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Underlying mechanism for the differences in heat-induced gel properties between thick egg whites and thin egg whites: Gel properties, structure and quantitative proteome analysis

Xin Liu [a,1], Jinqiu Wang [a,1], Qun Huang [b,**], Lei Cheng [c], Renyou Gan [d], Lili Liu [e], Di Wu [a], Hanmei Li [a], Lianxin Peng [a], Fang Geng [a,*]

[a] Key Laboratory of Coarse Cereal Processing (Ministry of Agriculture and Rural Affairs), College of Pharmacy and Biological Engineering, Chengdu University, No. 2025 Chengluo Avenue, Chengdu, 610106, China
[b] Provincial Key Laboratory of Quality Science and Processing Technology in Special Starch, College of Food Science, Fujian Agriculture and Forestry University, Fuzhou, 350002, China
[c] Beijing Advanced Innovation Center for Food Nutrition and Human Health, Beijing Technology and Business University (BTBU), Beijing, 100048, China
[d] Research Center for Plants and Human Health, Institute of Urban Agriculture, Chinese Academy of Agricultural Sciences, Chengdu, 610213, China
[e] College of Food and Bioengineering, Henan University of Science and Technology, Luoyang, 471023, China

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ABSTRACT

Fresh chicken egg whites can be easily categorized into two types, including the colloid-like egg white and the solution-like egg white, which are called thick egg white (TKEW) and thin egg white (TNEW), respectively. In the present study, the heat-induced gel properties of TKEW and TNEW were systematically analyzed and compared. The results showed that TKEW (72.51 °C) had higher temperature of heat denaturation than TNEW (67.01 °C). The texture profile analysis demonstrated that the TKEW gel appeared soft and tough (lower hardness and higher cohesiveness), while the TNEW gel appeared hard and brittle (higher hardness and springiness, but lower cohesiveness). SEM images showed that the TKEW gel exhibited a "mesh structure" with "mountain-like" protrusions and micropores, while the TNEW gel exhibited a "block structure" with a dense section and "rope-head" protrusions. Quantitative proteomic analysis revealed that a higher content of ovotransferrin in TNEW might be the main reason for its lower temperature of thermal denaturation, and a higher content of ovomucin in TKEW might regulate the microstructure of the gel during heating and cause the differences in the gel texture properties. Overall, this study provides insights into the mechanism for the differences of heat-induced gel properties between TKEW and TNEW from the comprehensive "composition-structure-function" perspective, and can give guidance for the regulation of egg white gel properties during its application.

1. Introduction

Chicken egg is not only an excellent protein source for human nutrition, but also an important food ingredient to provide excellent functional properties in the food industry. Egg white contains a variety of proteins, including ovalbumin, ovotransferrin, ovomucin, ovomucoid, lysozyme, etc. (Geng et al., 2012, 2019). These proteins have diverse physiochemical properties and biological activities, which greatly enrich the application of egg white (Abeyrathne, Huang, & Ahn, 2018; Mine, 2007). Especially, the heat-induced egg white has one of its most important applications. Heat treatment above 60–65 °C can denature the structure of egg white protein. As the protein unfolds, the denatured proteins can aggregate together *via* hydrophobic interactions, disulfide bonds, hydrogen bonds, etc., and subsequently form protein aggregates. Further bonding between the aggregates promotes the formation of a gel structure with a high degree ordering, which macroscopically appears as a stable egg white gel (Alleoni, 2006; Z.; Chen et al., 2015; Duan et al., 2018). Many factors can affect the gel formation process of egg white and therefore have a significant effect on gel properties, such as the pH and ionic strength of the egg white solution (Li

* Corresponding author.
** Corresponding author.
E-mail addresses: huangqunlaoshi@126.com (Q. Huang), gengfang@cdu.edu.cn (F. Geng).
[1] These authors contributed equally to this work.

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et al., 2018), the freshness of the egg white (Shan et al., 2020), chemical modifications (Geng, Huang, et al., 2018; Li et al., 2019), cold storage (Chen and Ma, 2020; Chen, Sheng, Gouda, & Ma, 2019), and high-intensity ultrasonic treatment (Sheng et al., 2018; Xie, Wang, Shi, et al., 2020; Xie, Wang, Wang, et al., 2020).

As is known that fresh egg white can be distinguished into two types as thick egg white (TKEW) and thin egg white (TNEW). The differences between the two types begin with the synthesis and secretion in the oviduct magnum of laying hen. TKEW and TNEW are secreted by different secretory cells, and the combined white can be divided into four layers from the outside to the inside according to their position in the assembly, including outer thin egg white, outer thick egg white, inner thin egg white, and inner thick egg white (Hiidenhovi, Ek-Kommonen, Järvenpää, Huopalahti, & Ryhänen, 2015; Juliet, 2004). Viscosity is the primary feature of differences between TKEW and TNEW. The viscosity of TKEW is significantly higher than that of TNEW, and an index for judging the freshness of chicken egg can be calculated based on the height of TKEW: Haugh unit. Furthermore, our previous research has shown the difference in the antibacterial properties of TKEW and TNEW (Fang et al., 2012).

