LWT - Food Science and Technology 163 (2022) 113591

Contents lists available at ScienceDirect

LWT

journal homepage: www.elsevier.com/locate/lwt

Controlling starch surface characteristics - Impact on dough formation in a reconstituted dough system

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ARTICLE INFO

Keywords:
Reconstituted dough
CLSM
Dough structure
Water distribution

ABSTRACT

The effects of starch granule-surface proteins (SGSPs) on dough formation and breadmaking quality were studied in a reconstituted dough. After removing the SGSPs by NaOH and SDS treatment, the dough development time, stability time, and resistance to extension were increased. CLSM exhibited a coarse and discontinuous gluten network in the dough containing NaOH- and SDS-treated starches. Meanwhile, gelatinization temperatures were shifted to higher values while the gelatinization enthalpy decreased. Removing SGSPs prevented the formation of gluten network and reduced dough extensional deformability, which showed that the dough structure became worse. Compared with the control group, the firmness of the bread sample without SGSPs was significantly increased ($p < 0.05$), which was manifested by small and dense pores in the bread. LF-NMR showed that the distribution of relative water content shifted from T_{21} (0–1 ms) to T_{22} (1–100 ms), indicating that removing SGSPs decreased the water-holding capacity of the bread.

This study suggested that starch surface characteristics were essential to maintain the structure and mechanical properties of dough and bread.

1. Introduction

Starch, the major component in wheat flour (about 700–800 g/kg), plays a crucial role in the transformation of dough to bread. When subjected to water hydration and mechanical mixing, gluten proteins are hydrated and become interconnected, forming a visco-elastic gluten network with the ability to retain gas during fermentation and baking. Simultaneously, the starch granules absorb water by up to about 46% and are entrapped in the three-dimensional matrix of proteins, acting as an inert filler (Hu et al., 2021; Jekle, Mühlberger, & Becker, 2016). The interaction of the "starch-gluten" matrix is related to the strength and extensibility of dough, which is very important to the texture of bread (Miyazaki, Van Hung, Maeda, & Morita, 2006).

Starch granule-surface proteins (SGSPs) are residues with molecular weights of 15 kDa, 30 kDa, and 60 kDa, which adhere to the granule surface (Bae et al., 2020). It contains high quantities of hydrophobic amino acids (e.g. tryptophan) and basic amino acids (e.g. lysine), resulting in a strong binding ability with starch (Baldwin, 2001). Sodium hydroxide (NaOH) and sodium dodecyl sulfate (SDS) are the effective extraction agents of SGSPs while the internal proteins are hardly extracted from the ungelatinized starch granules (Tao et al., 2021; Wang et al., 2014). NaOH leads to the exposure of hydrogen bonds and the dissociation of polar groups in SGSPs, thereby facilitating an intramolecular dissolution and separating SGSPs from starch granules. SDS destroys the non-covalent bonds between starch molecules and develops an SDS-protein complex, so SGSPs are separated from the whole granules (Bae et al., 2020). When SGSPs are extracted by these agents, the contact between starch granules and water increases, resulting in rapid swelling of the starch paste at gelatinization temperature (Wang et al., 2014). It accelerated the starch recrystallization that was manifested by an increase in retrogradation enthalpy and storage firmness (Tao et al., 2021). Meanwhile, SGSPs have an important function of supporting gas cells during the fermentation process. They develop continuous two-dimensional membranes at the interface between gluten matrix and starch, determining the dough extensionality (Pauly, Pareyt, Lambrecht, Fierens, & Delcour, 2013). Ryan and Brewer (2006) reported that biscuits without SGSPs showed a harder texture than the regular group.

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https://doi.org/10.1016/j.lwt.2022.113591
Received 9 January 2022; Received in revised form 15 May 2022; Accepted 21 May 2022
Available online 25 May 2022
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Abbreviations

NWS	native wheat starch
NaOH	sodium hydroxide
SDS	sodium dodecyl sulfate
DDT	dough development time
DST	dough stability time
CLSM	confocal laser scanning microscopy
LF-NMR	low field nuclear magnetic resonance
FITC	fluorescein 5-isothiocyanate
T_o	onset gelatinization temperature
T_p	peak gelatinization temperature
T_c	conclusion gelatinization temperature
ΔH	gelatinization enthalpy

Edwards, Dexter, and Scanlon (2002) showed the importance of starch surface to the dough rheology by adding glass powders instead of starch granules. They found that the linear viscoelasticity of the dough got weakened, indicating a decreased adhesion in the interface between gluten matrix and fillers (starch and glass powder). It suggested that SGSPs functioned as lubricants in dough which increased the strain hardening rate of the dough by promoting the interactions between starch and gluten (Rouille', Valle, Lefebvre, Sliwinski, & vanVliet, 2005). However, the impact of starch surface characteristics on dough functions and final product properties has not been fully understood due to the intricate multidimensional structure of dough and the complex chemical, biochemical and physical transformation processes that take place during bread-making.

In this study, a simple dough system, reconstituting with fractions of the same flour such as starch and gluten, was applied to explore the contribution of starch surface functionality to dough rheological properties and final bread quality. This study will provide new insights for understanding the mechanism of starch-gluten interactions and is of great value to enhance the dough structure and related products.

