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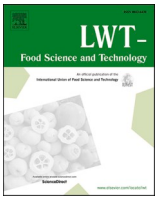

Seeds of *Coix lacryma-jobi* as a functional additive in steamed buns: Effects on bun quality and starch digestibility

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ABSTRACT

Seeds of *Coix lacryma-jobi* (SCL) are widely used as food and medicine in many Asian countries. This study examines the effects of SCL incorporation on steamed bun quality and starch digestibility. SDS-PAGE analysis suggests SCL disrupt gluten formation, reducing the buns' specific volume and gas-holding capacity. Furthermore, an increase in the SCL content of the buns results in a decrease in L^* values and an increase in a^* values. Despite these changes, the bun maintains acceptable general acceptability, scoring 4.8 ± 2.1 out of 10 for the bun containing 600 g/kg of SCL compared to 6.9 ± 1.7 for the one made entirely of flour. Moreover, increasing the SCL content of the buns from 200 g/kg to 800 g/kg reduces the hydrolysis index (HI) from 101.9 ± 3.9 to 74.6 ± 6.5 and the estimated glycaemic index (eGI) from 95.7 ± 2.1 to 80.7 ± 3.5. This decrease in HI and eGI is attributed, in part, to the increased protein and lipid content of the buns. These findings suggest that SCL incorporation warrants further investigation as a potential strategy for developing low-glycaemic-index, carbohydrate-rich products for consumers concerned about blood sugar levels.

1. Introduction

Coix lachryma-jobi (CL) is a grain-bearing perennial tropical plant belonging to the Poaceae family. It is commonly known as Job's tears, coix, Chinese pearl barley, and adlay (Devaraj et al., 2020). Indigenous to China, CL is widely cultivated across several Asian countries, including the Philippines, Burma, Sri Lanka, and Thailand (Chhabra & Gupta, 2015). In China, seeds of CL (SCL) are commonly used to make soups (Devaraj et al., 2020), while in Japan, they are utilized to produce "Dzu," a buttermilk- and cider-like flavoured liquid (Soni et al., 2023). SCL have a firm shell and a pear-shaped appearance (Chhabra & Gupta, 2015; Li & Corke, 1999). In terms of carbohydrate content, SCL has 610–830 g/kg of starch (Liu et al., 2016), and also contains non-starch carbohydrates such as fructooligosaccharides (Manosroi et al., 2014) and arabinoxylans (Yao et al., 2015). The non-starch polysaccharides in SCL have been shown to help lower blood cholesterol levels when consumed orally and may reduce the risk of cancer (Chhabra & Gupta, 2015; Wood, 2004).

CL has a higher content of essential amino acids than many other cereals (including rice) (Igbokwe et al., 2022). The primary protein components of SCL are prolamins, also known as coixin (Devaraj et al., 2020; Igbokwe et al., 2022; Liu et al., 2015). Some of the amino acids present in SCL are known to have antioxidant and anti-inflammatory properties (Weng et al., 2022; Zhu, 2017). In addition, SCL are rich in functional fats, including unsaturated fatty acids which are beneficial to cardiovascular health (Igbokwe et al., 2022). Examples of fatty acids present in SCL are oleic acid and linoleic acid (Omachi et al., 2024; Weng et al., 2022; Zhu, 2017). SCL also contain various essential minerals (including selenium, magnesium, phosphorus, sulphur, and potassium) (Liu et al., 2015), along with vitamin E (Choi et al., 2007) and β-carotene (Choi et al., 2007). It is a source of polyphenols [including flavonoids (such as apigenin, eriodictyol, formononetin, isoliquiritigenin, kaempferol, luteolin, naringenin, nobiletin, quercetin, rutin, and tangeretin), syringaresinol, 4-ketopinoresinol, syringic acid, coniferyl alcohol, and ferulic acid] (Huang et al., 2009, 2014), phytosterols (including β-sitosterol, campesterol, ergostanol, squalene, and tocopherol) (Wu et al., 2007), and policosanols (including docosanol, tetracosanol, and hexacosanol) (Wu et al., 2007).

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Table 1
Quantities of different ingredients added during the preparation of different SCL-incorporated buns.

Ingredients per steamed bun				
Bun	Mass of the SCL-flour mixture added (g)	Content of SCL in the SCL-flour mixture (g/kg)	Actual mass of yeast powder added (g)	Amount of water added (mL)
C00	60	0	0.20	35–42
C20	60	200	0.20	35–42
C40	60	400	0.20	35–42
C60	60	600	0.20	35–42
C80	60	800	0.20	35–42

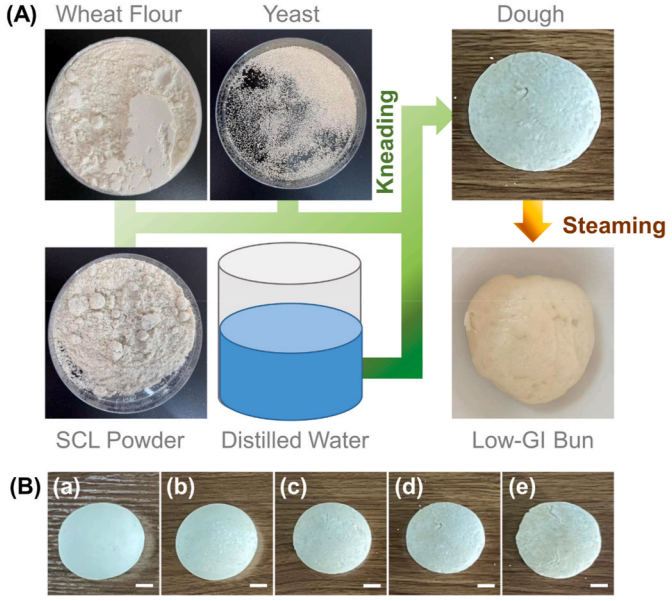

Fig. 1. Preparation of steamed buns with different SCL contents. (A) Schematic diagram showing the preparation of SCL-incorporated steamed buns. (B) Photos of (a) C00, (b) C20, (c) C40, (d) C60 and (e) C80. The scale bar represents 1 cm. Abbreviations: CSL, seeds of *Coix lacryma-jobi*; GI, glycaemic index.

In daily life, SCL are widely used as a cooking ingredient in Asia to enhance the nutritional value of foods. It is also a key ingredient in a porridge formulation—along with millets, Chinese yam rhizome, lotus seeds, and the fruit of *Ziziphus jujuba*—used to tackle spleen and stomach-related ailments (Yu et al., 2015). SCL contain coixans A, B and C, which have been reported to show hypoglycaemic effects as early as the 1980s (Takahashi et al., 1986). More recently, SCL have been administered orally to diabetic rats, followed by intravenous blood sampling and enzymatic analysis of total plasma cholesterol, with results indicating an improved lipid profile in diabetic rats (Cho & Lee, 1997). By fortifying yoghurt with SCL and comparing the glycaemic effect of the fortified yoghurt with the conventional one, SCL have been found to enhance high density lipoprotein (HDL) levels in type-2 diabetic patients (Djaja, 2022). Despite the diverse health-promoting effects of SCL mentioned above, so far SCL have been used mainly in folk medicine or as a food ingredient. Limited efforts have been directed toward exploring its potential as a functional food additive for modifying the properties and digestibility of carbohydrate-rich foods. This study aims to use steamed buns—commonly made from refined wheat flour and characterized by a high glycaemic index (GI)—as a model to examine the effects of SCL on starch digestion rates. Changes in the physical properties and sensory attributes of the SCL-incorporated buns will also be assessed to determine the potential of SCL as a functional additive for the development of carbohydrate-rich foods suitable for individuals who struggle with maintaining stable blood sugar levels.

