



Postharvest ripening of two varieties of corns: Structure, antioxidant activities and physicochemical properties of zein

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ABSTRACT

The newly harvested Jidan 66 (JD66) and Liangyu 99 (LY99) varieties of corns were stored for 56 days at constant temperature of 15 and 25 °C with relative humidity of 55%. The postharvest ripening resulted in more disordered secondary structure and less compact tertiary conformation of zein. The emulsifying activity and foaming stability reached maximum after storage of corns at 15 and 25 °C for 14 days, while the emulsifying stability and foaming capacity were the highest at two temperatures of storage for 7 days and 28 days, respectively. Furthermore, zein had the highest viscoelasticity as well as the strongest antioxidant activities after the storage of JD66 at two temperatures for 28 days and the storage of LY99 at 15 °C for 42 days and at 25 °C for 28 days. Therefore, appropriate postharvest ripening of corns changed the structure of zein, improving its antioxidant activities and physicochemical properties.

1. Introduction

Corn is the largest grain crop in China, its output reached 270 million tons in 2021, which is an important grain resource and plays a pivotal role in ensuring national food security and meeting nutritional and health needs. Corn is harvested after preliminary ripening and stored for a certain period of time (Zhao, Li, et al., 2022). During this process, the components in corns are further metabolized and transformed, so that their physiological and biochemical characteristics, nutrition and processing quality gradually reach a more stable state, which is usually called postharvest ripening (Wu, Wang, & Ma, 2018). Many grains, such as corn, wheat and rice, have obvious postharvest ripening characteristics, and the physicochemical characteristics and processing properties of grains that has finished the postharvest ripening are enhanced. Giorni (2015) found that the contents of various components in the newly harvested corn were changed during the postharvest ripening. Zhang et al. (2021) showed that the increased S—S and α -helix content induced by postharvest ripening enhanced the aggregation of gluten proteins for middle- and strong-gluten wheat, resulting in a denser network structure. The sulphhydryl-disulfide interchange reactions might lead to the changes in structure and function of proteins. Mu et al. (2021) reported

that the extensographic and farinographic properties of dough increased along with the storage time, indicating the improvement of rheological properties during the postharvest ripening of wheat. Díaz, Kawamura, and Koseki (2019) point out that the nutrient components of rice after storage were changed, especially protein content, and then affected its processing properties, appearance quality and palatability, which was related to the postharvest ripening characteristics of rice.

Zein is the main storage protein in corn, accounting for around 50% of the total storage proteins, which is mainly found in the endosperm, and a small amount in the germ (Calvez et al., 2021). The zein molecule contains more than 50% non-polar and hydrophobic amino acid residues, such as leucine, proline and alanine. This unique amino acid composition results in the strong hydrophobicity and high biocompatibility of zein (Li et al., 2021). Argos, Pedersen, Marks, and Larkins (1982) proposed a structural model for zein, depicting an antiparallel arrangement of 9–10 homologous helical segments forming an elongated prism structure. The prism side formed by the helical outer surface is hydrophobic, while the top and bottom surfaces of prism containing glutamine-rich bridges are hydrophilic. Therefore, zein is an amphiphilic protein. The zein has excellent physicochemical properties such as water/oil holding, emulsifying, foaming, and rheological properties due

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to this amphiphilic structure. Li and Yao (2020) found that deamidated zein nanoparticles presented excellent freeze-drying/re-dispersion stability and improved the antioxidant effect and curcumin bio-accessibility, which could be used to protection and delivery of curcumin. Sun et al. (2022) reported that appropriate acetic acid concentration increased the viscoelasticity of zein and formed the protein fibril by controlling its self-assembly behavior, which improved the rheological property and gel formation capacity of zein. In addition, zein has a wide application prospect in the fields of special food and biological medicine due to its antioxidant activity (Tran, Duan, Lee, & Tran, 2019). These improvements in the properties and functions of zein are mainly attributed to its structural changes.

However, to the best of our knowledge, there were few studies on the structure, antioxidant activities and physicochemical properties of zein during the postharvest ripening of corn. In this work, the changes and structure–function relationships of structure, antioxidant activities and physicochemical properties of zein during postharvest ripening of corn were investigated, and the functional improvement mechanism of zein during postharvest ripening was explored. The aim of this work was to improve the antioxidant activities and physicochemical properties of zein by controlling the postharvest ripening period of corn, which provided a theoretical basis for promoting the processing and application of zein in the food industry.

2. Materials and methods

2.1. Materials

Two varieties of corns were Jidan 66 (JD66) and Liangyu 99 (LY99), which were grown in Lishu County, Jilin Province, China. Sodium dodecyl sulfate (SDS), 1,1-diphenyl-2-picrylhydrazyl (DPPH), and 2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) were purchased from Sigma-Aldrich (St Louis, MO, USA). Soy oil was obtained from Yihai Kerry Arawana Holdings Co. Ltd. All reagents were analytical grade and used without further purification.

2.2. Storage of postharvest corn and preparation of zein

The storage of postharvest corn was performed according to the procedures of our previous study (Zhao, Li, et al., 2022). Two kinds of non-transgenic and cultivated stable corn varieties (JD66 and LY99)

$$\text{DPPH radical scavenging capacity (\%)} = \frac{\text{Absorbance of the blank without sample} - \text{Absorbance of the sample}}{\text{Absorbance of the blank without sample}} \times 100 \quad (1)$$

were selected, which were harvested after pollination for 68 days. The newly harvested corns were stored at 15 and 25 °C with 55% relative humidity, and the samples were taken out at 0, 7, 14, 28, 42 and 56 days to extract zein.

The corn was threshed and crushed through an 80 mesh sieve, and then extracted with *n*-hexane for 2 h three times. The precipitated solid was dried and mixed with 75% ethanol solution at a material:liquid ratio of 1:2 (w/v), which was extracted for 2 h and repeated three times. The mixture was centrifuged at 4000 g for 10 min, and the supernatant was mixed with 3% NaCl solution in a volume ratio of 1:4 (v/v). The samples stood for 12 h and were centrifuged at 4000 g for 10 min to obtain protein precipitates. The resulting precipitates were washed with deionized water to remove salt ions and then freeze-dried to obtain zein. The zein samples extracted at different storage times were labeled as ST0, ST7, ST14, ST28, ST42 and ST56, respectively. The protein contents in all the zein samples were approximately 90% as determined by the Kjeldahl method.

