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ORIGINAL PAPER

Modulating glycaemic response in steamed buns with chitosan: effects on bun properties and starch digestibility

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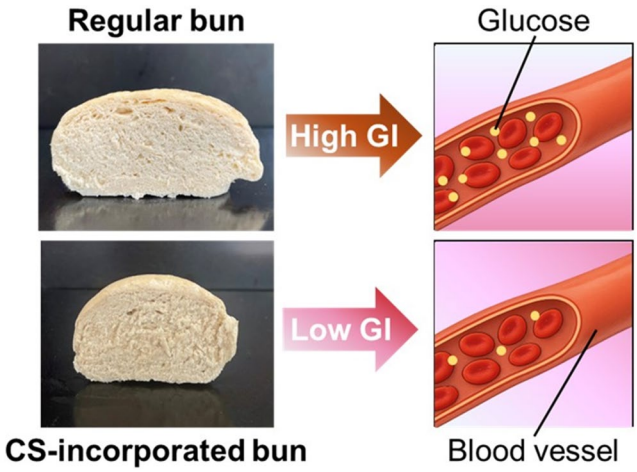
Abstract

Chitosan (CS) is a natural polysaccharide known for its antioxidant, antimicrobial, and non-toxic properties, and has been reported to lower blood glucose levels when orally administered prior to meals. Although widely utilized in industrial and biomedical contexts, its application in the development of low glycaemic index (GI) foods remains relatively unexplored. This study evaluates the potential of CS as a functional additive to reduce starch digestibility in starch-rich foods, using steamed buns as a model system. Incorporation of CS at 200 g/kg significantly reduced the area under the curve (AUC) of postprandial blood glucose response in participants consuming the CS-enriched bun (673.75 ± 101.7 mmol/L) compared to the control (747.0 ± 101.4 mmol/L). Sensory evaluation revealed modest declines in texture and taste scores; however, overall acceptability remained relatively high (5.7 ± 1.5 vs. 6.7 ± 1.6). CS addition also led to reductions in specific volume and fermented dough size, though SDS-PAGE analysis indicated no significant changes in gluten formation. Together with observed decreases in the hydrolysis index and estimated glycaemic index, these findings suggest that CS can effectively reduce starch digestibility while maintaining acceptable sensory quality in starch-rich food products.

Graphical Abstract

Chitosan (200 g/kg) in steamed buns reduced starch digestibility and postprandial glucose response, with modest impact on sensory quality, dough gluten structure, and overall bun acceptability



Keywords Chitosan · Steamed buns · Glycaemic index · Blood glucose · Food additive

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Introduction

Chitosan (CS), a natural polysaccharide consisting of D-glucosamine and N-acetyl-D-glucosamine units linked by β-1,4 glycosidic bonds, is derived from chitin [1, 2]. It exhibits antioxidant, antimicrobial, and non-toxic properties [3]. Numerous clinical and preclinical studies have highlighted the health benefits of CS, including its potential in preventing hypertension, dyslipidaemia, cancer, and inflammation [4, 5]. Several studies have also examined its antidiabetic effects, particularly its ability to lower blood glucose levels [6–8]. For example, a study by Kang and coworkers demonstrated an 11% reduction in blood glucose levels 30 min after bread consumption in participants who were administered 500 mg of CS oligosaccharide before eating [6]. A randomized controlled trial by Kim and coworkers also revealed that subjects with prediabetes or impaired fasting glucose levels who took 1500 mg of low-molecular-weight CS oligosaccharide daily for 12 weeks experienced significantly reduced postprandial glucose levels at 30 and 60 min post-meal compared to those receiving a placebo [7]. Additionally, Zhao and coworkers found that combining 100 mg/day of CS with 100 mg of the diabetes medication sitagliptin for 42 weeks resulted in better blood sugar control in diabetic patients compared to those who received only sitagliptin [8]. These findings are of practical importance given the global prevalence of hyperglycaemia and diabetes mellitus, which impose considerable health and economic burdens [9–11]. Managing postprandial blood glucose levels is a critical strategy in addressing this global health challenge [6]. Developing strategies to manage blood glucose levels through food products has, therefore, become an important focus in food product development.

While numerous studies have demonstrated the antidiabetic effects of CS, these have predominantly involved its administration as a dietary supplement in capsule or powder form, often isolated from real food systems [6–8]. Such approaches, while informative, do not account for the complex interactions between CS and food matrices, nor do they reflect typical consumption patterns. In contrast, the incorporation of CS directly into food products—particularly culturally significant, starch-rich staples like steamed buns—remains underexplored. This distinction is critical, as the functional performance of CS may differ when embedded within a food matrix, potentially influencing both its bioactivity and the sensory qualities of the final product. By investigating CS as a food ingredient rather than a supplement, this study offers a novel perspective that bridges nutritional science with practical food formulation, aiming to develop functional foods that are both effective and acceptable to consumers. The objective of this study is to investigate the potential of CS as a functional additive to control the postprandial blood glucose response induced by starch-rich food consumption. Steamed buns, which are typically made from refined wheat flour and have a high glycaemic index (GI) [12], are used as the model system in this study. Previous clinical research involving both healthy participants and individuals with diabetes has shown that steamed buns have a GI ranging between 65 and 97 [12, 13]. In clinical practice in China, steamed buns are frequently utilized as a reference food for blood glucose testing [12]. This further supports their suitability for this study. By evaluating both the GI and the physical characteristics of CS-enriched buns, this research provides a comprehensive assessment of CS's applicability in real-world food systems. The findings could inform the development of functional foods designed to support glycaemic control without compromising consumer acceptability.

Materials and methods

Reagents and materials

Tris-HCl (1.5 mol/L, pH 8.8), sodium dodecyl sulphate (SDS), acrylamide/bisacrylamide solution (300 g/L), dinitrosalicylic acid, ammonium persulfate solution (100 g/L), tetramethylethylenediamine (TEMED), Coomassie Brilliant Blue R-250, trypsin (EC 3.4.21.4, 1.7×10 –4 kat/mg, measured using N-Benzoyl-L-arginine ethyl ester as a substrate), pepsin (EC 3.4.23.1, 5.8×10^{-5} kat/mg), amyloglucosidase (EC 3.2.1.3, 7.51×10^{-7} kat/mg), hydrochloric acid (0.05 mol/L), sodium acetate buffer (0.5 mol/L, pH 5.2), propanol, potassium sodium tartrate solution (400 g/L), phenol, sodium sulphite, sodium hydroxide, glycerol, bromophenol blue, dithiothreitol and CS [Mn ≈ 250 kDa-350 kDa; degree of deacetylation = 90%; viscosity of its 2% (w/v) aqueous solution at ambient conditions = 100–200 mPa·s] were purchased from Macklin (Shanghai China). All reagents were of analytical reagent grade and were used as provided unless otherwise stated. Food-grade CS (Mn ≈ 250 kDa-350 kDa; degree of deacetylation = 90%, bulk density = > 0.6 g/mL, and viscosity [measured by using the 2% (w/v) aqueous solution at ambient conditions] = 100–200 mPa·s) was obtained from Oxford Vitality (Oxfordshire, UK). Wheat flour was sourced from Yihai Wheat Industry Co., Ltd. (Zhengzhou, China). The manufacturer provided the following composition details: 110 g/kg of protein, 16 g/kg of fat, 735 g/kg of carbohydrate, with the energy being 14,160 kJ/kg. Dry yeast (*Saccharomyces cerevisiae*) was obtained from Angie's Yeast Co., Ltd. (Yichang, China).