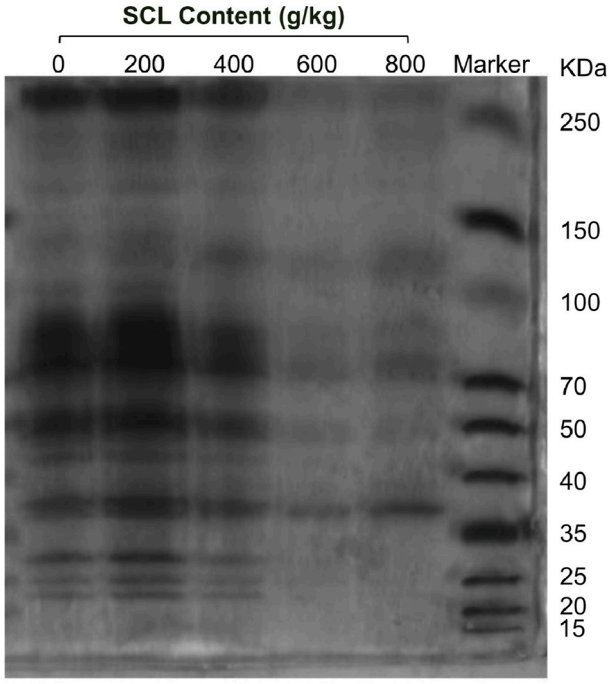

Fig. 2. Gluten formation in doughs with different SCL contents. SDS-PAGE patterns of dough samples containing different amounts of SCL powder. Abbreviation: SCL, seeds of *Coix lacryma-jobi*.

2. Materials and methods

2.1. Reagents and materials

Tris-HCl (1.5 mol/L, pH 8.8), sodium dodecyl sulphate, acrylamide/bisacrylamide solution (300 g/L), 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, dinitrosalicylic acid, phenol, sodium sulfite, sodium hydroxide, and potassium sodium tartrate solution (400 g/L) were purchased from Macklin (Shanghai China). All reagents were of analytical reagent grade and were used as provided unless otherwise stated. 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 (including 710 g/kg of starch, and 23 g/kg of dietary fibre), with the energy being 14,160 kJ/kg. Seeds of *Coix lacryma-jobi var. ma-yuen* (Rom. Caill.) Stapf., a variety of CL, were purchased from Guangdong Shizhen Pharmaceutical Co., Ltd. (Guangdong, China). The seeds contained 156 g/kg of protein, 56.6 g/kg of fat, and 726.6 g/kg of carbohydrate. Before processing, the seeds were thoroughly washed, then powdered using a laboratory-scale rotary pulverizer (800Y, Huangdai, Kunming, China) to obtain SCL powder with a size of 50–210 μm, with D10, D50 and D90 being determined by sieve analysis to be 70.45 ± 1.05 μm, 114.23 ± 2.21 μm and 189.72 ± 2.52 μm, respectively. Dry yeast (*Saccharomyces cerevisiae*) was obtained from Angie's Yeast Co., Ltd. (Yichang, China).

2.2. Dough and steamed bun preparation

A mixture (60 g) of wheat flour and SCL powder was prepared by

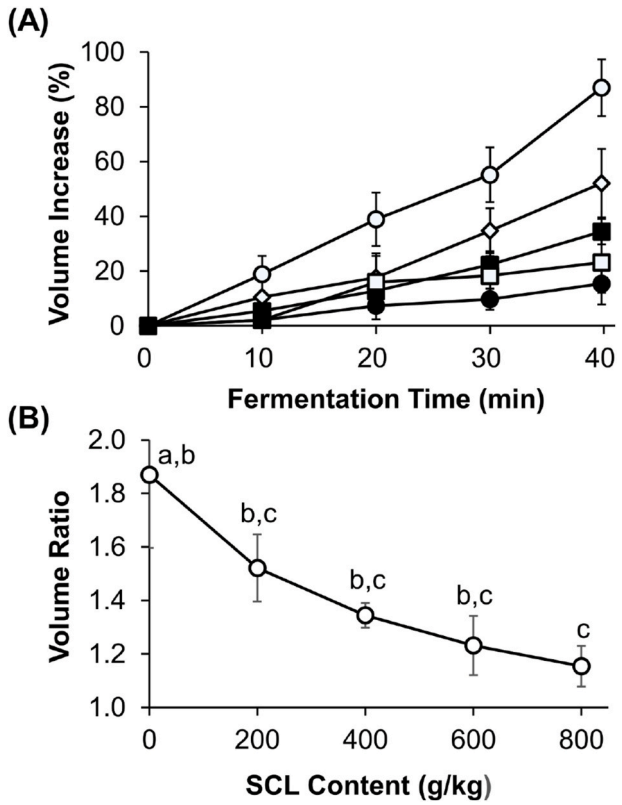

Fig. 3. Changes in dough volume with varying SCL contents. (A) Changes in the volume of SCL-incorporated doughs over time compared to their volume before fermentation. The SCL content in the SCL-flour mixture used to make the doughs designated by ◯, ⋄, ■, □, and ● was 0 g/kg, 200 g/kg, 400 g/kg, 600 g/kg and 800 g/kg, respectively. (B) The ratio of the volumes of fermented SCL-incorporated doughs to their pre-fermentation volumes as a function of SCL content in the flour-SCL mixture used for steamed bun preparation. The fermentation time was 40 min. The Kruskal-Wallis test conducted on all groups yielded a *p*-value of 0.018. A post-hoc Dunn's test with BH correction was performed. Groups assigned different lowercase letters are significantly different (adjusted $p < 0.025$, using $\alpha = 0.05$ and a two-tailed criterion). Abbreviation: CSL, seeds of *Coix lacryma-jobi*.

mixing wheat flour with the amount of SCL powder specified in Table 1. The dry yeast (0.2 g) was dispersed in distilled water (40 °C, 25 mL) and incubated at room temperature for 10 min. The dispersed dry yeast was mixed with the mixture of wheat flour and SCL powder, followed by the addition of 35–42 mL of water. The mixture was hand-kneaded for 3 min to form a dough, which was then fermented at 37 °C for 40 min at 80 % relative humidity. After proofing, the dough was hand-kneaded for 1 min, shaped and re-proofed at 37 °C for additional 15 min. The fully risen dough was steamed in a bread maker (DL-TM018, Guangdong Dongling Electric Appliances Co., Ltd., Guangdong, China) at 100 °C for 20 min and cooled at room temperature for 30 min before analysis. The steamed bun preparation was repeated three times.

2.3. Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE)

Dough samples were frozen at −80 °C for 48 h, lyophilized for two days, and ground into powder by using a pestle and mortar. After that, 50 mg of the sample powder was added to 1 mL of sample buffer, which was prepared by mixing 2 mL of Tris-HCl (1.5 mol/L, pH 8.8), 0.05 mL of a sodium dodecyl sulphate solution (100 g/L), 2 mL of a acrylamide/bisacrylamide solution (300 g/L), 0.05 mL of an ammonium persulfate solution (100 g/L), 0.002 mL of TEMED and 2.1 mL of distilled water. The suspension formed was heated in a water bath at 100 °C for 5 min and centrifuged at 10,000×*g* for 20 min. The supernatant (15 μL) was mixed with 10 μL of a loading buffer, followed by polyacrylamide gel electrophoresis conducted at 80 V for approximately 30 min and then at 100 V for additional 90 min as previously described (Weegels et al., 1995). Gels were stained with Coomassie Brilliant Blue R-250. After that, it was decolourised with methanol for 24 h.