2. Materials and methods

2.1. Materials

Native wheat starch (NWS) and gluten were obtained from Ante Food Co., Ltd. (Suzhou, Anhui, China). CBQCA (3-(4-carboxybenzoyl) quinolone-2-carboxaldehyde) Protein Quantification Kit (C6667) was purchased from Merck, USA. Rhodamin B and Fluorescein 5-isothiocyanate (FITC) were purchased from Yuanye Bio-Technology Co., Ltd (Shanghai, China). NaOH, SDS, KCN, sodium borate buffer, and phosphate-buffered saline (PBS) were provided by Sinopharm Chemical Reagent Co., Ltd. (Suzhou, China) which were analytical grade. Instant dry yeast (Angel Yeast Co., Ltd., Hubei, China), soft sugar (COFCO Fulinmen Co., Ltd. Beijing, China), and refined edible salt (China National Salt Industry Corporation, Beijing, China) were bought from a local market (Yong hui Superstore, Hefei, China).

2.2. Removal of SGSPs

The removal treatment was prepared according to Wang et al. (2014) and Tao et al. (2021) about NaOH and SDS methods, respectively. NWS (5 g) was added to 25 mL of 2 g/L NaOH solution and then mixed at 30 °C for 30 min. The mixture was centrifuged at *3000* × *g* for 20 min while the sediment was washed with distilled water 5 times, and centrifuged (*3000* × *g* for 20 min). The precipitates were dried in an oven at 37 °C for 24 h and passed through a sieve with a mesh size of 0.15 mm. The obtained starch powder was stored in a desiccator for the following analysis. In addition, SDS-treated starch samples were prepared in the same procedure as NaOH-treated starch, with 12 g/L SDS solution instead of NaOH solution.

2.3. Farinograph measurements

The dough mixing properties were investigated by Brabender Farinograph-E (Brabender, Duisburg, Germany) according to the AACC 54–21 (2002). The wheat starch-gluten reconstituted flour with a dry weight ratio of 86:14 was premixed in a 300 g mixing bowl of farinograph for about 2 min. An appropriate amount of water was continuously added until the farinograph unit (FU) arrived at the 500 ± 20 FU line. During the entire mixing process, the mixing bowl was operated at 63 rpm and 30 °C. To evaluate the role of SGSPs, the starch fraction in the reconstituted blends was individually replaced by the NaOH- and SDS-treated starch. The farinograph parameters were recorded as water absorption (%), dough development time (DDT, min), and dough stability time (DST, min).

2.4. Extensograph measurements

The reconstituted dough from Brabender Farinograph-E (8 min for mixing) was cut into two parts of 150 g each. After 45 min resting in a fermentation cabinet at 30 °C, the dough was stretched by Brabender Extensograph-E (Brabender, Duisburg, Germany), following the AACC 54-10 method (2002). The extensograph parameters such as area (cm^2), extensibility (E, mm), resistance to extension (R, BU), and ratio figure (R/E) were recorded.

2.5. Confocal laser scanning microscopy

The distribution of SGSPs in native and treated starch granules were stained with CBQCA Protein Quantification Kit according to Han, Benmoussa, Gray, BeMiller, and Hamaker (2005), with some modifications. Starch (14 mg) was incubated with CBQCA (20 mmol/L, 10 μL), sodium borate buffer (0.1 mol/L, 470 μL, pH 9.3), and 20 mmol/L KCN (20 μL) in a refrigerator at 4 °C for 10 h. Then, the dyed sample (2 μL) was placed on a microscope slide for their observation. A confocal laser scanning microscope (FV1000, Olympus, Tokyo, Japan) was operated in fluorescence mode with an Olympus inverted microscope (40 x, NA = 0.95). An argon laser at 488 nm was used to excite CBQCA-dyed SGSPs.

The reconstituted dough was prepared as described in Section 2.4, and then freeze-dried in a vacuum freeze-dryer (FD-1A-50, Biocool, Beijing, China). Dough samples were stained with a dye solution containing 0.2 g/L Rhodamine B and 1.5 g/L FITC in deionized water. The mixture was washed with 0.1 mol/L (pH 7.2) PBS buffer 3 times. Then, the participants were incubated at room temperature overnight in the dark before being subjected to CLSM observation (F. Jia et al., 2019; Mariotti, Lucisano, Ambrogina Pagani, & Ng, 2009). Starch and gluten stained with FITC and Rhodamine B were observed at excitation wavelengths of 488 and 543 nm, respectively.

2.6. Thermal properties

The gelatinization properties of freeze-dried dough were analyzed with a Q2000 DSC (TA Instruments, New Castle, USA). The freeze-dried dough was crushed and passed through a 100-mesh sieve. The sample was accurately weighed to 3.0 mg and placed in a measuring aluminum pan, and then 6.0 of deionized water was added, which was sealed and equilibrated at 4 °C overnight. The pans were heated from 25 to 90 °C at a rate of 10 °C/min, and an empty pan was used as a reference. The corresponding parameters were recorded as onset (T_o), peak (T_p), conclusion temperatures (T_c), and enthalpy (ΔH) of gelatinization.

