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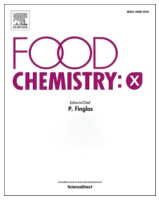

Impact of supplement of Qingke flours on physiochemical properties, sensory and *in vitro* starch digestibility of wheat bread and its enhancement by bread quality improvers

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ABSTRACT

The aim is to upgrade the formulation to produce wheat bread with lower starch digestibility by supplemented with Qingke flour. Physiochemical properties of multi-scale Qingke flours were examined to select the most satisfied Qingke flour for breadmaking. Data showed multi-scale Qingke samples differed in total starch content, water/oil binding capacity, freeze–thaw stability, but had similar swelling capacity and thermodynamic properties. Addition of Qingke flours significantly reduced the total *in vitro* starch digestion of bread from 80% to 41% and decreased the rapidly digested starch content from 53% to 27%. However, Qingke flours caused a worse bread quality, texture and sensory e.g. lower bread specific volume (4.26–3.3 mL/g), larger hardness (398–1170 g) and chewiness (296–707 mJ). Meanwhile, hydroxypropyl methylcellulose, sodium stearoyl lactylate and transglutaminase could improve the bread quality and sensory. Lastly, results revealed Qingke-supplemented bread could generate new volatile compounds, hence having a different aroma compared to original wheat bread.

1. Introduction

Wheat bread, a common staple in diets worldwide, predominantly relies on starch as its fundamental constituent. Due to its high level of starch digestibility and adsorption of starchy foods, postprandial blood glucose would increase rapidly. This characteristic prompt wheat bread to exhibit a high glycemic index (GI), which is further exacerbated by the tendency of its starch to gelatinize at elevated baking temperatures (Yuksel & Kayacier, 2022). In the recent years, the intake of low GI food has garnered significant attention, especially for the diabetics and subjects with impaired glucose tolerance. Consequently, the adjustment of starchy food formulation emerges as a feasible strategy for moderating starch digestion, glucose release and blood glucose intake. This has implications for various health concerns e.g. obesity, type-II diabetes and cardiovascular disease. Generally, the carbohydrate digestion of food is believed to be associated with the food constituents and structure, particularly the dietary fiber, resistance starch, as well as the ratio of amylose to amylopectin (Mikulec, Kowalski, Makarewicz, Skoczylas, & Tabaszewska, 2020). In fact, some researchers have tried to use other food sources as supplement to reduce the starch digestibility and glycemic response of high-starch bread. For instance, recent studies have demonstrated that gluten-free bread enriched with acorn or chickpea flours witnessed a substantial reduction in the *in vitro* starch digestibility. At the same time, the lower *in vivo* glycemic response was also observed in the acorn- and chickpea-modified bread (Gkountenoudi-Eskitzi et al., 2023). Similarly, pseudo-cereals e.g. amaranth, oat, buckwheat and quinoa have garnered attention for their potential in enhancing nutritional profiles and fostering hypoglycemic effects due to their elevated dietary fiber content (Cao et al., 2022; Gkountenoudi-Eskitzi et al., 2023; Melini, Melini, Luziatelli, & Ruzzi, 2017; Wang, Lao, Bao, Guan, & Li, 2021).

Qingke (*Hordeum vulgare Linn. var. nudum* Hook.f.), also namely highland hulless barley, holds a significant role as the staple food source in Tibet and has been under widespread cultivation for many years. Qingke is rich in many nutrition components e.g. dietary fiber, polyphenols, 1,3–1,4-mix-linked β-glucan, and is involved with various biological activities e.g. *in vitro* antioxidant activity and hyperglycemic effects (Guo et al., 2018; Lin et al., 2018a). In addition, β-glucan, as an important non-starch polysaccharide in Qingke, showed remarkable potential nutritional functions i.e. anti-immunomodulatory effects and

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anti-cancer effects as evidenced by prior investigations (Lin, Guo, Lu, Lu, Bu Gong, Wang, Zhang, Qin, & Wu, 2018b). Hence, it is hypothesized that adjustment of wheat bread formulation by supplement with Qingke flours could enhance the potential nutritional functions of wheat bread. It should be noted that the particle size of Qingke flour may affect its chemical compositions and physiochemical properties (Du, Zhu, & Xu, 2014; Zhu, Du, & Xu, 2015), and this could influence the bread quality. Thus, the selection of the Qingke flour with appropriate particle size appears to be necessary.