2.4. Scanning electron microscopy (SEM)

Microstructures of lyophilized dough samples were visualised using a scanning electron microscope (SEM, Inspect F50, FEI, Hillsboro, USA) at an energy of 10 kV after being sputtered with gold.

2.5. Determination of the dough volume

The exact volume was expressed in cubic centimeters, and the increase in dough volume was represented as a percentage difference (%). Changes in dough volume before and after fermentation were estimated based on changes in diameter (d). The dough volume (V) was calculated using the sphere volume formula (1), while the final volume increase (V_d) of the dough was determined using Eq. (2), where V_f and V_i denote the final volume and initial volume of the dough, respectively.

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

$$V_d = \frac{V_f - V_i}{V_i} \times 100\% \quad (2)$$

2.6. Examination of the microstructure and colour of the buns

The steamed buns were sliced in half from the center, and the cut surface was placed under a stereomicroscope (SZ680, Chongqing Optec Instrument Co., Ltd., Chongqing, China) to determine the microstructure. To examine the colour of the buns, a colourimeter (DS-620, Hangzhou CHNSpec Technology Co., Ltd., Hangzhou, China) was utilized. In brief, the buns were placed at room temperature. The colourimeter was placed on the crusts and crumbs of the buns for testing. Four randomly selected areas from the crust and an additional four randomly selected areas from the crumb of each sample were analysed. Prior to use, the colourimeter was calibrated using white and black standard tiles. The colour parameters L^*, a^*, and b^* were used as a measure of brightness, red/green, and yellow/blue, respectively.

2.7. Texture profile analysis

The texture of the steamed buns was evaluated using a texture analyser (TA-XTC-20, Shanghai Baosheng Industrial Development Co., Ltd., Shanghai, China). The TA/LKB probe was used. A trigger force of 0.05 N was adopted during analysis. The pre-test, test and post-test rates set for sample compression were 1.0 mm/s, 1.0 mm/s, and 2.0 mm/s, respectively. The compression ratio was set as 50 %. The analysis provided measurements for hardness, cohesiveness, springiness, and chewiness.

2.8. Nutritional analysis

The nutritional composition of both SCL and SCL-incorporated buns was determined following the analytical procedures outlined in the AOAC International method (Latimer, 2023). Specifically, the protein content was measured using the Kjeldahl method (AOAC 2001.11). This involved block digestion with a KJELDATHERM block digestion unit and a 12–0779 Scrubber VACUSOG (Gerhardt, Koenigswinter, Germany), followed by steam distillation using a VAPODEST 200 (Gerhardt, Koenigswinter, Germany). The fat content was determined using the Soxhlet method (AOAC 922.06), employing a SOXTHERM and Classic Laboratory Heater (Gerhardt, Koenigswinter, Germany). Moisture content was

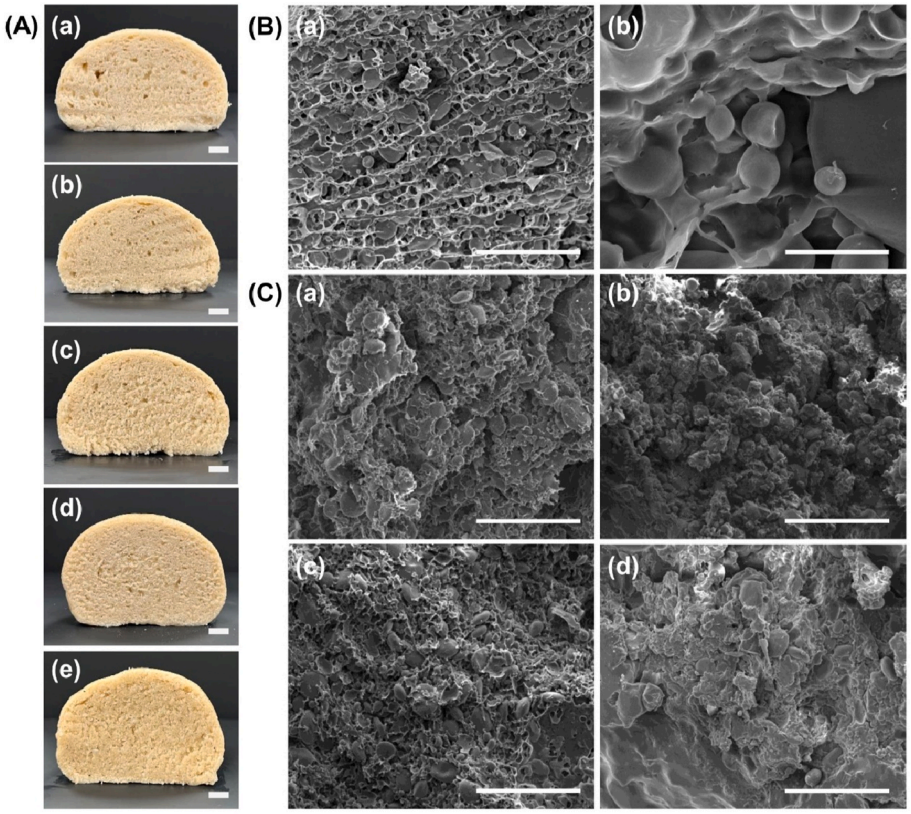

Fig. 4. Microstructures of steamed buns with different SCL contents. (A) Photos of the cross-sections of (a) C00, (b) C20, (c) C40, (d) C60, and (e) C80. The scale bar represents 1 cm. (B) (a) SEM micrograph of C00 and (b) a magnified view of its microstructure. The scale bar in (a) represents 100 μm; while that in (b) represents 10 μm. (B) SEM micrographs of (a) C20, (b) C40, (c) C60 and (d) C80. The scale bar represents 100 μm.

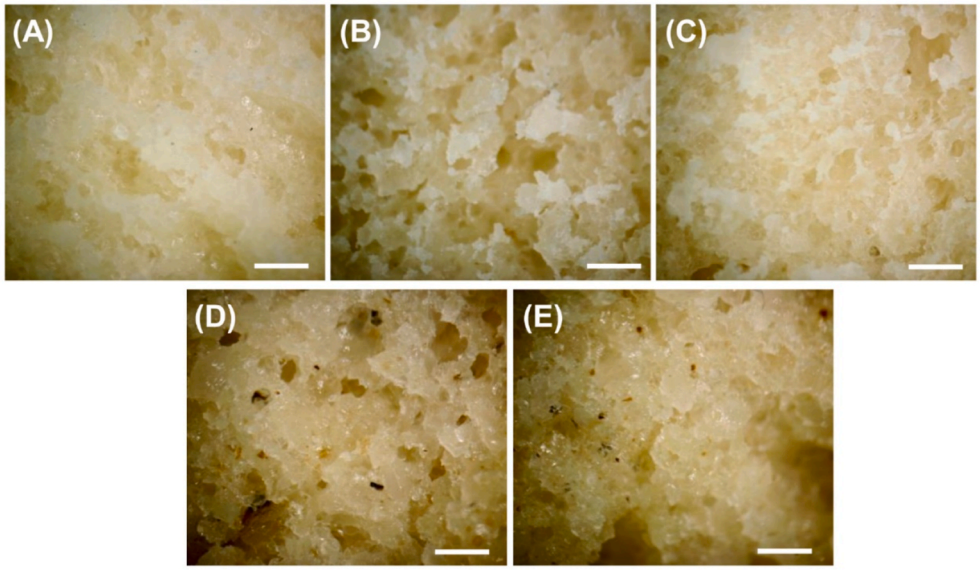

Fig. 5. Stereomicroscopic analysis of steamed buns with different SCL contents. Microscopic images of (A) C00, (B) C20, (C) C40, (D) C60, and (E) C80. The scale bar represents 1 mm.