Preparation of dough and steamed buns

A 60 g blend of wheat flour and CS was prepared by mixing the flour with CS at specified concentrations. Dry yeast (1.8 g) was dissolved in 25 mL of distilled water preheated to 40 °C and left to activate at room temperature for 10 min. Once activated, the yeast solution was added to the flour-CS mixture, followed by the gradual addition of water to achieve a workable dough consistency. The dough was manually kneaded for 3 min and then fermented at 37 °C for 40 min in a controlled environment with 80% relative humidity. After fermentation, the dough was kneaded again for 1 min, shaped into buns, and proofed at 37 °C for an additional 15 min. Steaming was performed at 100 °C for 20 min using a bread maker (DL-TM018, Guangdong Dongling Electric Appliances Co., Ltd., Guangdong, China). The steamed buns were cooled at ambient temperature for 30 min prior to further analysis. All procedures were conducted in triplicate.

Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) analysis

Dough samples were initially frozen at − 80 °C for 48 h and subsequently subjected to freeze-drying for two days. The dried material was finely ground using a mortar and pestle. 50 mg of the resulting powder was dispersed in 1 mL of sample buffer, comprising Tris-HCl (0.050 mol/L, pH 6.8), SDS (20 g/L), glycerol (100 mL/L), bromophenol blue (0.050 g/L), and dithiothreitol (0.100 mol/L). The mixture was heated in a water bath at 100 °C for 5 min and centrifuged at 10,000 × g for 20 min. Subsequently, 15 µL of the supernatant was combined with 10 µL of loading dye and loaded onto a pre-cast polyacrylamide gel consisting of a 5% stacking gel and a 12% separating gel. Electrophoresis was performed at 80 V for 30 min, followed by 90 min at 100 V, according to the method described by Weegels, Hamer, and Schofield [14]. Protein bands were visualized using Coomassie Brilliant Blue R-250 staining and destained in methanol for 24 h.

Scanning electron microscopy (SEM)

The internal structures of the lyophilized dough samples were examined using a scanning electron microscope (Inspect F50, FEI, Hillsboro, USA) operating at an accelerating voltage of 10 kV and a magnification of 1000×. Prior to imaging, samples were coated with a thin layer of gold using a sputter coater.

Measurement of dough volume

Dough volume was measured in cubic centimetres, and the percentage increase in volume was used to evaluate expansion. Volume changes resulting from fermentation were estimated based on variations in dough diameter (d). The initial volume (V) was calculated using the standard equation for the volume of a sphere (Eq. 1). The percentage increase in dough volume (V_d) was subsequently determined at four different time intervals using Eq. 2, where V_i and V_f represent the initial and final volumes of the dough, respectively.

$$V = \frac{4}{3}\pi\left(\frac{d}{2}\right)^3 \tag{1}$$

$$V_d = \frac{V_f - V_i}{V_i} \times 100\% \tag{2}$$

Analysis of viscoelastic properties

The viscoelastic properties of the dough samples were evaluated using a Kinexus Pro+ rheometer (Malvern Instruments, Worcestershire, UK) to determine the storage modulus (G'), loss modulus (G''), and damping factor (tan δ), calculated as the ratio G''/G'. Rheological measurements were conducted at 25 °C over an angular frequency (ω) range of 0–60 rad/s. A parallel-plate geometry with a 20 mm diameter and a 1 mm gap was employed for all tests.

Analysis of bun microstructure and colour

To evaluate the internal structure, the steamed buns were sliced longitudinally through the centre, and the exposed cross-sections were examined using a stereomicroscope (SZ680, Chongqing Optec Instrument Co., Ltd., Chongqing, China). For colour analysis, a colorimeter (DS-620, Hangzhou CHNSpec Technology Co., Ltd., Hangzhou, China) was utilized, using Illuminant D65 and a 10° standard observer. The buns were maintained at room temperature during assessment. Colour readings were taken by placing the device on both the outer crust and the internal crumb. Each sample was measured in seven randomly selected spots on the crust and another seven on the crumb. Prior to measurement, the instrument was calibrated using black and white standard tiles. The L^*, a^*, and b^* values were recorded to represent lightness, red-green spectrum, and yellow-blue spectrum, respectively. Based on the L^*, a^* and b^* values, the whiteness index was calculated based the distance of the colour

values from a standard white point in the colour space as previously described (Eq. 3) [15]:

$$Whiteness\ index\ =\ 100-\sqrt{(100-L^*)^2+a^{*2}+b^{*2}} \quad (3)$$

Assessment of the hydrolysis index (HI) and estimated glycaemic index (eGI)

Trypsin (2.7 g, 0.459 kat) was dissolved in 30 mL of distilled water, thoroughly mixed, and centrifuged at 9,000 × g for 10 min. Following centrifugation, 27 mL of the supernatant was collected and combined with 1 mL of amyloglucosidase and 4 mL of distilled water to prepare the final enzyme blend. Separately, freeze-dried steamed bun samples were ground into a fine powder using a mortar and pestle. A 0.4 g portion of the powder was mixed with 6 mL of 0.05 mol/L hydrochloric acid containing 0.02 g (1.16×10^{-3} kat) of pepsin. The mixture was sonicated at 40 kHz and 37 °C for 30 min. After sonication, 12 mL of sodium acetate buffer (0.5 mol/L, pH 5.2) was added, followed by the addition of 2 mL of the prepared enzyme blend one minute later. The reaction was maintained at 37 °C.

At predetermined time points, 50 µL aliquots were withdrawn and immediately mixed with 450 µL of propanol and 0.5 mL of DNS reagent. The DNS reagent was prepared by dissolving 1.5 g of dinitrosalicylic acid, 0.3 g of phenol, 0.075 g of sodium sulphite, and 1.5 g of sodium hydroxide in 150 mL of distilled water. After reagent addition, samples were heated at 90 °C for 10 min. Subsequently, 0.167 mL of potassium sodium tartrate solution (400 g/L) was added to stabilize the colour. The mixtures were cooled to room temperature, and absorbance was measured at 575 nm using a UV-visible spectrophotometer. The rate of starch hydrolysis, hydrolysis index (HI), and estimated glycaemic index (eGI) were calculated as previously described [16]. All eGI measurements were performed in triplicate to ensure reproducibility.

Sensory evaluation

A total of 40 untrained volunteers (29 females, 11 males; mean age: 24.7 ± 1.9 years; BMI: 22.9 ± 5.9 kg/m²) from the School of Food Science and Nutrition at the University of Leeds were recruited via convenience sampling to participate in the sensory evaluation of regular and CS-incorporated steamed buns. The CS content in the flour–CS mixtures used to prepare the buns was 0 g/kg for the control and 200 g/kg for the CS formulation. Ethical approval for the study was obtained from the Faculty Research Ethics Committee for Business, Environment, and Social Sciences (Reference No. 2425). All participants received detailed information about the sensory evaluation procedures, their role in the study, and their right to withdraw at any time without providing a reason. The buns were prepared and frozen one day prior to evaluation, and re-steamed one hour before the tasting session. Each participant received one regular bun and one CS-incorporated bun. Sensory attributes—including appearance, colour, aroma, texture, taste, and overall acceptability—were assessed using a 9-point hedonic scale, where 1 indicated "extremely dislike" and 9 indicated "extremely like."

Measurement of blood glucose response

The glycaemic response was recorded in mmol/L. Six healthy volunteers (4 females, 2 males; mean age: 24.0 ± 1.4 years; BMI: 23.1 ± 3.8 kg/m²; fasting glucose < 6.1 mmol/L) were recruited via convenience sampling from the School of Food Science and Nutrition at the University of Leeds. Participants monitored fasting and postprandial blood glucose levels over a two-hour period. Ethical approval for the study was granted by the Faculty Research Ethics Committee for Business, Environment, and Social Sciences at the University of Leeds (Reference No. 2425).