Despite the potential nutritional benefits of Qingke, the introduction of Qingke flour to wheat bread may cause a textural and sensorial difference compared to non-Qingke supplemented wheat bread. This phenomenon has been observed and reported in some previous study. For instance, the wheat bread with addition of wheat bran (i.e. whole-wheat bread) or faba bean flour led to reduced bread specific volume and increased hardness compared to original wheat bread (Coda, Varis, Verni, Rizzello, & Katina, 2017; Li, Tilley, Chen, Siliveru, & Li, 2023). Bread with altered characteristics and sensory attributes may not resonate well with potential consumers, thereby impacting acceptability. A general solution for bread quality improvement is to use quality improvers i.e. hydroxypropyl methylcellulose (HPMC), sodium stearoyl lactylate (SSL) and transglutaminase (TG) as elucidated in the present study. HPMC is a hydrocolloid and a food emulsion stabilizer, and it has been widely used as a food additive in baking for dough enhancement. It was also reported the bread improved by HPMC had the lower bread hardness (Kim & Yokoyama, 2010). Similarly, SSL, a food emulsifier, is able to strengthen dough by enhance the gluten protein network interactions. The SSL-improved bread product exerts better specific volume, better crumb structure and a slower bread staling (Gomes-Ruffi, da Cunha, Almeida, Chang, & Steel, 2012). TG is the key additional enzyme to enhance the gluten protein network interactions and is involved with many physiochemical properties of bread. The specific volume, crumb structure, hardness, elasticity and water-holding capacity of bread was improved by addition of TG (Boukid et al., 2018).

Nevertheless, the aim of the current work is to upgrade the wheat bread formulation by supplement with Qingke flour for a bread product with lower starch digestibility, as evaluated by an *in vitro* inhibition experiment against α-amylase. Multiple physiochemical properties and sensory of bread is assumed to be affected by addition of Qingke flours. Therefore, three bread quality improvers i.e. HPMC, SSL and TG were used to enhance the quality and sensory of Qingke-supplemented bread.

2. Methods and materials

2.1. Materials

Raw *Dingqing* blue Qingke flour was obtained from Gan Yu Cang Agroproduct Develop Inc. (Tibet, China), at Changdu, Tibet (95°38′12.19″E, 31°24′21.55″N, altitude > 4000 m), China, and then freeze-dried to remove the moisture. Afterwards, the Qingke flour was stored at −20 °C until further use. Other chemicals in analytical purity were purchased from Sigma-Aldrich (Merck group, Munich, Germany).

Dried Qingke flour was milled and filtered by a screen into different particle sizes (from 80 to 140 mesh), and their physicochemical properties were characterized and compared to select the most satisfied Qingke flours i.e. 120 mesh sample for further breadmaking (see supplementary document for detailed analytical method and the results were shown in Fig. S1 and Table S2).

2.2. Qingke-supplemented dough preparation and bread baking

Dough was prepared using different formulations of mixture of Qingke and wheat flour as the base flour. In brief, 6 sets of dough were made by replacing 0%, 10%, 20%, 30%, 40% and 50% wheat flour with Qingke flour, respectively. Each Qingke-wheat base flour was added to 60% distilled water in an auto-spiral mixer (SINMAG Inc., Jiang Su Province, China), and mixed for 20 min at room temperature. Each dough with different addition of Qingke flour was referred as D0, D10, D20, D30, D40 and D50, respectively. All dough samples were stored (<24 h) at 4 °C until further analysis. Bread was made of the Qingke-supplemented dough with different addition of Qingke flour (i.e. D0-D50) described above. The formulation contains 1.2% yeast, 12% glucose, 2% milk powder, 0.8% salt, 10% butter, distilled water and freshly prepared Qingke-supplemented dough. In short, yeast, glucose, milk powder, salt and butter were added into each Qingke-supplemented dough individually, and premixed for 1 min. The dough was mixed for 15 min in the auto-spiral mixer with continuous addition of distilled water. The butter was added into dough and mixed for further 15 min in the mixer. The dough was then transferred into pans (150 g/pan) and was proved in a bread proofer at 35 °C for 90 min with the 85% humidity (SOUTHSTAR Instrument, Guang Zhou, China). Each dough sample was baked in a pre-heated oven (HANKE Instrument, Shan Dong, China) at 190 °C for 30 min. Bread sample made of D0, D10, D20, D30, D40 and D50 were referred to B0, B10, B20, B30, B40 and B50, respectively. All bread samples were cooled into room temperature and stored at 4 °C until further analysis.