2.7. Bread making and analysis

The bread was made as described by Tao et al. (2016) with some modifications. Referring to farinograph experimental conditions, all fractions (300 g reconstituted flours + 2 g yeast+ 0.5 g sugar) were blent in the mixing bowl for 2 min and then 180 mL of water was added to develop dough (8 min for mixing). Afterwards, the resulting dough was molded into 60 g batters and fermented at 37 °C with 80% relative humidity for 90 min before the baking procedure (15 min, 210 °C). After cooling at room temperature for 2 h, a slice of bread 15 mm thick was cut into cubical pieces (30 × 30 × 15 mm) from the center portion of the bread. The bread sample firmness was evaluated using a TA-XT plus texture analyzer (Stable Micro Systems, Godalming, UK) with a probe P100 mm in diameter (Chen et al., 2021; Zhu, Xiong, Wang, & Ju, 2020). The detected conditions were set as follows: 50% strains, 3.00 mm/s of pre-test speed, 1.00 mm/s of test speed, and 5.00 mm/s of post-test speed.

2.8. Low field nuclear magnetic resonance (LF-NMR)

The spin-spin relaxation properties of bread were determined by NMI20-Analyst LF-NMR (Niumag Analytical Instrument Corporation, Suzhou, China) with a Carr-Purcell-Meiboom-Gill (CPMG) pulse sequence. Samples (0.7 g) were taken from the center of the bread and placed in a 10-mm NMR tube which was sealed with parafilm to prevent moisture loss (Zhu et al., 2020). The duration between successive scans was set at 800 ms while the echo time was 0.300 ms. For the CPMG sequence, the echoes were 2500.

2.9. Statistical analysis

Statistical analysis was performed using SPSS 16.0 Software (SPSS Inc., Chicago, USA). All samples were tested at least in triplicate. A one-way analysis of variance (ANOVA) was conducted on the collected data, and the significance between treatments was analyzed using Tukey's multiple range test at $p < 0.05$.

3. Results and discussion

3.1. Distribution of SGSPs

Fig. 1 showed the CLSM images of native and treated starch granules where the surface proteins were stained by CBQCA at excitation wavelengths of 488 nm. CBQCA, a protein-specific fluorescent probe fluorescent dye, only highlights the surface and empty space on starch granules (Kim & Huber, 2008). All NWS granules were stained with CBQCA dye solution to verify the distribution of SGSPs while the highlighted green fluorescence was no longer found on the surface of the starch granules after NaOH and SDS treatments (A2, B2, and C2). This indicated that NaOH and SDS treatment predominantly eliminated the surface proteins of wheat starch granules, as reported by Bae et al. (2020) and Q. Zhan et al. (2020) who found disappeared discrete points on the starch surface.

3.2. Farinograph properties of the dough

The farinograph curves and corresponding parameters of wheat dough were presented in Fig. 2 and Table 1, respectively. The water absorption of dough remained unchanged after removing SGSPs which was similar to the report by S. Jia et al. (2021). Low concentration (< 2 g/L) of alkaline and surfactant agents have no significant change in the amylose content of wheat starch, indicating that amylose molecules do not undergo leaching or degradation (Wang et al., 2014). Thus, during the mechanical stirring, there were no differences in swelling behavior between native and treated starch samples. However, the addition of NaOH- and SDS-treated starches prolonged the DDT and DST. DDT represents the tightness of gluten network and the strength of dough, while DST is defined as the tolerance of dough to mechanical stirring (Mehfooz, Mohsin Ali, Arif, & Hasnain, 2018). The increased DDT suggested that removing SGSPs inferred a reduction in the degree of interactions between starch and gluten matrix. SGSPs contain high