All participants received detailed information about the study, including their role and the right to withdraw at any time without providing a reason. On the day prior to testing, participants were instructed to fast for 8–12 h and avoid strenuous exercise and alcohol. A fasting blood glucose measurement was taken at time 0, followed by consumption of a 30 g portion of either the regular bun or the CS-incorporated bun within 10 min. The CS content in the flour–CS mixtures used to prepare the buns was 0 g/kg for the control and 200 g/kg for the CS formulation. Blood samples were subsequently collected at 15, 30, 45, 60, 90, and 120 min post-consumption. Glucose concentrations were measured using a portable glucometer (Accu-Chek Instant, Roche Diabetes Care Ltd., West Sussex, UK). A line graph was generated to illustrate changes in glucose concentration over time. This procedure was repeated three times for each participant to ensure reproducibility.

Statistical analysis

Unless otherwise stated, all experiments were conducted in triplicate using independent sample replicates. Results are presented as mean values accompanied by their respective standard deviations. Statistical analyses were performed using the Kruskal–Wallis test, followed by post hoc pairwise comparisons using Dunn's test with Benjamini–Hochberg (BH) adjustment to control the false discovery rate (FDR). The Wilcoxon signed-rank test was also applied

where appropriate. All analyses were conducted in R (version 4.4.2), with statistical significance defined as $p < 0.05$.

Results and discussion

Effect of CS incorporation on gluten formation

Incorporation of CS into the dough formulation at concentrations up to 300 g/kg did not result in significant changes to the gluten protein bands, as revealed by SDS-PAGE analysis (Fig. 1). The consistent banding patterns for gliadin and glutenin across all CS-containing samples suggest that CS does not alter the molecular structure or composition of gluten proteins. This is expected, as CS is not a protease and therefore does not cleave or chemically modify gluten proteins in a way that would affect their electrophoretic mobility. Although CS can influence the rheological and hydration properties of dough [17], these effects do not typically manifest as structural changes detectable by SDS-PAGE. The observed stability of gluten protein bands supports the hypothesis that CS's impact is more likely related to physical interactions within the dough matrix rather than direct modification of gluten proteins.

Despite the lack of observable changes in gluten protein profiles, dough expansion during fermentation was negatively affected by CS incorporation. Dough prepared with

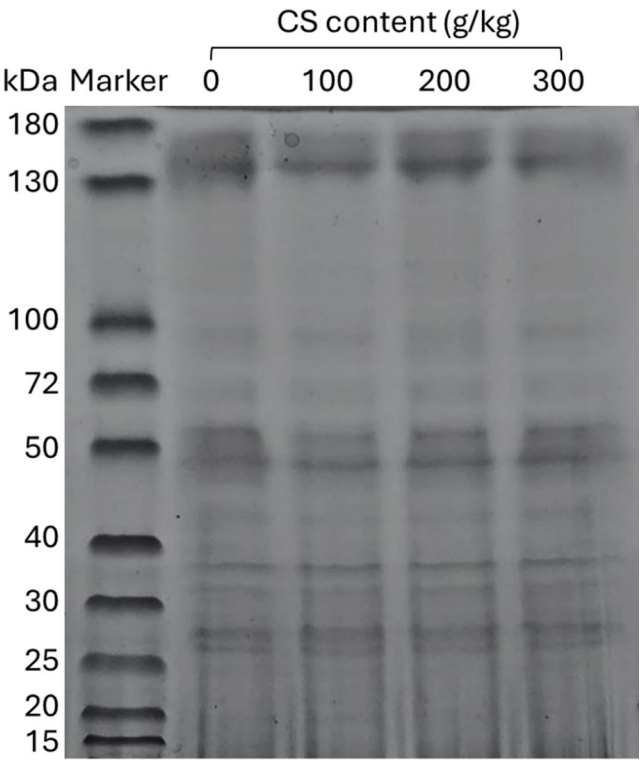

Fig. 1 SDS-PAGE patterns of dough made from flour–CS mixtures with different CS contents

200 g/kg CS expanded to only 1.37 ± 0.17 times its original volume after 40 min of fermentation, compared to a 1.98 ± 0.23-fold increase in the control dough (Fig. 2). This reduction in expansion also translated to a decrease in the specific volume of the final steamed buns (Fig. 3), which dropped from 0.0040 ± 0.000024 m^3/kg in the control to 0.0018 ± 0.000059 m^3/kg in the CS-enriched formulation. Similar reductions in specific volume have been reported in previous studies involving fungal-derived CS in bread [18], indicating a consistent effect on loaf expansion. However, another study has shown that when CS is added below a certain threshold, no significant differences in loaf weight or volume are observed [19]. At higher concentrations, volume reduction becomes more pronounced, suggesting that CS interacts with the dough matrix in ways that extend beyond gluten interference [19].

Although SDS-PAGE analysis did not reveal structural changes in gluten proteins in our current study, the rheological properties of the dough could still be affected by CS incorporation [17- 20]. CS is a hydrophilic polysaccharide capable of binding water [21], which can alter the dough's water retention capacity [22]. Previous research has shown that increasing levels of fungal CS in bread are associated with reduced moisture loss [18]. However, in complex food systems such as bread, CS's influence on water dynamics is multifaceted. CS and its oligosaccharides can modify water distribution and mobility within both the crust and crumb, affecting microstructure and functional properties [23]. By competing with starch and gluten for water, CS promotes relative dehydration of these matrices, driving moisture migration from crumb to crust. This shift influences Maillard reactions and accelerates staling [23]. Supporting this, earlier studies have observed faster reductions in freezable and total water in CS-treated crumbs during storage [24].

Molecular weight also plays a role in modulating these effects. Low-molecular-weight CS and CS oligosaccharides tend to cause less crumb firming than medium-molecular-weight CS, likely due to their preferential adsorption onto starch surfaces, which inhibits amylose–lipid complex formation during staling [23]. These findings may explain discrepancies in reported moisture content and texture outcomes across studies. In the present study, higher concentrations of CS appeared to increase water binding, resulting in a denser dough structure that restricted gas retention during fermentation. Additionally, interactions between CS and the gluten network may have contributed to increased dough rigidity, as evidenced by elevated G' and G'' values, which in turn reduced gas trapping efficiency and overall volume during both fermentation and steaming. These changes in viscoelastic properties are further explored in the following section.

