

Effect of Storage Conditions on the Protein Composition and Structure of Peanuts

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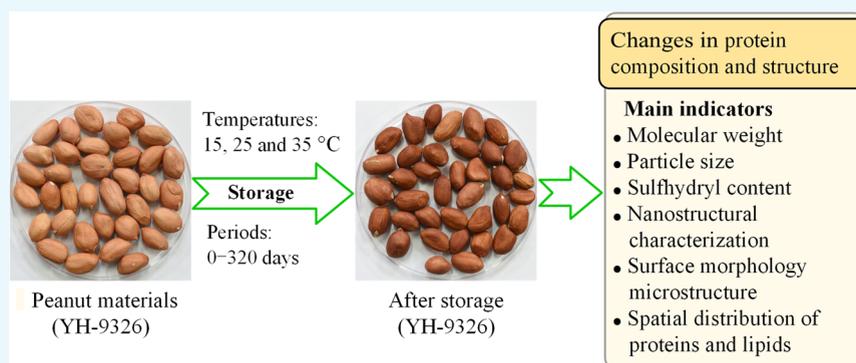
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ABSTRACT: Peanuts are important oil crops and plant protein source. This study evaluated the influence of storage temperature (15, 25, and 35 °C) and time (0, 160, and 320 days) on the protein composition and the molecular structure of peanuts through sodium dodecyl sulfate polyacrylamide gel electrophoresis, particle size, total sulfhydryl (–SH) contents, nanostructural characterization, surface morphology microstructure, and spatial distribution of proteins and lipid analysis. Results showed that the basic subunits and disulfide contents of peanut protein were not affected by storage temperature and time. However, the –SH contents decreased significantly ($P < 0.05$) in all samples except the 15 °C/160 day storage group. The protein particle size and graininess increased when stored at 25 and 35 °C for 160 and 320 days, respectively; however, there was no significant change ($P > 0.05$) when stored at 15 °C. In addition, significant changes ($P < 0.05$) on the microscopic morphology and spatial distribution of protein and lipids were observed when stored at 25 and 35 °C for 320 days.

1. INTRODUCTION

Peanuts (*Arachis hypogaea*) are one of the four major oil crops and an important food commodity worldwide. Peanut lipids supply the majority edible oil, and peanut proteins contribute 11% of the world's protein consumption.¹ Most grown peanuts are used for oil products, peanut butter, confections, and snack products. At present, this crop is cultivated on a large scale, with China being the largest producer, consumer, and exporter country in the world. According to the USDA's March 2022 report, the peanut production of China during 2020–2021 was 18.20 million tons, which ranked first and accounted for 36% of the world's total yield. Peanut seeds possess a high nutritional and commercial value because of their high fat (44–56%) and protein (22–30%) contents,^{2,3} reasonable fatty acid composition, essential amino acids, vitamins, calcium, and phosphorus. Peanut seeds have gained importance owing to its potential in lowering cholesterol, delaying human aging, and preventing cancer.²

Given that peanuts could only be planted in specific regions (mainly in Henan and Shandong provinces, accounting for 50% of the national output) and harvested in a particular

season (August to October) in China every year, peanuts are always needed to be stored for a period of time before being exported, processed, and consumed. Because of peanut's high fat contents (44–56%) and unsaturated fatty acid composition (80%), such as oleic acid (41%), linoleic acid (37%), and linolenic acid (0.7%), rancidification occurs easily during storage, which affects its nutritional value and agricultural importance.⁴ Therefore, studying the changes in the physicochemical characteristics and nutritional quality of peanuts during storage is of great significance for the efficient use of this crop.

During storage, the physiological properties, nutritional value, and sensory quality of peanut seeds change significantly.