The thinning of egg white, especially TKEW, during storage is a well-known phenomenon, and the accompanying changes have been studied in detail. During storage, the highly glycosylated ovomucin in egg white undergoes disaggregation as its glycan chain is hydrolyzed, accompanied by the thinning of egg white (Kato, Ogata, Matsudomi, & Kobayashi, 1981; Shan et al., 2020). Another study has suggested that the degradation of ovomucin undermines the electrostatic interaction by the lysozyme, and hinders the formation of protein aggregates, leading to the thinning of egg white (Kato, Wakinaga, Matsudomi, & Kobayashi, 1978). Additionally, ovalbumin is gradually converted into heat-stable S-ovalbumin during the storage, and the S-ovalbumin content is negatively correlated with the Haugh unit (Huang et al., 2012). Most of the above research mainly focused on the thinning of egg whites during the storage, but ignored a fundamental question about the molecular mechanism of the differences between TKEW and TNEW in the fresh eggs just produced.

The purpose of the current study is to explore the differences between TKEW and TNEW in the aspect of heat-induced gel properties. Combining the gel texture analysis, microstructure observation (SEM), and quantitative proteomic analysis, the methodology of "composition-structure-function" analysis is employed to reveal the underlying mechanisms for the differences in the gel properties of TKEW and TNEW.

2. Materials and methods

2.1. Preparation of samples

Fresh chicken eggs (egg weight 60.0 ± 2.0 g) laid within 24 h from German Roman hen were collected for research from Sichuan Sundaily Village Ecological Food Co., Ltd. (Mianyang, China). The eggs were manually broken, and the egg white was separated from the egg yolk, and the egg white was further separated by passaging through a 20-mesh sieve. The portion passing through the sieve was called the thin egg white (TNEW), and the portion entrapped on the sieve was thick egg white (TKEW). The obtained egg white samples were stored at 4 °C (<24 h) for further analysis. The proportion of TKEW in the eggs was 42.4%, and the moisture content of TKEW and TNEW were 88.95 ± 0.08% and 88.54 ± 0.06%, respectively. Total protein content of TKEW and TNEW were 10.15 ± 0.05% and 11.18 ± 0.04%, respectively. The pH of TKEW and TNEW were 8.19 ± 0.04 and 8.24 ± 0.03, respectively.

2.2. Differential scanning calorimetry (DSC)

The thermal properties of TKEW and TNEW were determined using differential scanning calorimetry (LF/1100 TG-DSC, Mettler Toledo, Switzerland) according to a previous study with some modifications (Rocha, Loiko, Gautério, Tondo, & Prentice, 2013). The lyophilized powders of TKEW and TNEW (10 ± 0.2 mg) were weighed and hermetically sealed in aluminum pans. The temperature was raised from 0 to 100 °C at a heating rate of 1 °C/min under a high-purity nitrogen gas (60 mL/min) purge. The DSC curves were recorded with an empty aluminum pan as the reference. The measurement was repeated three times and a representative curve was displayed.

2.3. Texture profile analysis (TPA)

TKEW or TNEW was placed in a beaker at room temperature and magnetically stirred for 10 min to make it uniform. The heat-induced gels of TKEW and TNEW were prepared in a cylinder of 12 mm × 12 mm (diameter × height) by heating in a water bath at 90 °C for 10, 20, 30, 40, and 50 min. Then, the gels were placed at 4 °C overnight. After returning to room temperature, the texture profiles of gels were performed using a texture analyzer (TA. TOUCH, BosinTech, China). The TA/36 cylindrical probe (diameter 36 mm) was used to compress the heat-induced gel twice with the following parameters: the pretest speed at 2 mm/s, the contact point pressure at 10 gf, the test speed at 1 mm/s, the posttest speed at 2 mm/s, the deformation of 40%, and the interval time of 5 s (Xie, Wang, Shi, et al., 2020). The texture analysis was repeated five times for each sample.

2.4. Water holding capacity (WHC)

The heat-induced gels were centrifuged at 10,000×*g* for 30 min at 25 °C. The ratio (%) of gel mass after and before centrifugation was calculated as the WHC. The determination was repeated five times.

2.5. Scanning electron microscope (SEM)

The microstructure of heat-induced gels was observed by an SEM (FEI Quanta 650, Thermo Fisher Scientific, Germany). TKEW and TNEW were heated at 90 °C for 30 min to form the heat-induced gels (12 mm diameter cylinder). The gel samples were fractured and lyophilized, and the fractured sections of the gels were coated with gold and observed with an acceleration voltage of 10 kV. The SEM imaging of each sample was performed three times, with three fields of view each time, and representative images were displayed.

2.6. Liquid chromatography-tandem mass spectrometry (LC-MS/MS)

The TKEW and TNEW samples were ground into powders with liquid nitrogen. Afterwards, the total protein extraction and digestion processes were performed as previously reported (Geng, Xie, et al., 2018).

The peptides were dissolved in 0.1% (v/v) of aqueous formic acid, and separated using a nanoElute UPLC system (Bruker Daltonics GmbH, Bremen, Germany). Mobile phase A was an aqueous solution containing 0.1% (v/v) of formic acid, and mobile phase B was acetonitrile containing 0.1% (v/v) of formic acid. The separation was carried out with the gradient: 0–70 min, 6–22% B; 70–84 min, 22–32% B; 84–87 min, 32–80% B; and 87–90 min, 80% B. The separation was performed at a constant flow rate of 300 nL/min. After the separation by UPLC, the peptides were injected into a capillary ion source for ionization, and then analyzed using a Bruker TIMS-ToF-MS/MS instrument (Bruker Daltonik GmbH, Bremen, Germany). The ion source voltage was 1.4 kV, and the secondary mass spectrometer scan range was set to 100–1700 m/z. The data acquisition mode uses the parallel accumulation series fragmentation mode (PASEF). The LC-MS/MS analysis of each sample was performed three times.

