

The effect of soluble dietary fiber from chia seeds on dough characteristics and steamed bread quality

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Abstract

The aim of this study was to investigate the effect of replacing medium-gluten wheat flour with different concentrations of chia soluble dietary fiber (C-SDF) on dough properties and the quality of steamed bread to treat dietary fiber deficiency among Chinese residents. The findings indicate that the mixed dough's storage modulus (G') and loss modulus (G'') increased with the increasing scanning temperatures but declined with the increasing shear stress. The secondary structures of dough proteins, including β -sheets and α -helices, exhibited a gradual increase in content as the C-SDF addition amount increased, followed by a decrease in β -turn structures. However, when the C-SDF addition amount reached 2.5%, the β -sheets and α -helices reduced dramatically, but β -turn structures increased, demonstrating that dietary fiber affected the compactness of the gluten network. As the quantity of C-SDF increased, the specific volume of steamed bread increased and then decreased; the height-to-diameter ratio increased; hardness and chewiness first reduced but later increased; and elasticity, cohesiveness, and adhesiveness first increased and then declined. The release of reducing sugars decreased, and sensory evaluation scores were first enhanced to 1.5% of C-SDF addition and then subsequently reduced. In summary, the findings of this study indicated that the optimal addition level does not exceed 1.5%.

Keywords: Chia soluble dietary fiber; wheat flour; dough characteristics; simulated in vitro digestion; steamed bread quality

Introduction

Chia seeds (*Salvia hispanica* L.) are nutrient-dense seeds containing dietary fiber, *n*-3 polyunsaturated fatty acids,

proteins, and lipids (Li *et al.*, 2018). They have received significant interest as a potential functional food ingredient. They were officially certified in China as a new food ingredient for food processing in 2014. Chia seeds

provide a variety of health advantages due to their 34.4% dietary fiber content, 31% of which is soluble. Soluble dietary fiber (SDF) may bind to cholesterol and carbohydrates, slowing their absorption and transit in the bloodstream (Abutair *et al.*, 2018). It also acts as a prebiotic, regulating gut microbiota and lowering obesity risk (Vily-Petit *et al.*, 2023). Additionally, it reduces the risk of ulcerative colitis through intestinal fermentation (Wang *et al.*, 2024). These unique physicochemical and functional properties make SDF particularly advantageous in food processing (Lazaro *et al.*, 2018).

Chia seeds are commonly used in bread, cereals, cookies, and meat products (Ozon *et al.*, 2023; Rani *et al.*, 2023; Wolever *et al.*, 2023), greatly enhancing food quality. For instance, Ran *et al.* (2021) discovered that modified chia dietary fiber enhanced the quality of tough biscuits, boosting water and oil retention, decreasing hardness and brittleness, and lowering the glycemic index. Angélica *et al.* (2019) observed that blending oat-chia mixes with wheat flour (35:65%) boosted protein content by 67.75%, total dietary fiber by 85.21%, and SDF by twofold, yielding softer bread with prolonged shelf life. In recent years, interest in chia seeds has grown domestically and internationally. While research on dietary fiber and its application has expanded, high-fiber products still face challenges regarding taste and appearance, hindering broader acceptance. Even though research suggests that chia soluble dietary fiber (C-SDF) has the potential to enhance the quality of wheat-based products, the effects of this material on dough characteristics and the quality of steamed bread have not yet been thoroughly examined. In order to provide theoretical insights into the development of chia seed-enriched wheat products, the purpose of this study was to have the following objectives: (1) investigate the effect of replacing medium-gluten wheat flour with various concentrations of C-SDF on the properties of mixed flour; (2) assess the effect of these concentrations on the rheological properties of dough; (3) produce a supplemented traditional steamed bread that is of high quality; and (4) treat dietary fiber deficiency among Chinese residents.

Materials and Methods

Preparation of chia soluble dietary fiber

Chia seeds (Beijing Fuhai Huakang Food Processing Co., Ltd., Beijing, China) were ground and subsequently passed through a 60-mesh sieve. A 1 g sample was mixed with 30 mL of 9% sodium hydroxide (NaOH) solution at a solid-to-liquid ratio of 1:30 (g/mL). The mixture was extracted using 300 W ultrasonic assistance (Ultrasonic Cleaner, SB25-12DTDN, Ningbo Xinzhi Biotechnology Co., Ltd., Ningbo, Zhejiang, China) at 50°C for

40 minutes. The supernatant was collected and mixed with three volumes of absolute ethanol (Tianjin Tianli Chemical Reagent Co., Ltd., Tianjin, China), allowed to stand for 10 minutes, and centrifuged at 4000 rpm for 5 minutes (High-Speed Centrifuge, 5702R, Honglong Biotechnology Co., Ltd., Hunghom, Hong Kong). The precipitate was dried at 60°C using an electric blast drying oven (Model 101-1AB, Tianjin Tester Instrument Co., Ltd., Tianjin, China) to yield C-SDF.

Preparation of mixed dough

Dough formulation

Medium-gluten wheat flour (Angel Yeast Co., Ltd., Yichang, Hubei, China) was partially replaced with C-SDF at concentrations of 0%, 0.5%, 1%, 1.5%, and 2% (weight by weight [w/w]). The final formulation included 100 g mixed flour, 1 g yeast (Angel Yeast Co., Ltd.), and 50 g distilled water.

Dough preparation

The ingredients were accurately weighed and kneaded in a multifunctional mixer (HMJ-A20E1, Bear Electric Appliance Co., Ltd., Foshan City, Guangdong Province, China) for 20 minutes. The dough was then placed in a fermentation chamber at 38°C and 85% humidity for the first fermentation lasting 50 minutes. After degassing and shaping for 5 minutes, 50 g portions of dough were prepared and subjected to a second fermentation under the same conditions for 15 minutes. The dough was then frozen at −20°C to stop fermentation, resulting in pre-prepared dough blanks.

Determination of mixed flour properties with C-SDF

Water-holding capacity

Water-holding capacity (WHC) was assessed using the methodology outlined by Ma *et al.* (2016), with minor modifications. A 2 g sample of mixed flour (M) was put in a centrifuge tube and the total weight of the tube and sample (M_1) was recorded. The sample was dissolved in 30 mL of distilled water, stirred in boiling water for 15 minutes, and then centrifuged at 3000 rpm for 25 minutes. The supernatant was removed, and the centrifuge tube was inverted over absorbent paper for 5 minutes to facilitate drainage. The total weight of the tube and the residual sample (M_2) was then recorded. WHC was calculated using the formula:

$$\text{WHC} = \frac{M_2 - M_1}{M}$$

Where M is the sample weight (g); M_1 is the total weight of the centrifuge tube and the sample before

centrifugation (g); and M_2 is the total weight of the centrifuge tube and the sample after draining (g).

