local Chinese breed, has not been intensively bred; thus, it is possible that this breed retained a better egg yolk texture. HTDH and RIR egg yolks showed significant differences in terms of hardness and resilience ($P < 0.05$), as well as springiness and cohesiveness ($P < 0.01$). These observations accounted for the differences in egg yolk texture among different flocks.

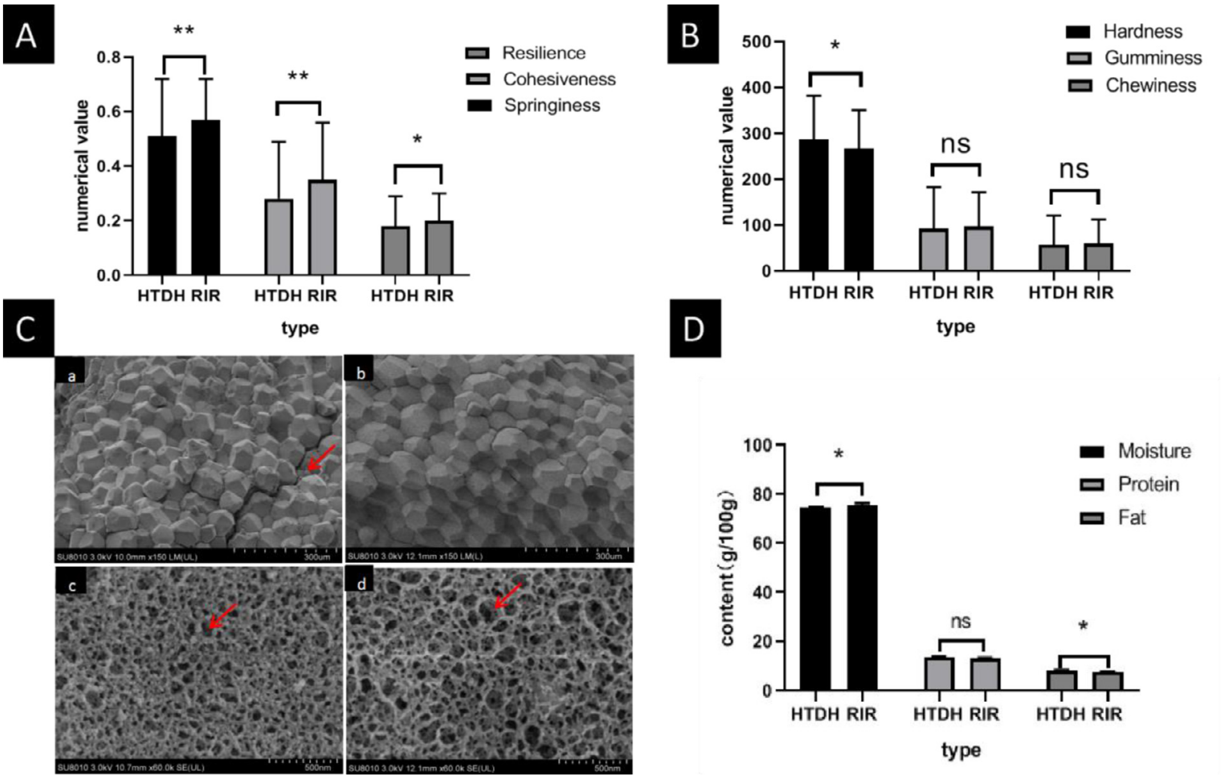

Figure 1. Texture, structure, and composition of HTDH and RIR yolks. (A) Resilience, cohesiveness, and springiness of HTDH and RIR egg yolks as analyzed using a texture analyzer. (B) Hardness, gumminess, and chewiness of HTDH and RIR egg yolks as analyzed using a texture analyzer. (C) Scanning electron microscopy images of boiled egg yolks from different breeds. (a) HTDH, magnification: 150 ×; (b) RIR, magnification: 150 ×; (c) HTDH, magnification: 60,000 ×; (d) RIR, magnification: 60,000 × . (D) Composition analysis of egg yolks from different breeds (n = 3). Abbreviations: HTDH, Hetian Dahei chickens; ns, not significant; RIR, Rhode Island Red chickens; $*P < 0.05$; $**P < 0.01$.