2.7. Data analysis of MS/MS

The MS/MS data were processed using Maxquant search engine

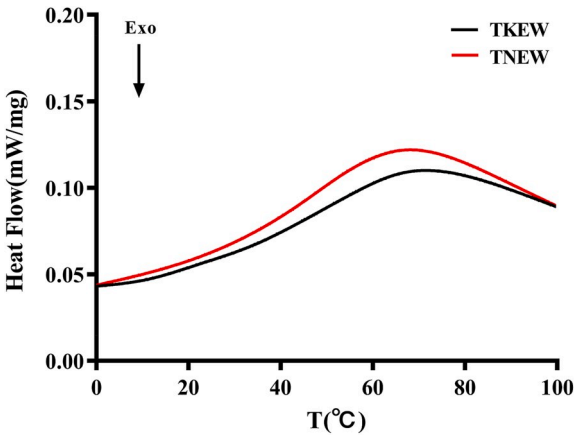

Fig. 1. Comparison of DSC curves of TKEW and TNEW.

(v.1.6.6.0), with search parameter settings as follows. Database was UniProt *Gallus gallus* (29,475 proteins), and an anti-library was added to calculate the false positive rate (FDR) caused by random matching, and a common pollution database was added to eliminate the potential contaminating proteins in the results. Trypsin was set as the cleavage enzyme, allowing up to 2 missing cleavages. The mass tolerance for the precursor ions was set at 40 ppm in the first search and main search, and the mass tolerance for the secondary fragment ions was set at 0.04 Da. Carbamidomethyl on Cys was set as the fixed modification, and the oxidation of methionine and the acetylation of the N-terminus of the protein were set as the variable modifications. The FDR for protein identification was set at 1%.

2.8. Statistical analysis

The results were expressed as mean ± standard deviation (SD). The statistical analysis was performed using GraphPad Prism 8.00 (GraphPad Software Inc., La Jolla, CA, US) by paired *t*-test. Statistical significance was defined at $p < 0.05$ (*) and $p < 0.01$(**).

3. Results and discussion

3.1. DSC analysis of TKEW and TNEW

A comparison of the DSC curves of TKEW and TNEW (lyophilized powder) is shown in Fig. 1. With the increase of temperature, the heat flow increased until the endothermic peak appeared. Based on the endothermic peak, the average thermal denaturation temperatures (T_d) of TKEW and TNEW lyophilized powder were 72.51 and 67.01 °C, respectively. The T_d is closely related to the protein thermal stability, and a higher T_d indicates a greater thermal stability (Hamdani, Wani, Bhat, & Siddiqi, 2018). The T_d of the main egg white proteins is quite different, and the T_d of native ovalbumin in PBS solution (10 mmol/L, pH 7.0, 2 mg/mL) is approximately 77.5 °C (Delahaije, Lech, & Wierenga, 2019; Takahashi et al., 2005), while the T_d of ovotransferrin is 60–65 °C in solution or hydrated state (Acero-Lopez, Ullah, Offengenden, Jung, & Wu, 2012; Iwashita, Handa, & Shiraki, 2019). It is speculated that the difference of T_d between TKEW and TNEW lyophilized powder may be closely related to the protein composition. In addition, the T_d of TNEW was lower than that of TKEW, indicating that TNEW should form a heat-induced gel earlier in the heating process. When eating the boiled eggs, the layering of gelled egg white can be occasionally observed, and this phenomenon may be caused by the differences of T_d between TKEW and TNEW.

The integrated area under the DSC curve of TKEW/TNEW represents the denaturation enthalpy (ΔH). The ΔH is related to the structure of protein. Research showed that the ΔH of whey protein concentrate decreased due to the destruction of bonds between proteins and increased with protein aggregation induced by prolonged sonication (Chandrapala, Zisu, Palmer, Kentish, & Ashokkumar, 2011). The ΔH of TNEW was larger than that of TKEW, suggesting that TNEW might have a more ordered protein structure than TKEW and absorb more heat during the thermal denaturation process. Overall, the differences in DSC curves suggest that TKEW and TNEW gels not only differ in gel formation temperature but also differ in gel strength and microstructure.

3.2. Texture properties of heat-induced TKEW and TNEW gels

A systematic analysis was carried out to compare the hardness, springiness, cohesiveness, and WHC of the TKEW and TNEW heat-induced gels formed under different heating time. Under the TPA mode, the hardness is the maximum force required to compress the sample during the first compression. This value represents the most important parameter for characterizing the gel strength (Saldaña et al., 2015). With the increase of heating time, the hardness of the TKEW gel reached a maximum of 342 gf when TKEW was heated for 30 min. Unlike the gel hardness of TKEW, the gel hardness of TNEW continuously enhanced and reached a maximum of 444 gf when the heating time was 50 min. Moreover, the hardness of the TNEW gel was significantly higher than that of TKEW during the heating time ($p < 0.01$) (Fig. 2A).

Springiness is the ability of a sample deformed after the first compression to return to the original status. The results showed that the TKEW gel overall had a comparable springiness compared to the TNEW gel during the heating time, except at 30 min of heating when the TKEW gel had a significant lower springiness ($p < 0.05$) than the TNEW gel (Fig. 2B). Cohesiveness represents the ratio of positive area during the second to that of the first compression cycle, and it is an indicator of whether the internal structure of the gel is easily damaged. It is calculated by dividing the energy required for the second compression by the energy required for the first compression (Quan & Benjakul, 2018). The cohesiveness of the TKEW gel was greater than that of the TNEW gel with the heating time of 10, 30, and 50 min, and the difference was significant at 10 and 50 min ($p < 0.05$) (Fig. 2C). In summary, the TNEW gel had a greater hardness and better springiness, but a weaker cohesiveness. This indicates that the TNEW gel appears “harder and brittler” and its internal structure can be more easily destroyed than the TKEW gel during the first compression cycle. In contrast, the TKEW gel appears “softer and tougher”, with a greater resistance to crush deformation.

