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Effect of grape seed proanthocyanidin on the structural and physicochemical properties of bread during bread fermentation stage

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ABSTRACT

Bread was prepared using wheat flour with grape seed proanthocyanidin (GSP) (0.4%). GSP improved the textural properties of bread including hardness, cohesiviness, gumminess and chewiness. At the last stage of fermentation, GSP reinforced the gluten microstructure with increased the disulfide bonds and hydrophobic interaction and α-helix in the secondary structures. Moreover, GSP addition could increase the total phenolics and antioxidative acitivity of the bread significantly. In addition, the degree of fermentation had a strong influence on the dough forces, and the reasonable control of bread fermentation time was beneficial to improve the bread quality, which provided a reference for the bread processing industry.

1. Introduction

Wheat-based products play a important role in the world people's food culture and daily life. However, many nutrients are lost or diminished during the wheat redining process, including vitamins, fibers, phytochemicals, etc., refined wheat was not suitable for providing balanced food nutrition for consumers (Cacak-Pietrzak, et al., 2023). Bread is one of the common staple foods in the world, mainly made from wheat flour. During the bread processing process, the gluten protein in the wheat flour mix the flour and water to form a dough that becomes more viscous and elastic. In recent years, a variety of phytochemical fortified wheat products have been produced, such as tea polyphenol bread (Kan et al., 2020), purple potato flour fortified buns (Zhu and Sun, 2019) and so on. Polyphenols, plant secondary metabolites, have also been mostly added to flours as functional ingredient (Roopchand et al., 2012). It has been shown that polyphenols can interact with the dough and influence the formation of gluten networks in the dough (Girard and Awika, 2020).

Polyphenols are often added to wheat-based foods to achieve different goals. A large portion of application are to help develop functional food. For example, flour was enhanced with eggplant addition to provide antioxidant capacity (Valerga et al., 2020). Some polyphenols have been used as natural pigments to enrich the color and product image. These include blue anthocyanin-enriched muffin (Ab Rashid et al., 2021) and purple pasta, whose color comes from purple wheat (Zanoletti et al., 2017). Grape seed proanthocyanidin (GSP) are formed by the aggregation of catechins, epicatechin, and epicatechin gallate ester C4–C8 or C4–C6 bonds, and has extremely strong biological activity and pharmacological effects, including antioxidant, anti-inflammatory, anti-tumor, antibacterial, lipid-lowering, and sleep improving effects. Considering the multiple functional properties of proanthocyanidin, there are many drugs, health products and comsmetics containing proanthocyanidin both domestically and internationally (Chen et al., 2022).

Gluten consists of linear high molecular glutenin subunits and spherical gliadin. The molecular ends of linear glutenin subunits form network and ring structures through disulfide bonds, and under the presence of various forces such as sulfur bonds, hydrogen bonds, and hydrophobic bonds, various subunits intertwine to form a network structure, the more entangled the points, the larger the molecular

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weight, and the more stable the network structure, ultimately giving the dough elasticity. Alcohol solution proteins form spherical structures under the action of disulfide bonds, hydrogen bonds, and van der Waals forces, and interact with each other through intermolecular hydrogen bonds. The interaction of glutenin further stabilized the network structure and gave the dough stickiness. There is no difference between the molecules of wheat gliadin sulfur bond. Grape seed oligomeric proanthocyanidins can affect gluten protein cross-linking through mechanisms such as disulfide/thiol exchange, free amino cross-linking, tyrosine cross-linking, and non covalent interactions, and GSP improved the wheat-based foods by regulating sulfhydryl/disulfide redox reactions in the gluten network and altering hydrophobic interaction sites with gluten proteins (Liu et al., 2018).

However, the effect of GSP on bread during fementation has not yet been studied in detail. We tried to reveal the impact of GSP on the fermentation process and quality of bread by micro and macro analysis methods, evaluate the effects of GSP on eating quality of bread including total phenolics and antioxidative activity.

2. Materials and methods

2.1. Reagents and samples

Potassium persulfate, sodium dodecyl sulfate, 8-Anilino-1-naphthalenesulfonic acid (ANS), L-cysteine, 5,5′-Dithiobis-(2-nitrobenzoic acid) (DTNB), Folin-Ciocalteu reagent, urea, catechin hydrate, ABTS (≥98.0%), and aminoacetic acid were purchased from Aladdin Reagent Co., Ltd., China. Phosphate-buffer (1 ×) was purchased from Biosharp Reagent Co., Ltd., China. The other chemicals used in this study were analytical grade from Sinopharm Chemical Reagent Co., Ltd. (Beijing, China). High-gluten wheat flour purchased from Jiangsu Nanshun Food Co. Grape seed proanthocyanidin (GSP) (proanthocyanidin content ≥98%) were from Shandong Shengjiade Biotechnology Co.Ltd.,China.