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It was reported that the germination percentage and the vitamin E content decreased, the acid value and the peroxide value increased, and some linoleic acid was oxidized to oleic acid after 10 months' storage at air-low temperatures.⁵ Storage time had a negative effect on seed quality parameters such as the oil content and lipid components, including fatty acids, α -tocopherol, and δ -tocopherol.⁶ High storage temperatures lead to a high degree of lipid oxidation and nutrient loss.⁷ When peanuts are stored in a normal atmosphere, 3-methylpyridine and 2,5-dimethylpyrazine decreased with storage time, which exhibited the highest increase in oxidized flavor and short shelf life (180 days).⁸

Peanut protein, the second nutrient component of peanut seeds, will be oxidized during storage. Meanwhile, the functional properties of peanut proteins changed significantly over the storage process. For example, the secondary structure, free sulfhydryl content, and functional properties of peanut proteins changed significantly, and protein aggregation occurred during storage.⁹ However, research focused on the structure change of peanuts protein during storage is limited. However, changes in the protein structure and function during storage have been reported in other materials. Ziegler et al.¹⁰ found that the extraction yields and functional and bioactive properties of soybean proteins changed significantly after storage at 32 °C for 12 months. The crude protein content of ginkgo nuts was generally increased while an opposite trend was observed for the concentration of total amino acids after storage for 5 months.¹¹ Some other research has been conducted on fillets and beef proteins, and it has demonstrated that the degree protein oxidation and degradation increased as the storage time increased.^{12–14} In short, the natural structure of proteins of various food materials changed significantly during storage, which led to the decrease in functional and nutritional properties. Actually, the active groups or oxidation products produced by lipid oxidation might interact with proteins and lead to protein decomposition or polymerization, which might damage the natural structure of proteins and even in turn affect their functional properties and nutritional value during storage.^{15–17} However, only a few studies have focused on this aspect. It remained unclear at peanut protein oxidization and changing of their internal structures during storage. Therefore, the current work aimed at evaluating the changes in the composition and structure of peanut proteins under different storage conditions and provided theoretical basis for the actual production and storage of peanuts.

2. RESULTS AND DISCUSSION

2.1. Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis. Figure 1 shows the results of sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) of peanut proteins under different storage conditions. Figure 1A indicates reductive SDS-PAGE analysis, and Figure 1B represents nonreductive SDS-PAGE analysis. As shown in Figure 1A, the peanut protein mainly contains four basic subunits (60, 40.5, 37.5, and 19.5 kDa), all of which did not change significantly ($P < 0.05$) during storage. Compared with Figure 1A, more high molecular bands and less low molecular bands were found, as shown in Figure 1B, indicating that a large number of disulfide bonds existed in peanuts. As shown in Figure 1B, no significant changes occurred in the disulfide bond contents of peanut protein during storage. Therefore, the basic subunits of peanut protein were not affected during storage, and the disulfide bonds in the peanut protein were

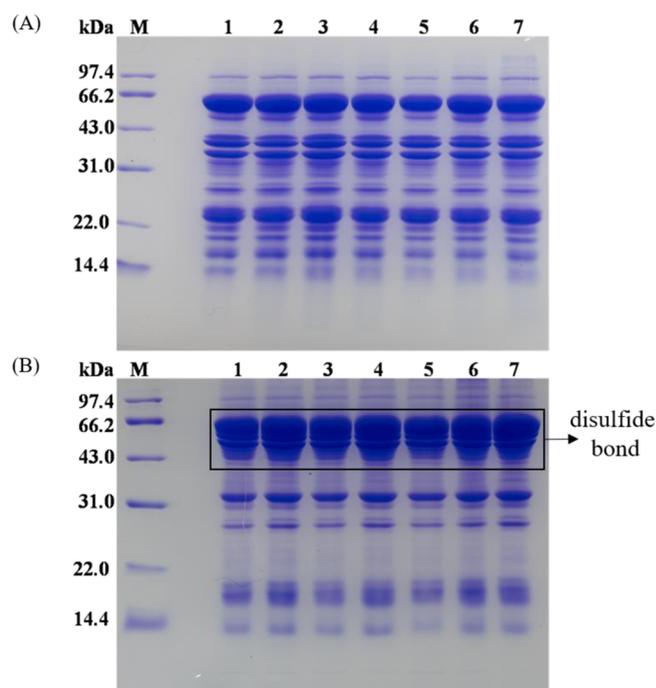


Figure 1. Reductive and nonreductive SDS-PAGE analysis of peanut proteins. A represents the reductive SDS-PAGE of peanut proteins, and B represents the nonreductive SDS-PAGE of peanut proteins. Lane M: protein molecular weight markers (kDa). Lanes 1 to 7 represent 0 d, 160 d/15 °C, 160 d/25 °C, 160 d/35 °C, 320 d/15 °C, 320 d/25 °C, and 320 d/35 °C, respectively.