3.3. WHC of heat-induced TKEW and TNEW gels

The WHCs of TKEW and TNEW were compared at different heating time. The results showed that the TKEW gel had a significantly lower WHC than the TNEW gel at the heating time of 10 min (Fig. 2D). However, as the heating time extended, the WHC of TKEW enhanced continuously, while the WHC of TNEW generally remained stable. After heating for 30 min, the WHC of the TKEW gel gradually became higher than that of the TNEW gel, and was significantly higher when the heating time reached 50 min ($p < 0.05$). Combined with the trend of gel hardness, it can be speculated that the gel structures of the TKEW and TNEW gels all continuously changed with the increase of heating time, but their changes were reflected in different properties. For the TKEW gel, the heating time mainly improved its WHC, while for the TNEW gel, the heating time mainly increased its hardness. The WHC of a gel was considered to be determined by both gel microstructure and stiffness (Urbonaite et al., 2016), therefore, the microstructures of heat-induced TKEW and TNEW gels were observed.

3.4. Microstructures of heat-induced TKEW and TNEW gels

The performance of heat-induced gel properties is usually closely related to the internal microstructure of a gel. The TKEW and TNEW gel

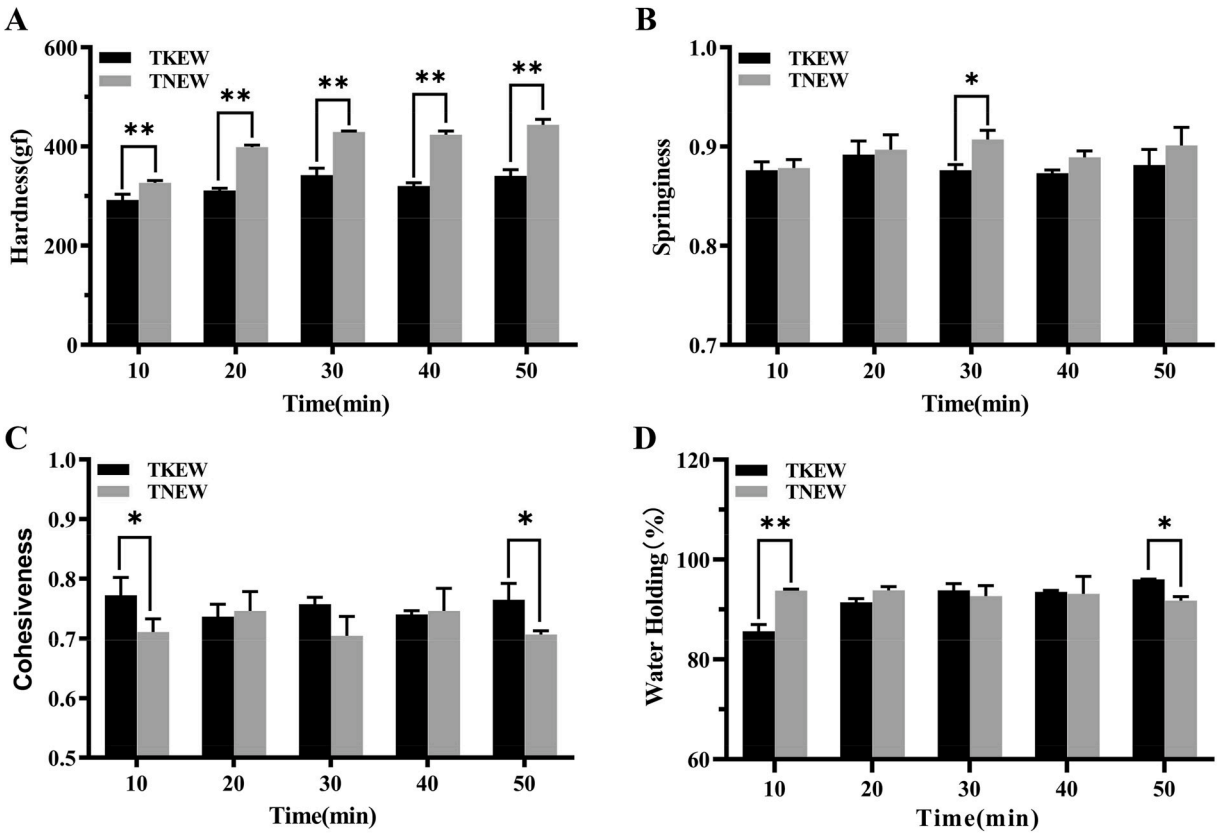

Fig. 2. Comparison of the TKEW and TNEW gel hardness (A), springiness (B), cohesiveness (C), and water holding capacities (WHCs, D). ($n = 5$, $^{*}p < 0.05$, $^{**}p < 0.01$).