2.3. Circular dichroism (CD)

The CD spectra were obtained using a J-820CD spectrometer (Jasco Co., Tokyo, Japan) according to the procedure reported by Zhao, Miao, et al. (2022) with slight modifications. The zein samples (0.2 mg/mL) were prepared with 75% (v/v) ethanol aqueous solutions. The mean residue ellipticity (θ , deg·cm²·dmol⁻¹) were recorded over the scanning wavelength range of 190–250 nm at a scanning rate of 100 nm/min with a constant nitrogen flow. The secondary structure, including α -helix, β -sheet, β -turn, and random coil, were quantified using CDPro software.

2.4. Ultraviolet (UV) spectroscopy

UV spectra were obtained using a UV-2550 ultraviolet–visible (UV–Vis) spectrophotometer (Shimadzu, Tokyo, Japan) according to the method of Sun et al. (2016) with slight modifications. The zein samples (2 mg/mL) were prepared with 75% (v/v) ethanol aqueous solutions. The ethanol aqueous solution was used as blank. The UV absorption spectra were recorded at the wavelength range of 200–400 nm with a slit width of 5 nm.

2.5. Intrinsic fluorescence spectroscopy

The intrinsic fluorescence spectra were obtained using a FR-5301PC fluorescence spectrophotometer (Shimadzu, Tokyo, Japan) according to the method described by Zhao et al. (2021) with slight modifications. The zein samples (0.2 mg/mL) were prepared with 75% (v/v) ethanol aqueous solutions. The emission spectra were recorded from 290 to 450 nm with an excitation wavelength of 280 nm and a slit width of 3 nm.

2.6. Measurement of antioxidant activities

2.6.1. DPPH radical scavenging capacity

The zein samples (0.2 mg/mL) were prepared with 75% (v/v) ethanol aqueous solution, and mixed with DPPH solution (0.2 mM, prepared in ethanol) in equal volume. The reaction proceeded for 30 min in the dark. The absorbance at 517 nm was measured using a UV-1800 spectrophotometer (Shimadzu Co., Tokyo, Japan) after reaction. DPPH solution without samples was used as a blank. The DPPH radical scavenging capacity was calculated as follows:

2.6.2. ABTS radical scavenging capacity

The ABTS (7 mM) was dissolved in the deionized water and mixed with potassium persulfate aqueous solution (2.45 mM) at the volume ratio is 2:1 (v/v). The mixture was incubated overnight in the dark at 25 °C to obtain ABTS solution. The zein samples (1 mg/mL) were prepared with 75% (v/v) ethanol aqueous solution. Subsequently, 200 μ L of sample solution was mixed with 800 μ L of ABTS solution, and incubated in the dark for 10 min at 25 °C. The absorbance at 734 nm was measured using a UV-1800 spectrophotometer (Shimadzu Co., Tokyo, Japan) after incubation. ABTS solution without samples was used as a blank. The ABTS radical scavenging capacity was calculated as follows:

$$\text{ABTS radical scavenging capacity (\%)} = \frac{\text{Absorbance of the blank without sample} - \text{Absorbance of the sample}}{\text{Absorbance of the blank without sample}} \times 100 \quad (2)$$

2.7. Measurement of emulsifying activity (EA) and emulsifying stability (ES)

The zein samples were dissolved in 75% (v/v) ethanol to obtain a 1 mg/mL protein solution, and mixed with soy oil at volume ratio of 3:1 (v/v). The mixture was homogenized at 10,000 rpm for 90 s using a high-speed homogenizer (Janus New-Materials Co. Ltd., Nanjing, Jiangsu, China) to obtain an emulsion. The 100 μL of emulsion was added to 5 mL of 0.1% (w/v) SDS. The absorbance at 500 nm of the diluted emulsion was measured using a UV-1800 spectrophotometer (Shimadzu Co., Tokyo, Japan) at 0 min and 10 min, respectively. The calculation equations for EA and ES were as follows:

$$\text{EA (m}^2/\text{g)} = \frac{2 \times 2.303}{C \times \varphi \times \theta (0.25) \times 10^4} \times \text{Absorbance of emulsion at 0 min} \times \text{DF (50)} \quad (3)$$

$$\text{ES (\%)} = \frac{\text{Absorbance of emulsion at 10 min}}{\text{Absorbance of emulsion at 0 min}} \times 100 \quad (4)$$

where C is the protein concentration (g/mL), φ is the optical path (1 cm), θ is the volume fraction of oil in the emulsion, and DF is the dilution factor.

2.8. Measurement of foaming capacity (FC) and foaming stability (FS)

The zein samples were dissolved in 75% (v/v) ethanol to obtain a 0.1 mg/mL protein solution. The 1 mL of protein solution was dropped into 25 mL of 0.02 M phosphate buffer (pH 7.0), and then homogenized at 12,000 rpm for 2 min using a high-speed homogenizer (Janus New-Materials Co. Ltd., Nanjing, Jiangsu, China). The volume of liquid level was immediately measured after homogenization, which was measured again after standing for 30 min. The calculation equations for FC and FS were as follows:

$$\text{FC (\%)} = \frac{\text{Volume of liquid level after homogenization} - 25}{25} \times 100 \quad (5)$$

$$\text{FS (\%)} = \frac{\text{volume of liquid level after standing for 30 min} - 25}{\text{Volume of liquid level after homogenization} - 25} \times 100 \quad (6)$$

2.9. Measurement of rheological properties

The rheological properties were measured using a MCR302 rheometer (Anton Paar Co., Graz, Austria) according to the procedure of Ma, You, and Lu (2014) with slight modifications. The zein samples were dissolved in 75% (v/v) ethanol to obtain a 200 mg/mL protein solution. The samples were placed between two parallel plates with a diameter of 40 mm. The gap of parallel plates was set to 1 mm. The exposed edges of the gels were covered with paraffin oil to prevent water evaporation. The apparent viscosity was measured at the shear rate range of 0.1–100