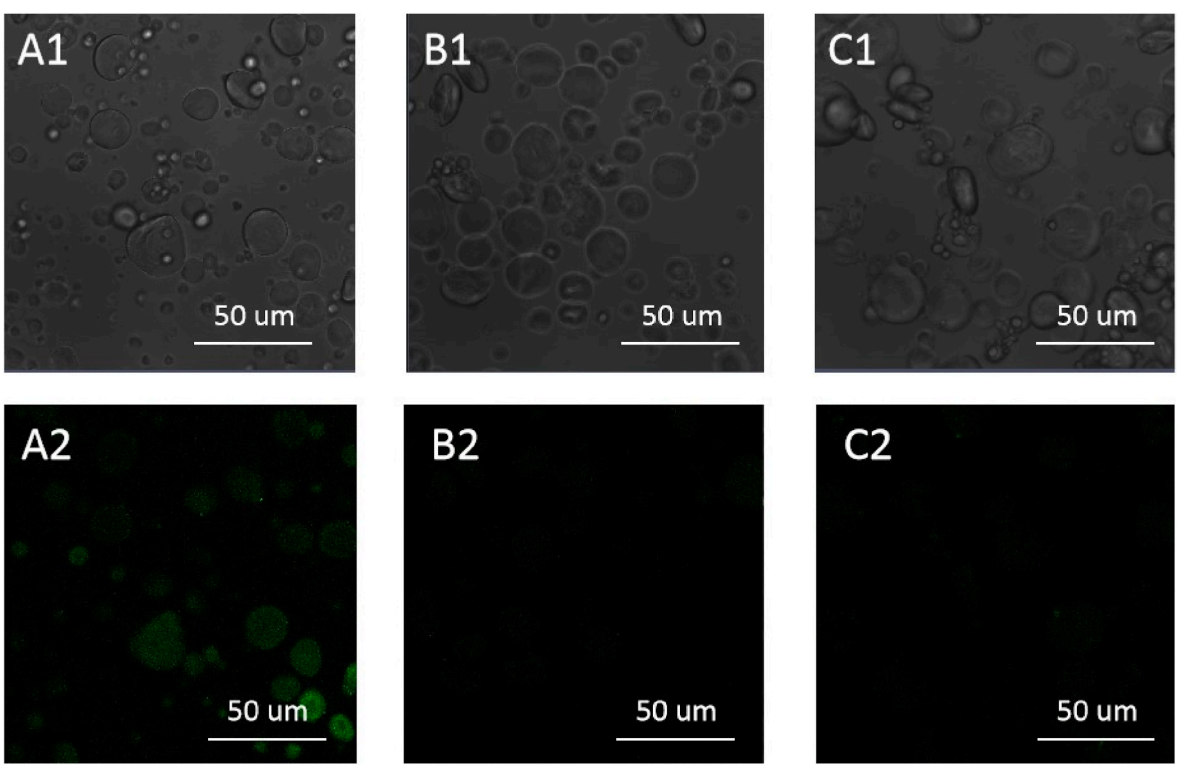

Fig. 1. The distribution of starch granule-surface proteins (SGSPs) of (A) NWS, (B) NaOH-treated starches and (C) SDS-treated starches was observed by CLSM. 1, 2 represent bright and fluorescence fields, respectively.
(NWS, native wheat starch; NaOH-treated starch and SDS-treated starch, wheat starch after removal of granule-surface proteins).

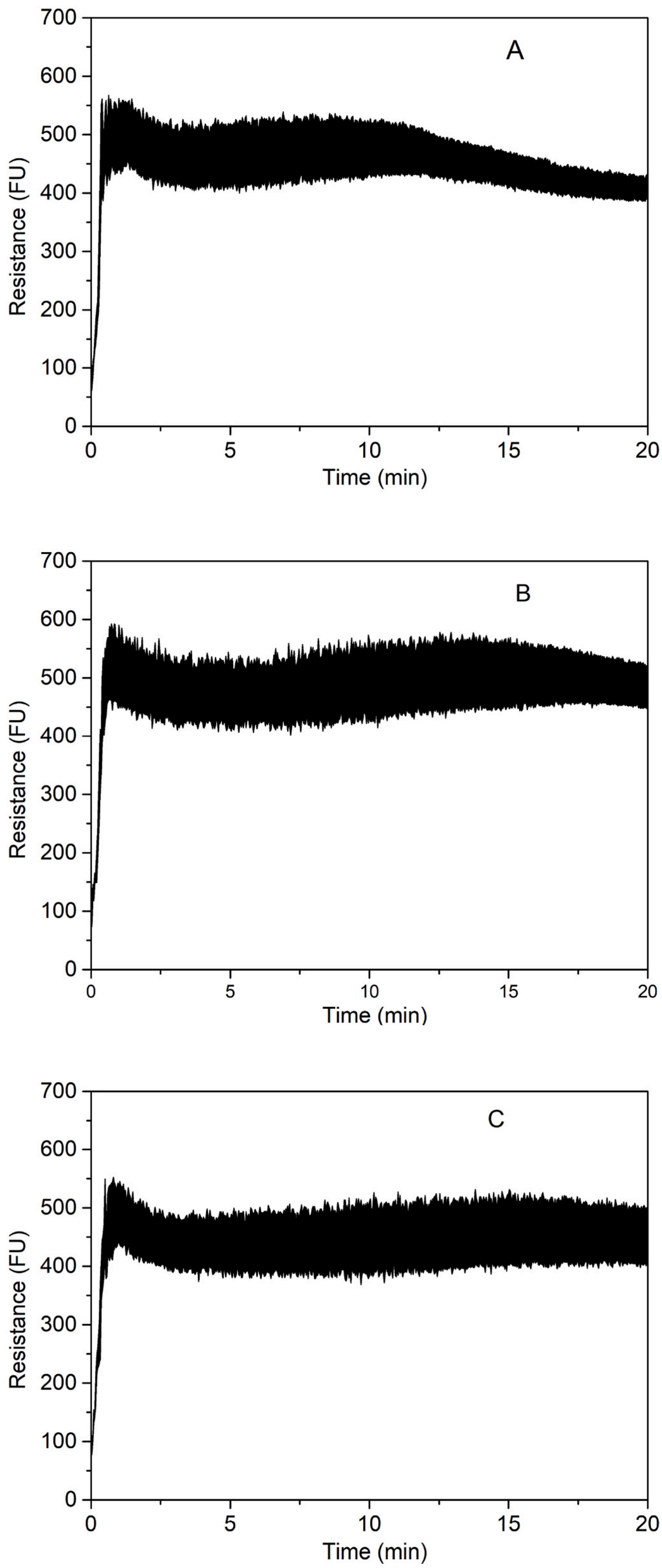

Fig. 2. Effect of removing starch granule-surface proteins (SGSPs) on farinograph curve of reconstituted dough. (A, reconstituted dough with native wheat starch; B, reconstituted dough with wheat starch after removing granule-surface proteins with NaOH; C, reconstituted dough with wheat starch after removing granule-surface proteins with SDS).

Table 1
The effect of starch granule-surface proteins (SGSPs) on farinograph parameters of reconstituted dough.