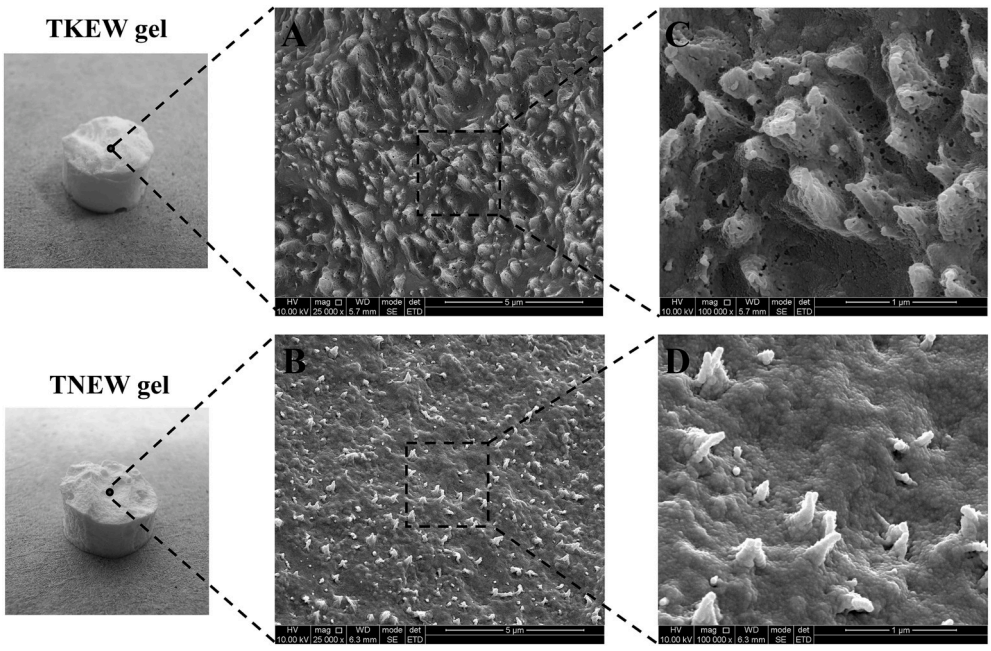

Fig. 3. Comparison of the microstructures (TEM) of the TKEW (A, B) and TNEW (C, D) heat-induced gels.

samples (90 °C, 30 min) were fractured and lyophilized, then the fractured sections of the gels were observed by SEM. At 25,000× magnification, the section surface of the heat-induced TKEW gel appeared as large undulations, with continuous "mountain-like" protrusions distributed on it (Fig. 3A). The microstructure of the TNEW gel section surface showed a morphology different from that of the TKEW gel, appearing as a relatively flat surface with "rope-head" protrusions distributed on it (Fig. 3B). More details and differences in the microstructures of the TKEW and TNEW gels could be observed at a magnification of 100,000-fold (Fig. 3C and D). Specifically, both gels could be

vaguely identified as having their microstructure formed by the accumulation of particles with a size of tens of nanometers. On the other hand, a large number of micropores were observed on the TKEW gel section, while the TNEW gel section surface appeared as a dense aggregation.

The above observation results indicate that the basic structure of the TKEW/TNEW gels consist of nanoparticles formed by egg white proteins. Furthermore, the "mountain-like" protrusions and the "rope-like" protrusions on the fractured gel surfaces suggest that there are some "linear skeletons" inside the TKEW/TNEW gels. Microscopically, the two gels are network-like gel structures with a "linear skeleton" interposed into the nanoparticle matrix. However, the difference is the number of "linear skeletons". The speculation based on the microtopography is that there are more "linear skeletons" in the TKEW gel. A large number of "linear skeletons" were removed when the TKEW gel was fractured, causing the displacement of the nanoparticle matrix, thereby forming the "mountain-like" protrusions and undulating surface. In contrast, the TNEW gel has fewer "linear skeletons", and its texture is relatively dense. Therefore, when the gel was fractured, the "linear skeleton" itself broke and formed the "rope-like" protrusions and flat surface. These differences imply that the microstructure of the TKEW gel is more like a "mesh structure" formed by interweaving a large number of "linear skeletons", while the TNEW gel is more like a "block structure" with "linear skeletons" sparsely distributed.

These differences in the microstructure were closely related to the gel properties of TKEW and TNEW. The "mesh structure" of the TKEW gel structure could provide a better toughness and cohesion, making it more resistant to external forces. Furthermore, the more microporous structure of the TKEW gel indicates that the retention of moisture in the TKEW gel is based on not only the hydration of protein molecules but also the capillary action and interception of micropores. This structural feature of the TKEW gel might be the main reason for its continued enhancement in WHC during the heating time. The "block structure" of the TNEW gel was consistent with its higher hardness and springiness, but lower cohesiveness (hard and brittle).

3.5. Quantitative proteomic comparison of TKEW and TNEW

To further explore the molecular basis of the TKEW and TNEW differences in gel properties and microstructure, a quantitative comparison of the proteins in TKEW and TNEW was performed using a label-free quantitative proteomic analysis. A total of 273,657 secondary spectra were obtained by mass spectrometry. After searching the protein database, a total of 27,316 spectra matched with 1131 peptide sequences. Among them, 708 sequences were identified as unique peptides and belonged to 133 proteins (Fig. 4A). A total of 80 proteins were identified in both TKEW and TNEW, with 19 and 34 proteins were identified in TKEW and TNEW, respectively (Fig. 4B). Furthermore, the protein profiles of TKEW and TNEW were distinguished by principal component analysis (PCA). The results showed that the samples of TKEW and TNEW were completely separated by their difference in the direction of PC1 (Fig. 4C), indicating that there was a significant difference in the protein profiles of TKEW and TNEW.