stable and could not be broken. However, some studies have different results. Michalczyk and Surówka¹⁸ stored a rainbow trout oval protein under 3 and 30 °C to observe the changes in the protein structure. SDS-PAGE analysis showed a significant decrease in the strength of troponin bands and a significant increase in the strength of alpha-mycoplasma myosin and myosin protein bands. In addition, the two bands of 255 and 135 kDa disappeared, and new bands with molecular weights of 163 and 117 kDa appeared after storage. Ceo et al.¹⁹ stored small molecule peptides at 20 °C and found the gradual formation of some polymer peptides with the prolonged storage as revealed by SDS-PAGE. The different results in this research might be due to the different materials and storage conditions.

2.2. Total –SH of Peanut Proteins. Figure 2 indicates the change in total –SH contents in peanut proteins stored at different temperatures (15, 25, and 35 °C). As shown in the graph, the contents of free –SH in peanut proteins decreased significantly ($P < 0.05$) with the increase in storage time and temperature. Compared with the initial –SH content value, significant decline occurred in all groups except 15 °C/160 days. In particular, the contents of total –SH in peanut proteins decreased from 8.140 to 6.707 $\mu\text{mol/g}$ after being stored at 35 °C for 320 days. This finding indicated that the natural structure of the peanut protein was destroyed during storage; hence, the exposed internal –SH might be oxidized and lead to the decline in total –SH contents. In food systems, –SH were easily converted to disulfide bonds, sulfonic acids, and sulfinic acids in an oxidizing environment.^{16,17,20} However, Figure 1 shows that the disulfide bond contents were not significantly altered by storage, providing that most of the oxidized –SHs were not generated to disulfide bonds, but

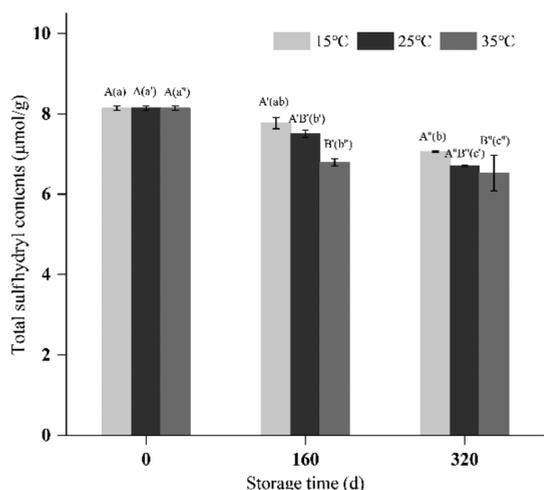


Figure 2. Effect of storage temperature and time on sulfhydryl contents of peanut. Each data column represents the mean of three replications. Vertical bars represent the standard errors of means. Values of each peanut cultivar followed by the same letter are not significantly different ($P > 0.05$).

might generate to oxidation products such as sulfonic acid and sulfinic acid. The results are in agreement with previous studies. Lund et al.¹⁶ stored meat under 4 °C for 14 days and observed the structural changes in pork protein and a significant decrease in the content of mercaptan in pork protein. Potes et al.¹⁷ mixed the whey protein separator and oil and then stored them at 20 and 40 °C and found that sulfhydryl contents in whey isolate protein decreased gradually during storage, accompanied by protein aggregation. Benjakul et al.²⁰ reported a decrease in the free sulfhydryl content of yellowfish, lizard fish, fin bream, and large-eyed snapper protein after being stored at 18 °C for 24 weeks.

2.3. Particle Size and Nanostructural Characterization Analysis of Peanut Protein. Figure 3 shows the particle size and distribution of peanut proteins during a 320 day storage period under different temperatures. As shown in Figure 3A, the particle size of all samples significantly ($P < 0.05$) increased with prolonged storage time and increased temperature. At the end of the storage period (320 days), the particle sizes of peanut proteins stored at 15, 25, and 35 °C

were 5.8, 16.1, and 21.9%, respectively, which were higher than that of the control. As shown in Figure 3B, the protein particle size of the nonstored exhibited two peaks at 10–100 and 100–1000 nm, respectively. Interestingly, a new intensity peak at 1000–10,000 nm was found at the end of the storage period (320 days) at 35 °C. Therefore, long-term (160 and 320 days) and high-temperature storage (25 and 35 °C) significantly affected the natural structure of peanut protein and may lead to produce large protein particles at the end of storage.^{21,22} For further verification, atomic force microscopy (AFM) was used to analyze the nanostructure of peanut protein. The results are shown in Figure 4. Similarly, the graininess of peanut protein increased significantly at 320 days under 35 °C (Figure 4D). However, the graininess did not change significantly under 15 and 25 °C.