2.3. Addition of HPMC, SSL and TG to bread baking

The Qingke-supplemented bread was prepared as the method described above, except that 0%-1.5% HPMC, 0%-0.25% SSL or 0%-0.025% TG was added while making the dough, respectively. Multiple physiochemical properties i.e. total free sulfhydryl group content, protein secondary structure, microstructure, textural parameters and sensory of each dough and bread sample with different addition of HPMC, SSL and TG were analyzed to evaluate their effect on bread quality.

Furthermore, the addition of HPMC, SSL and TG to bread baking was optimized *via* a three-factor Box-Behnken response surface methodology experiment comprising random organized 17 experimental runs. The methods performed and the optimal conditions of HPMC, SSL and TG were described in Supplementary document.

2.4. Analysis of multiple physicochemical properties of dough and bread

Textural analysis: each dough and bread sample was carried out using a TA.XTplus Texture Analyzer (Stable Micro Systems, Surrey, U.K.) according to a previous reported method with minor modifications (Corrado et al., 2023). Briefly, each dough slice (with same size) was tested on the Texture Analyzer and the test probe was P/36R. The test mode was as follows: test speed 1.0 mm/s, compression distance 50%, two compression cycle 5 s.

Microstructural analysis: The microstructure of each dough sample was analyzed by an ultra-high resolution scanning electron microscopy (SEM) (Regulus8100, Hitachi High-Tech Global, Tokyo, Japan) (Gómez, Ferrer, Añón, & Puppo, 2013). In short, each dough sample was freeze-dried and sputtered by gold. The microstructure of each sample was imaged by SEM using 6.0 kV accelerating voltage.

Free sulfhydryl group content determination: Free SH content of each dough sample was determined by spectrometer using Ellman's reagent i.e. DTNB regent according to a previous reported method with minor modifications (Gómez et al., 2013). Each freeze-dried dough sample (50 mg) was suspended in a buffer containing 2.5% sodium dodecyl sulfate, 10.4 g/L Tris, 1.2 g/L ethylenediaminetetraacetic acid and 6.9 g/L glycine. The DNTB regent was added, and incubated at dark for 30 min. After centrifugation at 10,000 r/min for 10 min, the absorbance of supernatant was measured at 412 nm. The free SH content was calculated by an equation as follows,

$$FreeSHcontent(\mu M) = 75.53 \times A_{412} \times \frac{D}{C} \quad (1)$$

where 75.53 is the molar absorption coefficience of DTNB; D is the

dilution times and C is protein concentration in sample (mg/mL).

Gluten analysis: The wet gluten content in each dough sample was determined according to a previous method based on the AACC International Approved Method (38–12.02) (Magallanes López, Ohm, Manthey, Rao, & Simsek, 2021).

Secondary protein structural analysis: The secondary structure of dough sample was analyzed using Fourier-transform infrared spectroscopy (FT-IR) (Thermo Fisher Scientific., Waltham, Massachusetts, America) according to a previous reported method (Gómez et al., 2013). Freeze-dried dough sample was mixed with potassium bromide and scanned by FT-IR at wavelength range from 400 to 4000 cm^{-1} with a resolution of 4 cm^{-1}. In Amide I region, the baseline passing through the ordinates at 1600 and 1700 cm^{-1} was smoothed and adjusted in order to calculate the band intensity. To calculate the secondary structure components, this region was truncated and fitted. The percentage contribution of each type of conformation, each band was divided by the total Amide I area (Gómez et al., 2013).

Specific volume analysis: The specific volume (as in mL/g) of each bread sample was measured and calculated by a method reported previously (Cao et al., 2022).