Based on the signal intensity of the corresponding peptide in the mass spectrometry, the protein abundance was further quantified. Proteins with significant differences in abundance ($p < 0.05$), and a change of more than 5% was filtered as different abundance proteins (DAPs). A total of 34 DAPs was filtered, of which 26 had a higher abundance in TNEW than in TKEW, and 8 had a lower abundance in TNEW than in TKEW.

3.6. Different abundance of ovotransferrin in TKEW and TNEW

Ovotransferrin is the second most abundant protein in egg white. Among the main egg white protein, the heat denaturation temperature of ovotransferrin is the lowest, between 60 and 65 °C, and the gelation of

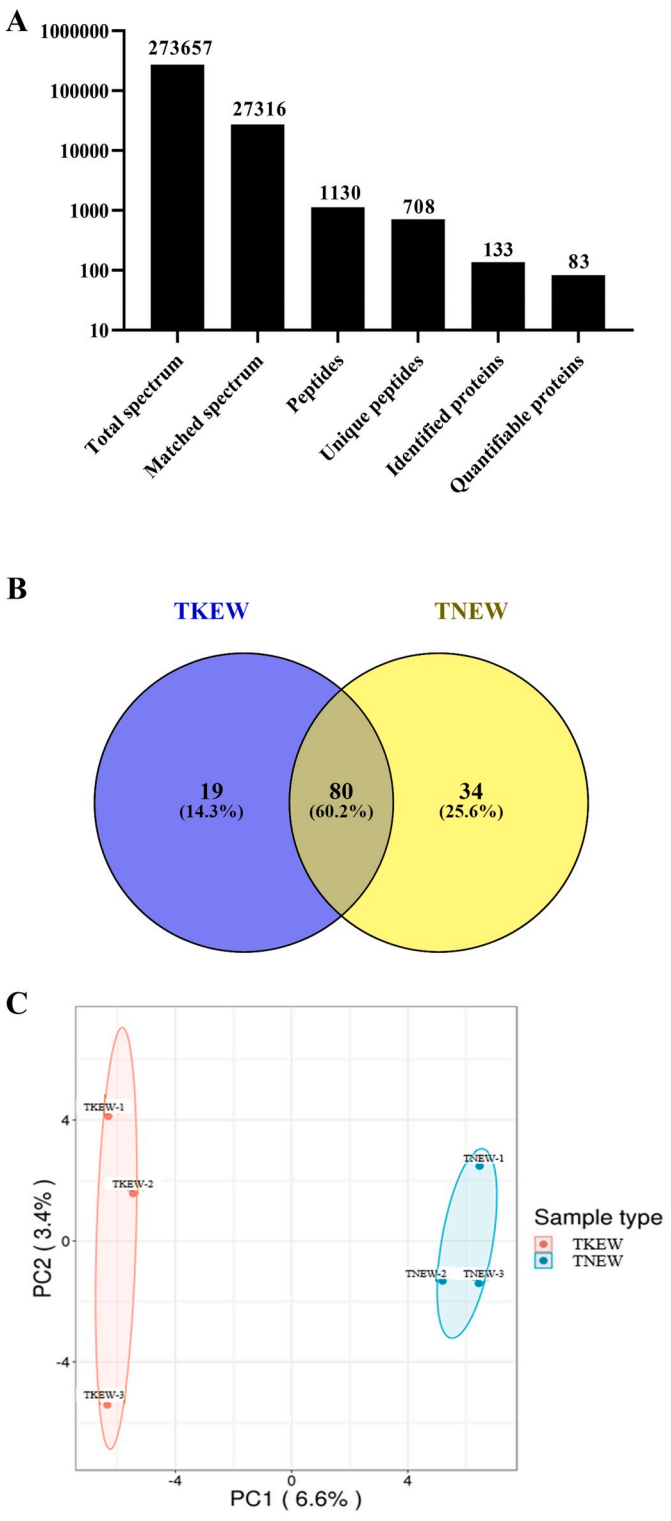

Fig. 4. Basic information of the quantitative proteomic analysis (A), protein Venn diagram (B), and principal component analysis (C) of protein profiles in TKEW and TNEW.

ovotransferrin occurs first during heating (Iwashita et al., 2019). The results of this study showed that the abundance of ovotransferrin in TNEW was 10.1% higher than that of TKEW ($p < 0.01$), which may be the main reason for a significant lower level of T_d in TNEW than in TKEW (Fig. 1). Furthermore, taking into account the high relative content of ovotransferrin in egg white, its different abundance in TKEW and TNEW might affect the gel properties beyond the denaturation temperature.