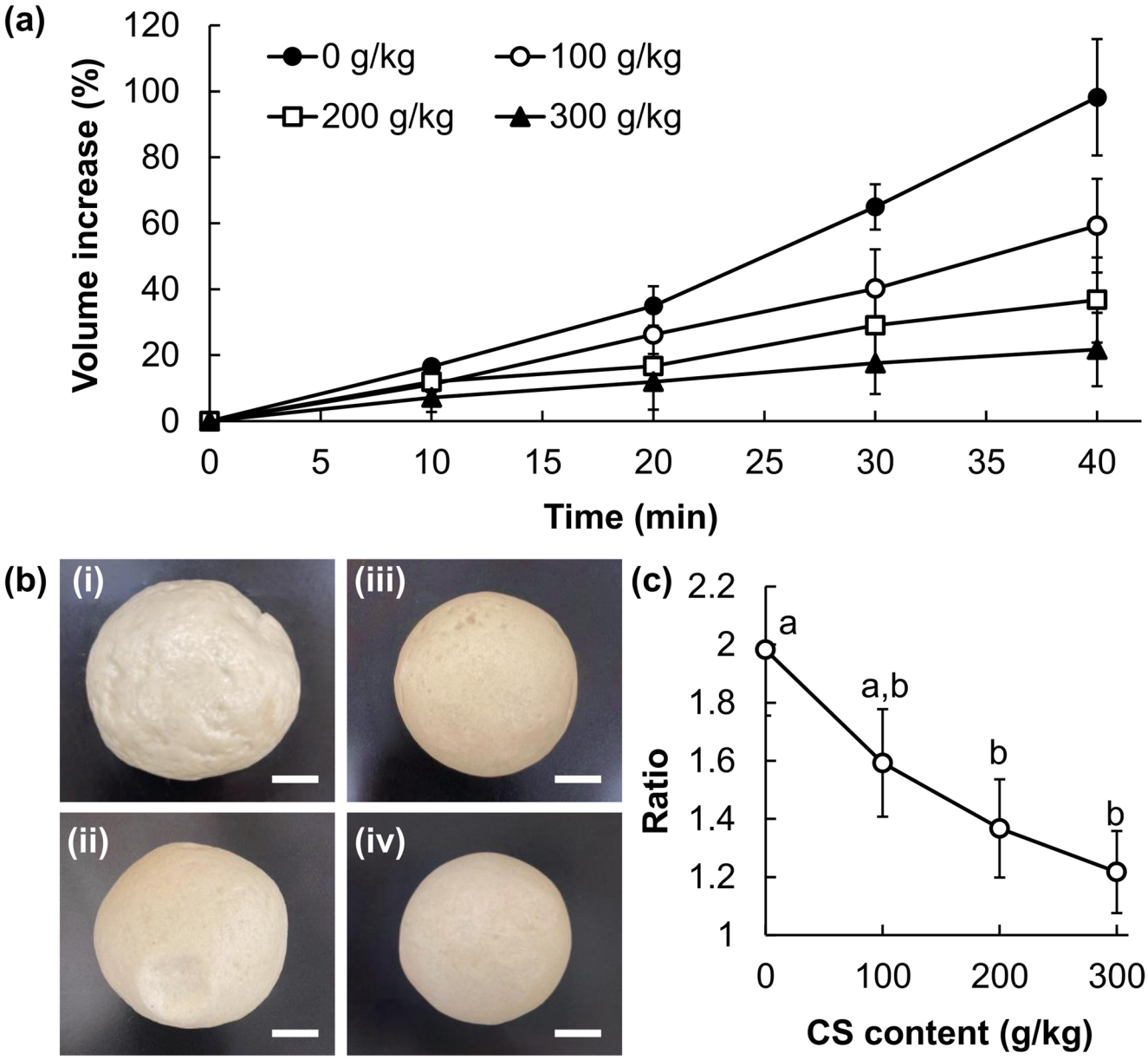

Fig. 2 (**a**) Changes in dough volume over time with different CS contents, relative to the initial volume before fermentation. (**b**) Photographs of doughs made from flour–CS mixtures with different CS contents: (i) 0 g/kg, (ii) 100 g/kg, (iii) 200 g/kg and (iv) 300 g/kg. The scale bar represents 2 cm. (**c**) The ratio of the volumes of fermented CS-incorporated doughs to their pre-fermentation volumes as a function of CS content in the flour-CS mixture used for bread making. The Kruskal-Wallis test conducted on all groups yielded a *p*-value of 0.03. A post-hoc Dunn's test with BH correction was performed. Data points assigned with different lowercase letters are significantly different (adjusted $p < 0.05$)

Viscoelastic properties of the dough after CS incorporation

The incorporation of CS into the dough formulation resulted in notable changes in its rheological behavior (Fig. 4). Rheological analysis showed that both *G'* and *G"* increased progressively with higher levels of CS addition. *G'* reflects the elastic or solid-like behavior of the dough, while *G"* corresponds to its viscous or liquid-like response. The concurrent increase in both moduli indicates that CS enhances the structural integrity of the dough, contributing to greater resistance to deformation and improved energy storage under oscillatory shear. This suggests that the dough becomes increasingly rigid and less prone to flow as CS concentration rises. Furthermore, the damping factor (*tan δ*), calculated as the ratio of *G"* to *G'*, remained consistently below 1 across all tested formulations, indicating that elastic behavior predominated over viscous behavior in

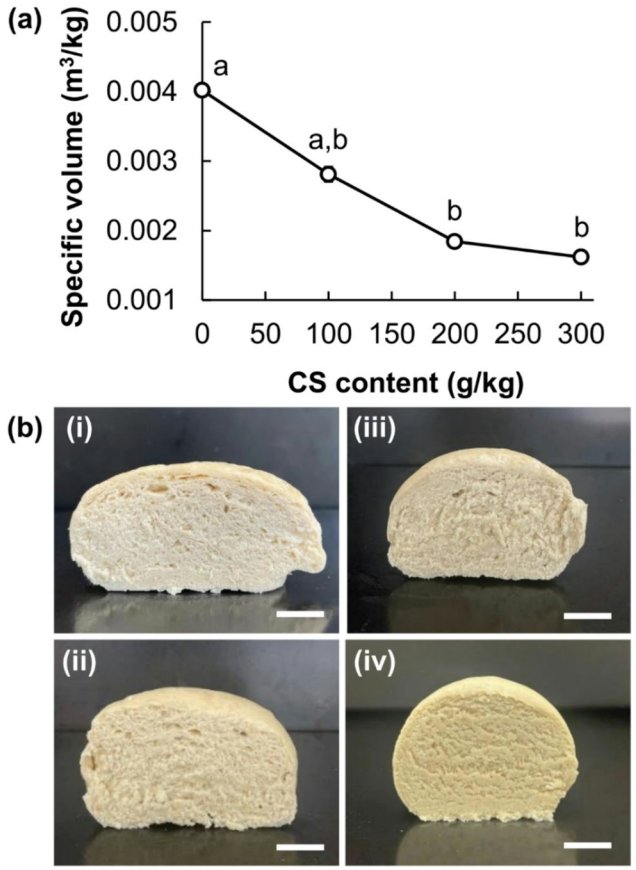

Fig. 3 (**a**) The specific volumes of the buns as a function of CS content in the flour-CS mixture used for bread making. The Kruskal-Wallis test conducted on all groups yielded a *p*-value of 0.02. A post-hoc Dunn's test with BH correction was performed. Data points assigned with different lowercase letters are significantly different (adjusted $p < 0.05$). (**b**) Photographs of steamed buns made from flour–CS mixtures with different CS contents: (i) 0 g/kg, (ii) 100 g/kg, (iii) 200 g/kg and (iv) 300 g/kg. The scale bar represents 2 cm