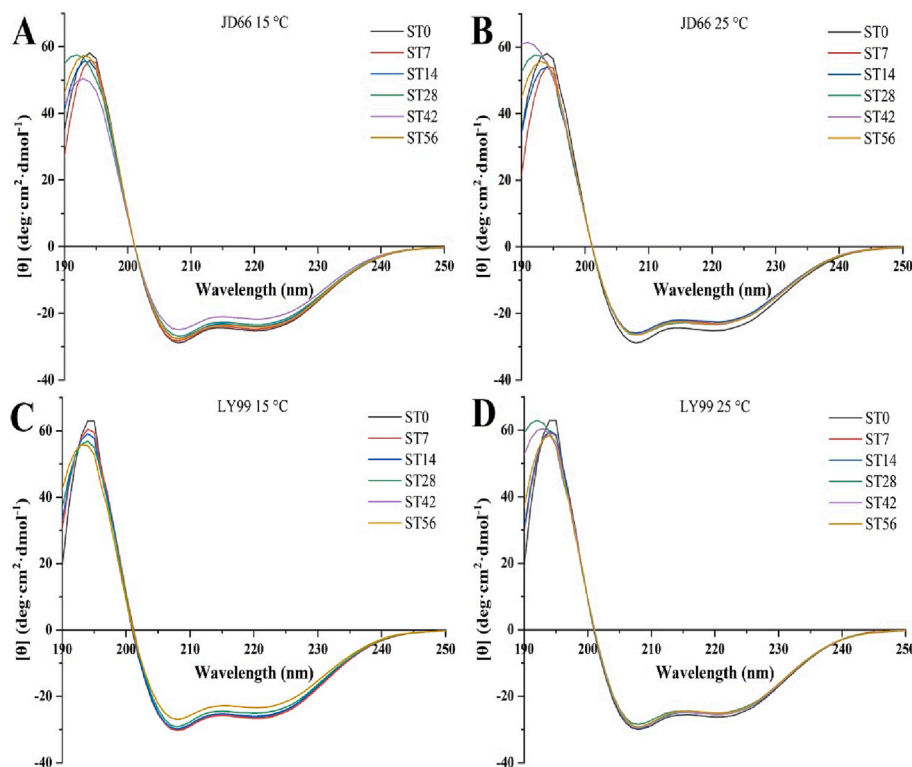


Fig. 1. Circular dichroism (CD) spectra of zein during postharvest ripening of two varieties of corns at two temperatures. A: JD66 15 °C; B: JD66 25 °C; C: LY99 15 °C; D: LY99 25 °C.

s^{-1} . The storage modulus (G') and loss modulus (G'') were recorded at the frequency range of 0.1–10 Hz. The temperature was 25 °C and the strain was 0.2%.

2.10. Statistical analysis

All the measurements were performed at least in triplicate and the results were expressed as mean \pm standard deviation. Significant differences were defined as $p < 0.05$, which were determined by analysis of variance (ANOVA) using SPSS software version 23.0 (SPSS Inc., Chicago, IL, USA). The graphs were drawn using Origin 2018 software (OriginLab Corporation, Northampton, MA, USA).

Table 1

Secondary structure contents of zein during postharvest ripening of two varieties of corns at 15 and 25 °C.

Samples		Content (%)			
		α -helix	β -sheet	β -turn	random coil
JD66 15 °C	ST0	50.88 \pm 0.23 ^c	15.31 \pm 0.16 ^f	15.66 \pm 0.04 ^a	18.15 \pm 0.13 ^a
	ST7	51.86 \pm 0.29 ^d	13.60 \pm 0.05 ^c	13.67 \pm 0.12 ^e	20.87 \pm 0.02 ^c
	ST14	51.01 \pm 0.37 ^a	13.23 \pm 0.15 ^c	15.48 \pm 0.09 ^b	20.28 \pm 0.11 ^b
	ST28	49.68 \pm 0.10 ^b	12.74 \pm 0.14 ^a	11.85 \pm 0.05 ^f	25.73 \pm 0.05 ^g
	ST42	49.74 \pm 0.11 ^b	13.76 \pm 0.08 ^d	12.94 \pm 0.08 ^e	23.56 \pm 0.04 ^e
	ST56	48.92 \pm 0.15 ^a	13.87 \pm 0.09 ^d	13.42 \pm 0.08 ^e	23.79 \pm 0.06 ^f
	ST0	50.88 \pm 0.13 ^c	15.31 \pm 0.16 ^f	15.66 \pm 0.06 ^a	18.15 \pm 0.13 ^a
JD66 25 °C	ST7	49.08 \pm 0.27 ^a	14.57 \pm 0.12 ^e	15.98 \pm 0.12 ^a	20.37 \pm 0.05 ^b
	ST14	50.08 \pm 0.38 ^b	14.53 \pm 0.07 ^e	14.53 \pm 0.11 ^c	20.86 \pm 0.07 ^c
	ST28	50.98 \pm 0.09 ^d	12.01 \pm 0.00 ^a	13.27 \pm 0.06 ^e	23.74 \pm 0.10 ^f
	ST42	50.82 \pm 0.16 ^c	13.74 \pm 0.14 ^d	14.23 \pm 0.13 ^d	21.21 \pm 0.09 ^c
	ST56	51.61 \pm 0.13 ^d	13.37 \pm 0.12 ^c	13.70 \pm 0.04 ^e	21.32 \pm 0.04 ^c
	ST0	52.85 \pm 0.13 ^e	14.92 \pm 0.09 ^f	11.07 \pm 0.07 ^f	21.16 \pm 0.08 ^c
	LY99 15 °C	ST7	51.92 \pm 0.14 ^d	13.45 \pm 0.04 ^c	11.19 \pm 0.14 ^f
ST14		49.81 \pm 0.05 ^b	13.37 \pm 0.12 ^c	13.08 \pm 0.09 ^e	23.74 \pm 0.05 ^f
ST28		50.47 \pm 0.12 ^c	13.18 \pm 0.07 ^c	11.57 \pm 0.04 ^f	24.78 \pm 0.10 ^f
ST42		49.71 \pm 0.06 ^b	12.66 \pm 0.02 ^b	10.80 \pm 0.07 ^g	26.83 \pm 0.13 ⁱ
ST56		49.24 \pm 0.14 ^a	12.71 \pm 0.04 ^b	12.74 \pm 0.05 ^e	25.31 \pm 0.12 ^g
ST0		52.85 \pm 0.09 ^e	14.92 \pm 0.08 ^f	11.07 \pm 0.07 ^f	21.16 \pm 0.08 ^c
LY99 25 °C		ST7	53.59 \pm 0.11 ^f	14.48 \pm 0.10 ^e	10.24 \pm 0.06 ^g
	ST14	49.40 \pm 0.10 ^b	13.29 \pm 0.07 ^c	14.93 \pm 0.11 ^c	22.38 \pm 0.05 ^d
	ST28	49.01 \pm 0.13 ^a	12.17 \pm 0.09 ^a	11.31 \pm 0.08 ^f	27.51 \pm 0.10 ^j
	ST42	49.18 \pm 0.11 ^b	12.30 \pm 0.08 ^a	12.82 \pm 0.06 ^e	25.70 \pm 0.13 ^g
	ST56	49.40 \pm 0.08 ^b	12.69 \pm 0.06 ^b	11.92 \pm 0.08 ^f	25.99 \pm 0.08 ^h
	ST0	52.85 \pm 0.09 ^e	14.92 \pm 0.08 ^f	11.07 \pm 0.07 ^f	21.16 \pm 0.08 ^c
	ST7	53.59 \pm 0.11 ^f	14.48 \pm 0.10 ^e	10.24 \pm 0.06 ^g	21.69 \pm 0.13 ^d
ST14	49.40 \pm 0.10 ^b	13.29 \pm 0.07 ^c	14.93 \pm 0.11 ^c	22.38 \pm 0.05 ^d	
ST28	49.01 \pm 0.13 ^a	12.17 \pm 0.09 ^a	11.31 \pm 0.08 ^f	27.51 \pm 0.10 ^j	
ST42	49.18 \pm 0.11 ^b	12.30 \pm 0.08 ^a	12.82 \pm 0.06 ^e	25.70 \pm 0.13 ^g	
ST56	49.40 \pm 0.08 ^b	12.69 \pm 0.06 ^b	11.92 \pm 0.08 ^f	25.99 \pm 0.08 ^h	