2.2. Preparation of bread, gluten protein and bread extract

The bread was prepared using the straight dough method. The amount of GSP addition was calculated as a percentage of flour (%, w/w of flour). Take the bread without GSP as an example, the ingredients included wheat flour (1156 g), butter (107 g), sugar (115.7 g), salt (19.5 g), yeast (15.1 g), milk powder (48.9 g), and purified water (711 g). All weighed ingredients except butter were put in flour roller (SM2-25, Sinmag, China) at low speed 190 rpm (2.1 min) and high speed 325 rpm (2.0 min) to mix, then added butter into dough and mixed at low speed (2.2 min) followed with high speed (2.5 min) (stage 1), put the dough into fermenting box (28 °C, 80%) for 20 min (stage 2). After that, divided the dough into pieces (250 g/piece), rolled the dough in round and continued to ferment for 20 min (stage 3). Next, rolled out the dough, continued to ferment for 20 min (stage 4) and put the dough into toast mold and fermented (38 °C, 80%) until 9/10 full (stage 5). At last, the dough was baked in preheated oven (SM2-521, Sinmag, China) (top at 190 °C, bottom at 200 °C) for 40 min.

Gluten protein was prepared by the traditional water washing method, in which the dough of different fermentation stages were washed in water, and then the gluten proteins were freeze-dried under vacuum, ground and passed through a 100-mesh sieve. The gluten from stage (1–5) of bread fermentation without GSP were named as W1, W2, W3, W4, W5, and the gluten from the stage (1–5) of bread fermentation with GSP were named as G1, G2, G3, G4, G5, respectively.

Referring to the method given by (Zhao et al., 2021) with few modifications, the bread was first dried and ground, then 2 g of bread powder was added into 50 mL of 80% methanol solution, extracted under shaking water bath for 2 h, and sonicated for 30 min, centrifuged at 1600×*g* for 10 min, and the supernatant was kept as bread extract. In order to compare the contribution of GSP to the antioxidative activity of bread, sample GSP (0.16 mg/mL) solution close to the amount of GSP addition in bread was prepared for antioxidative activity analysis.

2.3. The texture profile analysis (TPA)

Using the previous method (Alvarez-Jubete et al., 2009) with minor modifications, one or two slices of bread with a thickness of 12.5 mm were first excised from one side of the bread sample, and then third slice of bread with a thickness of 25 mm was cut out sequentially as the test sample. The bread texture profile was analyzed using a column probe TA/50 texturizer (TA.XTC-18,Shanghai, China), and the test parameters were set as follows: pre-test speed 1.00 mm/s, test speed 2.00 mm/s, post-test speed 1.67 mm/s, and target deformation 50%.

2.4. Scanning electron microscope (SEM) analysis

The samples of gluten were glued to the sample stage with conductive adhesive, sprayed with gold for 90 s, and the cross sections were observed with a cold-field SEM (Regulus 8230, Hitachi, Japan) at 5.0 KV (Chen et al., 2021). Imaging at magnification × 1.00 K.

2.5. Thermal analysis of gluten (DSC)

The thermal properties of the gluten samples were determined by differential scanning calorimetry (DSC) (Q2000, TA, America) according to reference (Chen et al., 2021). Samples were accurately weighed 3–5 mg, placed in a solid crucible and stored under refrigeration (4 °C) for 24 h. DSC was performed using nitrogen at a flow rate of 50 mL/min from 20 °C to 200 °C at 10 °C/min. Finally, DSC data were analyzed and processed using TA 60 software Muse to obtain denaturation temperature (T_p) and enthalpy (ΔH).

2.6. Total free sulfhydryl (-SH) contents

The total free sulfhydryl content in gluten protein was determined by Ellman's reagent method (Liu et al., 2018). Gluten protein (50 mg) was mixed with 2 mL Tris-glycine buffer (pH 8.0, 0.2 M Tris, 0.2 M glycine, 3 mM EDTA, 1% SDS, 8 M Urea), then were shaken for 1 h at room temperature and centrifuged at 5000×*g* for 10 min. After centrifugation, 50 μL of supernatant, 150 μL of L-tris-glycine buffer and 5 μL of Ellman's reagent were added to a 96-well enzyme plate and the reaction was carried out in the dark for 20 min, and the absorbance was measured at 412 nm by microplate reader (EPOCH2, BioTek Instruments, America). Different concentration gradients of L-cysteine were configured and the absorbance was measured according to the above experimental method to obtain the standard curve, the content of total free sulfhydryl in the sample was calculated by L-cysteine standard curve.

2.7. Fluorescence spectrum analysis

The surface hydrophobicity of gluten proteins was determined using 8-anilino-1-naphthalenesulfonic acid (ANS) fluorescent probe method (Liu et al., 2018). Gluten protein (2 mg) was mixed with 1 mL of 50 mM acetic acid solution, shaked for 1 h at room temperature, and then centrifuged for 10 min at 5000×*g*. The supernatant was diluted 5 times with acetic acid, and 20 μL of ANS (8.0 mM, acetic acid solution) was added to 4 mL of the dilution solution, and the fluorescence intensity was measured at 230 nm (excitation wavelength) and 340 nm (emission wavelength) using a fluorescence spectrophotometer (Perkin LS55, PerkinElmer, America).

2.8. FT-IR spectra and secondary structure analysis

Fourier transform infrared (FTIR) spectroscopy (Nicolet, Thermo Nicolet, America) was used to detect changes of the secondary structure of gluten protein in dough according to the reference (Chen et al., 2021). Briefly, the sample was mixed with potassium bromide in a ratio of 1:

100, then ground and formed into a film. Sixty-four scans were performed in the range of 400–4000 cm^{-1} with a resolution of 4 cm^{-1}. Secondary structures were quantified in Peakfit software based on the amide band at 1600–1700 cm^{-1}.