2.5. Sensory evaluation

The sensory evaluation of each bread sample was carried out according to a previous method with some modifications (Perri et al., 2021), by using a trained panel comprising 10 participants (5 males and 5 females) in triplicate. Randomized bread samples were tested by each participant at the same time. The sensorial traits were crumb color, taste, texture, aroma and overall acceptability, and the score scales from 0 to 20 presents the dislike to extreme like evaluated by participants.

2.6. Volatile compounds analysis

The key volatile compounds of bread samples were analyzed *via* a headspace extraction method combined with a gas chromatography-mass spectrometry (GC–MS) according to a reported method with some modifications (Xu et al., 2022). In brief, each sample (4.0 g) including B0 (i.e. wheat bread), B30 (i.e. wheat bread with 30% addition of Qingke flour) and the B30 with optimal addition of mixture of HPMC, SSL and TG (i.e. the optimal Qingke-supplemented wheat bread) were placed into a 30 mL headspace vial and equilibrated in a water bath at 60 °C for 45 min. The volatile compounds of each sample were then injected onto a RTX-5 ms column (RESTEK, Bellefonte, PA, America) and analyzed *via* GC–MS (Agilent GC–MS 5975, Agilent Technologies, Santa Clara, California, America) under the following conditions: helium was used as mobile phase at a flow rate of 1 mL/min; the initial temperature of GC–MS oven was set to 40 °C for 3 min; the heating program was a temperature increase at a rate of 4 °C/min until 150 °C; after 1 min holding, the temperature was further increased to 250 °C at a rate of 8 °C/min and the temperature was maintained for 6 min; the MS ion source and MS quadrupole temperature were set at 200 °C and 220 °C, respectively; the ionization mode was EI and the electron energy was 70 eV.

2.7. In vitro starch digestibility

The *in vitro* starch digestion assay was carried out using previous reported methods with minor modifications (Cao et al., 2022; Ratnaningsih, Suparmo, & E., & Marsono, Y. , 2020). In brief, each bread sample was freeze-dried, milled and filtered through 120 mesh, and each sample powder was dispersed with 50 mL distilled water (final concentration 100 mg/mL). Subsequently, sample was transferred to a 50 mL falcon tube and incubated with the pre-incubated porcine pancreatic α-amylase in sodium acetate buffer (500 mM, pH 7.0) (2.6 U) (Sigma-Aldrich, Merck group, Munich, Germany) at 37 °C with continuous shaking. Sample was aliquot during incubation from 0 min to 180 min, and the reaction was stopped by an immediate inactivation of α-amylase at 100 °C for 10 min. After cooling to room temperature, the reducing sugar content from each hydrolysate was measured using a DNS-colorimetric method with glucose as standard (Holck et al., 2019).

Total starch digestion (TSD) was calculated by equation as follow,

$$TSD(\%) = \frac{Totalrelseaseglucose \times 0.9}{Totalstarchcontent} \quad (2)$$

Note: glucose was converted into starch by multiplying 0.9 to the released glucose i.e. total reducing sugar content.

The *in vitro* starch digestion kinetics was fitted and calculated using a non-linear model, and the area under the hydrolysis curve (AUC) was calculated as described elsewhere (Ratnaningsih et al., 2020). The hydrolysis index (HI) (in %), was calculated by dividing the AUC of each sample by the AUC of a reference (white bread). The rapidly digested starch (RDS) was defined as the starch digested within first 20 min, and the slowly digested starch (SDS) was defined as the starch digested within the following 20–180 min. Estimation of glycemic indices (eGI) was calculated based on the HI by equation as follow according to the reported method (Ratnaningsih et al., 2020),

$$eGI(\%) = (0.549 \times HI) + 39.71 \quad (3)$$

2.8. Statistical analysis

All analytical experiments were carried out in triplicate, and the results were expressed as mean ± standard deviation of three independent replications. All data were analyzed statistically using S.P.S.S software (version 26.0, SPSS Inc., IL, USA). The significant difference ($p < 0.05$) was evaluated by one-way ANOVA test using Turkey's test.