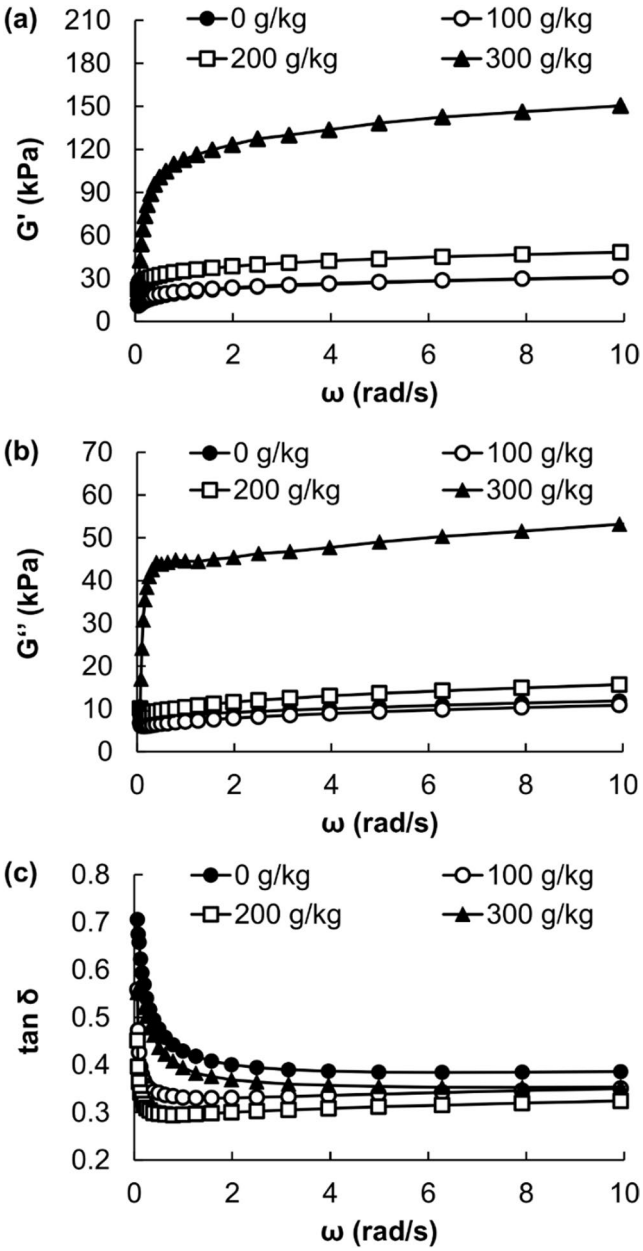

Fig. 4 Variation in (**a**) *G'*, (**b**) *G''* and (**c**) *tan δ* with angular frequency in doughs with varying CS content in the flour-CS mixture used for dough making

CS-containing dough systems. This trend reflects the formation of a more solid-like viscoelastic network, characteristic of strong gel-like materials.

The observed rheological enhancement can be attributed to the unique properties of CS. As a linear polysaccharide composed partly of β-(1→4)-linked D-glucosamine units, CS exhibits high molecular weight and cationic characteristics in acidic to near-neutral pH conditions due to the protonation of its amino groups [1, 25]. These features facilitate extensive hydrogen bonding and electrostatic interactions with negatively charged components in the dough matrix, such as gluten proteins. Specifically, CS may interact with the carboxyl and hydroxyl groups of gluten and starch [26, 27], thereby reinforcing the polymeric network and limiting molecular mobility. Additionally, CS may act as a filler that occupies space within the gluten-starch matrix [28], further enhancing the structural integrity and viscoelastic response of the dough. The cumulative effect of these interactions likely explains the observed increase in *G'* and *G''* and the maintenance of *tan δ* values below unity.

Microstructures of the buns after CS incorporation

SEM was employed to examine microstructural differences in the crumb matrices of steamed buns prepared with varying CS concentrations (Fig. 5). No distinct or systematic differences were observed across the samples, suggesting that CS-induced modifications may occur at a larger structural scale or through molecular-level interactions not readily

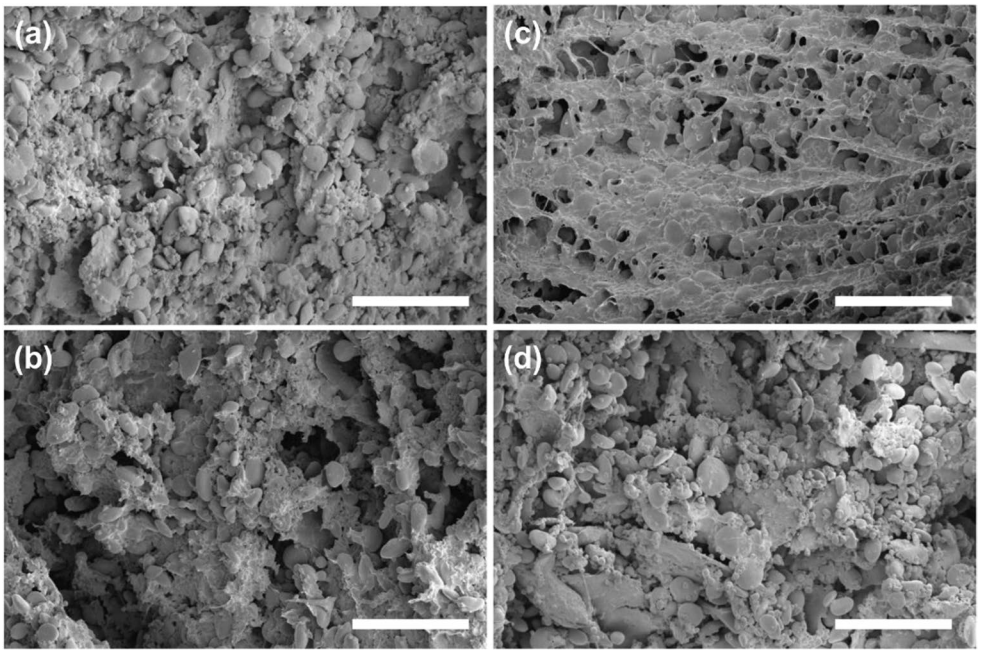

Fig. 5 SEM images of the cross-sections of the buns made from flour–CS mixtures with different CS contents: (**a**) 0 g/kg, (**b**) 100 g/kg, (**c**) 200 g/kg and (**d**) 300 g/kg. The scale bar represents 100 µm

detectable by SEM. Additionally, the high moisture content and soft, aerated texture of steamed buns present challenges for SEM sample preparation, potentially leading to surface artifacts or collapse that obscure subtle variations in pore wall morphology or starch-protein-CS interactions. To further investigate potential microstructural variations, stereomicroscopy was employed as a complementary technique (Fig. 6), offering a broader view of crumb architecture and enabling visual assessment of structural changes associated with CS incorporation.

Stereomicroscopic analysis of cross-sections of steamed buns containing varying levels of CS revealed notable differences in porosity and internal network homogeneity. As CS concentration increased, the crumb structure became

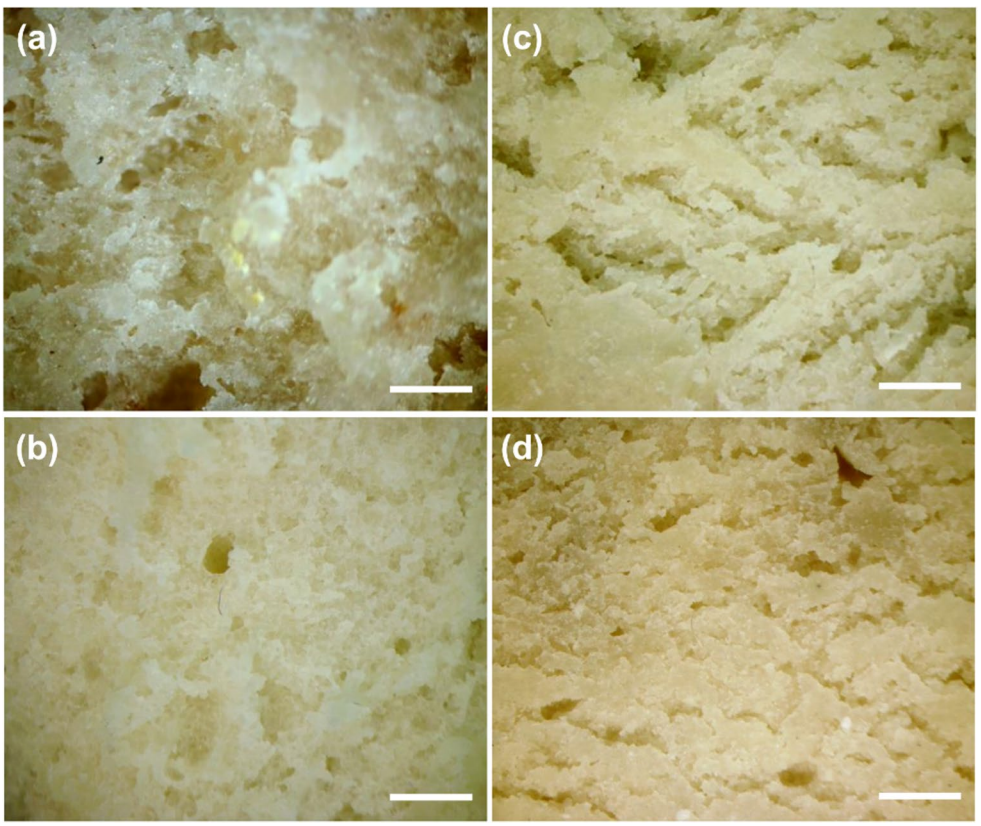

Fig. 6 Stereomicroscopic images of the cross-sections of the buns made from flour–CS mixtures with different CS contents: (**a**) 0 g/kg, (**b**) 100 g/kg, (**c**) 200 g/kg and (**d**) 300 g/kg. The scale bar represents 1 mm