Samples	Water absorption (%)	DDT (min)	DST (min)
NWS	60.3 ± 0.2 [a]	8.5 ± 0.3 [b]	13.3 ± 1.3 [c]
NaOH-treated starch	59.7 ± 0.1 [a]	13.3 ± 0.1 [a]	19.2 ± 0.4 [b]
SDS-treated starch	60.1 ± 0.1 [a]	13.7 ± 0.1 [a]	23.0 ± 0.1 [a]

NWS, native wheat starch; NaOH-treated starch and SDS-treated starch, wheat starch after removal of granule-surface proteins; DDT, dough development time; DST, dough stability time.
Means in the same column with different superscript lowercase letters indicate significant differences between different samples ($P < 0.05$).
All values are expressed as mean ± standard deviation ($n = 3$).

quantities of hydrophobic amino acids (e.g. tryptophan) and basic amino acids (e.g. lysine), promoting the strong hydrophobic interactions with the gluten network and strengthening the double helix structure of the “starch-gluten” matrix (X. Gao et al., 2020). Therefore, the removal of SGSPs decreased the affinity of starch to gluten network and required a longer time for dough formation. However, during the continuous mixing process, removing SGSPs resulted in higher stability (DST) which indicated the loss of extensibility. Correspondingly, the decrease of extensibility was also characterized by longer development time (DDT) and stability (DST) (Rosell, Rojas, & Benedito de Barber, 2001).

3.3. *Extensograph properties of the dough*

The extensograph properties of dough were shown in Fig. 3 and Table 2. After removing SGSPs, the area under the extensograph curve increased, indicating more energy required for the destruction of the dough structure (Zhang, Guo, Shi, & Ren, 2020). The extension resistance increased while the extensibility decreased, which manifested as a significant increase ($p < 0.05$) in the ratio figure. As the contact point between starch granules and gluten protein, the surface functionality of starch granules can determine their interaction, thus significantly affecting the viscoelasticity of dough (Brandner, Becker, & Jekle, 2019). In our study, removing SGSPs could reduce the adhesion of starch to the gluten network, making the dough not prone to extensional deformation and requiring greater stretching force. Furthermore, the removal of SGSPs could promote the internal friction between the treated starch granules which hindered the development of fine protein fibers from

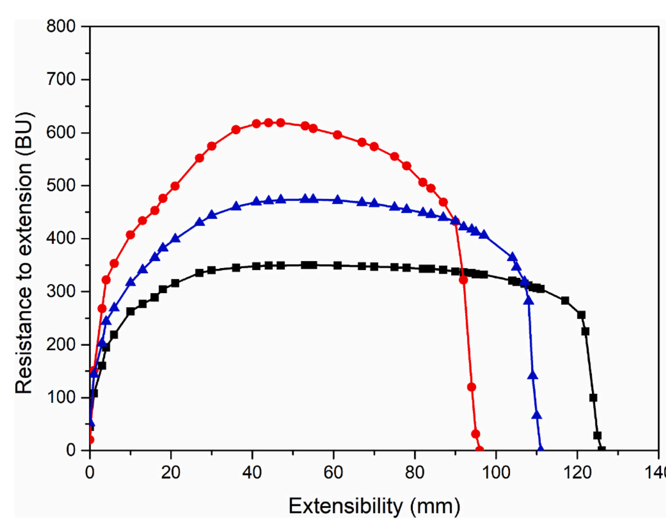

Fig. 3. Effect of removing starch granule-surface proteins (SGSPs) on extensograph curve of reconstituted dough. (—■—, reconstituted dough with native wheat starch; —●—, reconstituted dough with wheat starch after removing granule-surface proteins with NaOH; —▲—, reconstituted dough with wheat starch after removing granule-surface proteins with SDS).

Table 2
The influence of starch granule-surface proteins (SGSPs) on extensograph parameters of reconstituted dough.

Samples	Extensograph parameters			
	Area (cm^2)	Resistance to extension (BU)	Extensibility (mm)	Ratio figure (R/E)
NWS	68 ± 6 ^{c}	356 ± 23 ^{c}	123 ± 4 ^{a}	2.9 ± 0.1 ^{c}
NaOH-treated starch	86 ± 1 ^{a}	660 ± 64 ^{a}	91 ± 8 ^{c}	7.3 ± 1.3 ^{a}
SDS-treated starch	81 ± 1 ^{b}	483 ± 12 ^{b}	114 ± 4 ^{b}	4.2 ± 0.1 ^{b}

NWS, native wheat starch; NaOH-treated starch and SDS-treated starch, wheat starch after removal of granule-surface proteins.
Means in the same column with different superscript lowercase letters indicate significant differences between different samples ($P < 0.05$).
All values are expressed as mean ± standard deviation ($n = 3$).

gluten and resulted in poor extensibility (Li et al., 2021).

3.4. Microstructure analysis of dough

The distribution of gluten and starch in reconstituted dough was observed by CLSM (Fig. 4).