assessed gravimetrically (AOAC 926.08), utilizing an oven (Memmert GmbH + Co. KG, Schwabach, Germany). Ash content was determined through an ashing method (AOAC 923.03), performed in a Phoenix microwave furnace (CEM Corporation, Matthews, NC, USA). The total starch content and total dietary fibre content were determined using the Starch Assay Kit (Supelco, Merck Life Science UK Limited, Dorset, UK) and the Total Dietary Fiber Assay Kit (Supelco, Merck Life Science UK Limited, Dorset, UK), respectively, which were based on the AOAC 996.11 method and the AOAC 985.29 method. Finally, the carbohydrate content was calculated by subtracting the sum of protein, fat, moisture, and ash content from the total weight of the sample (AOAC 978.10).

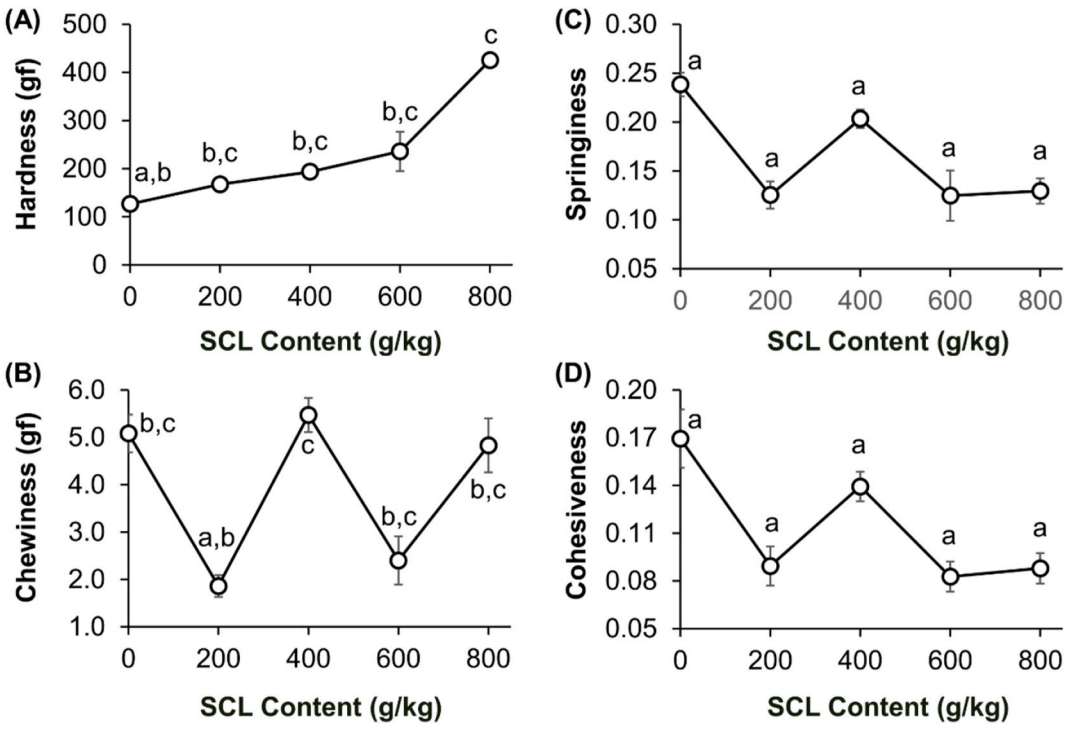

Fig. 6. Changes in the textural characteristics of steamed buns with varying SCL contents. (A) Hardness, (B) chewiness, (C) springiness, and (D) cohesiveness of steamed buns as a function of SCL content in the flour-SCL mixture used to make the buns. PERMANOVA using the Euclidean distance measure was performed to compare all groups, yielding a *p*-value of 0.001. A Kruskal-Wallis test was then conducted to compare hardness, chewiness, springiness, and cohesiveness of the buns, yielding respective *p*-values of 0.01, 0.02, 0.03, and 0.03. For each Kruskal-Wallis test, post-hoc pairwise comparisons were conducted using Dunn's test with BH correction. Groups assigned different lowercase letters are significantly different (adjusted $p < 0.025$). The post-hoc test identified significant pairwise differences for hardness and chewiness but did not identify significant differences for springiness and cohesiveness. Abbreviation: CSL, seeds of *Coix lacryma-jobi*.

2.9. Determination of in vitro GI values

An enzyme mixture was prepared by dissolving 2.7 g (0.459 kat) of trypsin in 30 mL of distilled water. This mixture was thoroughly mixed and subsequently centrifuged at 9000×*g* for 10 min. Following centrifugation, 27 mL of the supernatant was combined with 1 mL of an amyloglucosidase solution and 4 mL of distilled water to obtain the final enzyme mixture. Separately, a lyophilized steamed bun was ground into fine powder using a pestle and mortar. A 0.4 g portion of this powdered sample was dispersed in 6 mL of diluted hydrochloric acid (0.05 mol/L), in which 0.02 g (1.16×10^{-3} kat) of pepsin had been previously dissolved. This reaction mixture was sonicated at 40 kHz at 37 °C for 30 min. Following sonication, 12 mL of a sodium acetate buffer (0.5 mol/L, pH 5.2) was added to the mixture. After 1 min, 2 mL of the prepared enzyme mixture was added. The resulting reaction mixture was incubated at 37 °C. At regular time intervals, 50 μL of the reaction mixture was sampled. Each sample was immediately mixed with 450 μL of propanol and 0.5 mL of DNS reagent (which was prepared by dissolving 1.5 g of dinitrosalicylic acid, 0.3 g of phenol, 0.075 g of sodium sulfite, and 1.5 g of sodium hydroxide in 150 mL of distilled water). Following the addition of the DNS reagent, the mixture was heated at 90 °C for 10 min. After that, 0.167 mL of a potassium sodium tartrate solution (400 g/L) was added to stabilise the developed colour. The resulting mixture was cooled to room temperature. Its absorbance at 575 nm was measured using UV–vis spectroscopy. The starch digestion rate was determined as previously reported (Ding et al., 2017; Englyst et al., 1992):

$$\text{Starch digestion rate } (\%) = \frac{0.9 \times C_g}{C_s} \times 100\% \quad (3)$$

where Cg represents the glucose content and Cs represents the total starch content of the reaction mixture, which was calculated based on the total starch content of the steamed bun, as determined earlier in this study. The starch digestion rate was plotted against sampling time to generate a digestion curve. The hydrolysis index (HI) was then determined based on the area under the curve (AUC) using Eq. (4).

$$HI = \frac{AUC_s}{AUC_c} \times 100\% \quad (4)$$

where AUCs represents the area under the curve for the SCL-incorporated steamed bun, and AUCc represents the area under the curve for the control (viz., C00 in this study). Based on the HI calculated, the estimated GI (eGI) value of the sample was determined using Eq. (5) (Goñi et al., 1997). All eGI measurements were performed in triplicate.