progressively denser, characterized by reduced overall porosity and a more uniform distribution of smaller air cells. In contrast, control buns and those with lower CS levels exhibited a looser, more open crumb structure with larger and more irregular pores. This transition toward a finer and more cohesive internal architecture suggests that CS plays a significant role in modulating gas retention and expansion behavior during steaming [28]. The observed densification of the matrix may be attributed to CS's interaction with wheat flour components, particularly gluten and starch, forming a reinforced polymeric network that limits bubble coalescence and collapse [26, 27]. Additionally, the enhanced network homogeneity in CS-rich samples implies improved molecular compatibility between CS and native dough constituents. These microstructural observations are consistent with the rheological findings presented earlier, where increases in *G'* and *G"* indicated a stiffer and more elastic system. Collectively, the stereomicroscopic evidence supports the hypothesis that CS contributes to the formation of a more compact and mechanically stable dough structure, with potential implications for the texture and sensory quality of the final steamed buns, as discussed in subsequent sections.

Influence of CS incorporation on the colour of the buns

The visual appearance of food, especially its colour, plays a pivotal role in influencing consumer perception and acceptance, often serving as the initial criterion for evaluation prior to tasting or smelling [29, 30]. The CIELAB colour space offers a standardized method for quantifying food colour, with L^* denoting lightness, a^* representing the red–green axis, and b^* indicating the yellow–blue axis. Furthermore, the whiteness index was calculated by combining L^* with colour saturation and chromaticity (a^* and b^*) to provide a more complete picture of how white the bun is in comparison to pure white [31]. In this study, the incorporation of CS into steamed buns did not result in noticeable alterations to the colorimetric properties or whiteness indices of either the crumb or the crust until the CS content reached 200 g/kg or higher (Fig. 7). This was because CS itself is typically colourless or only slightly off-white. Its direct impact on the colour of the steamed buns would, therefore, not be evident at low CS concentrations.

As the CS content reached 200 g/kg or higher, the colour of CS in the bun became more pronounced. The cumulative effects of CS on the dough structure also became more apparent, influencing factors such as moisture distribution, crust formation, and the extent of Maillard reactions during steaming [28, 32]. These changes led to statistically significant alterations in the colour of both the crumb and the crust of the generated buns. In particular, a statistically significant decrease in the L^* value and whiteness index of the bun

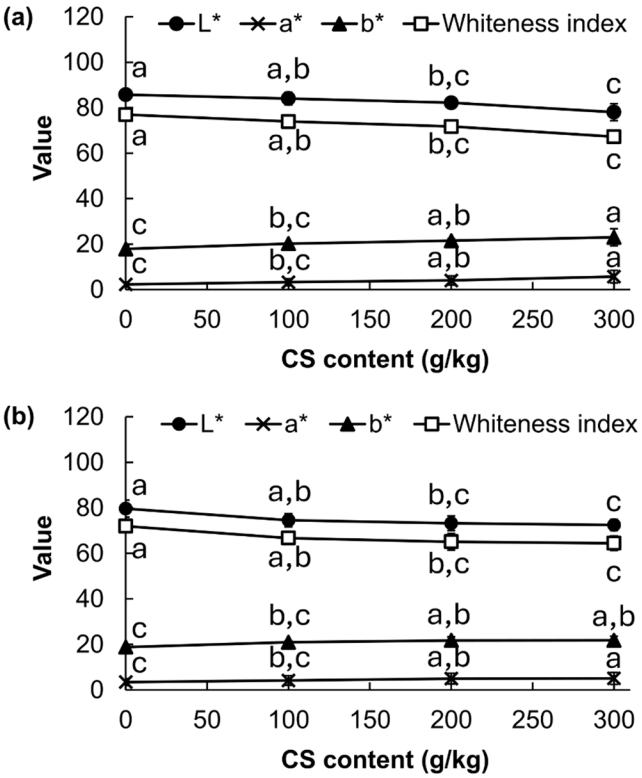

Fig. 7 Variations in the L^*, a^*, b^* values and whiteness indices of the (**a**) crust and (**b**) crumb of steamed buns as a function of CS content in the flour-CS mixture used for bread making. A Kruskal-Wallis test was conducted to compare the L^*, a^*, b^* values and whiteness indices of the crust and those of the crumb across all groups, yielding respective p-values of 0.001, 0.005, 0.001, 0.0001 for the crust and 0.003, 0.01, 0.004, 0.002 for the crumb. A post-hoc Dunn's test with BH correction was performed. Data points assigned with different lowercase letters along the same curve are significantly different (adjusted $p < 0.05$)

crust was observed, indicating a reduction in lightness and whiteness. Conversely, high CS concentrations resulted in a significant rise in the a^* and b^* values, suggesting a shift in crust color toward the red and yellow spectrum. Similar trends were observed in the crumb colour measurements. When CS content reached 200 g/kg and higher, a statistically significant decrease in the L^* value was noted, while the a^* and b^* values of the bun increased. These observations were consistent with those reported in earlier studies. For instance, Lafarga and coworkers reported similar darkening in CS-incorporated bread [33]. CS incorporation was also reported to lead to a decline in the whiteness of flat rice noodles [34]. Likewise, Kim and Han [35] found that the addition of CS led to an increase in yellowness in tofu.

Impact of CS incorporation on HI and eGI values of the buns

No statistically significant differences in HI or eGI were observed when CS was incorporated at levels of 200 g/kg or

below in the flour–CS mixture used for steamed bun preparation (Fig. 8). However, a significant reduction in both HI and eGI values was detected when the CS content reached 300 g/kg. One plausible explanation for this reduction is the inhibitory effect of CS on α-glucosidase, a key enzyme involved in intestinal starch hydrolysis [4]. By inhibiting α-glucosidase, CS slows the breakdown of disaccharides into oligosaccharides and monosaccharides, thereby delaying carbohydrate digestion and glucose release [9]. This mechanism reduces glucose absorption into the bloodstream and lowers postprandial blood glucose levels [9].

Supporting evidence comes from a previous study in which three CS oligosaccharide samples of varying molecular weights were evaluated in vitro for their inhibitory activity against rat small intestinal α-glucosidase and porcine pancreatic α-amylase [36]. All samples demonstrated comparable inhibition of both enzymes. In an in vivo experiment, Sprague–Dawley rats administered 0.1 g/kg of CS oligosaccharides prior to a sucrose loading test exhibited reduced postprandial blood glucose levels compared to controls [36]. Notably, CS oligosaccharides with a molecular weight below 1000 Da significantly lowered the glucose peak (C_{max}: 152 mg/dL vs. 193 mg/dL in control) and reduced the area under the blood glucose–time curve (AUC: 262 h·mg/dL vs. 305 h·mg/dL in control) [36]. Additionally, the time to peak plasma glucose concentration (T_{max}) was delayed (0.9 h vs. 0.5 h in control), indicating a slower glycaemic response [36]. More recently, an in vitro study demonstrated that 10 mg/mL of CS oligosaccharide inhibited α-glucosidase activity by 40% compared to the control group [9].