Swelling capacity

Swelling capacity (SC) was measured following the method of Anderson (1982) with minor modifications. A 2 g sample (Q_0) was mixed with 30 mL of distilled water in a preweighed centrifuge tube (Q_1). The mixture was heated in a 95°C water bath for 30 minutes and then centrifuged at 4000 rpm for 20 minutes. The supernatant was discarded and the centrifuge tube was inverted on absorbent paper to drain for 5 minutes. The weight of the tube and drained sample (Q_2) was measured. SC was calculated using the formula:

$$SC = \frac{Q_2 - Q_1}{Q_0}$$

Where Q_0 is the sample weight (2 g); Q_1 is the empty centrifuge tube weight (g); and Q_2 is the total weight of the centrifuge tube and sample after draining (g).

Freeze-thaw stability

Freeze-thaw stability was assessed based on the method described previously by Li *et al.* (2020) with modifications. A 2 g sample of mixed flour was combined with 20 mL of distilled water in a preweighed centrifuge tube (A_1). The mixture was boiled in a water bath for 8 minutes, cooled to room temperature, and frozen at -18°C for 24 hours. After thawing at room temperature, the sample was centrifuged at 3000 rpm for 15 minutes. The total weight of the centrifuge tube and sample (A_2) was recorded. The supernatant was discarded and the tube was inverted to drain for 30 minutes before weighing again (A_3). Freeze-thaw stability was expressed as the syneresis rate, calculated using the following formula:

$$\text{Syneresis rate} = \frac{A_2 - A_3}{A_2 - A_1} \times 100\%$$

Where A_1 is the weight of the centrifuge tube and sample before freezing (g); A_2 is the weight of the centrifuge tube and sample after freezing, thawing, and centrifugation (g); and A_3 is the weight of the centrifuge tube and drained sample (g).

Water solubility index

The water solubility index (WSI) was determined according to Li *et al.* (2020) with slight modifications. A 2 g sample (C_0) was mixed with 30 mL of distilled water in a centrifuge tube. After heating in a 95°C water bath for 30 minutes, the sample was centrifuged at 4000 rpm for 20 minutes. The supernatant was transferred to a predried evaporating dish (C_1), dried at 105°C to

constant weight, and weighed (C_2). WSI was calculated as follows:

$$WSI = \frac{C_2 - C_1}{C_0} \times 100\%$$

Where C_0 is the weight of the mixed flour sample (g); C_1 is the weight of the empty evaporating dish (g); and C_2 is the weight of the evaporating dish and dried sample (g).

Dough properties measurement

Wet and dry gluten content

After a few modifications, the wet gluten content was determined using the GB/T (Guóbìào/Tuìjiàn) 5506.1-2008 method. A 15 g sample (M_0) of the dough was washed with a 20 g/L NaCl (mixture of water and sodium chloride) solution to remove the starch. Following the kneading and washing of the dough under running water, the weight of the remaining mass was recorded as the wet gluten content when it stabilized (M_1). Then the wet gluten was dried in a forced air oven at 90°C until it reached a constant weight; this was the dry gluten content (M_2).

$$\text{Wet gluten content} = \frac{M_1}{M_0} \times 100\%$$

$$\text{Dry gluten content} = \frac{M_2}{M_0} \times 100\%$$

Where M_0 is the original sample weight (g); M_1 is the wet gluten weight (g); and M_2 is the dry gluten weight (g).

Determination of dough rheological properties

Dough rheological properties were determined using the dynamic rheology method described by Burešová & Kubínek (2016) with modifications. Frozen dough samples were thawed for 1 hour in the refrigerator. A 3 g portion of the thawed dough was placed on a rheometer's plate with a 50 mm gap and a spacing of 1.5 mm. The dough was subjected to temperature scanning (30–95°C) at a scanning frequency of 1 Hz and a strain of 1%. Amplitude scanning was also performed with an angular velocity of 10 rad/s and strain ranging from 0.001% to 100%.

Steamed bread preparation

The preparation process included the following steps: raw ingredients were precisely measured, mixed, and kneaded in a multifunctional mixer for 20 minutes. The dough was prepared as described in section 2.2.2.

Then the fermented dough was steamed directly at 100°C for 15 minutes and stewed for 5 minutes after turning off the heat.

Steamed bread properties measurement

Specific volume measurement

The specific volume of steamed bread was determined using the millet displacement method specified in GB/T 21118-2007 *Wheat Flour Steamed Bread*. Specific volume is defined as the ratio of volume (mL) to weight (g).

Height-to-diameter ratio

The height and diameter of the steamed bread were measured using a vernier caliper and the height-to-diameter ratio was calculated accordingly.

Color measurement

A spectrophotometer was used to measure the color (L^* , a^* , b^* values) of steamed bread with varying C-SDF levels. Measurements were taken for both the crust and crumb. Samples were ground into a powder using a multifunctional grinder for color analysis.

Texture profile analysis

The core texture of steamed bread was analyzed using a texture analyzer. A central portion of cooled bread was cut into 2.5 cm cubes and placed on the analyzer's platform. The test used the TPA-300 mode with a P/36R probe, a compression ratio of 50%, a test speed of 1 mm/second, an interval time of 3 seconds, and a trigger force of 5 g. Key metrics were determined, including hardness, elasticity, cohesiveness, adhesiveness, and chewiness.

In vitro starch digestibility assays

Simulated in vitro digestion was performed to predict the glycemic response following the methods described by Goñi *et al.* (1997) and Ma *et al.* (2013). A 50 mg steamed bread sample was mixed with 10 mL of 0.1 mol/L HCl-KCl (hydrochloric acid-potassium chloride) buffer (potential of hydrogen (pH) 1.5) and 0.2 mL of 0.1 g/mL pepsin solution stirred at 40°C for 1 hour. The solution was adjusted to 37°C and 15 mL of phosphate buffer (pH 6.9) and 0.01 mL of α -amylase solution (100,000 U) were added to initiate enzymatic hydrolysis. Digestion occurred at 37°C, with samples collected every 30 minutes for a duration of up to 210 minutes. The reducing sugar content in the hydrolysates was quantified using the DNS method. The glucose standard curve is $y = 1.5501 \times -0.0236$, $R^2 = 0.9964$.