Both HTDH and RIR egg yolks showed very large coefficients of variation for the 6 indicators, reflecting the lack of directional selection. The textures of egg yolks may be affected by the yolk nutrients, which are influenced by various factors, including genetic and environmental factors and feed components (Franco et al., 2020). Chewiness refers to the effort required to chew solid food to a consistency that will allow it to be swallowed. In this study, HTDH yolks had lower chewiness and were easier to swallow than RIR yolks. Cohesiveness, defined as resistance to food chewing, was lower in HTDH yolks than in RIR yolks ($P < 0.01$). Lower resistance to chewing was associated with more delicate egg yolks and easier chewing and swallowing. Therefore, the texture of HTDH egg yolks was considered to be superior to that of RIR egg yolks.

Comparison of the Microstructure of Cooked Egg Yolks

To further explain the texture characteristics between HTDH and RIR yolks, the microstructure of cooked eggs was analyzed using SEM. When magnified 150 times, the yolks of HTDH and RIR eggs appeared polygonal granular (Figure 1C), indicating that the eggs could gelatinize into a solid after heating. However, the yolk particles of HTDH eggs were smaller, and the chewing resistance was lower than that of RIR eggs, indicating reduced cohesiveness of HTDH yolks (Figures 1A and 1B). Furthermore, fatty acids, which may be denatured during the heating process to release lipids, were separated from the granules of HTDH yolks; substantial fat in HTDH overflowing egg yolk particles gathered around the granules (Kaewmanee et al., 2009). The accumulated fat increased the softness and reduced the cohesiveness of the egg yolk, consistent with our texture analysis results (Figures 1A and 1B).

When the surface of the egg yolk particles was enlarged, there was no obvious membrane structure around the egg yolk particles. The surface of the egg yolk particles exhibited a porous honeycomb shape (Figure 1C), which may have been caused by protein denaturation and aggregation. Compared with HTDH egg yolks, the surface pore size of RIR egg yolk particles was large (Figure 1C), possibly because of the evaporation of water during heating. The surface pore size of HTDH yolk particles was small (Figure 1C); therefore, the structure of HTDH egg yolk particles may be more compact, thereby increasing hardness (Figures 1A and 1B). Furthermore, the smaller pore size of HTDH yolk particles may have resulted in small HTDH yolk particles, which are easy to chew, thereby reducing cohesiveness and chewiness (Figures 1A and 1B). The above results showed that the microstructure of the cooked egg yolks was closely related to the texture of the yolks.