$$eGI = 39.71 + 0.549\ HI \quad (5)$$

2.10. Sensory evaluation

Fifty untrained participants (age = 24.6 ± 1.9 years, BMI = 21.9 ± 3.5 kg/m^2) were recruited by convenient sampling from School of Food Science and Nutrition at the University of Leeds to evaluate and analyse the sensory characteristics of C00 and C60. Ethical approval (reference number: 2425) was granted by Faculty Research Ethics Committee for Business, Environment, Social Sciences at the University of Leeds prior to the study. All participants were provided with information explaining the sensory evaluation, outlining their obligations, and informing them of their right to withdraw at any time without providing a reason. The buns were steamed and frozen one day prior to the evaluation. One hour before the sensory evaluation, the frozen C00 and C60 were re-steamed and made available to the participants. Each participant was provided with one C00 and one C60. A 9-point hedonic scale, ranging from 1 (dislike extremely) to 9 (like extremely), was used to assess the degree of liking for each characteristic, including appearance, colour, aroma,

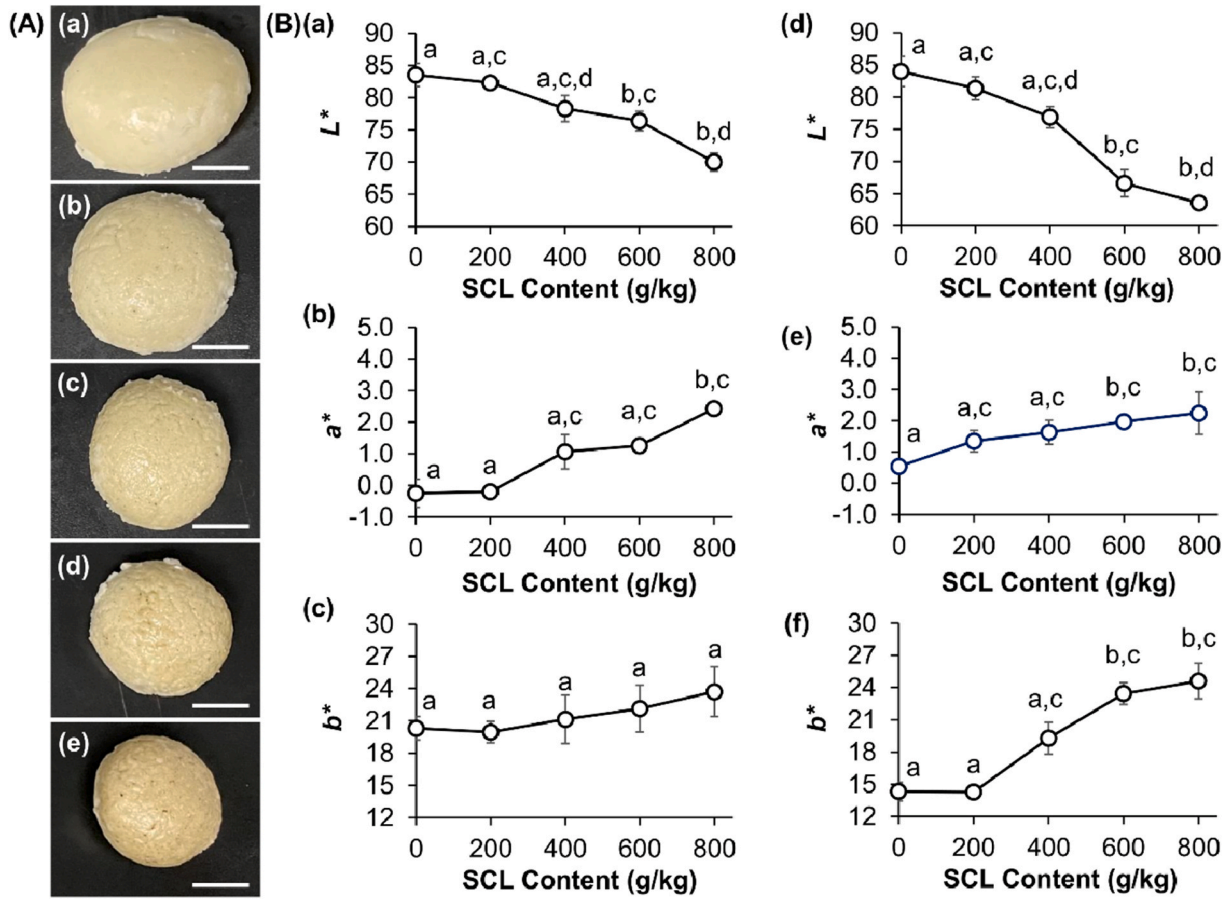

Fig. 7. Changes in the colour parameters of steamed buns with varying SCL contents. (A) Optical images of (a) C00, (b) C20, (c) C40, (d) C60, and (e) C80. The scale bar represents 2 cm. (B) (a, d) L^*, (b, e) a^* and (c, f) b^* values of the (a–c) crust and (d–f) crumb of steamed buns as a function of SCL content in the flour-SCL mixture used to make the buns. PERMANOVA using the Euclidean distance measure was performed to compare all groups, yielding a p-value of 0.001. A Kruskal-Wallis test was then conducted to compare the L^*, a^*, and b^* values of the crust and the L^*, a^*, and b^* values of the crumb across all groups, yielding respective p-values of 0.002, 0.002, 0.002 for the crust and 0.001, 0.008, 0.13 for the crumb. For each Kruskal-Wallis test, post-hoc pairwise comparisons were conducted using Dunn's test with BH correction. Groups assigned different lowercase letters are significantly different (adjusted $p < 0.025$). Abbreviation: CSL, seeds of *Coix lacryma-jobi*.

texture, taste, and general acceptability of the buns.

2.11. Determination of the blood glucose response

Glycaemic response was determined and expressed as mmol/L. Four female participants (age = 24.5 ± 1.3 years, BMI = 22.2 ± 2.7 kg/m^2, fasting blood glucose concentration <6.1 mmol/L) were recruited by convenient sampling from School of Food Science and Nutrition at the University of Leeds to assess both fasting and postprandial glucose response over a 2-h period. Ethical approval (reference number: 2425) was granted by Faculty Research Ethics Committee for Business, Environment, Social Sciences at the University of Leeds prior to the study. All participants were provided with information explaining the experiment, outlining their obligations, and informing them of their right to withdraw at any time without providing a reason. One day before the experiment, participants were instructed to fast for 8–12 h and to abstain from strenuous exercise and alcohol consumption. A fasting blood glucose measurement was taken and recorded as the baseline glucose level. Participants then consumed 30 g of either the C00 or C60 sample within 10 min. Subsequent blood samples were collected at 15, 30, 45, 60, 90, and 120 min post-consumption. Their blood glucose concentrations were determined using a blood glucose meter (Accu-Chek Instant meter, Roche Diabetes Care Ltd., West Sussex, UK). A line graph of blood glucose concentration over time was plotted, with the AUC being calculated using the trapezoidal method. Specifically, the trapezoidal areas between consecutive data points were calculated, and the sum of these areas was taken as the AUC. This blood glucose measurement procedure was repeated three times for each participant.

2.12. Statistical analysis

Experiments were conducted on three independent samples unless stated otherwise. Data were presented as mean ± standard deviation. Permutation-based ANOVA (PERMANOVA), the Kruskal-Wallis test followed by post-hoc Dunn's test with Benjamini-Hochberg (BH) correction to control the False Discovery Rate (FDR), and the Mann-Whitney U test with BH correction for ordinal data from the sensory evaluation test, were performed using R (version 4.4.2). All PERMANOVA analyses were performed with 999 permutations to generate a null distribution of F-statistics and assess statistical significance. Except for the post-hoc Dunn's test, in which a p-value less than $\alpha/2$ (where $\alpha = 0.05$) was considered statistically significant, the null hypothesis was rejected in all other tests when the p-value was less than 0.05.