2.9. Non-covalent interactions of doughs

Chemical interactions were operated using the steps described in the reference (Yang et al., 2023; Li et al., 2021). The sample powder (10 mg) was mixed with 1.0 mL of five reagents: NaCl (PA, 0.05 M), NaCl (PB, 0.6 M), NaCl (0.6 M) + urea (1.5 M) (PC), NaCl (0.6 M) + urea (8 M) (PD) NaCl (0.6 M) + urea (8.0 M) + DTT (50 mM) (PE), while vortex shaking. These reagents could break ionic bonds, hydrogen bonds and hydrophobic interactions. All reagents were prepared in phosphate buffer (0.05 M, pH = 7.5). The samples were fully reacted with the solution at least 2 h and then centrifuged at 5000×*g* for 10 min at 25 °C. 200 μL of the supernatant was added to 1.0 mL of Komas Brilliant Blue G-250 solution (filtered by filter paper) and mixed by vortexing. 200 μL of the mixture was taken and the absorbance was measured at 595 nm using an microplate reader. The soluble wheat gluten protein content was determined using the standard curve of bovine serum protein and expressed as mg/g (soluble protein/sample). The difference in soluble gluten protein content between PB and PA indicated ionic bonds; the difference in soluble gluten gluten protein between PC and PB denoted hydrogen bonds; hydrophobic interactions were expressed as the difference in soluble gluten protein between PD and PC; disulfide bonds was reflected as the difference in soluble gluten protein between PE and PD.

2.10. Total phenolic content assay

Total phenol content (TPC) was determined by traditional colorimetric method (Shang et al., 2022). Briefly, the bread extract (80 μL) was mixed with 20 μL Folin-Ciocalteu reagent and reacted for 5 min protected from light at room temperature, followed by the addition of 100 μL of Na_2CO_3 (20%). After 30 min of color development, the absorbance of the samples at 723 nm was measured. Catechin was used as a standard, and TPC was expressed as catechin equivalent (mg CE/g) per gram of each sample.

2.11. $ABTS^+$ radical scavenging assay

$ABTS^+$ radical scavenging activity was determined based on previous study (Shang et al., 2022). 11.8 mg of potassium persulfate and 13.7 mg of ABTS were dissolved in 100 mL water, stirred magnetically for 10 min and then reacted at room temperature and protected from light for 16 h. The bread extract was diluted twice, then added 10 μL of diluent, 90 μL of water and 100 μL of $ABTS^+$ radical solution in a 96-well plate. Finally, the absorbance of the sample was measured at 732 nm after 5 min of reaction in the dark. The scavenging capacity of the sample was calculated by the following formula:

$$Clearance\ rate\% = 1 - A_s/A_b$$

where A_s is the absorbance of the sample and A_b is the absorbance of control at 732 nm.

2.12. ·DPPH radical scavenging activity assay

The free radical scavenging activity of the bread extract was determined spectrophotometrically by reference (Özcan, 2022) with minor modifications. The method was as follows: 10 μL of sample, 90 μL of ethanol and 100 μL of·DPPH were added in a 96-well plate and reacted for 30 min at room temperature and protected from light, blank group was used as control with ethanol. Finally, the absorbance was measured at 515 nm. The scavenging capacity of the sample was calculated by the following formula:

$$Clearance\ rate\% = 1 - A_s/A_b$$

where A_s is the absorbance of the sample and A_b is the absorbance of control at 515 nm.

Table 1
Texture of dough with different GSP content.

Sample	Hardness/gf	Resilience	Cohesiveness	Gumminess/gf
0.0%	257.54 ± 5.53^a	1.03 ± 0.61^a	0.56 ± 0.20^a	144.53 ± 48.50^a
0.2%	255.71 ± 24.40^a	0.50 ± 0.02^{ab}	0.80 ± 0.01^b	203.43 ± 17.30^b
0.3%	251.58 ± 0.59^b	0.47 ± 0.00^b	0.76 ± 0.00^b	192.10 ± 0.50^b
0.4%	244.45 ± 9.34^a	0.47 ± 0.02^{ab}	0.78 ± 0.02^b	190.57 ± 8.99^{ab}
0.5%	252.98 ± 1.91^a	0.47 ± 0.00^{ab}	0.77 ± 0.01^b	193.60 ± 2.85^b

Note: 0.0%, 0.2%, 0.3%, 0.4%, 0.5%—control bread and bread with 0.2%, 0.3%, 0.4% and 0.5% of GSP, respectively. Data are presented as means with standard deviations (n = 3). Values of each parameter with different superscript letters a and b in the columns are significantly different ($P < 0.05$).

