Fortification of Chinese steamed bread with flaxseed flour and evaluation of its physicochemical and sensory properties

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\section{1. Introduction}
Consumer interest in healthy and nutritional diets has increased in recent years. While meeting hunger and basic nutritional needs, food is also expected to prevent diet-related diseases and improve consumer health (Mohamed et al., 2018). Chinese steamed bread (CSB), also known as Mantou or steamed bread, is a traditional staple food (Loong and Wong, 2018). CSB generally consists of wheat flour, water and yeasts/sourdough (Kim et al., 2019; Liu et al., 2020). CSB is widely consumed in North China and is popular worldwide. Recently, increasing attention has been devoted to improving the nutrition and consumer acceptance of CSB (Loong and Wong, 2018; Zhu and Chan, 2018). The development and evaluation of novel CSB with special flavors, tastes, and enhanced nutritional value has become increasingly popular and important (Kim et al., 2019).

Flaxseed \textit{(Linum usitatissimum)} is an oilseed rich in anti-inflammatory, gut health-promoting bioactive substances, including fermentable dietary fibers. It contains numerous essential fatty acids, especially polyunsaturated fatty acids (PUFAs), such as alpha-linolenic acid (\(\omega-3\)-ALA), linoleic acid (\(\omega-6\)), and oleic acid (\(\omega-9\)). These fatty acids play an important role in reducing cardiovascular disease, are anti-carcinogenic, and improve brain development in infants (Kaur et al., 2018; Sarabandi and Jafari, 2020; Morshedzadeh et al., 2021; Yari et al., 2021). In addition, flaxseed contains many biologically active substances, including dietary fiber, lignan (a class of phytoestrogens), high-quality proteins, vitamins, and minerals. These bioactivated components possess valuable benefits effects for humans, such as improving digestive health, relieving constipation, helping lower low-density lipoprotein cholesterol levels, and reducing the risk of diabetes (Huang et al., 2021; Xia et al., 2022). Due to the potential health benefits of its biologically active compounds, flaxseed has become an important functional food ingredient (Kaur et al., 2018; Zhu and Li, 2019; Santiago et al., 2019; Ardabili Bi Marandi et al., 2020).

Ground flaxseed (5–15\%) has been added to many bakery products as an egg substitute, which provides an alternative for vegan products (Wirkijowska et al., 2020; Oliveira et al., 2021; Cakmak, et al., 2021). Numerous studies have evaluated the improvements of adding flaxseed on improving the flavor, textural, antioxidant, and nutritional properties...
of various snack food products, including yogurt, muffins, sausages, breads cookies, wheat chips, and more (Hao and Beta, 2012; Yuksel et al., 2014; Sęczyk et al., 2017; Zhu and Chan, 2018; Santiago et al., 2019; Kaur et al., 2019; Ghafoori-Oskuei et al., 2020; Cakmak, et al., 2021). However, few studies have been conducted to explore the effects of adding flaxseed flour to staple foods. Recently, we investigated the physical and chemical changes resulting from the addition of flaxseed flour to wheat flour mixtures for yeast dough. The results showed that flaxseed flour change the sensory properties and rheology of the flour mix for yeast dough (Liu et al., 2020). However, the effects on flavor, texture, and antioxidant and nutritional properties resulting from adding flaxseed flour to CSB remained to be explored.

Therefore, the purpose of this study was to evaluate the nutritional composition of various flaxseed flours and investigate the effects of adding flaxseed flour on the edible acceptance (color, texture, aroma), amino acid, antioxidant, and digestion properties of CSB. Our study was intended to investigate the quality and nutritional characteristics of flaxseed flour as a functional ingredient in traditional steamed staple food production.

2. Materials and methods

2.1. Materials and reagents

Wheat flour and Angel yeast were purchased from the local market in Yangling (Shaanxi, China). Flaxseed was acquired from the county of Huining (Gansu, China). Flaxseeds were processed in a grinder and passed through a 40 mesh sieve (FW-100, Tianjin Taister Instrument Co., Ltd). Lutin and gallic acid were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Folin-Ciocalteu phenol reagent was obtained from Shanghai Lida Biotechnology Co., Ltd. (Shanghai, China). TPTZ, 2,4,6-tri(2-pyridyl)-s-triazin, DPPH•, and ABTS•+ were purchased from Shanghai Yuanye Biotechnology Co., Ltd. (Shanghai, China). Folin-Ciocaltel phenol reagent was obtained from Shanghai Lida Biotechnology Co., Ltd. (Shanghai, China). Methanol, ethanol, glacial acetic acid, sodium acetate, hydrochloric acid, potassium sulfate, sodium hydroxide, petroleum ether and other reagents were analytical grade and obtained from Tianjin Kemel Chemical Reagent Co., Ltd. (Tianjin, China).