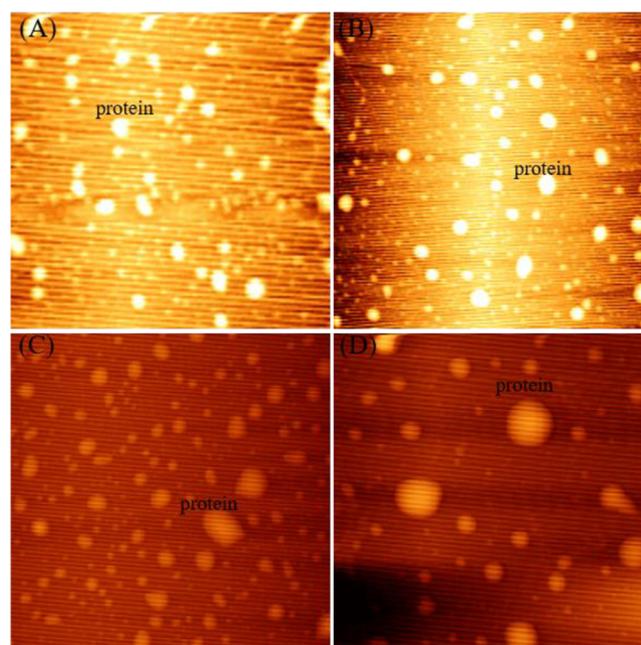


Figure 4. Changes in the nanostructure of peanut proteins under different storage conditions. A–D represent control, 320 d/15 °C, 320 d/25 °C, and 320 d/35 °C, respectively.

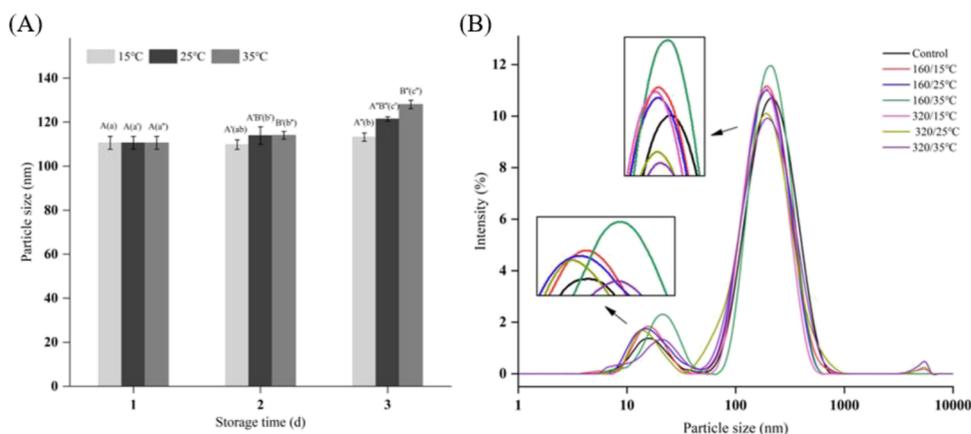


Figure 3. Changes in the particle size of peanuts as a function of storage. (A) Average particle size and (B) the particle size distribution of peanut proteins under storage. Results are expressed as the mean of three replications \pm SD. Values of each peanut cultivar followed by the same letter are not significantly different ($P > 0.05$).