Gluten network and starch granules were stained by Rhodamine B (red) and FITC (green), respectively (F. Jia et al., 2019). The reconstituted dough with NWS presented a relatively homogenous, smooth, and highly branched structure where the starch granules were evenly distributed in the gluten matrix (Fig. 4A). However, when NWS were replaced by NaOH- or SDS-treated starches, irregular and discontinuous gluten networks were observed in the reconstituted dough (Fig. 4B and C). Some embedded starch granules were exposed, separated, and detached from the gluten matrix, indicating that the absence of SGSPs weakened the adhesion of starch granules to the gluten matrix (Fig. 4B$_3$ and 4C$_3$). The "starch-gluten" matrix of dough with SGSPs was more compact than the dough without SGSPs. The results were in conformance with the dough rheological behaviors which were characterized

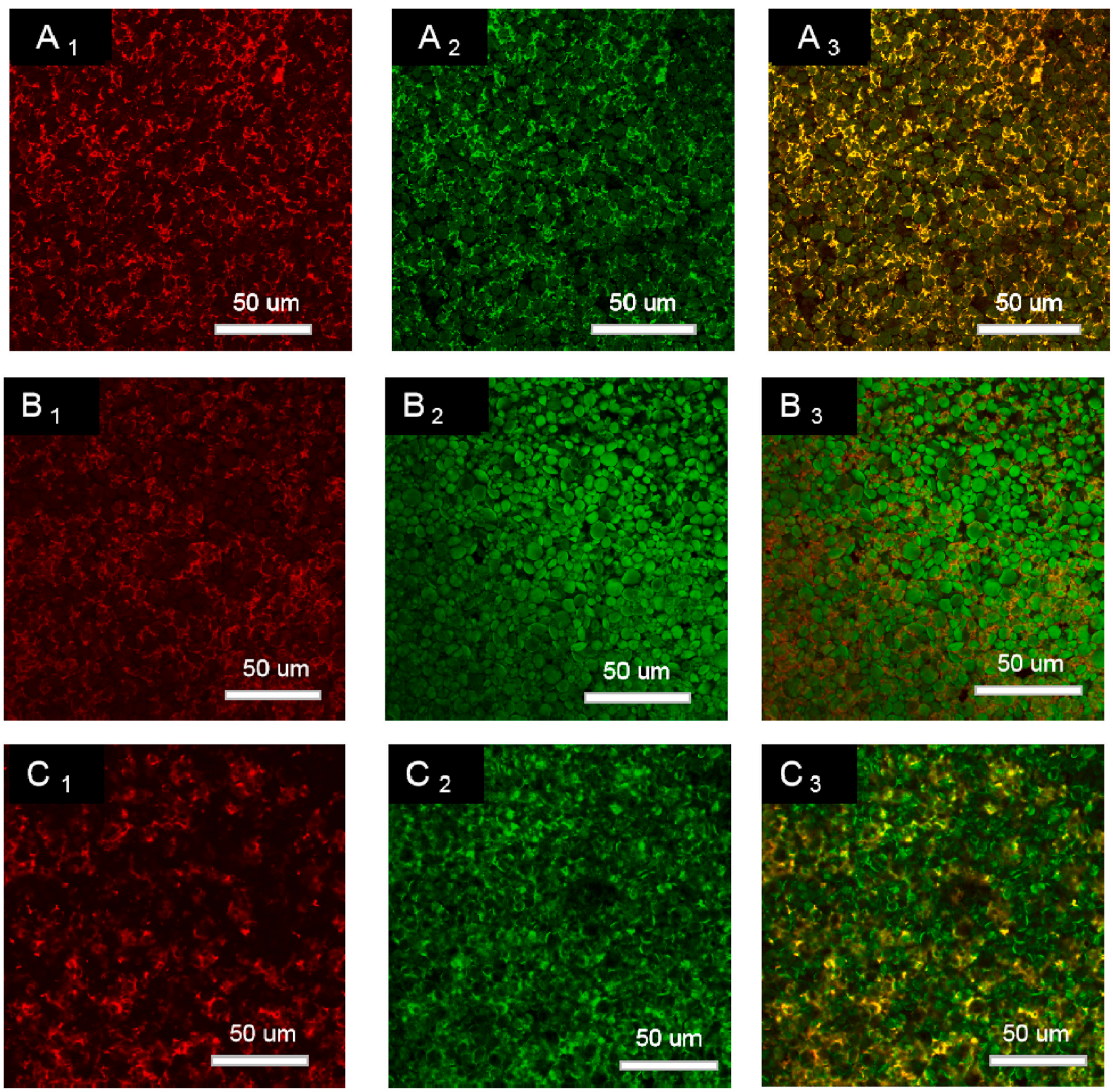

Fig. 4. The distribution of gluten network and starch granules in reconstituted dough was observed by CLSM. The gluten and starch were stained by Rhodamine B (1, red) and FITC (2, green), respectively, and the superimposition of 1 and 2 was 3 (yellow). (A, reconstituted dough with native wheat starch; B, reconstituted dough with wheat starch after removing granule-surface proteins with NaOH; C, reconstituted dough with wheat starch after removing granule-surface proteins with SDS). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

by greater extension resistance and lower extensibility (mentioned in section 3.3). Therefore, removing SGSPs made the gluten structure more scattered and less continuity, which could not be fully stretched.