2.2. Evaluation of various powder components of flaxseed

2.2.1. Antioxidant properties

Extraction of bioactive compounds. The extraction method that we used was described by Nithiyannatham et al. (2012) with slight modification. First, 5.0 g of each powder (defatted flaxseed flour (DFF), flaxseed kernel (FK), flaxseed peel (FP), and flaxseed flour (FF)) was weighed and each flour was placed in a separate centrifuge tube. Second, 80 mL methanol (80%) was added to each tube, and ultrasonic extraction was applied at 50 °C and 100 W for 60 min. Third, the mixture was centrifuged at 3800 rpm for 15 min, the supernatant was collected in a brown glass bottle, the residue was extracted under the same conditions by acetone (70%), and the supernatant was collected. Finally, the two supernatants were mixed and concentrated to 10 mL by evaporation. Then, the residue was filtered through a 0.22 μm organic filter membrane and stored at −20 °C until analyzed.

Total phenolic content (TPC). The TPC of different extracts was determined by the method described by Dini et al. (2010) with some modification. Specifically, the more gallic acid concentration gradients were used for standard curve; the solution was constant with methanol (80%) instead of double distilled water; the total volume was 10 mL instead of 25 mL. TPC was estimated by a colorimetric assay (765 nm) using gallic acid as a standard. The regression equation of gallic acid was

\[ y = 0.0947x + 0.0283 \]  \( R^2 = 0.9943 \)

The sample volume used for measurement was 100 μL. The TPC was indicated as the equivalent of gallic acid (as measured in the standard: mg gallic acid/100 g).

Total flavonoid content (TFC). The total flavonoids in various extracts were determined by the method of Dini et al. (2010) with some modification. Specifically, 0.5 mg/mL rutin standard solution was used instead of catechin solution; the volume was constant with methanol (80%) instead of double distilled water; the total volume was 25 mL instead of 5 mL; Al(NO₃)₃ solution was used instead of AlCl₃. The regression equation of rutin was

\[ y = 0.00952x + 0.00912 \]  \( R^2 = 0.9985 \)

The sample volume used for measurement was 5 mL. The TPC was indicated as the equivalent of rutin standard (mg rutin/100 g).

DPPH• radical scavenging assay. The DPPH• radical scavenging activity was measured by following the method described by Brand-Williams et al. (1995) with minor modifications. The modifications were as follows. First, standard curve was established by Trolox standard substance. About 1 mL Trolox standard reagents reacted with 2 mL DPPH• separately for 30 min. Finally, measured the absorbance value of reaction solutions at 517 nm. About 1 mL of sample extract was added with 2 mL DPPH (2 × 10⁻⁴ mol/L), made the mixture reacted fully in dark for 30 min before the determination of absorbance value. The regression equation of standard reagents and DPPH• radical scavenging activity was

\[ y = 4.4974x-0.0001 \]  \( R^2 = 0.9988 \)

The result of the sample to DPPH• was based on the equivalent of Trolox standard contained in 100 g dry weight sample (μmol Trolox/100 g).

ABTS•+ radical scavenging assay. The detection method was referred to Guedes et al. (2013). Specifically, the ABTS•+ solution was diluted and reacted with solvent (Trolox, K₂S₂O₈) until the absorbance at 734 nm is about 0.70. Accurately take 100 μL sample reactions with 3 mL fresh ABTS•+ solutions for the determination at 734 nm. The regression equation was

\[ y = 1.6335x + 0.0551 \]  \( R^2 = 0.9992 \)

The result of the sample to ABTS•+ was based on the equivalent of Trolox standard contained in 100 g dry weight sample (μmol Trolox/100 g).