The values with different letters in the same column are significantly different ($p < 0.05$).

3. Results and discussion

3.1. Circular dichroism (CD) spectra

The CD spectra can be used to characterize the structures of proteins, especially their different types of secondary structure (Wei et al., 2020). Fig. 1 shows the CD spectra of zein during postharvest ripening of two varieties of corns at two temperatures. There were positive peaks at 190–200 nm and negative peaks near 208 and 220 nm, indicating that the α -helix conformation was the main structure of zein (Venkateswarlu, Boopalan, Mohan, Das, & Sastry, 2006). During postharvest ripening of two varieties of corns at 15 and 25 °C, the absorption peak intensity of zein at 190–200 nm increased or decreased, and the peak position was shifted to a lower wavelength in different degree. The maximum shift of peak position occurred after the storage of JD66 at 15 °C for 28 days (Fig. 1A) and at 25 °C for 42 days (Fig. 1B), as well as the storage of LY99 at 25 °C for 28 days (Fig. 1D). However, the peak position did not shift significantly during the storage of LY99 at 15 °C (Fig. 1C). These changes indicated that the postharvest ripening of corns resulted in the changes in the secondary structure of zein, which might be related to the local sequence of amino acids and their interactions (Hu et al., 2013). Moreover, the intensities of absorption peaks near 208 and 220 nm were decreased in different degree after the postharvest ripening of corns, suggesting the breakdown of hydrogen bonds.

The secondary structure contents of zein during postharvest ripening of two varieties of corns at 15 and 25 °C were obtained by fitting CD spectra (Table 1). In the postharvest ripening process, the α -helix and β -turn of zein changed irregularly, and the β -sheet contents showed a decreasing trend, while the random coil contents showed an increasing trend. When JD66 was stored for 28 days at 15 and 25 °C, the β -sheet contents of zein reached the lowest values, which decreased from 15.31% to 12.74% and 12.01%, respectively; while the random coil contents reached the highest values, which increased from 18.15% to 25.73% and 23.74%, respectively. The similar secondary structure changes were also observed for the storage of LY99 at 15 °C for 42 days and at 25 °C for 28 days. This is consistent with the secondary structure results analyzed by FTIR in our previous report (Zhao, Li, et al., 2022). The transformation of β -sheet to random coil might be attributed to the disruption of hydrogen bonds and the occurrence of oxidation reaction. The unfolding of zein induced by the loss of β -sheet resulted in the increase of random coil and structural disorder, thus enhancing the molecular flexibility of zein during the postharvest ripening of corns, which might also improve the functional properties of zein. Huang, Yi, and Fan (2022) reported that the secondary structure of α -lactalbumin induced by peroxy radicals were changed from ordered β -sheet to disordered random coil, which improved protein functional properties such as emulsifying and foaming.

3.2. Ultraviolet (UV) spectra

UV absorption spectrum can provide the microenvironment state of protein in solution, so the changes in the microenvironment of aromatic amino acid residues will alter their absorption peaks (Kroll, Rawel, & Rohn, 2003). The UV spectra of zein during postharvest ripening of two varieties of corns at two temperatures are shown in Fig. 2. The maximum absorption peaks were observed near 285 nm for zein, which were attributed to the absorption of chromophoric amino acid residues such as tyrosine, phenylalanine and tryptophan in the protein (Sun et al., 2016). The postharvest ripening of JD66 at two temperatures resulted in a small shift in the peak positions of zein, which was unchanged for the postharvest ripening of LY99. The absorption peak intensities of zein were increased obviously after the postharvest ripening of two varieties of corns at two temperatures, suggesting the changes in the microenvironment of chromophoric amino acid residues in the protein. The absorption peak intensities of zein reached the maximum after the storage of JD66 for 28 days at 15 °C (Fig. 2A) and 25 °C (Fig. 2B), which were