3.5. Thermal properties

The DSC gelatinization curves and corresponding parameters of reconstituted dough were shown in Fig. 5 and Table 3, respectively. The gelatinization temperatures were shifted to higher values when the NaOH- and SDS-treated starches were added. This could be due to the reduction of free water as a plasticizer for starch molecules (Lapčíková, Burešová, Lapčík, Dabash, & Valenta, 2019). At the same water content, the dough without SGSPs might lack available water molecules to complete the starch gelatinization process, so the gelatinization temperatures increased to higher values. As for the enthalpy values of dough, the treated groups were significantly lower ($p < 0.05$) than the control group. It was possibly attributed to the starch-gluten interactions not the starch granules because NaOH and SDS treatments made the starch enthalpy unchanged (Tao et al., 2021; Wang et al., 2014). After removing SGSPs, the contact between starch granules and gluten became loose. The proteins developed a compact and poorly branched gluten network, which affected the relaxation of water in polymer chain conformations. Thus, it required less energy to break down the matrix structure during the gelatinization process (Jekle et al., 2016).

3.6. Bread firmness

Bread firmness was one of the key parameters that reflected the sensory of consumers. The firmness of the NWS group was 37.6 N, which was significantly increased to 54.2 N for the NaOH group and 42.5 N for the SDS group (Fig. 6), as consistent with the higher extension resistance in the extensograph experiment. Additionally, the scanning image of bread sample exhibited a worse crumb structure with fewer gas cells when the NaOH- or SDS-treated starches were incorporated into reconstituted dough (upper panel in Fig. 6). Bread characteristics are influenced by dough behavior during proofing and baking, especially the ability of the dough to expand along with gas cells (Yu et al., 2020; J.; Zhan, Ma, Wang, Li, & Zheng, 2019). Removing SGSPs resulted in a heterogeneous gluten network where some macromolecules were individually distributed in the dough. It reduced the interfacial areas in the "starch-gluten" matrix available for the mass transfer of CO_2 into the bubbles, thereby decreasing the CO_2 diffusion rate and dough growth during the mixing and fermentation process (J. Gao, Tay, Koh, & Zhou, 2017). Therefore, the baked bread made from SDS- and NaOH-treated starches had lower gas storage capacity with a denser and harder bread texture.

3.7. Water distribution in bread

The quantities and properties of water molecules in food matrix have been reflected as the spin-spin relaxation time (T_2), which indicated the water distribution and migration in bread (Curti, Bubici, Carini, Baroni, & Vittadini, 2011). Fig. 7 showed that ^1H T_2 was divided into T_{21} (0–1 ms) and T_{22} (1–100 ms) according to the length of relaxation time. The ratio of the area of each peak to the total integrated area (represented as A_{21} and A_{22}, respectively) was the relative water percentage of each component. T_{21} represented a part of the water that was tightly bound to gluten while T_{22} characterized the water that was bound to starch (Yu et al., 2019). Compared with NWS group, the removal of SGSPs shortened T_{22} relaxation time, and the corresponding A_{22} increased. For example, the relaxation time of T_{22} in the SDS-treated starch group was decreased from 12 ms to 8 ms where the value of A_{22} increased from 79.74% to 85.07% ($p < 0.05$). After removing SGSPs, the proportion and degree of starch bound water increased, while the proportion of gluten bound water decreased, which indicated that the water holding capacity of bread was weakened. When the NaOH-treated starch was added, the

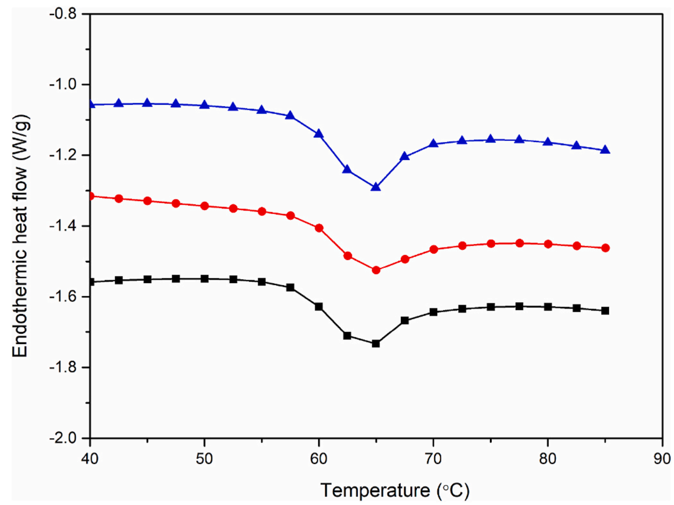

Fig. 5. Effect of removing starch granule-surface proteins (SGSPs) on DSC gelatinization curve of reconstituted dough. (—■—, reconstituted dough with native wheat starch; —●—, reconstituted dough with wheat starch after removing granule-surface proteins with NaOH; —▲—, reconstituted dough with wheat starch after removing granule-surface proteins with SDS).

Table 3
The influence of starch granule-surface proteins (SGSPs) on thermal properties of reconstituted dough.