Another proposed mechanism for the reduction in HI and eGI is the ability of CS to form hydrogen and covalent bonds with amylose molecules, creating a protective film around starch granules [22]. This coating reduces the granules' susceptibility to enzymatic hydrolysis [22]. The formation of CS–starch networks and aggregation has been observed via SEM imaging in previous studies [37]. Furthermore, the tight structure formed by CS–starch interactions may hinder starch swelling and leaching during gelatinization, limiting water penetration into the granules [37]. Reduced starch leaching results in fewer digestible carbohydrates being available, thereby contributing to a lower glycaemic response.

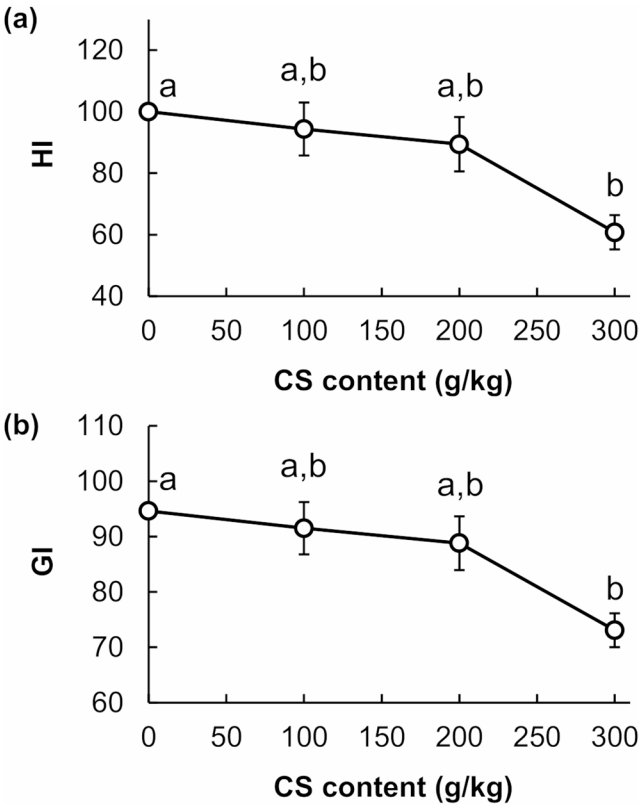

Fig. 8 (**a**) HI and (**b**) eGI of steamed buns as a function of CS content in the flour-CS mixture used to make the buns. A Kruskal-Wallis test was conducted to compare the HI and eGI across all groups, yielding respective *p*-values of 0.02 and 0.02. For each Kruskal-Wallis test, post-hoc pairwise comparisons were conducted using Dunn's test with BH correction. Data points assigned with different lowercase letters are significantly different (adjusted $p < 0.05$)

Postprandial blood glucose response after bun consumption

Despite the statistically significant reduction in the HI and eGI values of the steamed buns was observed only when the CS content reached 300 g/kg, the average postprandial blood glucose level of the six subjects was consistently lower after consuming a CS-incorporated bun (CB), which was made with a flour-CS mixture containing just 200 g/kg of CS, compared to the control group, which consumed a regular bun (RB) made without CS (0 g/kg) (Fig. 9). The peak postprandial blood glucose level after consuming the regular bun occurred at 60 min post-consumption. At this point, the postprandial blood glucose level following the consumption of the CS-incorporated bun was already lower (5.60 ± 0.9 mmol/L) compared to the control (7.0 ± 1.2 mmol/L). Although the peak postprandial blood glucose level for the CS-incorporated bun occurred earlier, at 30 min, compared to the group consuming the regular bun, the postprandial blood glucose level at this time point (6.03 ± 1.1 mmol/L) was still lower than that of the control group at the same interval (6.10 ± 0.6 mmol/L). It is worth noting that, while the difference in postprandial blood glucose levels between the two groups at 30 min was not statistically significant, the average fasting blood glucose level of the subjects was slightly higher on the day of testing on the CS-incorporated bun.

In addition to the findings described above, a reduction in blood glucose levels was observed at 45, 90, and 120 min

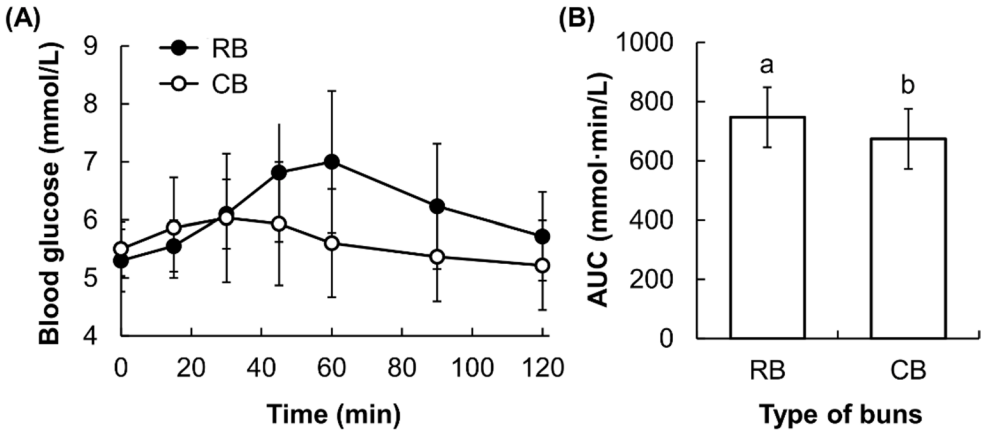

Fig. 9 (**A**) Blood glucose response of participants after consuming the regular bun and CS-incorporated bun. (**B**) AUCs of postprandial blood glucose response over 120 min for subjects consuming the regular bun and CS-incorporated bun. A paired Wilcoxon signed-rank test was conducted to compare the AUCs, revealing a statistically significant difference between the two groups ($V=21$, $p=0.0355$). Data points labelled with different lowercase letters indicate significant differences

following consumption of the CS-incorporated bun. Analysis of the area under the curve (AUC) revealed that the CS-enriched bun resulted in a significantly lower cumulative postprandial blood glucose level over 120 min (673.75±101.7 mmol/L) compared to the control bun (747.0±101.4 mmol/L). Notably, at 120 min post-consumption, the average blood glucose level in the CS group had returned to baseline (i.e., fasting levels), whereas the control group remained slightly elevated. These results clearly demonstrate that CS incorporation can effectively modulate glucose release and significantly attenuate postprandial glycaemic response. Importantly, this glycaemic-lowering effect was achieved with a CS concentration of 200 g/kg, despite the absence of statistically significant changes in HI and eGI at this level. This suggests that CS may exert its glucose-regulating effects through mechanisms not fully captured by in vitro digestion models. The observed discrepancy between the HI and eGI measured in vitro and the significant changes in postprandial blood glucose response in humans can be attributed to several factors that differentiate in vitro conditions from the physiological environment of the human body. In vitro GI and HI tests simulate aspects of digestion, primarily focusing on the rate of starch hydrolysis and glucose release [38]. However, these tests are limited as they do not fully replicate the complexities of the digestive system. Consequently, in vitro tests cannot fully account for the physiological processes that occur during digestion and glucose absorption in vivo. In fact, apart from inhibiting α-glucosidase and interacting with amylose to retard starch digestibility [39], CS, at a concentration of 200 g/kg, may affect the gastric emptying rate to slow the passage of food from the stomach to the small intestine [40]. This delayed gastric emptying can result in a slower absorption of glucose into the bloodstream, which may lead to a more gradual postprandial glucose rise and a reduced peak glucose concentration. Moreover, CS may influence intestinal motility and interact with gut microbiota [41–43], both of which can modify glucose metabolism in ways not reflected in the in vitro models. All these complex physiological interactions affect the rate at which glucose is absorbed into the bloodstream and its subsequent metabolism, contributing to changes in the postprandial blood glucose response. In contrast, in vitro GI and HI tests primarily focus on the rate of starch digestion and do not account for these broader factors influencing glucose dynamics.