3. Results and discussion

3.1. Preparation and fermentation of SCL-incorporated doughs

The objective of this study is to investigate the potential of SCL as a functional additive for controlling starch digestibility in steamed buns

Table 2
Specific volume and nutritional composition of SCL-incorporated steamed buns.

Bun	Specific volume (mL/g)		Protein (g/kg)		Lipid (g/kg)		Carbohydrate (g/kg)		Starch (g/kg)		Dietary fibre (g/kg)		Moisture (g/kg)	
	Mean ± SD	95 % CI	Mean ± SD	95 % CI	Mean ± SD	95 % CI	Mean ± SD	95 % CI	Mean ± SD	95 % CI	Mean ± SD	95 % CI	Mean ± SD	95 % CI
SCL	N/A	N/A	156.03 ± 0.98 ^{b,d,e}	(153.60, 158.46)	56.56 ± 0.90^{b,d,e}	(54.33, 58.80)	726.65 ± 1.30 ^{a}	(723.41, 729.88)	696.72 ± 3.21 ^{a}	(688.75, 704.69)	28.32 ± 1.06 ^{a}	(25.69, 30.95)	45.95± 0.69 ^{b,c}	(44.23, 47.68)
C00	2.81 ± 0.14^{a}	(1.51, 4.10)	89.79± 0.34 ^{a}	(88.94, 90.63)	12.14 ± 2.42 ^{a}	(6.13, 18.16)	502.87 ± 2.28^{a,b}	(497.22, 508.5)	479.87 ± 3.29 ^{a,b}	(471.69, 488.05)	22.81 ± 2.12 ^{a}	(17.54, 28.08)	389.87 ± 0.48 ^{a}	(388.67, 391.07)
C20	2.75 ± 0.09^{a,c}	(1.94, 3.56)	94.36± 0.54 ^{a,c}	(89.54, 99.18)	18.02 ± 2.29 ^{a,c}	(12.32, 23.71)	503.45 ± 2.42^{a,b}	(481.73, 525.16)	477.83 ± 2.96 ^{a,b}	(465.09,490.57)	23.85 ± 1.85 ^{a}	(19.25, 28.45)	379.09 ± 0.25 ^{a,c}	(378.46, 379.72)
C40	2.66 ± 0.19 ^{a,c}	(0.95, 4.37)	96.02± 1.90^{a,c,d}	(91.30, 100.73)	22.54± 2.18 ^{a,c,d}	(17.12, 27.97)	489.22 ± 3.42^{a,b}	(480.73, 497.72)	464.46 ± 3.86 ^{a,b}	(447.85, 481.07)	24.59 ± 2.32 ^{a}	(18.82, 30.36)	385.15 ± 0.59 ^{a,d}	(383.68, 386.62)
C60	2.05 ± 0.09 ^{a,c}	(1.20, 2.90)	97.04± 0.23 ^{a,e}	(96.46, 97.61)	24.29± 1.70 ^{a,e}	(20.07, 28.51)	486.09 ± 1.40 ^{a,b}	(482.62, 489.56)	460.72 ± 4.32 ^{a,b}	(442.13, 479.31)	25.22 ± 1.75 ^{a}	(17.69, 32.75)	384.66 ± 0.26 ^{a,c}	(384.01, 385.31)
C80	1.72 ± 0.00 ^{b,c}	(1.71, 1.73)	105.52 ± 3.02 ^{b,c,e}	(100.21, 110.84)	35.81± 6.28 ^{b,c,e}	(20.21, 51.41)	482.90 ± 4.69^{b}	(440.73, 525.08)	455.83 ± 4.92 ^{b}	(443.62, 468.04)	26.74 ± 2.12 ^{a}	(21.47, 32.01)	370.08 ± 0.28 ^{b,c,d}	(369.38, 370.76)
Pooled SD	0.12		1.54		3.14		2.84		3.83		1.91		0.46	

PERMANOVA using the Euclidean distance measure was performed to compare all groups, yielding a *p*-value of 0.001. A Kruskal-Wallis test was then conducted to compare the specific volume, protein content, lipid content, carbohydrate content, starch content, dietary fibre content and moisture content across all groups, yielding respective *p*-values of 0.02, 0.006, 0.007, 0.009, 0.01, 0.09 and 0.006. For each Kruskal-Wallis test, post-hoc pairwise comparisons were conducted using Dunn's test with BH correction. Groups assigned different lowercase letters in each column are significantly different (adjusted $p < 0.025$). Abbreviation: CI, confidence interval; SD, standard deviation.

(Fig. 1). In an earlier clinical study on both healthy and diabetic individuals, steamed buns were reported to have a GI value of 65–97 (Lau et al., 2015; Zhu, 2019). In China, steamed buns are among the most widely used reference foods for blood glucose testing in clinical settings (Zhu, 2019). This makes steamed buns an ideal food model for examining the effect of SCL incorporation on the GI of starch-rich foods.

As far as the volume expansion and texture formation of buns are concerned, gluten plays a key role. During hand-kneading, it interacts to form an elastic gluten network that imparts elasticity and extensibility to the dough (Biesiekierski, 2017; Wieser, 2007). The ability of the gluten network to capture and retain carbon dioxide produced during fermentation is essential for dough expansion (Cauvain, 1998; Chevallier et al., 2012). The effect of SCL on gluten formation in the dough was examined using SDS-PAGE, which enables subunits of gluten to be separated based on their molecular weight and mobility in an electric field (Roy, 2014). The width and intensity of the protein bands decreased as the SCL content of the dough increased (Fig. 2). The incorporation of SCL into the dough may, therefore, affect the effectiveness of gluten formation, with an increase in the SCL content leading to a decrease in dough volume after fermentation (Fig. 3). After 40 min of fermentation, the C00 dough exhibited the largest volume increase, with the final volume reaching 1.8 times of its initial volume. On the other hand, the volume of the fermented C80 dough was only around 1.15 times its initial volume. Similar observations were made in the case of barley (Cleary et al., 2007; Škrbić et al., 2009; Sullivan et al., 2010), where its incorporation into the dough disrupts gluten network formation, leading to a decline in gas-holding capacity and a subsequent reduction in the size and number of gas cells in the fermented dough (Chauhan & Sharma, 2000; Sullivan et al., 2010). Apart from disruption of gluten formation, components such as arabinoxylan in SCL cannot be effectively metabolized and absorbed by *Saccharomyces cerevisiae* as a carbon source (Bieniek & Buksa, 2024). Instead, they compete with other carbohydrates (such as starch), causing a decrease in available sugars that can be utilized by the yeast cells. As the concentration of SCL polysaccharides increases, the polysaccharides potentially inhibit carbon dioxide production and limit dough expansion.

3.2. Physical characterization of SCL-incorporated steamed buns

The effect of SCL incorporation on the cross-sections of the generated steamed buns is shown in Fig. 4A. Their microstructures were further examined using SEM (Fig. 4B). The gluten network of C00 was found to be complete, continuous, compact, and uniform. It contained starch granules with smooth surfaces and spherical shapes. These granules varied in size and were embedded within the gluten network. As the SCL content of the steamed bun increased, shrinkage of the starch granules was observed. Additionally, the gluten network became less continuous with the addition of SCL powder, and the size and number of gas cells decreased compared to C00. This is consistent with the results of stereomicroscopic examination (Fig. 5), where the size and number of pores in the crumb of SCL-incorporated steamed buns decreased in the order of C00 > C20 > C40 > C60 > C80. As the SCL content of the bun increased, the originally homogeneous, well-established, compact, and continuous microstructure of the crumb was gradually replaced by a more discontinuous structure. This largely resulted from the effect of SCL incorporation on the integrity of the gluten network, consistent with SDS-PAGE analysis results.