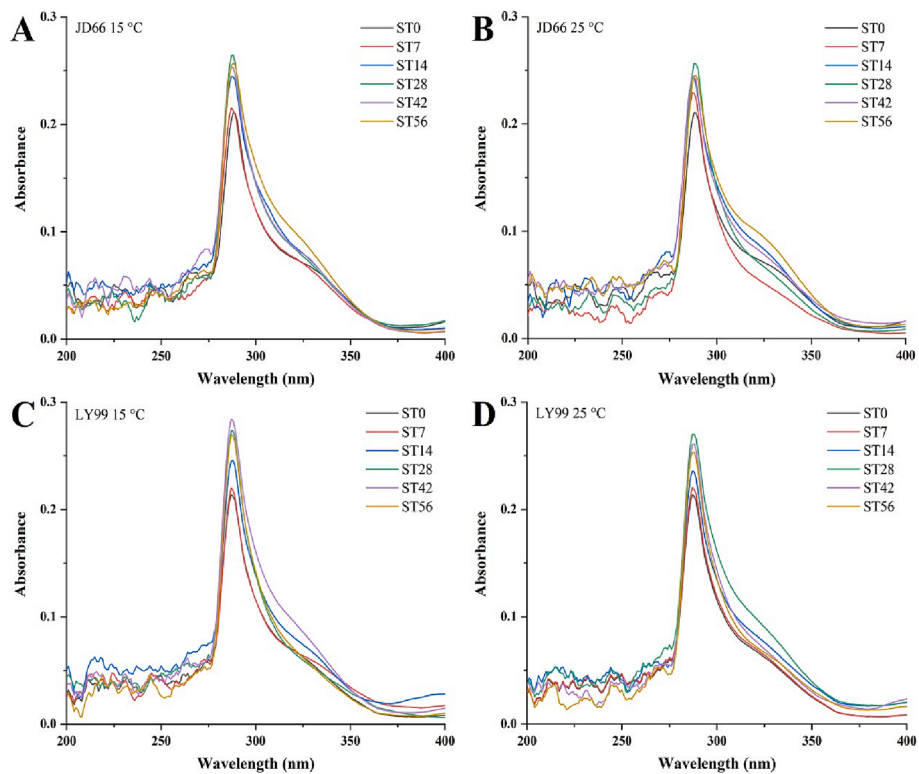


Fig. 2. Ultraviolet (UV) spectra of zein during postharvest ripening of two varieties of corns at two temperatures. A: JD66 15 °C; B: JD66 25 °C; C: LY99 15 °C; D: LY99 25 °C.

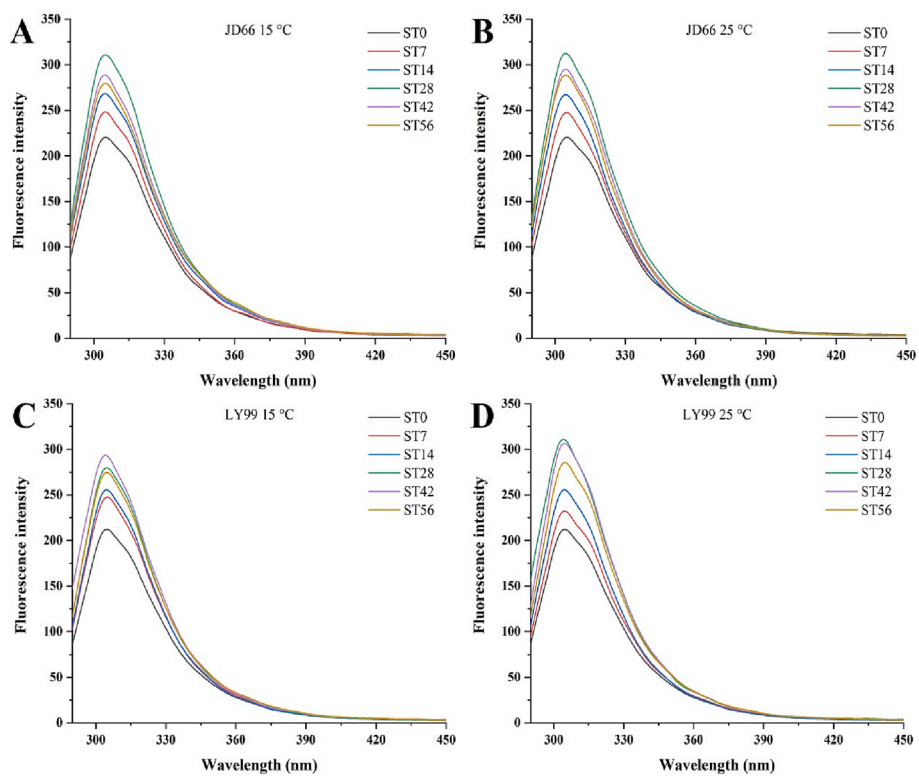


Fig. 3. Intrinsic fluorescence spectra of zein during postharvest ripening of two varieties of corns at two temperatures. A: JD66 15 °C; B: JD66 25 °C; C: LY99 15 °C; D: LY99 25 °C.

the similar changes for the storage of LY99 for 42 days at 15 °C (Fig. 2C) and for 28 days at 25 °C (Fig. 2D). It was possible that the zein structure tended to be disordered and became less compact, which exposed the aromatic chromophoric groups inside the protein, leading to the increase in the peak intensities. The enhancement of UV absorption peak indicated that the postharvest ripening of corns changed zein structure. Chen, Chen, Ren, and Zhao (2011) found that the structural unfolding of protein promoted the exposure of chromophoric amino acid residues, thus increasing the UV absorption peak intensity.

3.3. Intrinsic fluorescence spectra

The intrinsic fluorescence spectroscopy is used to characterize the tertiary conformation of proteins through providing the information of the polarity around the chromophoric amino acids residues in the microenvironment (Zhang, Chen, Qi, Sui, & Jiang, 2018). Fig. 3 shows the intrinsic fluorescence spectra of zein during postharvest ripening of two varieties of corns at two temperatures. The highest fluorescence intensity of zein was appeared near 304 nm, indicating the most contribution of the tyrosine residues for the fluorescence absorption among three chromophoric amino acids (Shukla & Cheryan, 2001). The fluorescence intensities of zein were increased in different degrees during postharvest ripening of corns, which was attributed to a more hydrophilic and stronger polar microenvironment of tyrosine residues (Wei, Sun, Dai, Zhan, & Gao, 2018), suggesting a less compact tertiary conformation of zein. However, the position of fluorescence absorption peak did not shift during the postharvest ripening process. The fluorescence intensities of zein reached the maximum after 28 days of storage at 15 °C (Fig. 3A) and 25 °C (Fig. 3B) for JD66, as well as 42 days of storage at 15 °C (Fig. 3C) and 28 days of storage at 25 °C (Fig. 3D) for LY99. It was possible that the postharvest ripening caused the unfolding of zein molecular structure due to the disruption of intramolecular hydrogen bonds induced by the respiration effect of corn and oxidation reaction of protein (Zhao, Li, et al., 2022), leading to a less compact tertiary conformation and the exposure of more chromophoric amino acid residues, which enhanced the fluorescence intensity (Chen et al., 2019). In addition, the increased fluorescence intensity might also be associated with structural unfolding and exposure resulting from the reduction in the intramolecular hydrogen bonds of zein, as demonstrated by the results of CD (Fig. 1). However, the fluorescence intensity of zein decreased with further prolongation of the postharvest ripening time, which might be due to the protein aggregation induced by its excessive oxidation. Ye, Liao, Sun, and Zhao (2015) also found that increasing the oxidation degree of protein resulted in the decrease of its fluorescence intensity, which was mainly attributed to the formation of the oxidation aggregates through the disulfide bonds and hydrophobic interactions after protein oxidation, forcing the fluorescent amino acids to be re-embedded in the hydrophobic environment inside the molecules.