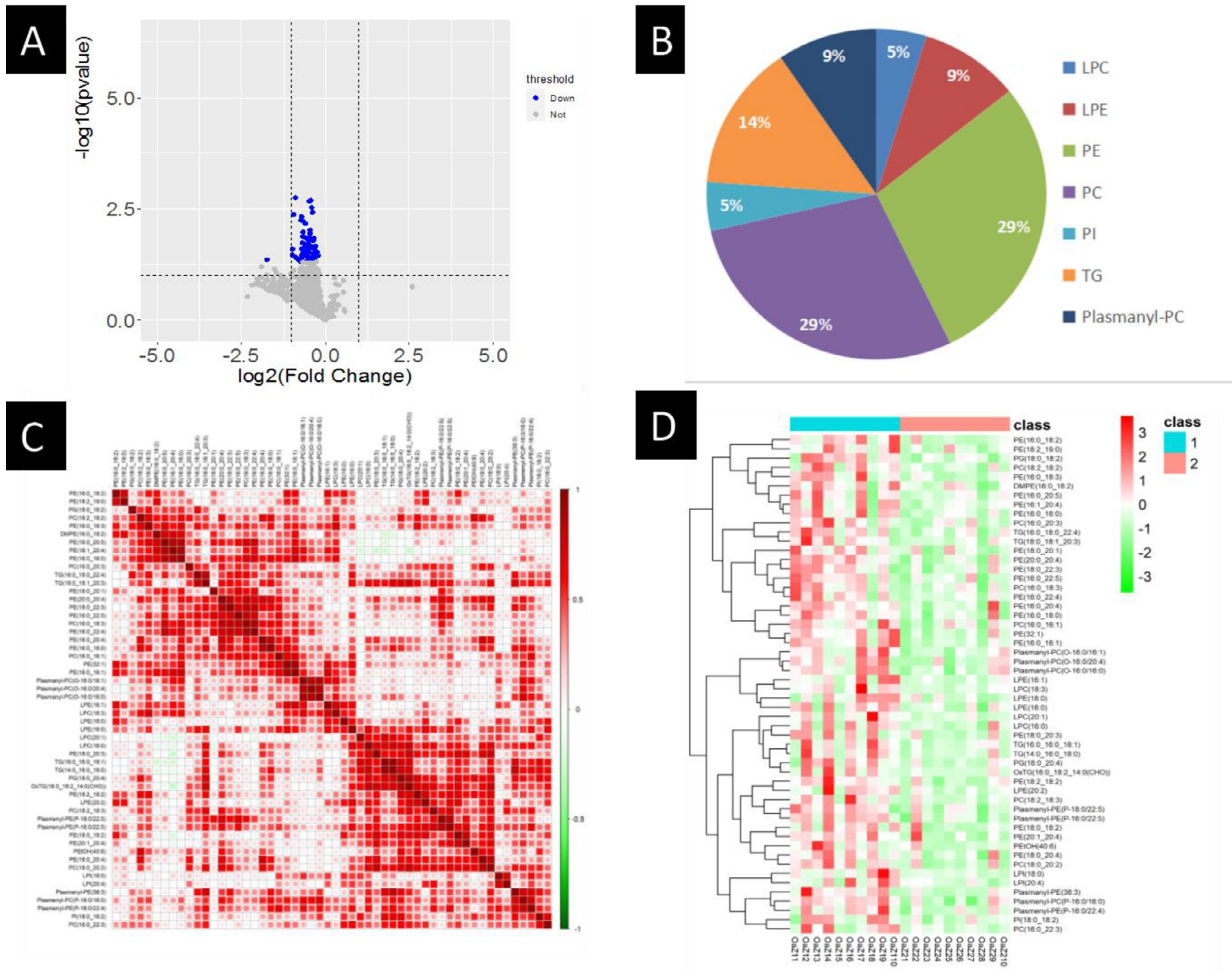

Figure 2. Association of lipid contents with cohesiveness. A value of 1 indicates low cohesiveness, whereas 2 indicates high cohesiveness. (A) Volcano map of differential lipids. Blue indicates lipid downregulation in the second group compared with that in the first group. (B) Group fan chart of different lipids. (C) Correlation matrix chart of the different metabolites between groups 1 and 2. Each row and column represent differential metabolites. The correlation coefficient is shown on the right. (D) Heatmap of the different metabolites between groups 1 and 2. Rows represent the differential metabolites, columns represent sample numbers, and the tree structure on the left represents the similarity clustering relationship between the differential metabolites. Abbreviations: LPC, lysophosphatidylcholine; LPE, lysophosphatidylethanolamine; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; plasmanyl-PC, plasmanyl phosphatidylcholine; TG, triacylglyceride.

Comparison of the Components of Uncooked Egg Yolks

From the above results, we speculated that water, protein, and fat contents may affect the texture and microstructure of egg yolks. Therefore, we analyzed the water, protein, and fat contents in raw egg yolks from the 2 chicken breeds (Figure 1D). Water levels were higher in RIR yolks than in HTDH yolks. During heating, more water evaporated, increasing the grid gap on the particle surface (Figure 1C). By contrast, protein levels were higher in HTDH yolks than in RIR yolks; however, the difference was not significant. This increase in protein levels may promote the aggregation of a grid structure (Causeret et al., 1992). Additionally, these changes may be related to the reduced egg yolk particle surface area and egg yolk cohesiveness observed in this study (Figure 1A−1C). Fat levels were also higher in HTDH yolks than in RIR yolks, consistent with the observed accumulation of free fatty acids around the HTDH yolk particles in our SEM analysis (Figure 1C). The fat in egg yolks increases the fineness and chewability and reduces the cohesiveness of egg yolks.