Such changes in gluten formation were expected to alter the textural characteristics of the generated buns. As shown in Fig. 6, the hardness of the buns increased with the SCL content. Although changes in hardness were not statistically significant when the SCL content increased from 200 g/kg to 600 g/kg, a significant difference was observed when the content was further increased to 800 g/kg. This trend was partially attributed to the reduced gas-holding capacity of the buns upon SCL incorporation. On the other hand, variations in the springiness, chewiness, and cohesiveness of the buns did not appear to correlate with

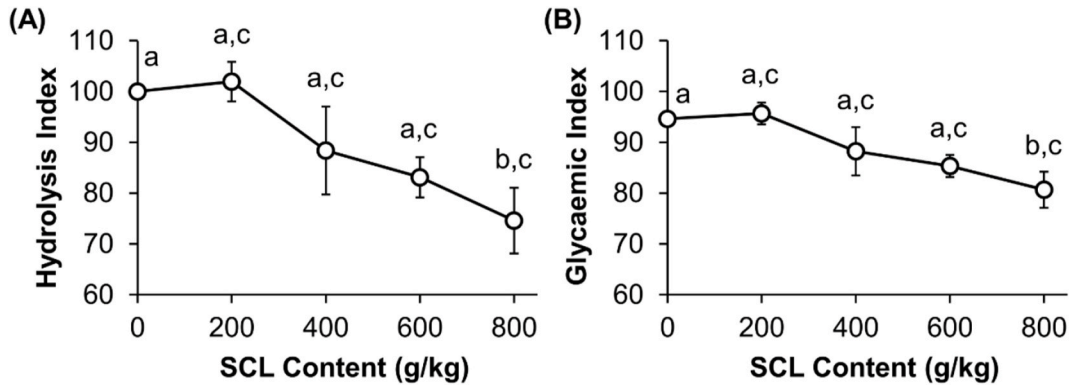

Fig. 8. Changes in the starch digestibility of steamed buns with varying SCL contents. (A) Hydrolysis index and (B) estimated glycaemic index of steamed buns as a function of SCL content in the flour-SCL mixture used to make the buns. PERMANOVA using the Euclidean distance measure was performed to compare all groups, yielding a *p*-value of 0.001. A Kruskal-Wallis test was then conducted to compare the hydrolysis index and glycaemic index 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. Groups assigned different lowercase letters are significantly different (adjusted $p < 0.025$). Abbreviation: CSL, seeds of *Coix lacryma-jobi*.

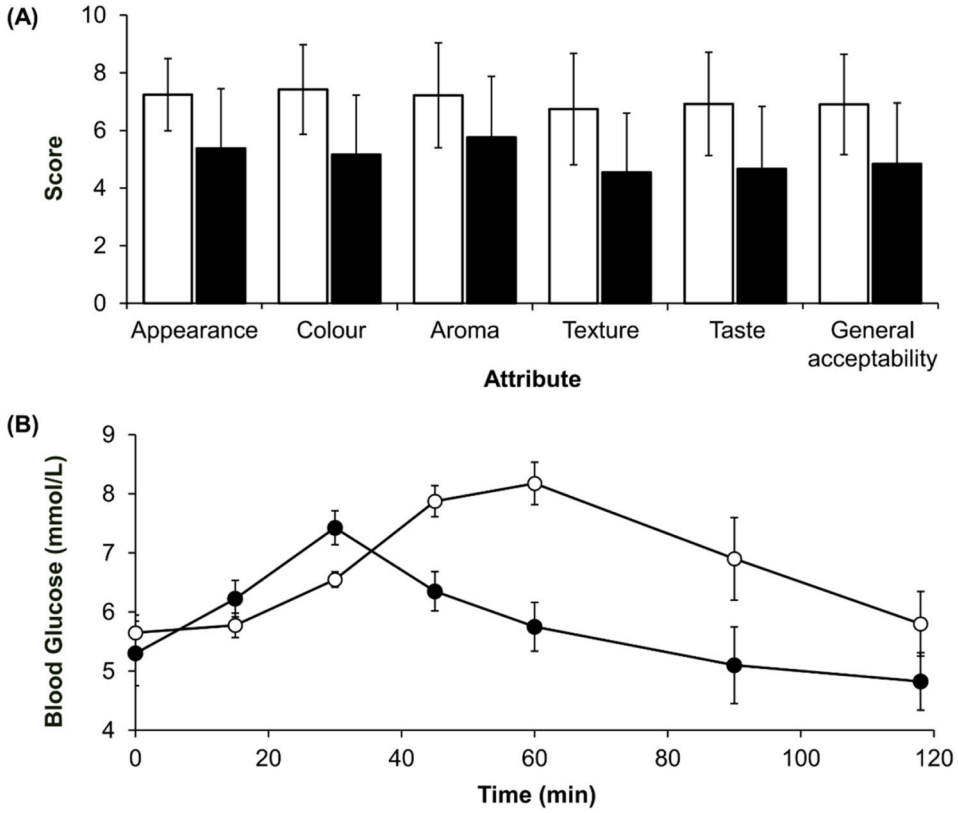

Fig. 9. Sensory evaluation of steamed buns with or without SCL incorporation and blood glucose response in human subjects. (A) Satisfaction scores obtained from a sensory panel assessment for different sensory attributes of C00 and C60. The SCL content in the SCL-flour mixture used to make the buns designated by □ and ■ was 0 g/kg and 600 g/kg, respectively. PERMANOVA using the Bray-Curtis dissimilarity measure was performed to compare all groups, yielding a *p*-value of 0.001. Sensory satisfaction scores, collected on an ordinal scale, were treated as compositional data, where each sample's sensory profile was analysed in a multivariate space. Bray-Curtis dissimilarity was chosen as it could capture differences in overall sensory profiles based on relative attribute intensities. A Wilcoxon rank-sum test, with BH correction for multiple comparisons, was then conducted to compare C00 and C60 in terms of appearance, colour, aroma, texture, taste, and general acceptability. All adjusted *p*-values were less than 0.01. (B) Blood glucose response of participants after consuming C00 and C60. The SCL content in the SCL-flour mixture used to make the buns designated by ◯ and ● was 0 g/kg and 600 g/kg, respectively. A Wilcoxon rank-sum test was conducted to compare the AUC of the two curves, yielding a *p*-value of 0.03.

increments in the SCL content of the buns. Beyond textural properties, the colour of the buns plays a crucial role in determining consumer acceptability (Cornejo & Rosell, 2015; Morreale et al., 2018). The incorporation of SCL into the buns led to changes in the L^* values of the crumb (Fig. 7). These changes were noticeable when the SCL content reached 600 g/kg and above. A similar trend was observed in the crust, which became darker as the SCL content increased. In addition to lightness, SCL incorporation affected the overall colour of the buns. Buns with a higher SCL content exhibited a redder hue in both the crust and crumb, with C80 being the reddest among all samples tested. While SCL

incorporation did not significantly affect the blueness and yellowness of the crust, the b^* value of the crumb increased with higher SCL content. Given that SCL are rich in polyphenol oxidase (Zeng et al., 2007), it is expected that the increase in yellowness, along with the decrease in lightness after SCL incorporation, is due to polyphenol oxidase-induced browning during steaming.