FRAP assay. The specific detection method was described by Sczyżk et al. (2017) with minor modification. Specifically, during preparation of FRAP working solutions, the potassium ferricyanide K₃[Fe(CN)₆] was replaced by TPTZ (20 mmol/L) and FeCl₃·6H₂O (20 mmol/L). The Trolox was acted as fluorescent reactant and the absorbance value was determined at 593 nm instead of Fe³⁺ at 700 nm. The regression equation was

\[ y = 2.3536x + 0.0336 \]  \( R^2 = 0.9989 \)

The volume of sample extract applied for measurement is 0.5 mL. The result of the sample to FRAP was based on the equivalent of Trolox standard contained in 100 g dry weight sample (μmol Trolox/100 g).

2.2.2. Chemical composition

Fatty acid composition: The sample was prepared according to GB 5009.168-2016. Fatty acid methyl esters (FAMES) were prepared by the transesterification of triglycerides. First, approximately 5 g fat extract of flaxseed flour (wheat flour) was weighed and placed in a tube with 4 mL n-hexane. Second, 4 mL of a methanolic sodium hydroxide solution (0.4 mol/L) was added. Third, the tube was mixed thoroughly by a vortex and left 30 min for complete reaction. Fourth, 4 mL deionized water was added and mixed totally for extraction. Finally, the supernatant was collected for analysis.

The fatty acid composition of the flaxseed flour (wheat flour) was determined by gas chromatography. A DB-Wax (30 m × 0.25 mm × 0.25 μm) capillary column was used in this analysis, and the injection volume was 1 μL. The column temperature was set at 70°C for 5 min, ramped to 200°C at a rate of 25 °C/min, and finally heated to 230 °C at 2 °C/min. Helium (purity 99.999%) was used as the carrier gas with a flow rate of 1 mL/min and split ratio of 5:1. Fatty acids were identified by comparison with external standards and quantified using the normalization method. All the samples were analyzed in triplicate.

Mineral components. The analyses of Mg, Ca, Mn, Cu, Zn, and Fe were conducted by flame atomic absorption spectrometry (FAAS) using an Analyst 800 atomic absorption spectrometer (Perkin Elmer, USA) equipped with an AS 800 autosampler (Perkin Elmer, USA). The specific pretreatment steps of samples were referred to GB/T 14924.12-2001. The analyses of Na and K were performed using flame atomic emission spectrometry (FAES). An air-acetylene flame was used for both machines. Each sample was analyzed in triplicate.
2.3. Sensory and textural properties of Chinese steamed bread

Preparation of Chinese steamed bread: Wheat-flaxseed flour (1000 g) was prepared by mixing varied amounts of flaxseed flour and wheat flour such that flaxseed flour was added to yield 0% (control), 5%, 10%, 15% and 20% of the total flour (mass). The preparation of CSB was conducted according to the previous research of our lab (Liu et al., 2020). Specifically, yeast (10 g) was mixed with 300 mL distilled water for 5 min under low-speed (50 rpm) stirring. Next, total flour and 300 mL distilled water were added to the yeast solution and mixed until a smooth dough was formed. Then, the dough was stored in a fermenting box and incubated at 35 °C and 85% relative humidity for 90 min. After fermentation, the dough was divided into small pieces (50 g), rounded and molded. The dough was steamed at 100 °C for 20 min at atmospheric pressure. The five CSBs described above were produced in each trial. Each piece of CSB was freeze-dried, ground, and passed through a 100-mesh sieve for further use. The experimental groups were named according to their flaxseed flour content as follows: CSB-0 (0%), CSB-5 (5%), CSB-10 (10%), CSB-15 (15%) and CSB-20 (20%).

2.3.1. Color

Color parameters (L*, a*, b*) for CSB (crumb and crust) were determined using a colorimeter. The colorimeter was calibrated with a standard whiteboard (L*: 97.42, a*: 0.41, b*: 1.84). The calculation formula of ΔE was expressed as follows:

\[ \Delta E = \sqrt{(L_a - L_d)^2 + (a_a - a_d)^2 + (b_a - b_d)^2} \]

where \( L_a \) was the brightness value, \( a_a \) was the red-green value, and \( b_a \) was the yellow-blue value.