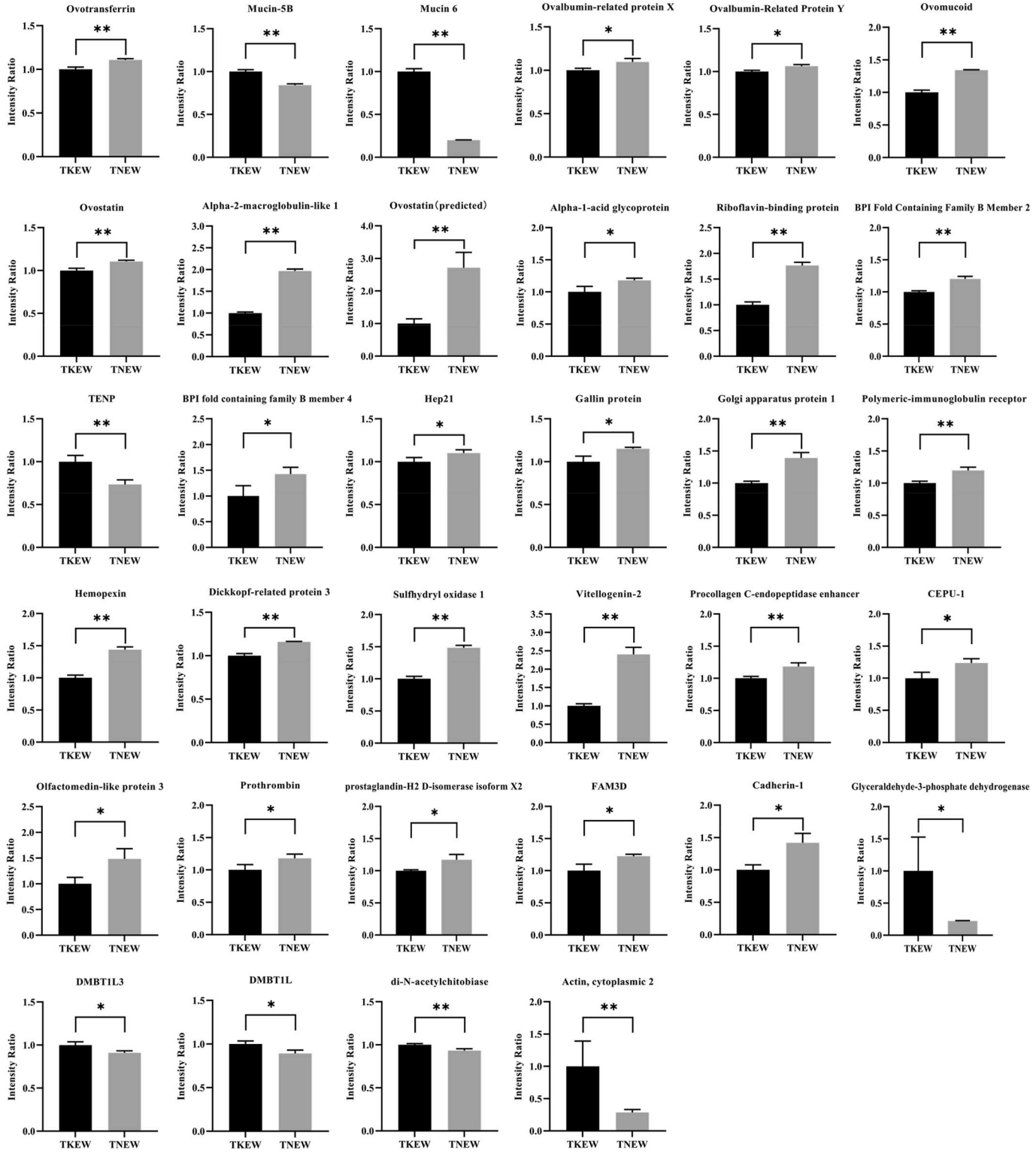

Fig. 5. The intensity ratios of DAPs in TKEW and TNEW ($n = 3$, $^{*}p < 0.05$, $^{**}p < 0.01$).

Therefore, it can be speculated that ovotransferrin is a key protein associated with the difference in the heat-induced gel properties of TKEW and TNEW.

3.7. Different abundance of ovomucin in TKEW and TNEW

Ovomucin is composed of two subunits: α-ovomucin (Mucin 5B) and β-ovomucin (Mucin 6) (Shan et al., 2020). As shown in Fig. 5, the abundances of α-ovomucin (Mucin 5B) and β-ovomucin (Mucin 6) in TKEW were 18.9% and 400.0% higher than those in TNEW ($p < 0.01$), respectively. A previous study reported that TKEW resulted in a higher yield of ovomucin (2.46 g/L) than TNEW (1.27 g/L) (Hammershoj, Nebel, & Carstens, 2008). The results of the current study were consistent with previous research and provided more detailed information. The difference in the abundance of the two components of ovomucin was quantified.

Ovomucin is the most heavily glycosylated protein in chicken egg white and is considered the major contributor to egg white viscosity (Geng, Wang, Liu, Jin, & Ma, 2017). However, the structures of α-ovomucin and β-ovomucin are significantly different. In detail, α-ovomucin mainly exists as N-glycosylation, and the N-glycan is relatively short with an average of 10 monosaccharide units. The glycan moiety in α-ovomucin is 15%. In contrast, β-ovomucin mainly occurs as an O-glycosylation modification, and the O-glycan is long and complex. Therefore, β-ovomucin is similar to the proteoglycan with carbohydrate content approximately 60% (Robinson & Monsey, 1971). These structural differences in α/β-ovomucin, and their abundant differences in TKEW and TNEW, might have an important effect on the gel properties and microstructure. First, the linear high-molecular-weight polymeric structure is likely to provide the core of the "linear skeleton" structure, which was observed in SEM microscopic images. Accordingly, the ovomucin content in TNEW was less than that in TKEW, so there was less "linear skeleton" formation in the TNEW gel. Furthermore, TKEW contained substantially more β-ovomucin, and the longer O-glycans could enhance its interaction with other egg white proteins. Consequently, during the gel formation process, the binding force was enhanced between the "linear skeleton" and other parts of the gel. Finally, when the gel was fractured, the undulating sections formed because of the stronger mutual involvement.