3. Results and discussions

3.1. Effect of addition of Qingke flour on bread quality and in vitro starch digestion of Qingke-supplemented bread

Specific volume: It is expected that the supplement of Qingke flour could affect the quality and multiple physiochemical properties of wheat bread. It was shown that with the increase in addition of Qingke flour (from 0% to 50%), the bread specific volume was decreased from 4.26 to 3.3 mL/g (Fig. S2). The addition of Qingke flour significantly affected the specific volume of bread compared to white bread i.e. the B0 as control but higher addition (i.e. 30%-50%) of Qingke flour appeared not to further reduce the bread specific volume (Fig. S2). The decrease in bread specific volume could also be supported by the bread slice images shown in Fig. 1A. Compared to the white bread, bread supplemented with 10–20% Qingke flour exhibited slightly smaller size, while bread supplemented with 40–50% Qingke flour had an obvious reduction in bread slice size. The results were in accordance with some previous research that addition of other plant flours to wheat bread e.g. the whole-wheat bread, had lower specific volume compared to their white bread counterparts (Alzuwaid, Pleming, Fellows, Laddomada, & Sissons, 2021; Li et al., 2023; Packkia-Doss, Chevallier, Pare, & Le-Bail, 2019). The difference in bread specific volume is considered to be the decrease in wet gluten in dough. The dietary fiber in cereals may be able to disrupt the gluten network and hinder the dough extension, and result in a reduced bread volume (Gan, Galliard, Ellis, Angold, & Vaughan, 1992). Low gluten content may affect the formation of gluten network, and hence showed and negative effect on bread specific volume. Interestingly, we observed a significantly ($p < 0.05$) reduction in wet gluten content in dough with higher addition of Qingke flour (Fig. S3). Dough made of pure wheat flour showed 37% gluten content, and it was decreased to 23% while the addition of Qingke flour increased to 50% (Fig. S3). At this point, dough with 50% Qingke flour could barely form a good gluten network compared to other dough samples. Thus, an extreme high addition of Qingke flours to wheat bread is regarded to be