Comparison of the Lipidomic Profiles of Cooked Egg Yolks

From SEM and liquid-egg yolk composition analyses, we concluded that fats affected the texture of the cooked egg yolks. Thus, to reduce the influence of chicken breed on lipid contents, we selected 10 HTDH yolks with high cohesiveness and 10 HTDH egg yolks with lower cohesiveness for lipidomic analysis. The abundances of all 54 lipids decreased in the highly cohesive group compared with those in the less cohesive group (Figure 2A). In this experiment, nontargeted lipidomics was used, and the lipid levels in eggs were not determined. Using the variable importance projection (**VIP**) value of the first principal component (VIP > 1) of the orthogonal projections to latent structures discriminant analysis model, combined with the P value from one-dimensional tests ($P < 0.05$), 21 statistically significant differentially expressed lipids were screened and qualitatively identified as potential lipid components to distinguish less cohesive and highly cohesive groups. All 21 substances were less abundance in the highly cohesive group (Figure 2A).

The identified differential fats could be divided into 7 categories (Figure 2B). The differentially expressed lipids identified between the less cohesive group and highly cohesive group were mostly phospholipids. The hydrophobic portion of the phospholipid combines with the protein to facilitate gel formation (Li et al., 2020). Phospholipids in egg yolks are primarily composed of unsaturated fatty acids. Lipids rich in unsaturated fatty acids are related to the gelatin of yolks. This may account for the high levels of phospholipids in the less cohesive group. The most significant difference observed between groups was in the level of phosphatidylcholine (**PC**; 16:0_22:3), which may be because of the effects of unsaturated bonds in PC on the noncovalent interactions between protein molecules in egg yolks (Lin et al., 2020). Compared with the egg yolk in the highly cohesive group, the egg yolk in the less cohesive groups was denser (Figure 1C), with more bound phospholipids, which may lead to high levels of egg yolk phospholipids. Lysophospholipids are products of phospholipase hydrolysis. Compared with phospholipids, lysophosphatides are hydrophilic. The hydrophilicity of lysophospholipids can increase the binding of water and lipids in the egg yolk, facilitate the retention of water in egg yolks after thermal gelation, and reduce the chewing resistance of the egg yolk.

Triacylglyceride (**TG**; 14:0_16:0_18:0), phosphatidylethanolamine (**PE**; 18:0_20:3), and TG (16:0_16:0_18:1) can be clustered together. PC (16:0_22:3) and PC (16:0_20:3) can be clustered with PE and phosphatidylinositol (**PI**; Figure 2D). According to Pearson correlation analysis, the abundances of various metabolites were positively correlated (Figure 2C). Different types of lipids are related, and phospholipids interact with TG to affect egg yolk texture. TG contents also differed between the highly and less cohesive groups. During the heating process, TG accumulates around egg yolk particles and acts as a lubricant, reducing the friction between egg yolk particles and decreasing cohesiveness. Furthermore, TG affects the texture of egg yolks by regulating lipoproteins.

Overall, based on the texture, structure, and nutritional composition, we determined the differences between the cooked eggs obtained from 2 chicken breeds. The texture can be used to effectively distinguish the egg yolks between the breeds, indicating that the texture of yolk of the Local Chinese breed is superior to that of the highly selected lines of broiler chicken breeds. The microstructure of the cooked-egg yolks, which was observed using SEM, also illustrated the differences in the texture between the breeds. Less cohesive egg yolks and highly cohesive egg yolks exhibited significant differences in lipid composition and contents, suggesting that lipid levels were related to egg yolk cohesiveness. However, the factors and mechanisms underlying this difference remain unclear and require further investigation.

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DISCLOSURES

The authors have no conflicts of interest to declare.

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