2.3.2. Volatile substances

The volatile composition was determined by headspace solid-phase microextraction/gas chromatography–mass spectrometry (HS-SPME/GC–MS) (50/30 μm DVB/CAR/PDMS; USA, SUPELCO). The volatile components of CSB were extracted via the HS-SPME method. After extraction of the volatiles, the SPME fiber was immediately inserted into the GC–MS sample inlet desorbed at 250 °C for 3 min. GC–MS equipment (Shimadzu-QP2010, Kyoto, Japan), which matched with a DB-17MS chromatographic column (60 m × 0.25 mm × 0.25 μm, Agilent, Santa Clara, CA, U.S.A.) was employed to separate and identify the volatile components of CSB. The volatile compounds were tentatively identified using the GC–MS spectra. Compounds with ≤ 85% similarity to the NIST library were not considered.

2.3.3. Texture

The textural properties of CSB were studied using a TA.XT plus texture analyzer with a cylinder probe (P/36R) (Stable Micro Systems Ltd). Briefly, whole CSB after 20 min of cooling was placed on the center of the platform for compression analysis. A cylindrical probe (36 mm in diameter) was used to compress the CSB. The speeds of the probe were set at 2.0, 1.0 and 1.0 mm/s during the pretest, test and posttest of the samples, respectively. The trigger force was set to 5.0 g, and the compression ratio was set to 50.0%. The measurement of each sample was repeated eight times. The hardness, cohesiveness, springiness, and chewiness were recorded.

2.4. Chemical compositions of Chinese steamed bread

2.4.1. Amino acids

The amino acids of CSB were analyzed using an amino acid analyzer (Babcock Hitachi, L-8900). First, the sample was hydrolyzed by hydrochloric acid. Then, ninhydrin was used to make a full reaction solution, and the amino acids of CSB were detected automatically using the amino acid analyzer.

2.4.2. Starch compositions

In vitro digestion of CSB was measured by using the method of Qi Li et al. (Qi Li et al., 2021), with minor modification. The main enzymes were α-amylase (3000 U/mL) and glucoamylase (2500 U/mL). The results were indicated as rapid digestible starch (RDS), slowly digestible starch (SDS) and resistant starch (RS).

2.5. Antioxidant properties of Chinese steamed bread

The TPC, TFC, DPPH•, ABTS•+, and FRAP values of CSB were estimated as described above for the detection methods of flaxseed flours.

2.6. Statistical analysis

All experiments and measures were repeated at least three times. Statistical analysis was performed using ANOVA, with \( p < 0.05 \) considered significant. Statistical differences among values are indicated by different letters according to the Tukey test. All statistics were performed in R v4.0.2. The violin plot was generated by the ggpubr package. The stacked barplot was generated by ggplot2 v3.3.3. A heatmap was generated by the pheatmap package in R using the average amino acid contents.

3. Results and discussion

3.1. Evaluation of various powder components of flaxseed

3.1.1. Antioxidant properties of flaxseed flour

Recently, flaxseed has become an important functional food ingredient. Various components of flaxseed (including flaxseed flour, flaxseed oil, and flaxseed hull) have been added to several food products (breads, muffins, biscuits, cookies, etc.). To obtain the optimal composition of the flaxseed additive for CSB, the antioxidant properties (including TPC, TFC, DPPH•, ABTS•+, and FRAP) of each flaxseed product (defatted flaxseed flour (DFF), flaxseed kernel (FK), flaxseed peel (FP) and flaxseed flour (FF)) were evaluated (Fig. 1). As shown in Fig. 1 A-E, the antioxidant capacity of each flaxseed flour could be ranked as follows: defatted flaxseed flour > flaxseed peel > flaxseed flour > flaxseed kernel. Specifically, for the content of TPC in various powders, the flaxseed flour had the highest content of TPC, 78.58 mg gallic/100 g. The flaxseed peel and defatted flaxseed flour had small differences. The flaxseed kernel contained the lowest content of TPC, approximately 43.3% of flaxseed flour. From this, the primary source of TPC was flaxseed peel. Flaxseed peel could be used as a nutrient fortifier in various daily foods. For TFC, there were significant differences among the various types of flaxseed flour prepared for this study. The defatted flaxseed flour had the highest TFC content. One plausible reason was that TFC from flaxseed is mainly a nonfat soluble substance. Similarly, the defatted flaxseed flour possessed the highest DPPH•, ABTS•+, and FRAP radical scavenging activities. This may be due to the high content of TFC in the defatted flaxseed flour. The DPPH•, ABTS•+, and FRAP radical scavenging activities of flaxseed flour were higher than those of defatted kernel and lower than those of flaxseed peel. Factoring the economic and nutritional value and the efficiency of CSB processing, flaxseed flour was chosen as the CSB flour additive for investigation.