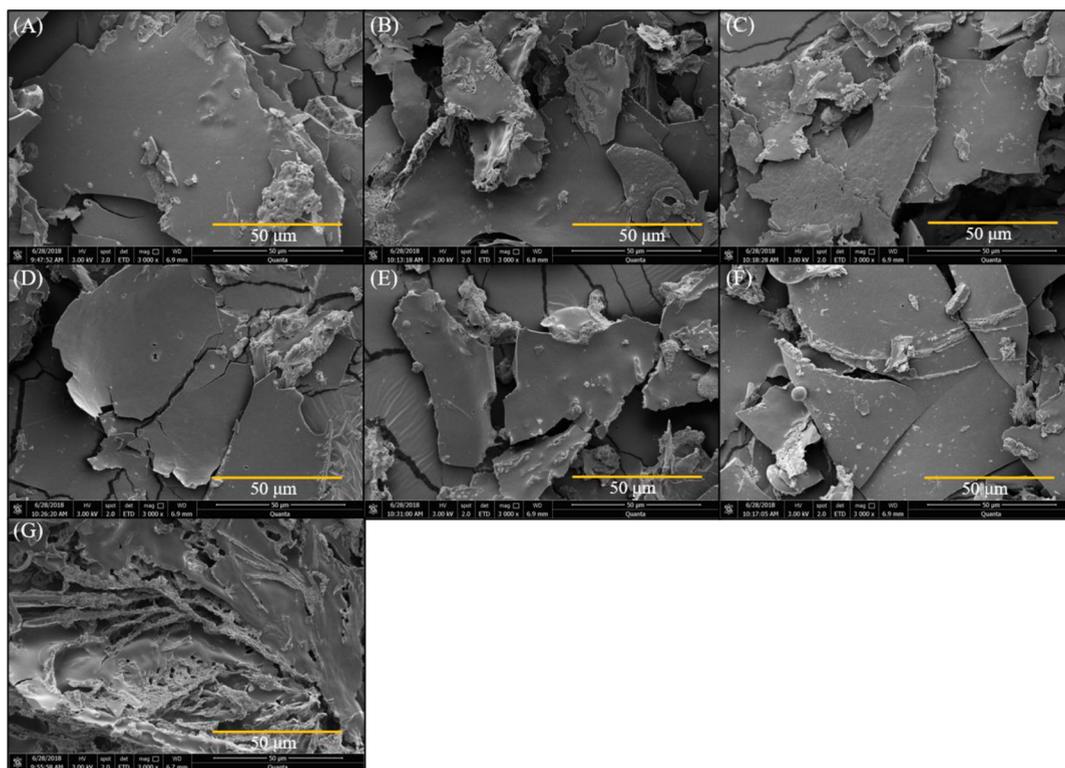


Figure 5. Surface microscopic morphology of peanut protein during storage. Figures A–G represent 0 d, 160 d/15 °C, 160 d/25 °C, 160 d/35 °C, 320 d/15 °C, 320 d/25 °C, and 320 d/35 °C, respectively.

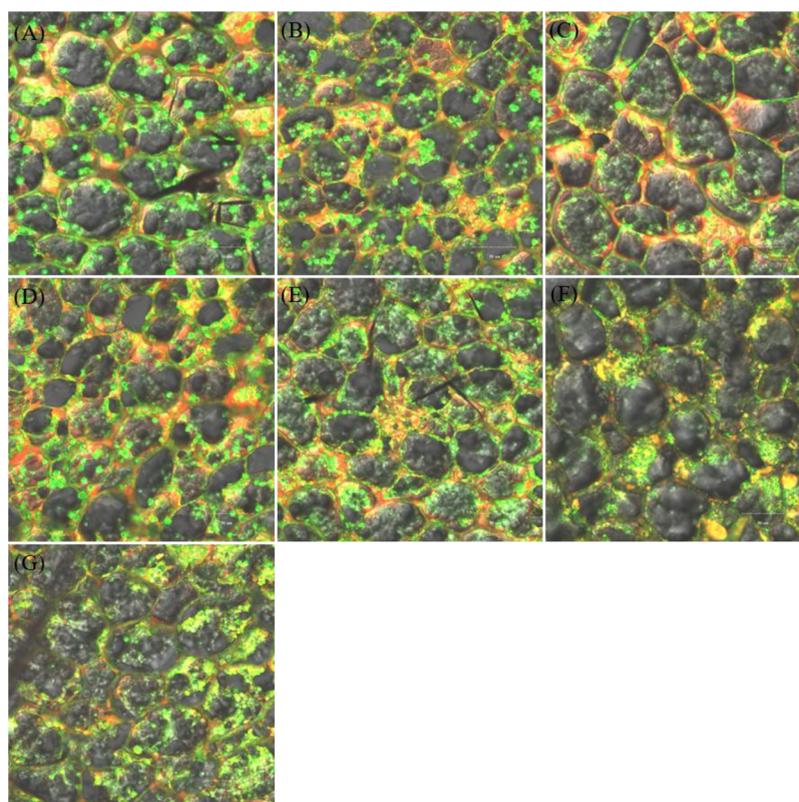


Figure 6. Changes in the spatial distribution of proteins and lipids under different storage conditions. Figures A–G represent 0 d, 160 d/15 °C, 160 d/25 °C, 160 d/35 °C, 320 d/15 °C, 320 d/25 °C, and 320 d/35 °C, respectively.