3.8. Other DAPs in TKEW and TNEW

Ovalbumin and ovalbumin-related proteins. There have been many studies that show the abundance of ovalbumin changes during storage or processing. Based on a two-dimensional electrophoresis analysis, Omana and Qiu et al. have found that ovalbumin is degraded and its abundance changes greatly under different storage time and temperature conditions (Omana, Liang, Kav, & Wu, 2011; Qiu et al., 2012). These findings suggest that the abundance of ovalbumin is related to the thinning of egg white and imply that there may be a different abundance of ovalbumin in TKEW and TNEW. However, the current results showed that the abundance of ovalbumin in TNEW was 97.6% of that in TKEW, but there was no significant difference ($p = 0.089$). In addition to ovalbumin, the abundances of ovalbumin-related protein X and Y in TNEW were significantly higher (9.7% and 6.2%, respectively, $p < 0.05$) than those in TKEW. Previous studies found that both ovalbumin-related protein X and Y underwent a phosphorylation modification and N-glycosylation modification (Geng et al., 2017; Yang et al., 2019). These posttranslational modification of protein structures could affect their functional properties by changing the physicochemical properties of the two proteins. Therefore, the difference in the abundances of the two proteins makes them have a potential impact on the differences in the properties of TKEW and TNEW heat-induced gels.

Lysozyme. The present study found that there was no significant difference in the abundances of lysozyme between TKEW and TNEW (fold change = 1.018, $p = 0.471$). As an important egg white protein, lysozyme has a unique isoelectric point at 10.7. Therefore, lysozyme can interact with other egg white proteins through electrostatic interactions in the natural egg white solution environment (Le Floch-Fouéré et al., 2009). Moreover, Kato et al. have found that lysozyme and ovomucin are usually present in aggregates in TKEW, which is an important cause of egg white viscosity (Kato, Imoto, & Yagishita, 2014). Combined with the results of this study, it is speculated that lysozyme may have potential effects on the characteristics of TKEW and TNEW through its different interaction modes with other egg white proteins rather than difference in abundance.

Macroglobulin family proteins. A total of three members of the macroglobulin family were identified in the present study, including ovostatin (P20740), α-2-macroglobulin-like 1 (A0A1D5P2X2), and OVST (A0A1D5P3R8). Their abundance in TNEW were all significantly higher than that in TKEW ($p < 0.01$). As protease inhibitors, ovostatin and its homologous proteins play an important role in the defense of microorganisms. Furthermore, the high molecular weight makes them likely play a potential role in the formation of egg white heat-induced gel.

Ovomucoid. The abundance of ovomucoid in TNEW was 34.1% higher than that in TKEW ($p < 0.01$). Ovomucin is considered the primary allergen in egg white (Zhu, Vanga, Wang, & Raghavan, 2018). Therefore, the results suggest that TNEW has a higher risk of allergy.

Alpha-1-acid glycoprotein. It was observed that the abundance of α_1-acid glycoprotein in TNEW was 17.9% higher than that of TKEW ($p < 0.05$). This protein is also known as ovoglycoprotein and has an average molecular weight of 30 kDa with a carbohydrate portion approximately 30%. However, the biological, biochemical, and functional properties of α_1-acid glycoprotein in egg white are still unclear. Some studies have discovered their chiral recognition function for the separation of drug enantiomers (Yutaka, Hisami, Kazuya, Yasumaru, & Jun 2002).

Bactericidal permeability-increasing (BPI) protein family members. TENP, a protein with strong homology to the BPI protein family (Kinoshita et al., 2016), had a 26.6% higher abundance in TKEW than in TNEW ($p < 0.01$). Another two BPI protein family members, BPI folding protein family member 2 and BPI folding protein family member 4 were identified and quantified in TKEW and TNEW. Unlike TENP, the abundances of these two proteins in TKEW were significantly lower than those in TNEW ($p < 0.05$). The BPI protein family is suggested to be involved in the innate immune system and to provide defense against bacteria (Bingle, Seal, & Craven, 2011).

Vitellogenin-2. The abundance of vitellogenin-2 in TNEW was 140.2% higher than that in TKEW ($p < 0.01$). The presence of vitellogenin-2 in egg white at a low abundance may be due to its residue in the outer layer of the yolk membrane.

4. Conclusion

In this study, DSC analysis, gel texture analysis, microstructure observations, and quantitative proteomic analysis were used to reveal the underlying mechanism of the differences in the heat-induced gel properties between TKEW and TNEW. Results demonstrated that the TKEW gel appeared softer and tougher with a "mesh structure", while the TNEW gel appeared harder and brittler with a "block structure". The quantitative proteomic analysis suggested that the higher ovotransferrin abundance in TNEW may be the main reason for its lower average thermal denaturation temperature, and the different abundance of ovomucin in TKEW and TNEW may be the main reason for the differences in the gel microstructure and gel water holding capacity. Overall, these findings provide insights into the different mechanisms of heat-induced gel formation in TKEW and TNEW, and can guide the regulation of egg white gel properties during application. In addition, different from the well-defined food hydrocolloid system constructed *in vitro*, this work provide a paradigm for studying the natural complex food colloidal systems.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Xin Liu: Investigation, Data curation, Writing - original draft. **Jinqiu Wang:** Data curation, Project administration, Writing - review & editing. **Qun Huang:** Writing - review & editing, Funding acquisition. **Lei Cheng:** Methodology. **Renyou Gan:** Writing - review & editing. **Lili Liu:** Funding acquisition, Resources. **Di Wu:** Conceptualization, Formal analysis. **Hanmei Li:** Methodology. **Lianxin Peng:** Resources. **Fang Geng:** Supervision, Project administration, Writing - original draft, Writing - review & editing.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodhyd.2020.105873.

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