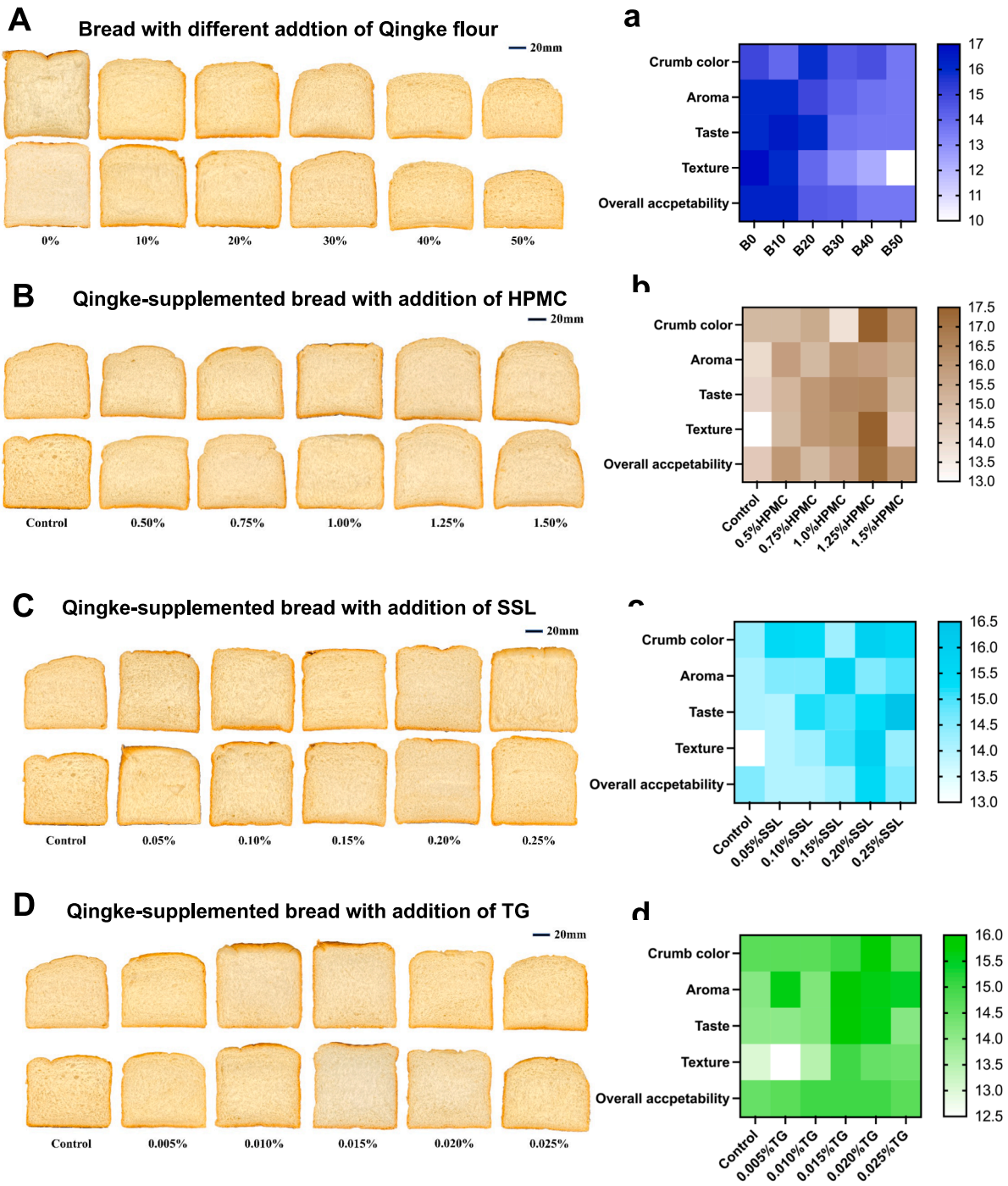

Fig. 1. Bread slices appearance image (A) and sensory (a) of Qingke-supplemented bread with different addition of Qingke flour. Bread slices appearance image (B-D) and sensory (b-d) of Qingke-supplemented bread improved by different addition of HPMC, SSL and TG, respectively. Control is the original Qingke-supplemented wheat bread sample without any addition of bread quality improvers. Sensory data for heatmap was collected from sensory evaluation in 10 replicants and the heatmap legends of a-d indicates the sensory data range and the lowest and highest sensory scores.

a negative effect on wheat bread quality.

Texture and sensory: The addition of Qingke flour clearly changed the texture of bread (Table 1 and Fig. 1). Compared to the white bread, the hardness and chewiness were significantly increased with addition of Qingke flour. Up to 4.8-fold and 3.4-fold higher hardness and chewiness were found in bread with 50% of Qingke flour. These results were in accordance with some previous study that bread made of whole cereal or bran had higher values of hardness and chewiness (Li et al., 2023; Wang, Hou, & Dubat, 2017). Hardness and chewiness are important index for bread product, and the ideal hardness and chewiness values could influence the acceptance by costumers. The high addition of Qingke flours caused significantly higher hardness and chewiness and is assumed to negatively affect the sensory characteristics. Surprisingly, the springiness appeared not to be affected (or only in a very slight level) by addition of Qingke flour. With 50% of Qingke flour addition, bread still showed a good springiness of 0.88 mm compared to the white bread (0.92 mm). As shown in Fig. 1A, the white bread i.e. control, showed good external characteristics and the gas cell was evenly distributed. Along with the increase in addition of Qingke flour, the gas cell structure of bread was changed. High addition of Qingke flour caused a higher cell

Table 1
Textural parameters of Qingke-supplemented bread and the bread improved by bread quality improvers.