3.4. Antioxidant activities

Antioxidant effects mainly involve two mechanisms of scavenging radical and inhibiting radical production (Giustarini et al., 2016). DPPH is a nitrogen-centered fat-soluble radical, while ABTS is a water-soluble radical, and they can form stable substances by obtaining the electrons and reactive hydrogens from proteins, respectively (Brand-Williams, Cuvelier, & Berset, 1995). The antioxidant activities of zein during postharvest ripening of two varieties of corns at two temperatures were characterized by DPPH radical scavenging capacity (Fig. 4A–B) and ABTS radical scavenging capacity (Fig. 4C–D). The scavenging capacities of DPPH radical and ABTS radical of zein were significantly increased by the postharvest ripening of corns. The DPPH radical scavenging capacities of zein reached the highest (increasing from 10.37% to 14.43% and 14.50%, respectively) after the storage of JD66 at 15 °C and 25 °C for 28 days (Fig. 4A), which were the similar changes (increasing

from 11.45% to 14.45% and 14.15%, respectively) for the storage of LY99 at 15 °C for 42 days and at 25 °C for 28 days (Fig. 4B). The variation trend of ABTS radical scavenging capacity of zein was similar to that of DPPH radical scavenging capacity during the postharvest ripening process of corns. The ABTS radical scavenging capacities of zein were the highest after the storage of JD66 at 15 °C and 25 °C for 28 days (Fig. 4C) and the storage of LY99 at 15 °C for 42 days and at 25 °C for 28 days (Fig. 4D), which increased from 19.20% to 24.89% and 24.16%, as well as from 18.31% to 24.18% and 23.89%, respectively. The enhancement of scavenging capacities of DPPH radical and ABTS radical might be attributed to the better dispersion of zein in solvents, such as ethanol aqueous solution and deionized water after postharvest ripening (Zhao, Li, et al., 2022), which was conducive to the interactions between zein and free radicals, thus leading to the increase in scavenging capacity of free radicals. In addition, the postharvest ripening unfolded the structure of zein and increased the molecular flexibility (Fig. 1–Fig. 3), leading to the exposure of more free radical binding sites, which was also responsible for the improved antioxidant activities. However, the formation of zein aggregates caused the sites in the proteins that could bind to free radicals to become embedded within the molecule again as the further extension of postharvest ripening time, thus inhibiting their antioxidant activities to a certain extent.

3.5. Emulsifying properties

Zein is an amphiphilic protein containing hydrophilic and hydrophobic groups, which can be adsorbed on the oil–water interface to form an interface film and play a role in stabilizing the emulsion (Khan et al., 2011). The emulsifying properties of zein during postharvest ripening of two varieties of corns at two temperatures are shown in Fig. 4E–F. The emulsifying activity (EA) and emulsifying stability (ES) of zein increased first and then decreased as the extension of postharvest ripening time. When JD66 (Fig. 4E) and LY99 (Fig. 4F) were stored at 15 °C and 25 °C for 14 days, the EA of zein reached the maximum, both of which were approximately 30 m²/g. The ES of zein increased to about 52% at 15 °C and 25 °C of storage of two varieties of corns for 7 days, which were unchanged significantly until 14 days for JD66 or 28 days for LY99 of storage at two temperatures. It was possible that appropriate postharvest ripening caused the disordered secondary structure and loose tertiary conformation of zein (Fig. 1–Fig. 3), and exposed polar groups to increase hydration, which resulted in a better hydrophilic/hydrophobic balance, thereby improving the emulsifying properties (Khan et al., 2011). Zhang, Zhang, Wang, and Guo (2012) found that the unfolding of protein structure promoted its emulsification. However, the prolonged postharvest ripening time broke this hydrophilic/hydrophobic balance, resulting in a decrease in emulsifying properties. The emulsifying properties of proteins were closely related to their structural exposure and hydrophilic/hydrophobic balance, which played a major role in their adsorption capacity at the oil–water interface and the stability of the interface films (Lam & Nickerson, 2013).

3.6. Foaming properties

In the foam systems, the proteins can form a layer of viscoelastic film with considerable thickness at the air–water interface, thus contributing to the production and stabilization of foam (Bora, 2002). The foaming properties of zein during postharvest ripening of two varieties of corns at two temperatures are shown in Fig. 4G–H. The foaming capacity (FC) and foaming stability (FS) of zein increased first and then decreased as the extension of postharvest ripening time. When JD66 (Fig. 4G) and LY99 (Fig. 4H) were stored at 15 °C and 25 °C for 28 days, the FC of zein were the highest (57%–62%). The FS of zein reached the maximum (48%–51%) when the two varieties of corns were stored at 15 °C and 25 °C for 14 days. The improvement of foaming properties was mainly due to the enhanced interface interactions resulting from the structural changes of zein such as the unfolding of secondary structure and the