3.1.2. Fatty acid and mineral compositions of flaxseed flour

To fully understand the composition of the prepared flaxseed flours, we measured the main fatty acids and mineral components of each of the various flour types (Supplementary Table A.1). This showed that there were five types of fatty acids in flaxseed flour. Of these, oleic acid, linoleic acid and linolenic acid accounted for 90.21% of the total fatty acid content. Linolenic acid showed the highest content at 53.58 ± 0.76%, followed by oleic acid, and finally linoleic acid, which was the lowest. Generally, the flaxseed flour was rich in unsaturated fatty acids. Thus, it may play a key role in improving the nutritional value of CSB.
In addition, flaxseed flour is rich in various mineral elements, especially K (5137.48 ± 7.82 mg/kg), Mg (3814.99 ± 12.72 mg/kg), Ca (2171.46 ± 21.92 mg/kg), Fe (71.52 ± 1.26 mg/kg), and Zn (41.32 ± 0.20). It is generally known that K, Mg, Ca, Fe, and Zn are indispensable elements for the human body. The content of K in flaxseed flour was twice that of wheat flour, and the content of Ca and Mg was more than 10 times that found in wheat flour. In addition, flaxseed flour with a high K/Na ratio is good for patients with hypertension, which may indicate how flaxseed can reduce the incidence of cardiovascular and cerebrovascular diseases (Morshedzadeh et al., 2021). Therefore, it can be concluded that flaxseed flour was an optimal dietary supplement for its contribution to the unsaturated fatty acids and minerals of full flour CSB.

### 3.2. Sensory and textural evaluation of Chinese steamed bread

#### 3.2.1. Color

Color is one of the most important quality indicators of food. CSB is the staple food of Chinese residents’ daily consumption. As an intuitive indicator of CSB, color plays a decisive role in consumption and popularity. The crust and core colors of CSB are shown in Table 1. The results showed that for both crust and core, as the amount of added flaxseed flour increased, the lightness value (L) decreased, the red–green

| Type         | Items         | CSB-0       | CSB-5       | CSB-10      | CSB-15      | CSB-20      |
|--------------|---------------|-------------|-------------|-------------|-------------|-------------|
| Color        | Crust L*      | 87.40 ± 0.21 | 73.50 ± 0.35 | 62.16 ± 0.62 | 55.69 ± 0.89 | 50.95 ± 0.21 |
|              | a*            | 0.06 ± 0.04  | 2.95 ± 0.10  | 5.30 ± 0.08  | 6.71 ± 0.14  | 7.44 ± 0.01  |
|              | b*            | 20.09 ± 0.27 | 17.55 ± 0.31 | 18.02 ± 0.20 | 17.76 ± 0.12 | 17.14 ± 0.14 |
|              | Core L*       | 85.08 ± 0.21 | 72.65 ± 0.34 | 64.91 ± 0.27 | 61.56 ± 0.57 | 57.62 ± 0.23 |
|              | a*            | 0.13 ± 0.01  | 2.16 ± 0.03  | 3.62 ± 0.10  | 4.41 ± 0.07  | 4.93 ± 0.11  |
|              | b*            | 18.39 ± 0.12 | 15.54 ± 0.24 | 15.20 ± 0.23 | 15.46 ± 0.13 | 15.25 ± 0.20 |
| Texture properties | Hardness/g | 1569 ± 94e | 1753 ± 63d | 1985 ± 10b | 2213 ± 74b | 2512 ± 109a |
|              | Cohesiveness  | 0.82 ± 0.004 | 0.81 ± 0.002 | 0.79 ± 0.001 | 0.77 ± 0.003 | 0.77 ± 0.012 |
|              | Springiness   | 0.10 ± 0.012 | 0.10 ± 0.004 | 0.95 ± 0.001 | 0.94 ± 0.002 | 0.94 ± 0.004 |
|              | Chewiness/g   | 1283.62 ± 87.38 | 1419.03 ± 50.54 | 1495.86 ± 18.25 | 1607.68 ± 88.89 | 1811.50 ± 90.81 |
value increased, and the yellow–blue color changed just slightly, which indicated that as the lightness of CSB decreased, the red value increased, and the transparent color became darker. One likely reason was that because flaxseed is reddish-brown the natural color of flaxseed flour itself might affect the color of the steamed bread. From this we concluded that the addition of flaxseed flour is of excellent value to human health, but the amount of flaxseed should be appropriate to maintain consumer preferences. As income increases and living standards improve, consumers pay more attention to their health with the result that food color preference is gradually diminished and the inherent nutritional value is more keenly considered. The results of this study are similar to those of our earlier work (Liu et al., 2020). Additionally, a sensory quality evaluation with flaxseed flour indicated that adding 12% flaxseed flour had negligible effect on the sensory quality of cookies (Kaur et al., 2019). Thus, we believe that using 10% flaxseed flour in CSB would be easily accepted by consumers.