Samples	Hardness (g)	Springiness (mm)	Chewiness (mJ)
Qingke-supplemented bread samples			
B0	245 ± 21^{z}	0.92 ± 0.0^{x}	211 ± 42^{z}
B10	398 ± 101^{yz}	0.91 ± 0.1^{x}	296 ± 85^{z}
B20	485 ± 140^{y}	0.91 ± 0.1^{x}	345 ± 102^{y}
B30	895 ± 136^{y}	0.90 ± 0.0^{x}	427 ± 51^{y}
B40	1099 ± 241^{x}	0.90 ± 0.0^{x}	545 ± 90^{y}
B50	1170 ± 100^{x}	0.88 ± 0.0^{x}	707 ± 62^{x}
Addition of HPMC (%)			
Control	895 ± 136^{a}	0.90 ± 0.0^{ab}	427 ± 51^{a}
0.5	641 ± 106^{b}	0.87 ± 0.1^{b}	434 ± 68^{a}
0.75	605 ± 116^{b}	0.88 ± 0.0^{ab}	379 ± 74^{a}
1.0	404 ± 103^{c}	0.92 ± 0.0^{ab}	290 ± 69^{b}
1.25	342 ± 79^{c}	0.93 ± 0.0^{a}	235 ± 65^{b}
1.5	347 ± 60^{c}	0.93 ± 0.0^{a}	244 ± 36^{b}
Addition of SSL (%)			
Control	895 ± 136^{A}	0.90 ± 0.0^{AB}	427 ± 51^{A}
0.05	429 ± 64^{B}	0.89 ± 0.0^{C}	283 ± 44^{B}
0.10	392 ± 84^{B}	0.91 ± 0.0^{BC}	262 ± 54^{B}
0.15	377 ± 48^{B}	0.91 ± 0.1^{BC}	257 ± 42^{B}
0.20	355 ± 64^{B}	0.94 ± 0.0^{AB}	253 ± 45^{B}
0.25	358 ± 49^{B}	0.94 ± 0.0^{A}	259 ± 33^{B}
Addition of TG (%)			
Control	$895 \pm 136^{\alpha}$	$0.90 \pm 0.0^{\alpha\beta}$	$427 \pm 51^{\alpha}$
0.005	$929 \pm 217^{\alpha}$	$0.91 \pm 0.0^{\alpha\beta}$	$593 \pm 101^{\alpha}$
0.010	$778 \pm 138^{\alpha\beta}$	$0.91 \pm 0.0^{\alpha\beta}$	$522 \pm 86^{\alpha\beta}$
0.015	$524 \pm 121^{\gamma}$	$0.93 \pm 0.0^{\alpha}$	$374 \pm 77^{\delta}$
0.020	$749 \pm 93^{\beta}$	$0.90 \pm 0.0^{\beta}$	$497 \pm 46^{\beta\gamma}$
0.025	$773 \pm 120^{\alpha\beta}$	$0.92 \pm 0.0^{\alpha\beta}$	$508 \pm 74^{\alpha\beta\gamma}$

Values represent mean ± standard deviation, and superscripts × to z differ significantly ($p < 0.05$) among different Qingke-supplemented bread samples with different addition of 100-mesh Qingke flour; a to c differ significantly ($p < 0.05$) among different Qingke-supplemented bread samples with different addition of HPMC; A to C differ significantly ($p < 0.05$) among different Qingke-supplemented bread samples with different addition of SSL; α to δ differ significantly ($p < 0.05$) among different Qingke-supplemented bread samples with different addition of TG.

density and porosity compared to white bread and the distribution of gas cell was more unevenly (Fig. 1A). In addition, as discussed above, high addition of Qingke flour could also reduce the bread size.

In order to understand the acceptability of each Qingke-supplemented bread, sensory evaluation was carried out by 10 trained participants. As expected, the white bread was the best-accepted bread i.e. having the highest sensory score in almost all parameters including crumb color (15.2 ± 0.1), aroma (16.0 ± 0.3), taste (16.0 ± 0.4), texture (17.0 ± 0.2) and overall acceptability (16.3 ± 0.5) (Fig. 1a). In general, increase in addition of Qingke flour clearly decreased all sensory parameters. The texture score of Qingke-supplemented wheat bread was significantly ($p < 0.05$) lower than the white bread, and this agreed with the lower bread specific volume and worse bread appearance (Fig. S2 and Fig. 1A). The sensory result of each bread product was in accordance with their physiochemical properties that with the addition of Qingke flours, smaller bread specific volume and worse textural properties caused lower acceptability of wheat bread. Interestingly, bread with 20% of Qingke flour displayed significantly higher ($p < 0.05$) crumb color (15.8 ± 0.3) and taste score (16.0 ± 0.3) compared to the wheat bread with higher addition of Qingke flours.