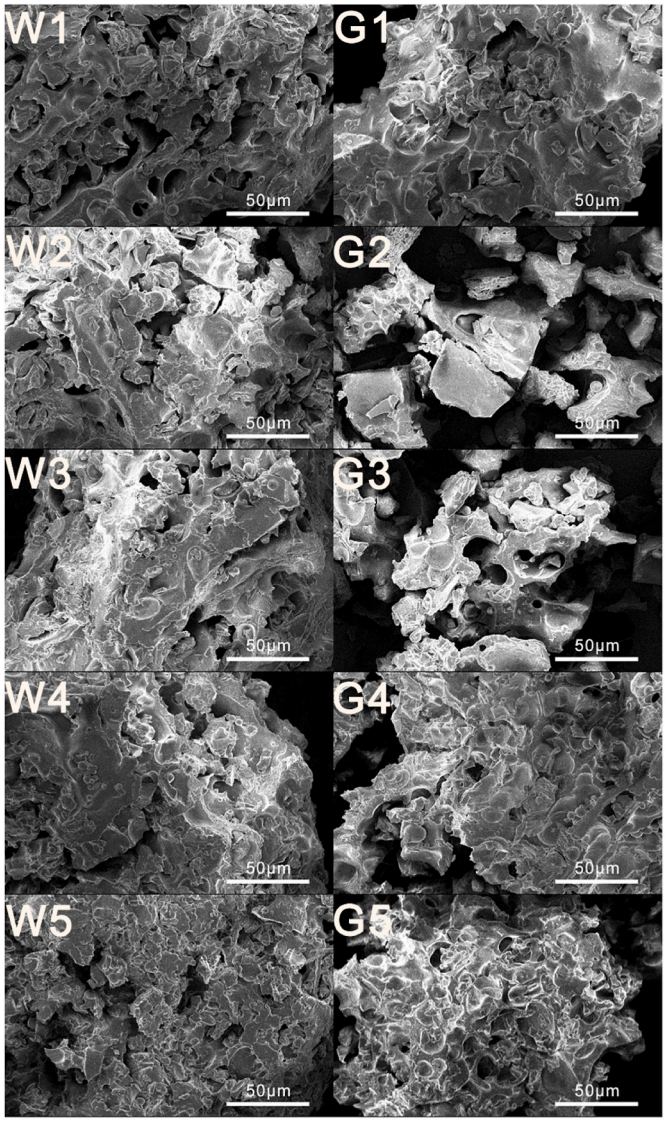

Fig. 1. SEM micrographs of bread in different stages. The stages of bread fermentation without GSP were named as W1,W2,W3,W4,W5, and the stages of bread fermentation with 0.4%GSP were named as G1,G2,G3,G4,G5, respectively.

2.13. Statistical analysis

The results were presented as the mean value ± SD (standard deviation) of three replicates, which were analyzed using one-way analysis of variance (ANOVA) and Duncan's test, with a significant difference ($P < 0.05$).

3. Results and discussion

3.1. The effect of GSP about textural properties on bread

TPA simulates the chewing behavior of food in the mouth to derive objective physical parameters. The textural parameters of the bread after the addition of different levels of GSP were presented in Table 1. The taste of the bread is negatively correlated with its hardness and gumminess. On the contrary, when the cohesiveness and resilience of the bread are higher, the softer and more elastic in the mouth with better quality (Culetu et al., 2018). With the increase of GSP addition, the cohesiveness and gumminess showed a significant increase and then decreased and remained stable, with the final values significantly higher than those in control group, while the hardeness and resilience decreased and remained stable. When GSP was added at 0.4%, the hardness and gumminess of bread were minimal compared to other GSP addition. This might be due to the fact that GSP enhanced the gluten network at lower addition, while excessive amounts (>0.5%) can adversely affect the bread quality. This was in agreement with the results reported in the study of Liu (Liu et al., 2018). Additionally, the millet weight exclusion method was used to determine the change in the volume of bread with the addition of GSP in the range of 0.5%, and no significant difference was found, so no data was presented. When the GSP addition was 0.5%, the bread tasted bitter and astringent. Overall, 0.4% GSP addition was selected for further research.

3.2. Evaluation of dough microstructure

To investigate the effect of GSP on the microscopic network structure of gluten proteins, SEM was used to observe five samples during the fermentation process. The effects of different fermentation stages on the microstructure of the dough were shown in Fig. 1. W1 to W5 showed the microstructure of gluten got more compact during fermentation process. For 0.4% GSP addition gluten, the gluten structure was corrupted during second and third fermentation stages, and resumed again with more compact and denser at the last stage. For gluten, the subunits of glutenin are connected by disulfide bonds and hydrogen bonds to form a three-dimensional network like backbone structure, and the smaller globular gliadin is embedded into the network backbone structure formed by glutenin through non covalent bond (hydrogen bonds and hydrophobic bonds). As the fermentation progressed, the three-dimensional microstructure of both the control group and the 0.4% GSP addition group showed an increase in voids, which might be due to the gradual decrease in disulfide bond content. However, the last stage of fermentation of 0.4% GSP addition group showed continuous gluten network (G5 in Fig. 1), which was consistent with the change in disulfide bond content in the gluten (Fig. 5). As the disulfide bonds in the protein mainly maintain a protein conformation and reinforced the gluten network structure (Wang et al., 2023).