Fourier transform infrared spectroscopy

Dry steamed bread samples were pulverized, mixed with potassium bromide at a ratio of 100:1 (sample: potassium bromide), and pressed into pellets for Fourier transform

infrared spectroscopy (FTIR) analysis. The scanning range was 400–4000 cm^{-1} with 32 scans at a resolution of 4 cm^{-1} . The data were processed using Peakfit 4.12 for baseline calibration and Gaussian deconvolution to identify peak positions and relative sub-peak areas.

Microstructure observation

According to Sun *et al.* (2023), the core of steamed bread was sectioned into 2.5 cm^3 cubes, subjected to freeze-drying, coated with gold, and subsequently analyzed via scanning electron microscopy (SEM, Zeiss Sigma 300).

Sensory evaluation

A trained panel of 10 members evaluated the sensory attributes of steamed bread in a well-lit, comfortable environment. Panelists rinsed their mouths between samples. A control sample with 0% C-SDF was used and sensory scoring criteria were adapted from GB/T 35991-2018. Refer to Table 1 for specific scoring rules.

Statistical analysis

All experiments were conducted in triplicate and the data were shown as mean \pm S.D. The resulting data were statistically analyzed by ANOVA (Analysis of Variance) using SPSS (Statistical Package for the Social Sciences) 26.0, Excel, Origin 8.5, and Peakfit 4.12.

Results and Discussion

Effects of chia soluble dietary fiber on mixed flour

Water-holding capacity and swelling capacity

Figure 1A shows that WHC and SC of mixed flour initially increase and then decrease with rising C-SDF addition levels up to 1.5%. This trend can be attributed to the hydroxyl groups present in C-SDF, which can form hydrogen bonds with water, giving it a strong hydration capacity (Hamdani *et al.*, 2019; Huo *et al.*, 2024; Yang *et al.*, 2021). Additionally, C-SDF can adsorb and retain water, minerals, and other substances effectively, enhancing its swelling ability and facilitating integration into the network structure of mixed flour (Muñoz *et al.*, 2021). However, when the C-SDF addition exceeds a certain threshold, the high molecular weight of the C-SDF competes with starch and protein for water, and the stronger aggregation effect disrupts the protein and network structure in wheat flour. Consequently, WHC and SC are reduced. The optimal SC is observed with an addition of 1.5% C-SDF, while the highest WHC occurs at 2% C-SDF.

Freeze-thaw stability and water solubility

As shown in Figure 1B, the syneresis rate of mixed flour significantly decreased as C-SDF levels increased,

Table 1. The sensory quality rating of steamed bread.

Item	Maximum score	Evaluation criteria	Score range
Elasticity	10	Finger press elasticity is good	8–10
		Weak rebound of finger press	6–7
		Finger pressure does not bounce back or is difficult to press;	4–5
Surface color	10	Good luster	8–10
		Slightly darker	6–7
Internal structure	10	Pores are fine and uniform	8–10
		The pores are fine and basically uniform, with individual bubbles;	6–7
		Uneven pores or very rough structure;	4–5
Toughness	10	Strong bite	8–10
		The bite is average	6–7
		Poor bite strength, slag off when cutting or chewing dry;	4–5
Stickiness	10	Refreshing without sticking teeth	8–10
		A little sticky	6–7
Aroma and taste	10	The natural fragrance of normal wheat	8–10
		Taste plain;	6–7
Comprehensive score	10	–	–

indicating enhanced freeze-thaw stability. Generally, amylose molecules, with their limited spatial structure, tend to rearrange and form hydrogen bonds during freezing and thawing. Thus, a lower amylose content typically corresponds to better freeze-thaw stability (Li *et al.*, 2012; Srichuwong *et al.*, 2012). It is hypothesized that the addition of C-SDF reduces amylose formation or dilutes its relative content, thereby improving freeze-thaw stability. The samples retained a paste-like consistency even after freeze-thaw cycles, suggesting that C-SDF improves antifreeze properties, making it suitable for frozen food applications.

The WSI, which reflects the ease of digestion and absorption, initially increased and subsequently decreased as C-SDF levels rose (Figure 1B). The optimal WSI occurs with the addition of 1.5% C-SDF. The inherent solubility of C-SDF contributes to an initial increase in soluble content. When the addition of C-SDF exceeds 1.5%, enhanced aggregation modifies the gluten network, which leads to the capture of minerals and soluble components, consequently decreasing WSI (Wu *et al.*, 2018).

Effects of C-SDF on dough characteristics

Wet and dry gluten content

Wet and dry gluten contents somewhat reflect the quality of wheat flour. Figure 2A shows that these values initially increase and then decrease with rising C-SDF levels. Between 0% and 1.5% C-SDF, the gluten content increased significantly due to the hydrophilic properties of C-SDF, which enhance the gluten network structure and resistance to extension (Roussanova *et al.*, 2014; Shu *et al.*, 2021). Beyond 1.5%, the dilution of gluten protein caused by excess C-SDF reduces its percentage and

weakens the gluten network (Zhang, 2021), thereby lowering the gluten content. Different C-SDF addition levels can be chosen based on production needs to create high dietary fiber wheat-based foods. Consistent with the research findings by Gao *et al.* (2022), an increase in SDF in rice bran enhances dough volume; however, when the addition exceeds 5%, the volume declines. This suggests that excessive SDF dilutes gluten protein, affecting the dough's capacity to establish a gluten network structure, thereby reducing its volume (He, Ma, & Wang, 2009).

Rheological properties of dough

Figure 3A and B shows that the storage modulus (G') and loss modulus (G'') of mixed dough increased sharply around 60°C and reached their maximum values between 80°C and 90°C. The G' increase at temperatures above 60°C indicated heat-induced gelation (He *et al.*, 2022), enhancing starch gelatinization and protein denaturation. Gelatinized starch particles absorb water and integrate into the gluten network, causing a loss of water from gluten and increasing the elasticity and viscosity of the dough (Wang *et al.*, 2020). This aligns with the findings by Xu *et al.* (2017), who reported that starch is a primary factor influencing rheological properties during dough heating.