3.3. Analysis of the nutritional composition

Comparing the measured nutritional content of SCL shown in Table 2 with that of wheat flour, the protein and fat contents of SCL (156 g/kg and 56.6 g/kg, respectively) were higher than the values provided by the manufacturer for wheat flour (110 g/kg and 16 g/kg, respectively). The protein and fat contents of SCL aligned with the findings of Liu et al. (2015), who reported that the protein and fat levels of eleven Coix seed samples, as determined by near-infrared spectroscopy analysis, range from 121.8 to 166.5 g/kg and 51.4 to 94.0 g/kg, respectively. In this study, both the protein and fat contents of the steamed buns were found to increase progressively with higher SCL content (Table 2). The protein content in C80 was 105.5 g/kg, which was significantly higher than that of C00 (89.8 g/kg). Likewise, the fat content rose from 12.1 g/kg in C00 to 35.8 g/kg in C80. These changes were attributed to the high protein and moderate fat content of SCL. Replacing wheat flour with SCL led to an increase in fat content in the dough, enriching the generated buns with functional fats and enhancing the overall nutritional value.

The moisture content of the buns decreased as the SCL content increased, from 389.9 g/kg in C00 to 370.1 g/kg in C80 (Table 2). In addition, both the carbohydrate content and starch content of the buns showed a general trend of reduction with increasing SCL content, though the changes were not statistically significant. This was likely due to the carbohydrate content of SCL (726.6 g/kg) being similar to the value provided by the manufacturer for wheat flour (735 g/kg), meaning that replacing wheat flour with SCL has little effect on the resulting carbohydrate and starch contents. Regarding dietary fibre content, it tended to increase with increasing SCL content, but this increase was not statistically significant.

3.4. In vitro starch digestibility of SCL-incorporated steamed buns

Starch digestibility of a food product is influenced by multiple factors (Singh et al., 2010), including food processing conditions, structural features of starch (such as amylose/amylopectin ratio, molecular weight, and degree of gelatinization), and interactions between starch and other food components (e.g., proteins and lipids). Increasing the SCL content of the buns resulted in a progressive decrease in HI and eGI values (Fig. 8), with HI decreasing from 101.9 ± 3.9 to 74.6 ± 6.5 and eGI decreasing from 95.7 ± 2.1 to 80.7 ± 3.5 as the SCL content increased from 200 g/kg to 800 g/kg. This decrease in HI and eGI is attributed, in part, to the increase in the protein content of the buns, as shown in our nutritional composition analysis (Table 2). The higher protein content could contribute to reduced starch digestibility. This is supported by a previous study, which found that proteins in wheat protect starch from gelatinization, leading to a decrease in the GI value (Jimenez-Pulido et al., 2022).

Furthermore, as observed in earlier parts of this study, increasing the SCL content of the buns led to a higher lipid content. Among these lipids, an earlier study reported that SCL contain 380–510 g/kg of oleic acid, 140–180 g/kg of palmitic acid, and 20–30 g/kg of stearic acid (Xi et al., 2016). In terms of triglycerides, SCL were reported to possesses 180–260 g/kg of 1,2-linolein-3-olein, 230–270 g/kg of 1,2-olein-3-linolein, 90–150 g/kg of 1-palmitin-2-olein-3-linolein, 140–300 g/kg of triolein, and 70–100 g/kg of 1,2-olein-3-palmitin (Xi et al., 2016). Moreover, oils extracted from SCL were found to be rich in 1,2,3-trioleylglycerol, 1,2-dilinoleyl-3-oleylglycerol, 2,3-dioleyl-1-palmitoylglycerol, 1,2-dilinoleyl-3-palmitoylglycerol, 2-linoleyl-3-oleyl-1-palmitoylglycerol, 2,3-dioleoyl-1-linoleylglycerol, and 1,2,3-trilinoleylglycerol (Yu et al., 2011). These fatty acids could potentially complex with amylose in the buns, as observed in a previous study (Singh et al., 2010), reducing starch solubility and increasing resistance to α-amylase activity during digestion (Crowe et al., 2000).

3.5. Sensory evaluation and postprandial blood glucose response

As SCL were investigated as a functional additive to lower the glycaemic index of steamed buns, assessing both general acceptability and the blood glucose response to these buns was essential to evaluate the practical feasibility of SCL incorporation in food production. Considering the balance of properties, HI value, and eGI value of steamed buns with different SCL contents, as presented in the sections above, C60 was selected for further evaluation to assess its acceptability and the blood glucose response following its consumption by human subjects. Even though SCL incorporation led to a detectable change in the appearance, colour, aroma, texture and taste, C60 received a score of 4.8 ± 2.1 out of 10 for general acceptability, compared to 6.9 ± 1.7 for the one made entirely of flour (Fig. 9A). With targeted refinements in aspects such as taste and texture, a more favorable consumer response can be anticipated.

After consumption, there was a significant increase in blood glucose levels in both groups. The participants consuming C00 reached a peak blood glucose value of 8.2 ± 0.4 mmol/L at 60 min, while those consuming C60 reached a peak of 7.4 ± 0.3 mmol/L at 30 min (Fig. 9B). The onset time of the peak was earlier in the C60 group than in the C00 group, but the peak value was lower for C60. This could be due to the presence of monosaccharides in SCL, which may be easily broken down by enzymes and absorbed. After reaching the peak, blood glucose levels began to decline in both groups, with notable differences in the rate of decline. Blood glucose in the C00 group decreased rapidly, while in the C60 group, it decreased more gradually. After 120 min, blood glucose levels in both groups returned to near fasting levels (5.5 ± 0.4 mmol/L); however, the blood glucose level of the C60 group was noticeably lower than that of the C00 group. Additionally, the area under the blood glucose curve (AUC) for the C60 group was calculated to be 694.5 ± 23.1 mmol L^{-1}•min, which was substantially lower than that of the C00 group (823.3 ± 29.5 mmol L^{-1}•min). The lower overall glycaemic response observed in the C60 group indicated that the incorporation of SCL helped to reduce postprandial blood glucose fluctuations by diminishing spikes and accelerating recovery. The lower peak blood glucose value and shorter duration of blood glucose rise in the C60 group contribute to the observed smaller AUC.

4. Conclusion

We investigated in this study the potential of SCL as a functional additive for controlling starch digestibility in steamed buns. Although SCL incorporation led to changes in sensory attributes of the bun used for testing, the bun maintained a reasonable level of overall acceptability among human subjects and resulted in a lower glycaemic response. Given the variations in the nutritional composition of the seeds of different CL varieties, future research should focus on comparing their effects on starch digestibility. This could help identify the most effective variety for use as a functional additive in carbohydrate-rich food. Nevertheless, findings of this study confirm the

effectiveness of SCL in retarding the rate of starch digestion. Along with its established use as a food ingredient and herbal medicine, SCL demonstrate strong potential as an additive for the development of low-GI food products, catering to individuals concerned about blood sugar levels in the coming decades.

CRediT authorship contribution statement

Wing-Fu Lai: Writing – review & editing, Writing – original draft, Validation, Supervision, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Weijie Fang:** Writing – review & editing, Writing – original draft, Validation, Data curation, Formal analysis, Conceptualization. **Tong Fu:** Validation, Formal analysis, Data curation. **Sreekanth Reddy Obireddy:** Writing – review & editing, Validation.

Declaration of competing interest

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

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lwt.2025.117721.

Data availability

The generated data during the analysis has been included in the article.

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