3.3. Effects of fermentation on the thermal properties of gluten proteins

Food components undergo certain changes in their physical and chemical properties during heating, and these changes determine the thermal properties of the food. In DSC, this denaturation temperature (Tp) and enthalpy of denaturation (ΔH) can be determined by the temperature and peak area corresponding to the largest peak, respectively (Wang et al., 2016). The denaturation temperature can reflect the stability of the protein, while the enthalpy change is related to the hydrophobicity, hydrophilicity and degree of aggregation of the protein, which is directly related to the protein denaturation. Table 2 showed the Tp and ΔH values for bread. W samples showed higher SD values, possibly due to experimental operation reasons, but the overall SD value does not exceed 10% of the mean, which still has credibility in statistics. For white bread, there was no significant change in Tp during fermentation, but there was a significant change in enthalpy change (Table 2). In the later stage of fermentation, the enthalpy significantly increased with the stability of the protein reached its optimal state. However, the enthalpy change of bread with GSP gradually decreased with fermentation, and the trend of change was consistent with Tp. This result indicated that GSP reduced the stability of bread gluten protein, Although the hydrophobicity between polyphenols and gluten protein increased after the addition of GSP (Fig. 5), the interaction between polyphenols and gluten protein affected the aggregation between gluten and the network structure of gluten protein, leading to a decrease in thermal stability of gluten protein. This was consistent with the previous study, due to excessive addition of grape seed power, macromolecular was formed between polyphenols and proteins to hinder the formation of gluten networks and leading to a decrease in the thermal stability of gluten protein (Chen et al., 2021).

Table 2
Tp and ΔH in different fermentation stages of white dough and GSP dough.

Sample	Tp (°C)	ΔH (J/g)
W1	130.68 ± 0.76^{a}	47.12 ± 4.01^{a}
W2	134.09 ± 0.26^{a}	47.88 ± 4.61^{a}
W3	128.39 ± 0.45^{a}	75.40 ± 0.44^{b}
W4	130.17 ± 1.03^{a}	42.89 ± 0.57^{a}
W5	137.09 ± 0.31^{a}	79.15 ± 2.87^{b}
G1	96.83 ± 0.56^{A}	56.76 ± 0.56^{A}
G2	94.82 ± 0.70^{B}	53.95 ± 0.71^{B}
G3	93.19 ± 0.19^{C}	50.16 ± 0.70^{C}
G4	91.07 ± 0.11^{D}	44.53 ± 0.93^{D}
G5	91.87 ± 0.44^{D}	45.15 ± 0.44^{D}

Note: The stages of bread fermentation without GSP were named as W1, W2, W3, W4, W5. The stages of bread fermentation with GSP were named as G1, G2, G3, G4, G5, respectively. Data are presented as means with standard deviations (n = 3). Values of each parameter with different superscript letters a, b, A, B, C, D in the columns are significantly different ($P < 0.05$).

3.4. Free sulfhydryl content and surface hydrophobicity

Wheat gluten protein is a polymer formed by the interaction of disulfide bonds between discontinuous polypeptide molecules, while alcoholic protein is the result of the bonds of disulfide bonds within hydrophobic polypeptide molecules (Chen et al., 2021). The presence of disulfide bonds facilitates the formation of the gluten protein network structure, and its reduced content in the dough affects the formation of the gluten protein network structure and ultimately weakens the quality of the dough product. The free sulfhydryl content of white bread and GSP bread at different fermentation stages was shown in Fig. 2 white bread showed an increase and then decrease with fermentation time, and the free sulfhydryl significantly increased at the last fermentation stage, indicating a decrease in gluten strength, which was consistent with the SEM results. Compared with the white bread group, the free sulfhydryl content of the GSP addition significantly increased, and increased from 0.89 μmol/dough to 2.55 μmol/dough in the first stage of fermentation, due to the fact that GSP contains phenolic groups, which are highly reductive, they can break the disulfide bonds in the dough thereby reducing the gluten protein crosslink strength (Wang et al., 2015). However, in the final stage of fermentation, the number of free sulfhydryl groups significantly changes, which is significantly lower than other stages of fermentation and white bread,and perhaps oxidation of GSPs to quinones promoting the formation of gluten network through sulfhydryl/disulfide exchange (Fujimoto and Masuda, 2012). In agreement with the results obtained from TPA data, namely that the