Sensory evaluation of the steamed bun after CS incorporation

Incorporation of CS at a concentration of 200 g/kg led to changes in all sensory attributes (including appearance, colour, aroma, texture, and taste) of the steamed bun (Fig. 10). The average appearance scores for the CS-incorporated bun (6.3 ± 1.9) and the regular bun (6.9 ± 1.2) were

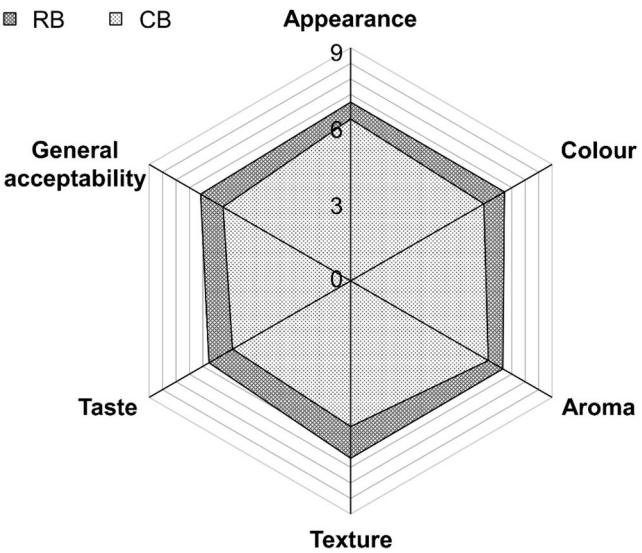

Fig. 10 Radar plot comparing sensory panel satisfaction scores for various sensory attributes of the regular bun and the CS-incorporated bun. The Wilcoxon signed-rank test was conducted to compare the appearance, color, aroma, texture, taste, and general acceptability between the regular bun and the CS-incorporated bun. The respective test statistics (V) were 257.5, 401, 329.5, 435.5, 486.5, 568, yielding raw p-values of 0.04, 0.0004, 0.01, 0.001, 0.005, and 0.0009. After applying the BH correction for multiple comparisons, the adjusted p-values were 0.04, 0.002, 0.02, 0.002, 0.007, and 0.002

statistically different, though the absolute difference was modest, suggesting a relatively similar level of visual acceptability. Colour, a key factor influencing consumer perception [33], also differed between the two samples. Consistent with instrumental colour measurements (L^*, a^*, b^*, and whiteness index), the CS-incorporated bun received a lower colour score (6.0 ± 1.7) compared to the regular bun (6.9 ± 1.4), likely due to panelists' expectations of a traditional white, smooth crust [44]. Aroma scores were also lower for the CS-incorporated bun, attributed to CS's subtle intrinsic odour, which was often described as earthy or marine and could interfere with the characteristic aroma of steamed buns [45]. Additionally, CS's film-forming ability [2, 46] and its interaction with volatile compounds may have limited the release of key aromatic components. Structural changes in the crumb, such as increased density and altered moisture retention, may have further inhibited aroma diffusion during consumption.

In the literature, studies on the sensory impact of CS have produced mixed results. One study reported minimal effects on taste, texture, and overall acceptability when fungal CS was added to bread, with crust colour being the primary attribute affected [18]. In contrast, other studies have shown that CS can increase firmness in bread crusts, hardness in goat sausages [17, 47], and density in barley noodles [22]. These discrepancies are likely due to differences in CS concentration and molecular weight. In the present study, buns containing CS exhibited reduced specific volume—a trait associated with increased internal density and altered texture [48]. This likely contributed to the lower texture score (5.6 ± 1.8) compared to the regular bun (6.9 ± 1.5). Taste scores also declined (from 6.3 ± 1.9 to 5.3 ± 1.9), possibly due to CS's characteristic astringency [49, 50]. Consequently, the overall acceptability score for the CS-incorporated bun (5.7 ± 1.5) was lower than that of the regular bun (6.7 ± 1.6). Although statistically significant, the absolute differences in scores were modest, suggesting that CS's impact on consumer acceptability may be moderate in practical terms.

Beyond sensory attributes, CS and chitooligosaccharides have demonstrated antimicrobial activity in food systems [51]. When used to replace flour in bread formulations, both compounds suppressed the growth of *Bacillus cereus* and *Rhizopus* spp., delaying odour development [51]. Chitooligosaccharides, due to their lower molecular weight and higher degree of deacetylation, were more soluble and exhibited stronger inhibitory effects against spoilage organisms [51]. Similarly, Lafarga and coworkers reported that CS (124,000 ± 10,000 g/mol; degree of deacetylation: 19%) inhibited *Bacillus cereus* growth and prevented rope spoilage in bread over a 3–5 day period [33]. At 1% flour substitution, CS significantly delayed mold development compared to control loaves, which showed visible mold within 72 h at 30 °C [33]. This preservative effect is likely due to CS's antimicrobial activity and its influence on water availability within the bread matrix.

In addition to shelf-life extension, CS has been shown to suppress the formation of advanced glycation end products (AGEs) [52]. In sponge cakes, CS reduced AGE levels while improving texture, moisture retention, and colour [52]. Specifically, levels of free Nε-carboxymethyllysine and Nε-carboxyethyllysine were significantly decreased [52]. Mechanistic studies suggest that CS inhibits AGE formation by limiting glycation substrates [52]. In particular, it interacts with proteins and competes with lysine and arginine residues for reducing sugars, thereby reducing protein oxidation and slowing dicarbonyl–protein reactions. Although the present study focuses on CS's role in modulating glycaemic response and the physical and sensory properties of steamed buns, these additional functionalities—antimicrobial activity and AGE inhibition—highlight CS as a promising multifunctional ingredient for bakery applications, with potential to enhance both nutritional quality and shelf-life.

Conclusion

This study investigated the potential of incorporating CS into steamed buns to reduce starch digestibility and lower postprandial blood glucose levels. The observed reductions in eGI, HI, and postprandial glycaemic response highlight CS's ability to modulate carbohydrate digestion in starch-rich foods. While previous studies have demonstrated the hypoglycaemic effects of CS in healthy, prediabetic, and diabetic individuals [6–8], these typically involved direct administration of CS as a supplement, either prior to or over the course of a test meal. In contrast, the present study evaluated CS as a functional food ingredient within a realistic dietary context, by incorporating it directly into steamed buns. Although higher concentrations of CS may induce more pronounced changes in dough and bun properties, the influence on product quality at 200 g/kg was modest. These findings contribute to the growing body of evidence supporting CS as a multifunctional ingredient for glycaemic control, and offer practical insights for the development of functional foods aimed at individuals at risk of or living with diabetes.

Author contributions **Wing-Fu Lai**: Writing – review & editing, Writing – original draft, Validation, Supervision, Investigation, Formal analysis. **Weijie Fang**: Writing – review & editing, Writing – original draft, Validation, Investigation, Formal analysis. **Miranda Sekarsari Dewi Herrakartika**: Writing – review & editing, Writing – original draft, Validation, Investigation, Formal analysis. **Sreekanth Reddy Obireddy**: Writing – review & editing, Validation, Visualization.

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Data availability Data supporting this study are included within the article.

Declarations

Conflict of interest The authors declare no conflicts of interest.

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