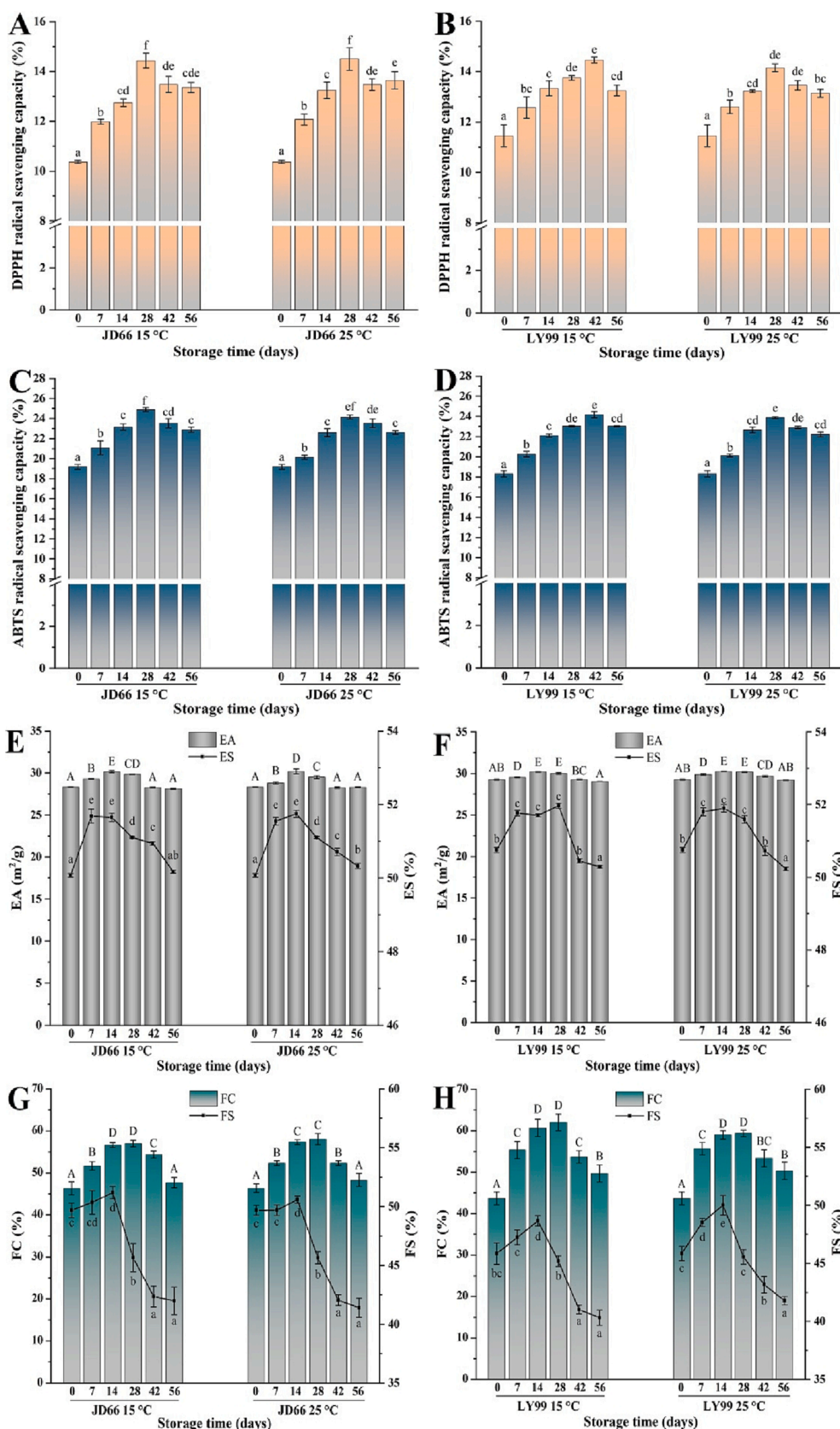


Fig. 4. DPPH radical scavenging capacity (A, B), ABTS radical scavenging capacity (C, D), emulsifying properties (E, F) and foaming properties (G, H) of zein during postharvest ripening of two varieties of corns at two temperatures. The different lowercase or uppercase letters in the graphs indicate that the results are significantly different ($p < 0.05$).