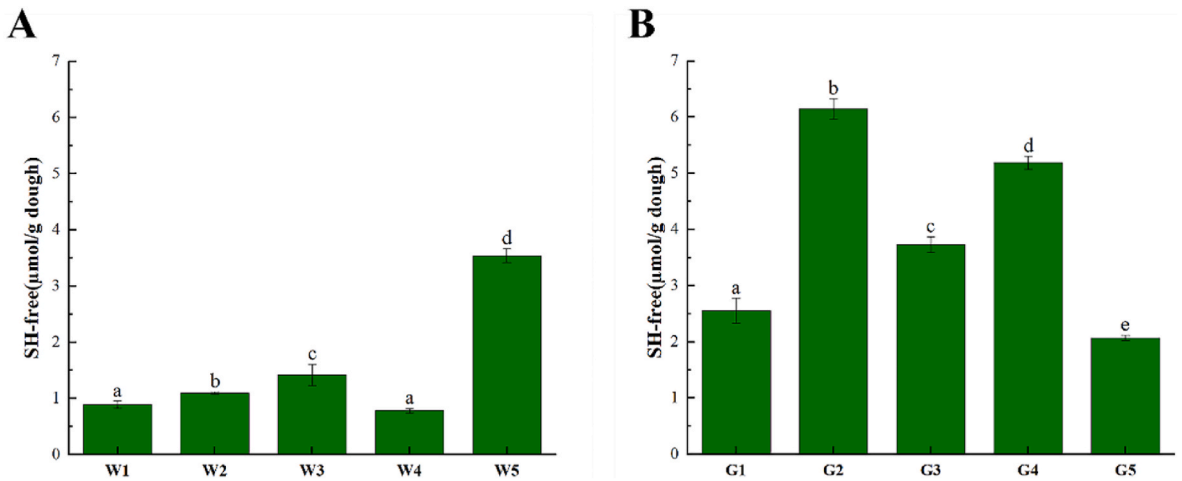

Fig. 2. Free sulfhydryl (SH) contents of different stages about about white dough (A) and GSP dough (B). The stages of bread fermentation without GSP were named as W1,W2,W3,W4,W5, and the stages of bread fermentation with 0.4% GSP were named as G1,G2,G3,G4,G5, respectively. a, b, c, d indicates a significant difference ($P < 0.05$).

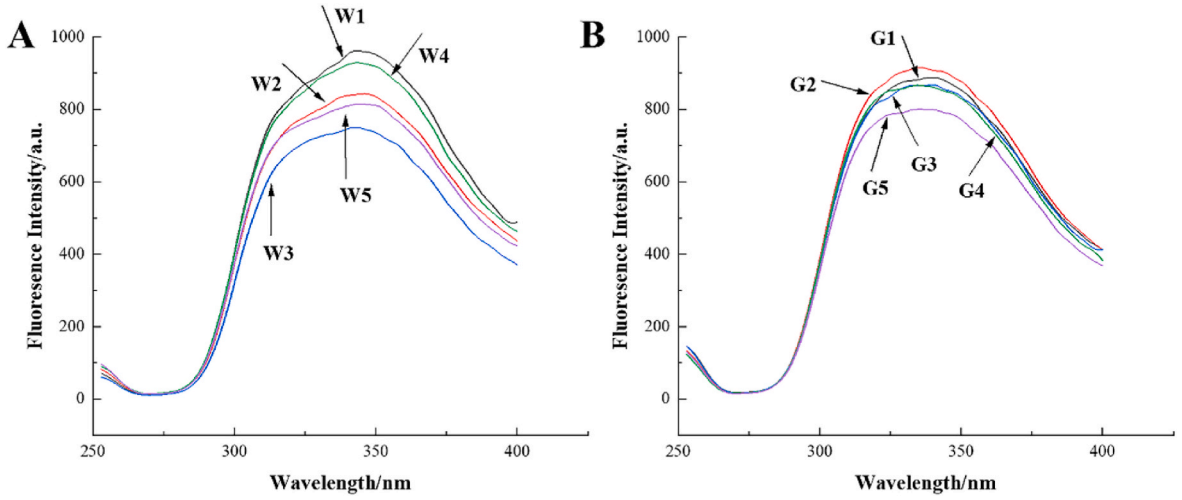

Fig. 3. Extrinsic emission fluorescence spectrum of different stages about white dough (A) and GSP dough (B). The stages of bread fermentation without GSP were named as W1,W2,W3,W4,W5, and the stages of bread fermentation with 0.4% GSP were named as G1,G2,G3,G4,G5, respectively. a, b, c, d indicates a significant difference ($P < 0.05$).

gluten strength in the sample with 0.4% addition was higher than in the white bread after fermentation. The total free sulfhydryl content of W5 was 3.54 μmol/dough higher than 2.07 μmol/dough in G5 also supports the above conclusion.

Meanwhile, Girard reported that GSP can promote cross-linking of gluten proteins through hydrogen bonds and hydrophobic interactions to increase the density of gluten networks (Girard and Awika, 2020). The number of hydrophobic groups available on the surface of a protein can be determined by the hydrophobicity of the surface. ANS is a fluorescent dye with high affinity for the hydrophobic surface of proteins and binds to the less polar regions of the protein surface with increased fluorescence intensity. If proteins aggregate with each other through hydrophobic interactions and reduce ANS binding sites, this leads to a decrease in surface hydrophobicity. The fact that the hydrophobic interaction increased and then decreased in white bread and that the hydrophobic interaction did not differ significantly in the first four fermentation stages and increased significantly at the last stage of GSP bread indicated that GSP addition can change the gluten hydrophobic interaction (Fig. 3), which was similar to the study of Tian (Tian et al., 2021), where catechin monomers enhanced the hydrophobic interaction in the gluten system. It was presumed that GSP and proteins converge into protein aggregates through newly formed hydrophobic bonds.