Figure 3C illustrates a significant decrease in G' with increasing shear stress, indicating a transition to a non-linear region resulting from structural breakdown at elevated shear stress levels. The degree of particle structure disruption reduced as C-SDF levels increased (Korus *et al.*, 2020), suggesting that C-SDF enhances the stability of the dough structure. Figure 3D indicates that G'' exhibits a trend consistent with G' , as most samples maintain a linear viscoelastic range following the addition of C-SDF. This behavior is likely attributed to the

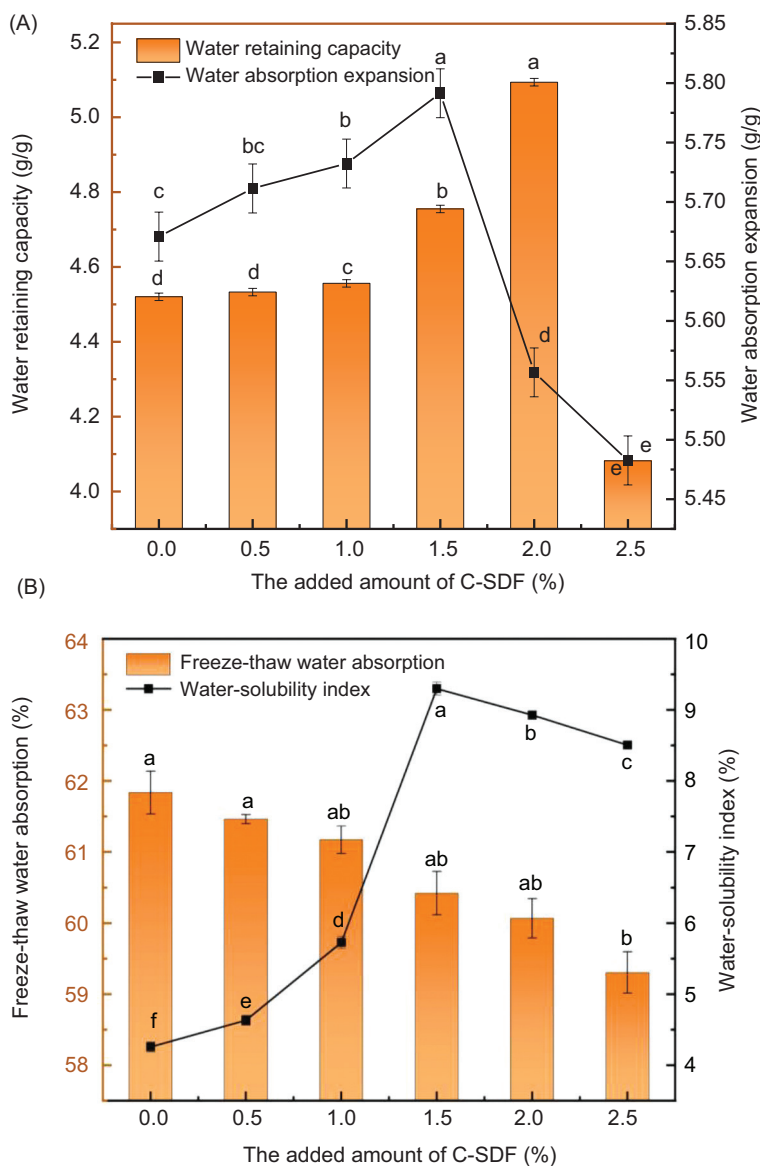


Figure 1. Effect of different amounts of chia seeds soluble dietary fiber (C-SDF) on mixed flour: Water-holding capacity and swelling capacity (A); Freeze-thaw water absorption and water-solubility index (B).

uniform distribution of particles within the dough's network (Yan *et al.*, 2024). This result can be attributed to the strong hydrophilicity of C-SDF, enabling it to interact with starch molecules via hydrogen bonds, thereby creating a more stable three-dimensional network structure, consistent with research by Ma *et al.* (2019), Wang *et al.* (2023), and Zhang *et al.* (2024).

Effects of C-SDF on steamed bread quality

Specific volume and height-to-diameter ratio

Figure 2B demonstrates that the specific volume of steamed bread initially increased and subsequently decreased as the levels of C-SDF rose. Specific volume

increases between 0% and 1.5% C-SDF due to the water retention properties of C-SDF, which maintain the gluten network and capture fermentation gases (Shao *et al.*, 2023). Exceeding C-SDF up to 1.5% adversely affects dough extensibility and dilutes gluten protein, thereby compromising the gluten network and diminishing specific volume. The height-to-diameter ratio correlates with the dough's extensibility (Gül *et al.*, 2009; Nandeesh *et al.*, 2011). The ratio increases with a specific volume between 0% and 1.5% C-SDF, indicating enhanced extensibility. Exceeding 1.5% leads to a deterioration in extensibility, resulting in a reduction in diameter and adversely affecting the ratio. In agreement with studies by Lin *et al.* (2012), Liu *et al.* (2017), Rawat and Darappa (2015), and Wenjun *et al.* (2018), the addition of SDF led to a notable