### 3.2.2. Flavor

Flavor is another important indicator that determines the perception of overall quality attributes and consumer acceptance. Therefore, the flavor components of CSB with flaxseed flour were evaluated. The specific compounds are shown in Fig. 2 (Supplementary Table A.2). Seventy-two kinds of volatile compounds were detected in CSB, and they can be divided into ten classes, including alkanes, acids, esters, ketones, aldehydes, alcohols, olefins, nitrogen-containing compounds, oxygen-containing heterocyclic compounds, and benzene ring compounds. As shown in Fig. 2, the main volatile compounds in all CSB groups, regardless of varying proportions of flaxseed flour, were alcohols, nitrogen-containing compounds, ketones, alkanes, and aldehydes, accounting for more than 95% of the total volatiles. As the proportion of flaxseed flour increased, the content of alcohols decreased slowly, while the content of ketones and aldehydes increased significantly, and both groups reached a maximum when the addition proportion of flaxseed flour was 10%. It is commonly reported that the pathway of flavor substance formation in CSB mainly includes fatty acid oxidation, heat treatment, and microbial metabolism. Therefore, added flaxseed flour might slightly affect the volatile composition of CSB. The main flavors of the control CSB were fruity and grassy fragrances, which was due to volatile alcohols (Dong et al., 2018). In addition, owing to their lower aroma activity threshold, aldehydes played a significant role in the flavor of the flaxseed flour CSB, which carried fatty and fruity aromas. The results suggested that the flavor of CSB may be appreciably improved by adding 10% flaxseed flour.

### 3.2.3. Texture

The texture of food mainly refers to its tissue characteristics and this quality is related to the sensory and edible properties of the food. The textural results of CSB are shown in Table 1. The results show that with the addition of flaxseed flour, the hardness and chewiness of CSB gradually increased, while the cohesiveness and springiness decreased slightly. The results indicated further that with the addition of flaxseed flour, the CSB formed a more stable and chewy texture, while the properties of cohesiveness and springiness were slightly reduced. Protein denaturation and starch gelatinization are major factors affecting the texture of CSB (Liu et al., 2020). The principal components of flaxseed flour are dietary fiber, lipids, and proteins, which can interact with wheat gluten and starch (Hao and Beta, 2012; Zhu and Li, 2019; Liu et al., 2020). Therefore, flaxseed flour may affect the formation of the gluten network, which could reduce the gas-holding capacity of dough, decrease the specific volume, and increase the hardness of CSB. In addition, flaxseed gum is present in flaxseed flour, which has high viscosity and likely contributed to the high chewiness of CSB. Therefore, the optimal flaxseed flour addition ratio of CSB, again, appears to be 10%.

### 3.3. Composition evaluation of Chinese steamed bread

#### 3.3.1. Amino acids

Amino acids, which are the basic units of proteins, are vital dietary compounds for humans. From the perspective of human nutrition, the 20 amino acids that make up human proteins can be divided into essential amino acids (EAAs), delicious amino acids (DAAs), and nonessential amino acids (NEAAs). The amino acid composition of flaxseed flour and CSB is shown in Fig. 3 and Supplementary Table A.3. A total of 17 amino acids were detected, and the total protein content was 22.19 ± 0.03 g/100 g of flour. Trp was not detected in any samples. One probable reason was that the concentration was below the detection limit. The content of Glu acid in CSB-15 was relatively high. The specific reason needs further investigation. For most of the amino acids in CSB, as the amount of flaxseed flour increased, their content also increased gradually. Their distribution ranged from 12.38 to 22.76 g/100 g protein. The most abundant amino acids were Glu and Pro, accounting for 3.13 ~ 10.73 and 3.13 ~ 3.53 g/100 g protein, respectively. The addition of flaxseed flour increased the content of amino acids in CSB, especially the concentration of EAA. Therefore, to obtain a more nutritional CSB, the addition of an appropriate amount of flaxseed flour was suggested.