3.5. Fourier transform infrared (FTIR) spectroscopy characterization

The secondary structure of gluten proteins measured by FTIR. A set of amide absorption bands can reflect the secondary, namely amide I-VII, amide A and B. The amide I band (1700-1600 cm^{-1}) is the most valuable for research, whose absorption peaks are mainly C═O vibrations and a small amount of N–H in-plane bending (Yuan et al., 2021). 1650-1660 cm^{-1} range of peaks corresponds to α-helix, 1660-1700 cm^{-1} and 1610-1640 cm^{-1} represent β-turn and β-sheet, respectively, and 1640-1650 cm^{-1} indicates random coil (Huang et al., 2018). The characteristic bands of FTIR spectra of the gluten protein amide I were given in Fig. 4. Among the gluten protein secondary structures, β-sheet was the highest, followed by β-turn, α-helix and random coil. During the fermentation of white bread, the content of different structures was not significant ($P < 0.05$), although they were different. In contrast, in GSP bread, there was a significant difference ($P < 0.05$) between G1 and G5 for α-helix and random coil, which increased from 15.58% to 20.37% and 14.53%–11.21%, respectively. α-Helix is a stable protein secondary structure formed by hydrogen bonding, which brings rigidity and

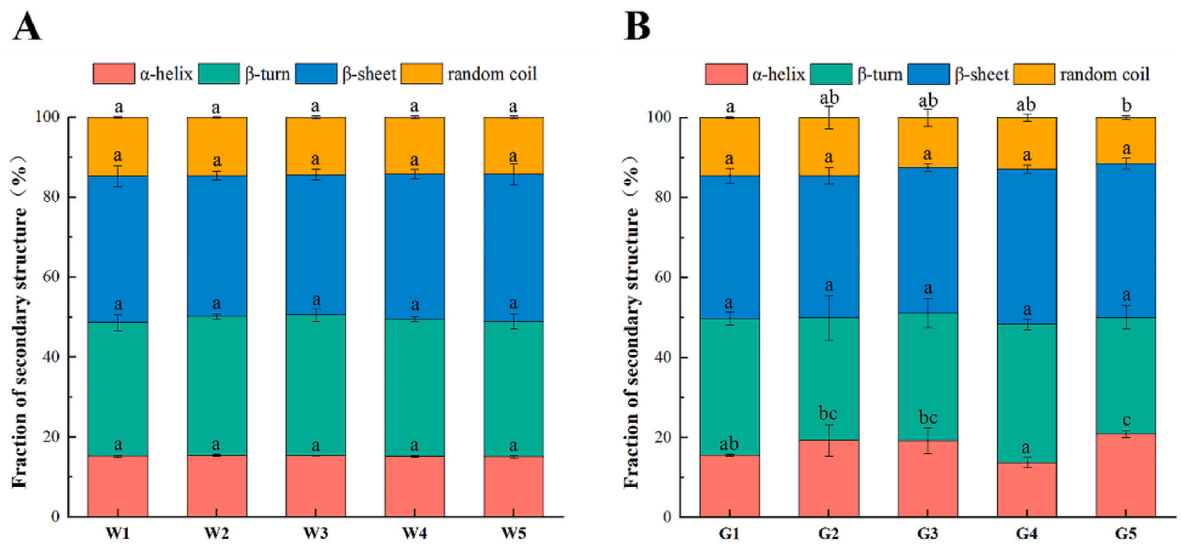

Fig. 4. Effects of GSP on α-helix, β-sheet, β-turn, and random coil secondary structure contents of protein in white dough (A) and GSP dough (B); The stages of bread fermentation without GSP were named as W1,W2,W3,W4,W5, and the stages of bread fermentation with 0.4% GSP were named as G1,G2,G3,G4,G5, respectively. a, b, c, d indicates a significant difference ($P < 0.05$).

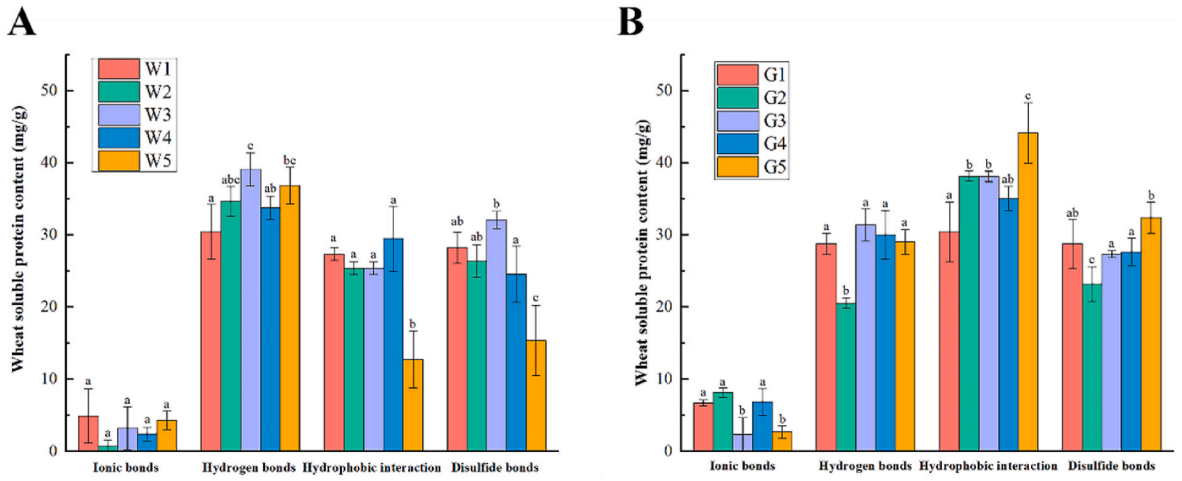

Fig. 5. Contributions of chemical interactions of doughs about white dough (A) and GSP dough (B). The stages of bread fermentation without GSP were named as W1,W2,W3,W4,W5, and the stages of bread fermentation with 0.4% GSP were named as G1,G2,G3,G4,G5, respectively. a, b, c, d indicates a significant difference ($P < 0.05$).