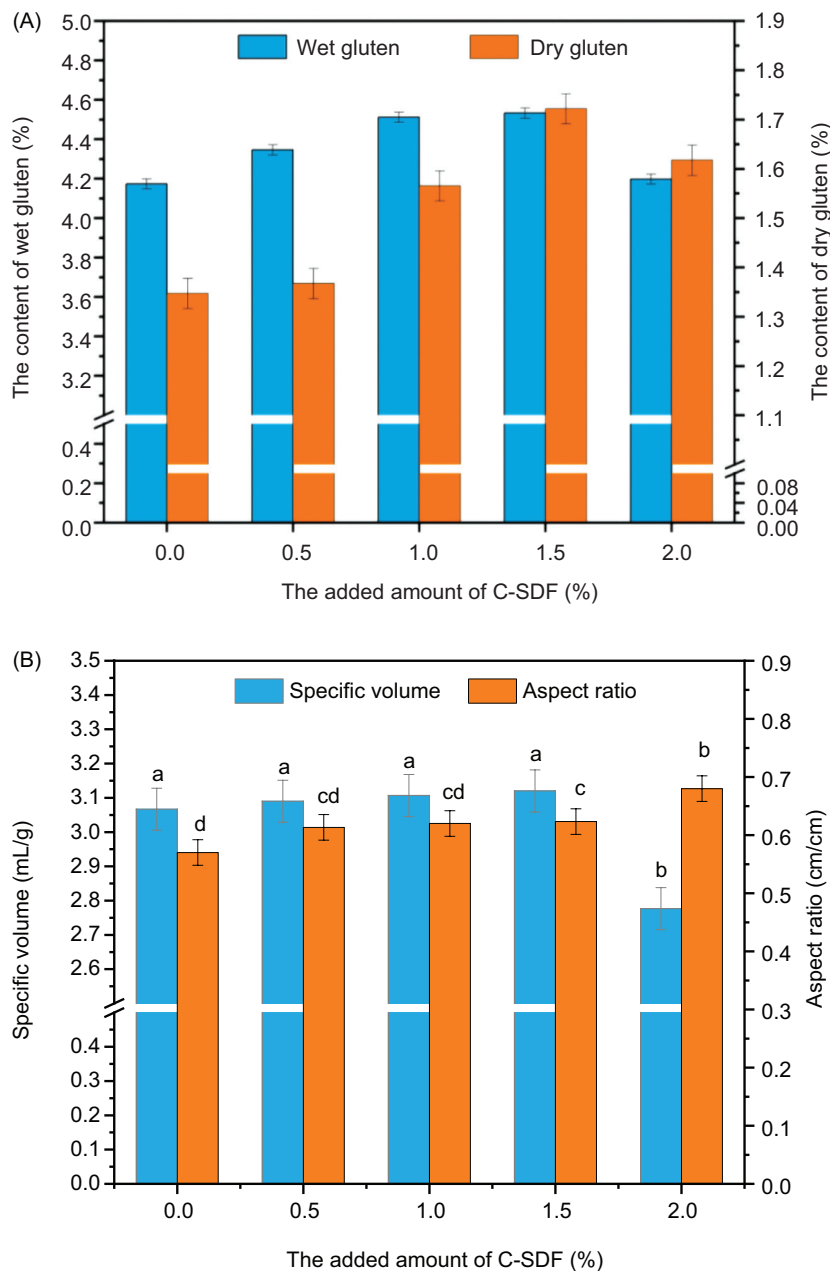


Figure 2. Effect of different amounts of C-SDF on the properties of dough and steamed bread: Content of dry and wet gluten for dough (A); Specific volume and aspect ratio to diameter of steamed bread (B).

decrease in bread volume and decreased ductility, attributable to the dilution of gluten in wheat flour dough and the reduction of gluten hydration.

Color changes

Color is a key sensory attribute for evaluating steamed bread quality. As shown in Figure 4A, increasing C-SDF levels from 0% to 2% decreases L^* values (lightness) while increasing a^* (redness) and b^* (yellowness) values for both crust and crumb. The dark brown color of C-SDF contributes to these changes. More considerable color changes are observed in the crust due to faster moisture

evaporation during steaming and cooling, causing slight wrinkling and collapse. In alignment with studies by Gao *et al.* (2022) and Huang (2024), an increase in dietary fiber content in rice bran correlates with a gradual decrease in the L^* value, indicating reduced brightness, while the a^* and b^* values significantly increase, resulting in a yellow coloration of the steamed bread.

Textural properties

Within certain limits, textural properties such as hardness, elasticity, cohesiveness, adhesiveness, and chewiness reflect the quality of steamed bread (Lan *et al.*, 2023).

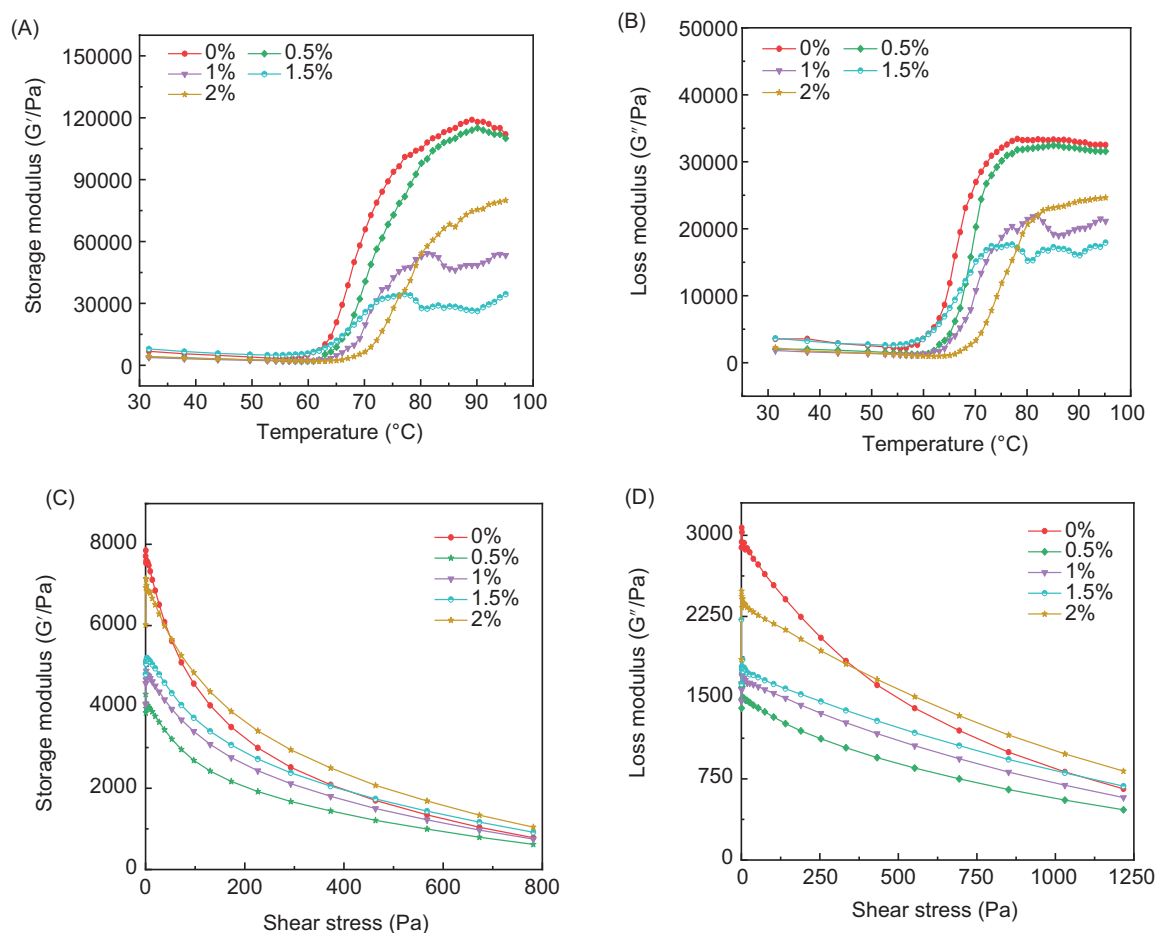


Figure 3. Rheological properties of dough with different amounts of C-SDF: Dough temperature-storage modulus diagram (A); Temperature-loss modulus of dough (B); Dough shear storage modulus diagram (C); Shear loss modulus of dough (D).