#### 3.3.2. Starch

Starch is the main component of CSB, and the consumption of starchy food is closely related to diet-related chronic metabolic diseases, including hypertension, obesity, and type II diabetes. It is generally believed that excessive intake of RDS is not conducive to the stability of postprandial blood glucose, while the ingestion of SDS is beneficial to the balance of postprandial blood glucose, and the intake of RS is beneficial for preventing colon cancer and improving intestinal health (Li et al., 2021). The starch types of various CSB are shown in Fig. 4. Specifically, as the added amount of flaxseed flour increased, the content of RDS fluctuated, but the overall trend was downward, and the content of SDS and RS increased with the addition of flaxseed flour (±15%). The RS content ranged from 34.89 ± 0.80 to 54.64 ± 0.23%. Compared to previous research, the change expectations of RDS and SDS are inconsistent. With increasing flaxseed flour content, the RDS content decreased, while the SDS content first increased and then decreased (Zhu and Li, 2019). This phenomenon might be attributed to the various levels of flaxseed flour added to CSB. In short, after adding flaxseed flour, the content of RS and SDS in CSB increased. This would be more helpful for the digestion and digestive absorption of CSB.
3.4. Antioxidant evaluation of Chinese steamed bread

Phenolic compounds are important secondary plant metabolites that help promote the health of crops and foods. TPC and TFC are significant nutrients in flaxseed flour (Morshedzadeh et al., 2021; Yari et al., 2021). The concentrations of TPC, TFC, DPPH•, ABTS+•, and FRAP in CSB are shown in Fig. 5. The results showed that as the amount of flaxseed flour increased, the concentration of TPC in CSB increased. The content of TPC in CSB-20 (5.79 mg gallic/100 g) was 3.18 times that of CSB-0 (1.82 mg gallic/100 g). This phenomenon was probably due to the high content of antioxidants (including lignans, ferulic acid, and coumaric acid) in flaxseed flour. The content of TFC, DPPH•, ABTS+•, and FRAP in CSB showed the same trend as TPC. The same phenomenon has been reported in a previous investigation of flaxseed addition to steamed bread. DPPH increased approximately four-fold with the addition of flaxseed flour (Zhu and Li, 2019). Therefore, the addition of flaxseed flour to CSB could promote the intake of functional compounds and increase the antioxidant capacity of ingested CSB.

In summary, flaxseed flour is rich in unsaturated fatty acids (USFAs), mineral elements, and antioxidants (TFC, TPC, etc.). Our results suggest that flaxseed flour is an optimal ingredient of CSB, which can considerably increase the nutritional content of CSB while slightly improving its color, flavor, and texture. Of course, our goal was to achieve healthy and tasty CSB. Therefore, through comprehensive consideration of these
factors, we suggest adding 10% flaxseed flour when preparing a novel and nutritionally fortified CSB.

4. Conclusion

The experimental results indicated that CSB made from flaxseed flour was more nutritious than non-flaxseed CSB. The addition of an increased concentration of flaxseed flour significantly affected the sensory, textural, and chemical composition of CSB. Flaxseed flour is rich in unsaturated fatty acids, essential minerals, and strong antioxidant capacity. With added flaxseed flour, the resulting CSB exhibited slight color deepening, acceptable texture, aroma, and amino acid features as well as significantly increased antioxidant capacity and digestive properties. Therefore, flaxseed flour appears to be an excellent dietary supplement for various food products due to its excellent quality improvement and antioxidant and nutritional properties.

CRediT authorship contribution statement

Yuan Gao: Data curation, Writing – original draft. Tingting Liu: . Caihong Su: . Qi Li: . Xiuzhu Yu: .

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.fochx.2022.100267.

Fig. 5. The antioxidation ability of various CSB (n = 3) Antioxidant activity of total phenolic content (TPC), total flavonoids content (TFC), DPPH radical scavenging activity (DPPH), ABTS radical scavenging activity (ABTS), and Ferric Reducing Antioxidant Power (FRAP) were measured.

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