elasticity to the protein and is closely related to the hardness and elasticity of the dough, α-Helix also participates in the formation of hydrophobic interactions. In GSP bread, the change trend of α-helix in the fermentation process was consistent with the change of hydrophobic interaction, while the change of hydrogen bond in the fermentation process was not obvious, indicating that after the addition of GSP, hydrophobic interaction played a leading role in the change of the protein secondary structure of gluten protein, which was consistent with the research structure of Meng et al., the extension of fermentation could strengthen the hydrophobic interaction, enhancing the non-covalent crosslinking of oligomeric procyanidins with gluten proteins (Meng et al., 2022).

3.6. Analysis of chemical interactions in wheat flour dough

Using the principle that specific chemical reagents can be directed to break specific chemical bonds of proteins, the change in solubility of proteins after the addition of GSP can be studied with different denaturing agents as a way to infer the change in covalent and non-covalent forces. It was shown that hydrophobic interactions contributed most in GSP bread, while hydrogen bonds contributed most in white bread. The ionic bonds force became stronger with the addition of GSP (Fig. 5). Hydrogen bonds is important for maintaining protein secondary structure, and intramolecular hydrogen bonds is important for structural firmness of globular proteins. The addition of GSP resulted in a weaker hydrogen bonds force but an enhanced hydrophobic force, which was consistent with the fluorescence results. The previous study introduced that gluten had strong hydrophobic and hydrogen bonds interactions with polyphenols (Girard and Awika, 2020), and it was possible that the addition of GSP affected the hydrophobic interaction more significantly. During the fermentation of white bread, it can be seen that the degree of contribution of hydrogen bonds, hydrophobic interaction and disulfide bonds did not differ much compared to the beginning, but as the fermentation proceeded, the role of hydrogen bonds gradually became important and the percentage of both hydrophobic and disulfide bonds decreased to a great extent, which was consistent with the previous study.

Table 3
Total phenolics content and antioxidant capacity.

	$ABTS^+$ radical scavenging activity	·DPPH radical scavenging activity	TPC (mg CE/g)
White bread	39.199% ± 0.045[a]	1.784% ± 0.024[a]	0.682 ± 0.220[a]
GSP bread	52.003% ± 0.017[b]	10.241% ± 0.002[b]	1.013 ± 0.287[a]
GSP	75.412% ± 0.025[c]	33.366% ± 0.023[c]	455.820 ± 45.982[b]

Note: a, b, c, d indicates a significant difference ($P < 0.05$). White bread is the bread without GSP, GSP bread is the bread with 0.4% GSP added. $ABTS^+$and·DPPH radical scavenging activity is expressed as clearance rate. TPC is expressed as catechin equivalent (mg CE/g) per gram of each sample.

3.7. Total phenolics content and antioxidant capacity

The TPC and antioxidative capacity of white bread, GSP bread, GSP can be derived from Table 3. The intake of certain amount of polyphenols can reduce the harm of free radicals to some extent and delay the occurrence of many chronic diseases (Silva et al., 2019). As it can be seen in the results, the bread without GSP also contained some polyphenols and had antioxidant activity. Both TPC and antioxidative capacity were significantly increased in bread with GSP addition compared to white bread ($P < 0.05$). GSP solution showed strong antixidative acvtivity, adding an almost equal amount of GSP to bread can to some extent enhance the antioxidant activity of bread. GSP bread had lower TPC and antioxidative capacity than GSP solution, which may be due to the oxidation of polyphenols during the baking process (Peng et al., 2010). In conclusion, the addition of GSP was beneficial in increasing the antioxidant capacity of the bread and enhancing the functional properties of the finished bread (Özcan, 2022).

4. Conclusion

In summary, the addition of a small amount of GSP could improve the degree of gluten protein cross-linking. The addition of GSP increased the content of free sulfhydryl groups in gluten proteins, indicating that GSP could break the disulfide bonds in proteins and enhance hydrophobic interactions at the same time, and the most significant effect was observed at the end of the fermentation process. The secondary structure of the proteins indicated that the fermentation process could make the GSP dough more orderly, while there was no significant difference in the white bread during fermentation ($P > 0.05$). The results of dough intermolecular interactions indicated that the fermentation time had a significant effect on the covalent and non-covalent forces between the doughs. The impact of GSP on the fermentation process and quality of bread deserves in-depth research.

CRediT authorship contribution statement

Tong Jiang: Conceptualization, Formal analysis, Software, Validation. **Hong Wang:** Formal analysis, Methodology, Software, Validation. **Peihua Xu:** Software. **Yifan Yao:** Software, Validation. **Yilong Ma:** Software, Validation. **Zhaojun Wei:** Methodology, Writing – review & editing, Data curation. **Xiangli Niu:** Software, Validation. **Yafang Shang:** Conceptualization, Resources, Supervision, Writing – review & editing, Project administration. **Dong Zhao:** Methodology, Writing – review & editing, Data curation.

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

Data will be made available on request.

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