Figure 4B shows that hardness and chewiness decreased initially and then increased with rising C-SDF levels, while elasticity, cohesiveness, and adhesiveness follow the opposite trend. Between 0% and 1.5%, reduced hardness and chewiness are linked to C-SDF's hydrophilic groups (Cao *et al.*, 2023), which enhances hydration and softening, improving texture. Beyond 1.5%, excessive C-SDF creates dense aggregates, reducing the gluten network's flexibility and increasing hardness and chewiness as well as decreasing elasticity, cohesiveness, and adhesiveness. This negatively affects the overall quality. Consistent with studies by Torres *et al.* (2024), incorporating dietary fiber may enhance wheat bread's hardness, cohesion, and elasticity. Zhang *et al.* (2024) reported that incorporating rice bran dietary fiber into the dough enhances the elasticity, viscosity, and stability of the frozen dough gluten structure, which aligns with this research's findings.

In vitro simulated digestion of steamed bread

Figure 5 illustrates that the rate of reducing sugar release for all digestion samples reached its height during the initial 30 minutes of *in vitro* digestion, indicating a rapid

digestion phase characterized by the quick conversion of starch into glucose. The variation in digestion rate among the steamed bread samples with different levels of C-SDF was insignificant during this phase. At the 30-minute mark, the levels of reducing sugar released were as follows: 2% > 0% > 0.5% > 1% > 1.5%. In the later stages of simulated digestion, differences in the release of reducing sugars became more evident. The findings indicated that the rate of reducing sugar release was predominantly affected by the gradual digestion of starch (Kim and White, 2012). Throughout the 3.5-hour *in vitro* digestion process, the total release of reducing sugars was higher in control (0% C-SDF) bread compared to bread with 0.5%, 1%, and 1.5% C-SDF. Furthermore, the release of reducing sugars tended to decrease as the C-SDF content increased. This is likely because higher C-SDF content forms a temporary network structure that envelops the starch granules, acting as a barrier and effectively reducing the number of enzyme-active sites, thereby slowing down the starch digestion rate (Qin *et al.*, 2023). In contrast, during the overall digestion process, the total reducing sugar released from the bread

with 2% C-SDF was greater than that from the control (0% C-SDF) bread. This may be due to the presence of water molecules surrounding the starch molecules in the bread. During steaming, these water molecules disrupt the tight structure of resistant starch, converting it into digestible starch, which releases more reducing sugars (Lou *et al.*, 2022). For bread with less than 1.5% C-SDF, the breakdown of resistant starch was limited, resulting in lower reducing sugar release. Therefore, adding appropriate amounts of C-SDF in bread can help delay glucose release, suggesting its potential for glycemic control.

Fourier transform infrared spectroscopy analysis

The secondary structure of proteins plays a crucial role in food quality, as protein structure is closely linked to its various groups and bonding types. Liu *et al.* (2014) found that different vibrational modes of C = O, C-N, and N-H bonds in proteins absorb infrared radiation across multiple wavelengths, with the amide I band being particularly well studied. Thus, FTIR can be used to examine the relative content of protein secondary structures by

analyzing specific absorption peaks associated with different C-SDF levels.

As shown in Figure 6A, no new characteristic peaks appeared in the FTIR spectra of samples with added C-SDF, indicating that the interaction between starch molecules and C-SDF involved intermolecular forces rather than the formation of new chemical bonds (Tu *et al.*, 2024). The addition of C-SDF caused slight changes in the peak width, intensity, and position across different samples, suggesting that C-SDF altered the secondary structure of gluten protein molecules. As the amount of C-SDF increased, the peak width at the O-H group region increased, which was broader than that of the control (0% C-SDF) bread. This suggests that hydrogen bonding interactions between molecules in the bread were enhanced (Zhang *et al.*, 2021).

Further analysis showed shifts (Figure 6B) in the absorption peaks as C-SDF content increased. In the amide I band (1600–1700 cm^{-1}), there was a noticeable change

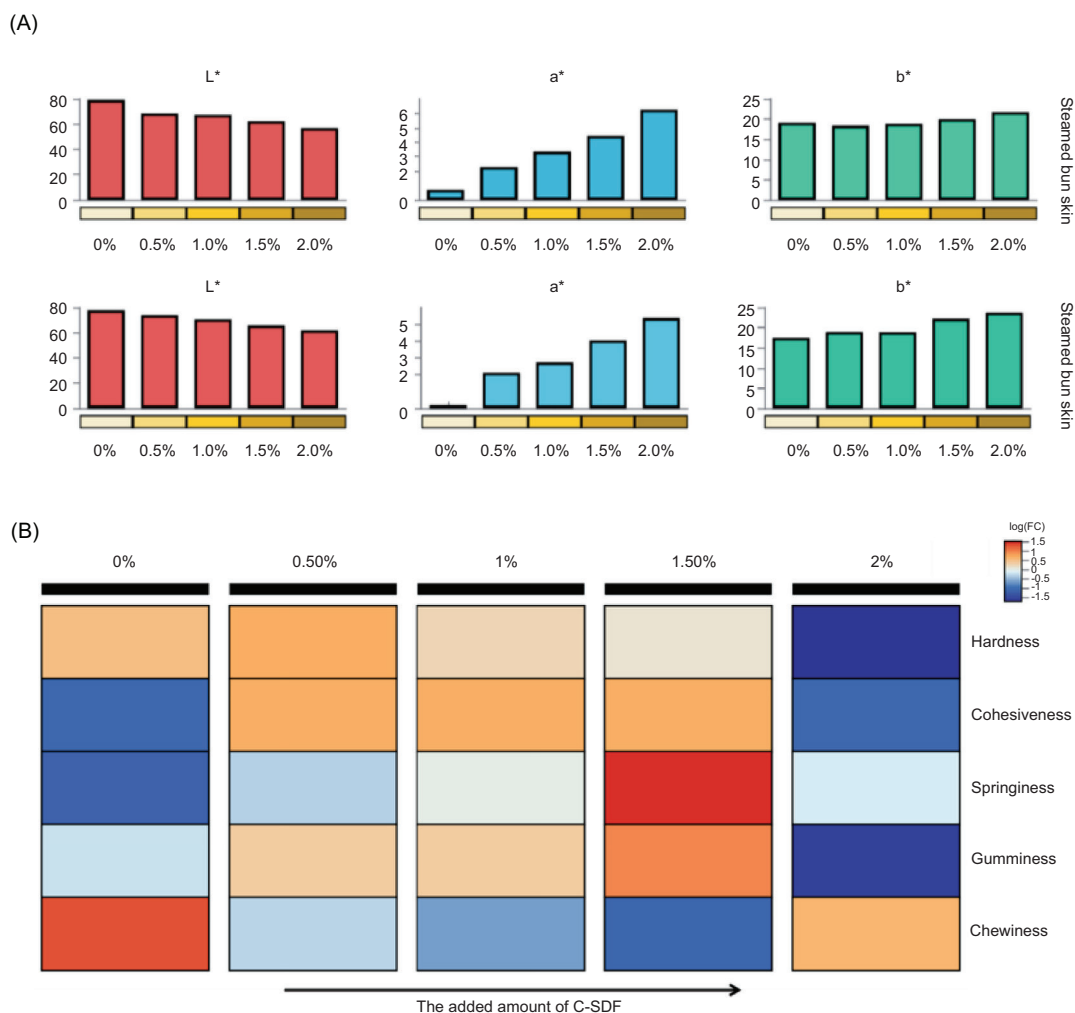


Figure 4. Effect of different amounts of C-SDF on the chromaticity (A) and the texture of steamed bread (B).