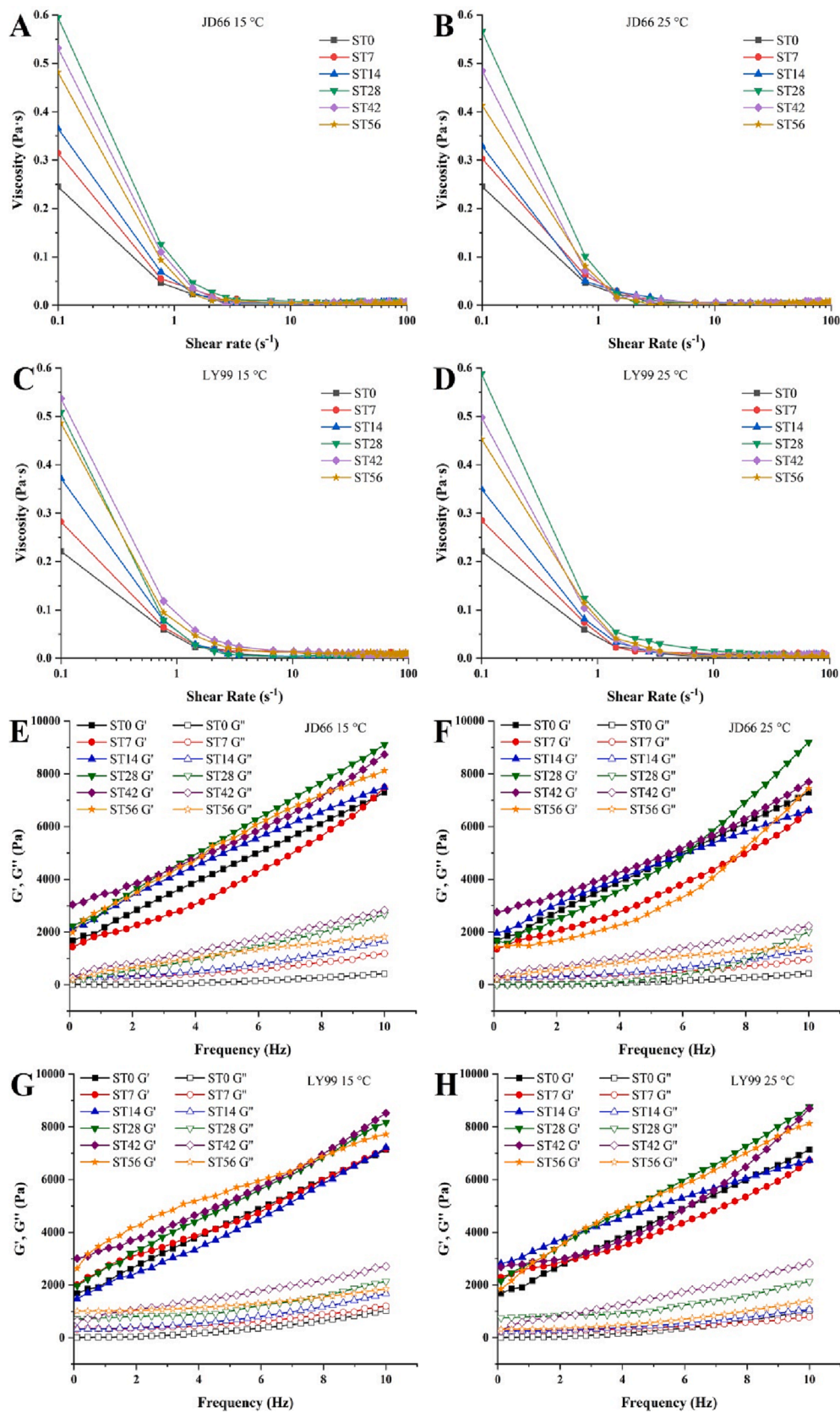


Fig. 5. Apparent viscosity as a function of shear rate (A: JD66 15 °C; B: JD66 25 °C; C: LY99 15 °C; D: LY99 25 °C) and storage modulus (G') and loss modulus (G'') as functions of frequency (E: JD66 15 °C; F: JD66 25 °C; G: LY99 15 °C; H: LY99 25 °C) for zein during postharvest ripening of two varieties of corns at two temperatures.

exposure of tertiary structure during the postharvest ripening of corns (Figs. 1–3). Zhao, Xiong, Chen, Zhu, and Wang (2020) found that the heat treatment resulted in the unfolding of protein structure and the increase of molecular flexibility, which improved its FC and FS. However, excessive postharvest ripening of corns formed insoluble protein aggregates and reduced the intermolecular interactions, which changed the surface tension of the molecules and also destabilized the air–water interface, thus leading to the reduction of foaming properties.

3.7. Rheological properties

Viscosity can be defined as a measure of the resistance of a fluid to motion when the shear stress is applied (Vlierberghe, Dubruel, & Schacht, 2011). The apparent viscosity as a function of shear rate for zein during postharvest ripening of two varieties of corns at two temperatures is shown in Fig. 5A–D. All zein samples showed the decrease trends in viscosity with increasing the shear rate, exhibiting pseudo-plastic or shear thinning behavior, which were the typical non-Newtonian fluid phenomena (Özkan, Xin, & Chen, 2002). The postharvest ripening of corns increased the viscosity of zein, which reached the maximum after the storage of JD66 at 15 °C (Fig. 5A) and 25 °C (Fig. 5B) for 28 days as well as the storage of LY99 at 15 °C for 42 days (Fig. 5C) and at 25 °C for 28 days (Fig. 5D). It was possible that the postharvest ripening of corns unfolded the structure of zein and enhanced random coil (Fig. 1) and intrinsic fluorescence intensity (Fig. 3), which exposed the hydrophilic groups and increased hydration of protein, leading to an increase in its apparent viscosity (Vestergaard et al., 2007).

The quality and processing characteristics of food are closely related to its viscoelastic properties. The storage modulus (G') and loss modulus (G'') as functions of frequency for zein during postharvest ripening of two varieties of corns at two temperatures are shown in Fig. 5E–H. The protein system exhibits a higher elasticity and is a solid-like gel state when $G' > G''$; while the system shows a higher viscosity and is a solution state when $G'' > G'$ (Puppo & Añón, 1998). The G' of all zein samples were greater than G'' over the entire frequency range, indicating the formation of three-dimensional gel network structure by protein cross-linking. The G' and G'' gradually enhanced as increasing the frequency, and the increase in G' is more significant than that in G'' , suggesting that the solid properties and elasticity of zein became stronger (Nonthanum, Lee, & Padua, 2012). The different postharvest ripening periods of corns resulted in the different G' and G'' curves of zein. The G' of zein reached the maximum after the storage of JD66 at 15 °C (Fig. 5E) and 25 °C (Fig. 5F) for 28 days as well as the storage of LY99 at 15 °C for 42 days (Fig. 5G) and at 25 °C for 28 days (Fig. 5H), thus exhibiting the strongest elastic properties, which might be attributed to structural changes of zein caused by biochemical reactions during the postharvest ripening of corns. Therefore, appropriate postharvest ripening increased the cross-linking of zein molecular, thus promoting the establishment of three-dimensional network structure, which might contribute to the improvement of gelation ability of zein (Banerjee & Bhattacharya, 2012).

4. Conclusions

The postharvest ripening of corns caused the secondary structure of zein to transform from β -sheet to random coil and resulted in more disordered and less compact tertiary conformation of zein. The DPPH radical and ABTS radical scavenging capacities of zein were the highest after the storage of JD66 at 15 °C and 25 °C for 28 days and the storage of LY99 at 15 °C for 42 days and at 25 °C for 28 days. When JD66 and LY99 were stored at 15 °C and 25 °C for 14 days, the EA of zein reached the highest. The ES of zein increased to maximum at 15 °C and 25 °C of storage of two varieties of corns for 7 days. The FC and FS of zein reached the maximum after the storage of two varieties of corns at two temperatures for 28 days and 14 days, respectively. In addition,

appropriate postharvest ripening of corns also improved the apparent viscosity and elastic properties of zein, exhibiting a strong solid gel behavior. The results suggested that controlling the postharvest ripening time of corns could modify the structure of zein, thus improving its antioxidant activity and physicochemical properties, which were helpful to understand the relationship between structure and function of zein during postharvest ripening.

CRedit authorship contribution statement

Chengbin Zhao: Conceptualization, Writing – review & editing. **Jinying Zhu:** Writing – original draft, Methodology. **Hao Zhang:** Supervision. **Qi Qi:** Visualization. **Nannan Hu:** Investigation. **Runzhi Han:** Resources, Software. **Mingzhu Zheng:** Data curation. **Xiuying Xu:** Formal analysis. **Yuzhu Wu:** Project administration. **Jingsheng Liu:** Funding acquisition, Validation.

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.

Data availability

The authors do not have permission to share data.

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