Samples	T_o (°C)	T_p (°C)	T_c (°C)	△H (J/g of dry dough)
NWS	58.0 ± 0.5 [b]	63.1 ± 0.7 [b]	72.1 ± 1.4 [a]	5.7 ± 0.3 [a]
NaOH-treated starch	59.7 ± 0.2 [a]	64.1 ± 0.3 [ab]	71.6 ± 1.6 [a]	4.9 ± 0.2 [b]
SDS-treated starch	59.7 ± 0.1 [a]	64.3 ± 0.1 [a]	70.2 ± 0.5 [a]	4.9 ± 0.3 [b]

NWS, native wheat starch; NaOH-treated starch and SDS-treated starch, wheat starch after removal of granule-surface proteins; T_o, onset gelatinization temperature; T_p, peak gelatinization temperature; T_c, conclusion gelatinization temperature; ΔH, gelatinization enthalpy.
Means in the same column with different superscript lowercase letters indicate significant differences between different samples ($P < 0.05$).
All values are expressed as mean ± standard deviation ($n = 3$).

water distribution of bread was further modified. The peaks of T_{21} and T_{22} got merged in one peak at 1.74 ms of relaxation time, suggesting that lacking SGSPs prompted the migration of water from bound and weakly-bound conditions to semi-bound conditions. One of the reasons was that removing SGSPs could enhance the contact between water molecules and starch granules, thus promoting the water migration in the bread. On the other hand, the weakening of the adhesion between starch granules and gluten matrix caused by removing SGSPs might affect the redistribution of water during bread baking (Sui, Xie, Liu, Wu, & Zhang, 2018).

4. Conclusions

Removing SGSPs significantly modified the mixing, structural, thermal, and baking properties of the reconstituted dough. The lack of SGSPs weakened the adhesion of starch granules to gluten matrix, making the dough more dispersed and discontinuous. This led to higher extension resistance, gelatinization temperatures, and lower enthalpy. In addition, it reduced the ability of dough to expand along the gas cells and bread to hold water, thus exhibiting a denser and harder bread texture. These results are helpful to clarify the surface functionality of starch granules, among which SGSPs, as the contact medium between

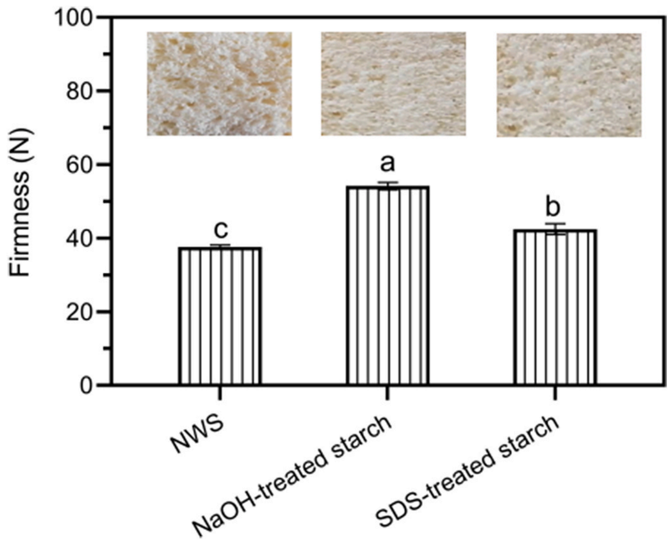

Fig. 6. Removing starch granule-surface proteins (SGSPs) modified the bread firmness of reconstituted dough (Bread samples depicted in the upper panel). Means in the same index with different superscript lowercase letters indicate significant differences between different samples (P < 0.05).

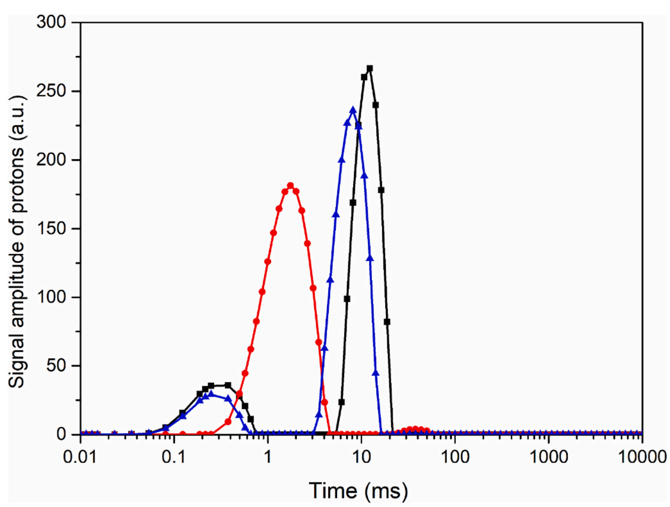

Fig. 7. Effect of starch granule-surface proteins (SGSPs) on water distribution in bread. (—■—, reconstituted dough with native wheat starch; —●—, reconstituted dough with wheat starch after removing granule-surface proteins with NaOH; —▲—, reconstituted dough with wheat starch after removing granule-surface proteins with SDS).

starch granules and gluten matrix, have a significant impact on the quality of dough and bread.

CRediT authorship contribution statement

Fan Lu: for, Investigation, Formal analysis, and, Writing – original draft. **Xue-Feng Zhu:** for, Methodology, and, Data curation. **Han Tao:** for, Writing – review & editing, and, Funding acquisition. **Hui-Li Wang:** for, Supervision, and, Project administration. **Zhi Yang:** for Revision and English Expression Improvement, English Revision.

Declaration of interest statement

The authors have no conflict of interests related to this study.

Acknowledgment

This study was financially supported by National Science Foundation of China (No. 31901727) and the Fundamental Research Funds for the Central Universities (JZ2020HGTB0043 & PA2021KCPY0054).

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