in peak position and intensity, with intensity initially increasing and then decreasing (Table 2). Based on studies of protein secondary structure, the various sub-peaks within the amide I band are attributed to β -sheets (β), random coils (r), α -helices (α), and β -turns (t) (Figure 6C) (Bock and Damodaran, 2013; Choi and Ma, 2007). When the C-SDF addition ranged from 0.5% to 2%, the content of β -sheets and α -helices gradually increased while β -turn structures decreased. However, when the C-SDF content reached 2%, this trend reversed, with a significant reduction in β -sheets and α -helices and an increase in β -turn structures. This suggests that the addition of C-SDF affects the relative amounts of β -sheets, α -helices, and β -turns. Bock and Damodaran (2013) indicated that some β -turn structures may convert to β -sheets, while Choi and Ma (2007) found that β -sheets and α -helices are more ordered secondary structures associated with hydrogen bonding, while random coils and β -turns are more disordered.

Thus, when C-SDF addition is 1.5%, the increase in β -sheets and α -helices indicates more ordered and stable protein secondary structures. The strong aggregation of C-SDF enhances the polymerization of protein molecules, leading to a more robust gluten network (Zhang *et al.*, 2019). When the C-SDF content exceeds 2%, the high molecular weight of C-SDF negatively impacts the secondary structure of gluten proteins. High-molecular C-SDF has a stronger water absorption capacity and forms highly viscous gels that attach to the surface of gluten, hindering the formation of the gluten network (Qian *et al.*, 2022). This leads to the dispersion

and depolymerization of protein molecules, resulting in reduced β -sheets and α -helices. The appearance of random coils at 2% C-SDF and their reduction at 2.5% C-SDF suggest that the high molecular weight of C-SDF introduces more noncovalent bonds, disrupting the protein's secondary structure.

Microstructure observation of steamed bread

As shown in Figure 7, the ellipsoid and spherical particles represent large and small starch granules, respectively. These granules exhibit good adhesion and are encapsulated by the gluten network. The voids observed in the image are likely due to air incorporation during dough mixing, possibly aided by carbon dioxide produced during fermentation. Comparing the microstructures of steamed bread with different C-SDF levels reveals that at 0% C-SDF, the starch granules are embedded within the gluten protein membrane, forming a compact gluten network with unclear boundaries between starch and gluten (Tian *et al.*, 2014). However, as C-SDF content increases, the continuity and encapsulation of the gluten network change. At 0.5% C-SDF, voids increase and starch granules extrude with a weaker bond to the gluten protein. At 1–1.5% C-SDF, the voids decrease and the contact area between starch granules and gluten protein increases, forming a more continuous and compact gluten network. However, when C-SDF content exceeds 1.5%, the damage to starch granules increases, with surface depressions and deformations observed. This may be due to excessive C-SDF, which dilutes the protein in the dough, causing partial dehydration of the gluten protein and reducing its elasticity and extensibility, leading to a weaker gluten

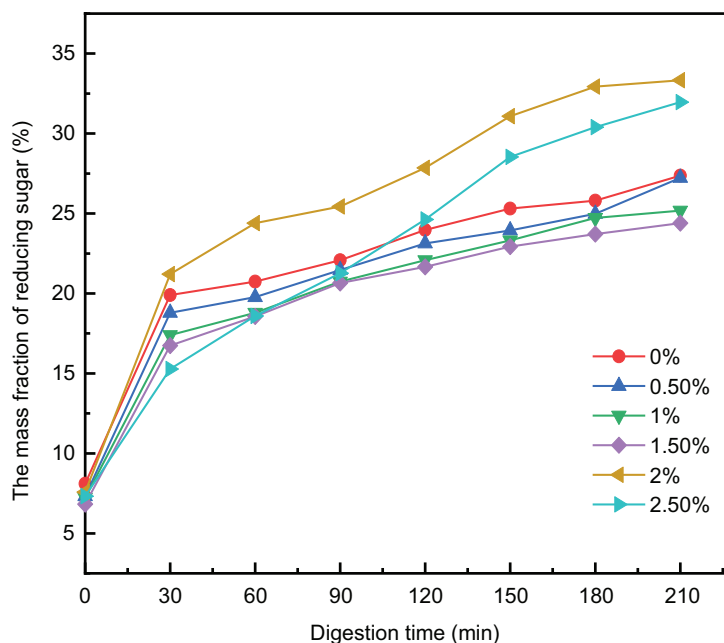


Figure 5. Comparison of in vitro digestion rate of steamed bread with different amounts of C-SDF.