In conclusion, peanut protein aggregation occurred and formed larger particles after storage at 25 and 35 °C for a long

time (160 and 320 days). Two main reasons might account for the phenomenon. On the other hand, the free –SH groups

inside the peanut protein easily oxidize to form disulfide bonds or other sulfonic acid during storage. These substances destroy the protein natural structure and increase the internal repulsion of the protein, which in turn causes the structure to stretch in a certain direction.⁹ On the other hand, peanut protein is affected by lipid oxidation products and polymerized with small molecules or macromolecules such as lipids and sugars to form macromolecular protein polymers, resulting in a large particle size.^{23,24} Badii and Howell¹⁵ explored the structural changes of muscle proteins during frozen storage and found that fish protein was significantly denatured after storage as manifested by the expansion of muscle protein molecules and the formation of macromolecular aggregates connected by noncovalent bonds and covalent bonds. Potes et al.¹⁷ stored whey protein isolates and oils at 20 and 40 °C and found the formation of large molecular proteins.

2.4. Peanut Protein Surface Microscopic Morphology.

Scanning electron microscopy was used to investigate changes in the surface microscopic morphology of peanut protein, and the results are shown in Figure 5. Initially, the fraction of peanut protein presented a complete and smooth flake structure (Figure 5A) that is continuous and compact. When stored at 15 and 25 °C, the surface microscopic morphology of the protein did not change significantly after 320 days. However, a significant difference was observed during storage at 35 °C for 320 days. The surface morphology was no longer a complete smooth flake structure but presented a loose and nonsmooth stripe form with more tiny pores in the middle of the protein structure.

These results showed that a long period of storage at high temperature (35 °C) seriously destroyed the complete and smooth surface morphology of peanut protein. The changes in the protein surface structure could be attributed to the oxidative degeneration of proteins.²⁵

2.5. Spatial Distribution of Proteins and Lipids in Peanut Cells. Figure 6 indicates the spatial distribution of proteins and lipids in peanut cells under different storage conditions. The red particles in the picture represent lipids, green particles represent proteins, and yellow particles represent the part of protein and lipid overlapped.²⁶ As shown in the graph, the proteins were dispersed in peanut cells in a granular form, and the lipids presented in a continuous flake form. Before storage, the green particles in peanut cells could be clearly observed, and the red part was inlaid in the middle of green particles, indicating that the proteins and lipids were relatively independent of each other. No significant change was observed in the microscopic morphology and spatial distribution during storage at 15 °C for 160 and 320 days, 25 °C for 160 days, and 35 °C for 160 days. However, a significant change in the microscopic morphology and spatial distribution was observed when stored at 25 and 35 °C for 320 days. In particular, the green protein particles gradually disappeared and formed a continuous polymer. Most of the fluorescent red lipids disappeared and might have been covered by large protein aggregates.²⁷ A possible reason is that the cell walls of peanuts have been destroyed during storage under high temperature (35 °C), leading to the outflow and interaction of proteins and lipid components during storage.^{28,29} These findings suggested that the natural peanut lipid and protein were destroyed after storage under the temperature of 25 and 35 °C, but 15 °C was suitable for peanut storage.

3. CONCLUSIONS

Storage temperature and time significantly affected the natural structure of peanut proteins. When stored at 15 °C for 320 days, the particle size, surface morphology, spatial morphology, and distribution of peanut proteins showed no significant changes, but only the –SH content decreased significantly. As for high temperatures (25 and 35 °C), the –SH content, particle size, surface morphology, spatial morphology, and distribution of peanut proteins changed significantly after storing for 160 and 320 days, and the greatest impacts on protein structural properties were found at 35 °C. Therefore, the conditions of 15 °C within 160 days were recommended for peanut storage. This work will provide a theoretical basis for the actual production and storage of peanuts.