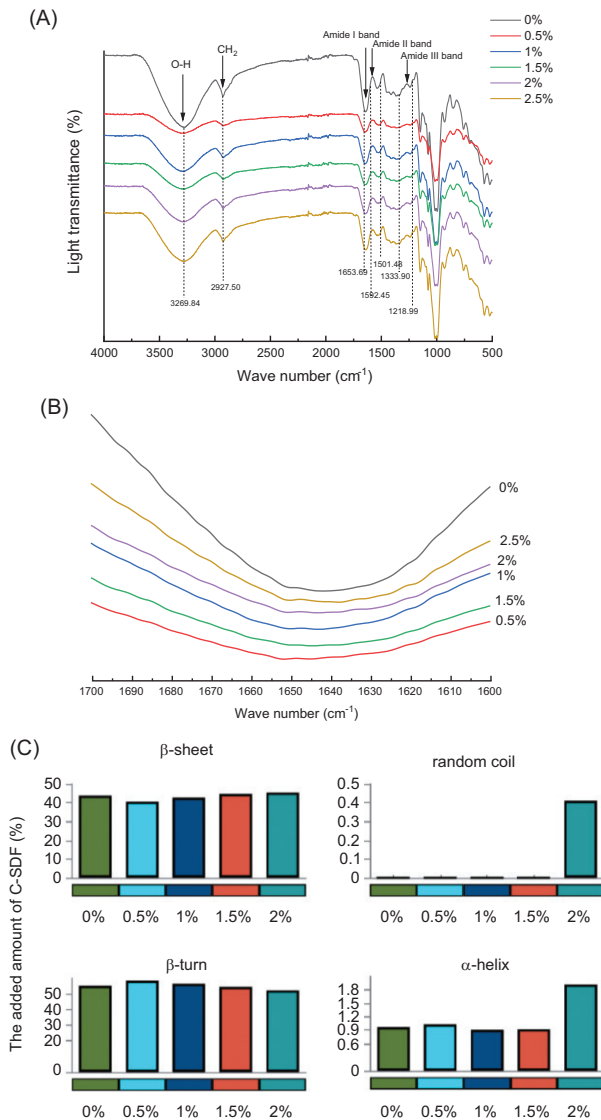


Figure 6. Comparison of FTIR spectra of steamed bread with different content of C-SDF: Infrared spectra of steamed bread (A); Infrared spectrum of amide I band of steamed bread (B); The secondary structure of steamed bread in protein (C).

network incapable of supporting the entire structure. Thus, as C-SDF content increases, the gluten structure is increasingly disrupted. The gluten structure remains relatively intact when the C-SDF content is below 1.5%, maintaining a tight protein-starch network. However, beyond 1.5%, the gluten network's density significantly decreases, reducing its encapsulation capacity and affecting the formation of gluten network proteins, thereby altering the dough properties.

Sensory evaluation of steamed bread

Figure 8 illustrates that increased C-SDF content results in a notable darkening of the steamed bread's color. The

Table 2. Assignment of deconvoluted amide I bands in the FTIR spectrum of protein.

Wave number	Structure name	Structural feature
1600–1639	β	β -sheet
1640–1650	r	Random coils (C = O to form hydrogen bonds with water)
1651–1660	α	α -helix
1661–1700	t	β -turn

elasticity and chewiness initially increase, followed by a subsequent decrease. Other sensory attributes exhibited an initial decline followed by a subsequent increase. At 1.5% C-SDF, the elasticity, internal structure, chewiness, and adhesiveness of the steamed bread closely resembled those of the control bread with 0% C-SDF. When C-SDF surpasses 1.5%, sensory scores for various attributes declined sharply, presumably due to the swelling and space formation induced by C-SDF, which hinders effective gluten stretching (BeMiller, 2011; Zhou *et al.*, 2021). Excessive C-SDF dilutes gluten protein, reducing certain quality attributes (Han, 2023). Incorporating C-SDF reduces the overall sensory score, especially when the C-SDF content is between 1.5% and 2%, with the most pronounced decrease observed at these concentrations. Based on the findings of this study, it can be concluded that the optimal addition of C-SDF for steamed bread production is 1.5%.

Conclusion

The incorporation of C-SDF significantly influences dough properties and steamed bread quality. With increased C-SDF content, the WHC, SC, and WSI of the mixed flour initially rise before declining. The syneresis rate exhibits a decreasing trend, whereas the wet and dry gluten content of the mixed flour initially increased before subsequently decreasing. In the mixed dough, both the storage modulus (G') and loss modulus (G'') exhibited a sharp increase of around 60°C and reached maximum values at 80°C and 90°C, respectively. As shear stress increases, G' decreases sharply. Adding C-SDF helps stabilize the dough structure and prevents significant damage to the dough particles. It is recommended that C-SDF should not exceed 1.5% in the dough mixture. Over this level, the specific volume, elasticity, cohesiveness, and adhesiveness of the steamed bread decrease and the release of reducing sugars increase. The secondary structure of proteins and the gluten network formation are also affected. Based on these findings, adding up to 1.5% C-SDF is optimal for producing enhanced quality steamed bread. The findings from this study can serve as a foundation for further research on modifying C-SDF, expanding its applications, and

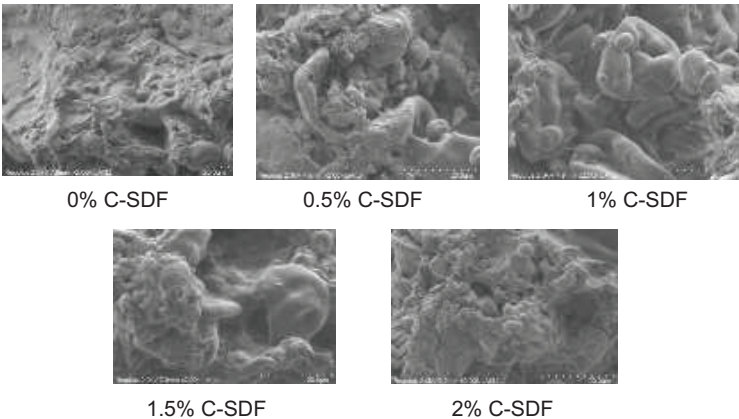


Figure 7. Microstructure of steamed bread supplemented with different amounts of C-SDF.

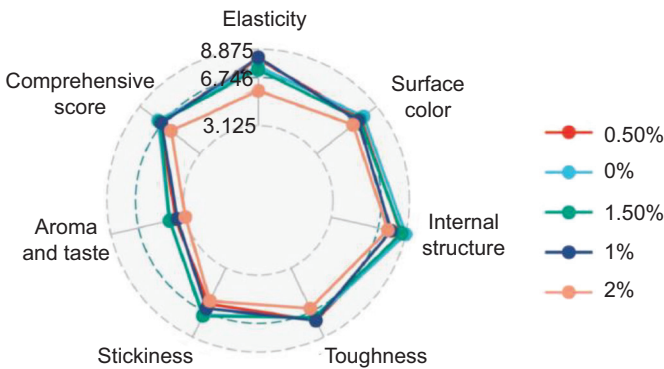


Figure 8. Effect of different amounts of C-SDF on sensory quality of steamed bread.

optimizing chia-based products to enhance their industrial value and nutritional benefits.

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Declaration of Competing Interest

The authors declare no conflict of interest, and this manuscript has not been submitted to any other journal in parallel or published previously.

Ethical Approval

This article contains no studies performed by authors with human participants or animals.

Informed Consent

Not applicable.

Data Availability

Data will be made available on request.

Author Contribution

All authors contributed equally in preparing, writing, and revising this manuscript.

Conflicts of Interest

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

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