4. EXPERIMENTAL PROCEDURE

4.1. Materials. Newly harvested peanuts YuHua-9326 (YH-9326) were purchased from the Henan Academy of Agricultural Sciences, China. Samples were shelled and stored at –20 °C for further treatment.

4.2. Experimental Design. For storage, peanut seeds were grouped into three equal portions (250 g in each and wrapped in cloth bags) placed individually in a controlled temperature incubator of 15, 25, and 35 °C with a humidity of 70%. These samples were collected for further analysis after 0, 160, and 320 days of storage.

4.3. Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis. SDS-PAGE was performed using the method of Zhang et al.³⁰ with slight modifications. Two different experiments, namely, reductive and nonreductive SDS-PAGE, were conducted. Peanut protein powder was diluted with phosphate buffer, heated in boiling water for 5 min, and centrifuged for 20 min (10,000 g). The obtained supernatant (10 μL) was loaded into each electrophoresis well. SDS-PAGE was performed with a vertical system at a constant current of 20 mA in the concentrated glue and 40 mA in the separation glue. Upon completion, the glue was removed, fixed for 1 h, dyed for 2 h, and discolored to the background clear. The standard protein molecular weight (MW) ranges 14.4–97.4 kDa.

4.4. Total Sulfhydryl (–SH). Sulfhydryl (–SH) contents were determined according to the method of Beveridge et al.³¹ Peanut proteins were added to 30 mL of Tris-glycine buffer (containing 0.086 M Tris, 0.09 M glycine, 4 mM EDTA, pH 8.0) in a blender containing 8 M urea, stirred for 1 h, and centrifuged for 10 min (10,000 g). The supernatant was mixed with 160 μL of Ellman's reagent (4 mg/mL) and left in the dark for 15 min. Absorbance was measured at 412 nm using a spectrophotometer (722 s, Instruments and Electronics Associates, Shanghai, China). Total free –SH were calculated as follows:

$$\mu\text{MSH/g} = \frac{73.53 \times A_{412}}{C}$$

4.5. Particle Size. The protein particle size of peanuts was determined using the method of Anema et al.³² The peanut proteins were dissolved in phosphate buffer to an appropriate concentration (2%), stirred for 1 h at room temperature, and concentrated for 15 min (10,000 g). The supernatant was collected for determination. Particle size distribution and the average particle size were analyzed using a Malvern static light scattering instrument (ZS 90, Malvern Company, USA).

Related spectral analysis was performed using Malvern Zetasizer software.

4.6. Atomic Force Microscopy. The peanut protein nanostructure was examined using a nanoscope AFM (Digital Instruments, Santa Barbara, CA, USA) under tapping mode in a glovebox with 30–40% of relative humidity and 25 °C, as described by Xin et al.³³ The samples were controlled to an appropriate concentration (10–15 µg/mL) and pipetted (10 µL) on freshly cleaved mica sheets. The solution on the mica was dried in air at room temperature and then located to the sample stage. The tapping mode function for AFM imaging was performed using a Si3N4 cantilever with a resolution of 0.1 nm in vertical and 1–2 nm in horizontal positions. The particle characteristics of peanut protein such as graininess, diameter, and circumference were examined using the AFM software.

4.7. Scanning Electron Microscopy. The surface microstructure of the peanut protein was observed using a SEM microscope.³⁴ Briefly, a small amount of peanut protein powder was placed on a microscope slide on the sample table for observation.

4.8. Confocal Laser Scanning Microscopy. Confocal laser scanning microscopy (CLSM) is widely used for food microstructure analysis. In this experiment, the state and the relative position of protein and fat in cells were observed by CLSM during storage.³⁵ The peanut seeds were soaked in deionized water for 2 h and then cut into slices (10–15 µm) used a frozen slicer. The flakes were placed on a transparent slide and dropped for 20 min with Nile red (0.1%) and fluorescein isothiocyanate (FITC) (0.02%). Finally, the presence and the relative position of proteins and lipids in peanut cells were observed using a laser cofocusing system.

4.9. Statistical Analysis. Values were expressed as means ± standard deviations, and measurements were obtained in triplicate. Significant difference was determined at the $P < 0.05$ level for Duncan's multiple range test by SPSS software (version 20.0).

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Notes

The authors declare no competing financial interest.

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