In vitro starch digestion: One of the most key targets of the current work is to reduce the starch digestion of wheat bread by supplement with Qingke flour. Results displayed a typical exponential increase curve in the *in vitro* starch digestion experiment of Qingke-supplemented wheat bread (Fig. 2a). Starch was rapidly and massively hydrolyzed by α-amylase at the first 20 min of total digestion period in all bread samples. The starch digestion of the white bread and bread with 50% Qingke flour was gradually reached a near-plateau phase with only slight subsequent increase in digestion after 60 min, and the total starch digestion was ended at 82% and 41%, respectively, while starch digestion of other bread samples was slowed down after 20 min (Fig. 2a). Generally, all bread samples showed less *in vitro* starch digestibility compared to the white bread, and the total starch digestion after the exhaustive enzymatic hydrolysis (i.e. 180-min incubation) ranged from 41% to 45%. Significantly lower starch digestibility may suggest that the addition of Qingke flour could contribute to the less intake of blood glucose when taking Qingke-supplemented wheat bread as food. The rapidly digested starch (RDS) and slowly digested starch (SDS) were further displayed and discussed (Fig. 2e). Resistance starch (RS) was defined as the non-digested starch after 180-min incubation with α-amylase. It was demonstrated that the highest RDS (53%) was found in the white bread, and it was decreased to 27% with the increase in addition of Qingke flour up to 50% (Fig. 2e). Except for the bread with 50% of Qingke flour, other Qingke-supplemented bread samples showed significantly lower SDS. Additionally, all Qingke-supplemented bread samples showed higher level of RS but no discrimination among all samples, and this agreed with the results observed in the starch digestion curve (Fig. 2a). These results revealed that the starch in Qingke-supplemented bread may be more difficult to be digested. The reduced starch digestibility could be explained by the reduced bread specific volume (Fig. 1A). The more compact microstructure e.g. less gas cell distance, is believed to hinder the binding and interaction between substrate and the α-amylase (Gkountenoudi-Eskitzi et al., 2023). The non-digestible dietary fiber was also increased with the increase in Qingke flour addition, and this could also affect the enzyme's access to substate, therefore resulting in lower RDS values. A previous study also reported that non-digestible and insoluble dietary fiber may limit the starch swelling and gelatinization and hence hinder the *in vitro* starch digestibility and glucose release (Brennan & Tudorica, 2008). The lower eGI observed in wheat bread samples with high addition of Qingke flour could also support that the glucose release *in vitro* was inhibited (Fig. S4). Taking results together, despite the wheat bread with 30% of Qingke flour did not show the greatest sensory properties (Fig. 1a), it was considered as the most satisfied bread product based on the *in vitro* starch digestion results, and it was used for further experiment.

3.2. Effect of quality improvers on quality, sensory and in vitro starch digestion of Qingke-supplemented bread

Specific volume and texture: In the present study, three quality improvers i.e. HPMC, SSL and TG were used, and their effects on Qingke-supplemented wheat bread quality were investigated. As shown in Table 1, in general, HPMC, SSL and TG were all able to improve the textural parameters of Qingke-supplemented wheat bread sample. By adding the HPMC and SSL, the hardness and chewiness of Qingke-supplemented bread samples were significantly ($p < 0.05$) reduced, while the springiness was slightly increased (Table 1). Interestingly, TG could also reduce the hardness and chewiness of bread samples while the addition of TG was only up to 0.15%. The further addition of TG could cause higher hardness and chewiness of bread samples than other TG-added bread samples, but still lower than original bread sample (Table 1). As expected, the addition of HPMC and SSL could increase the bread specific volume. The specific volume was increased to highest level of 4.2 g/mL with addition of 1.25% of HPMC (Fig. 3a). The specific volume was significantly increased to about 4.9 g/mL by addition of SSL in all concentrations with no obvious discrimination (Fig. 3b). Interestingly, the specific volume of Qingke-supplemented bread appeared only to be increased by 0.010% and 0.015% of TG (Fig. 3c). The change of specific volume could be supported by the bread appearance and texture shown in Fig. 1B-D. For instance, bread sample with 1.0% HPMC showed larger size of bread compared to the control. The same result

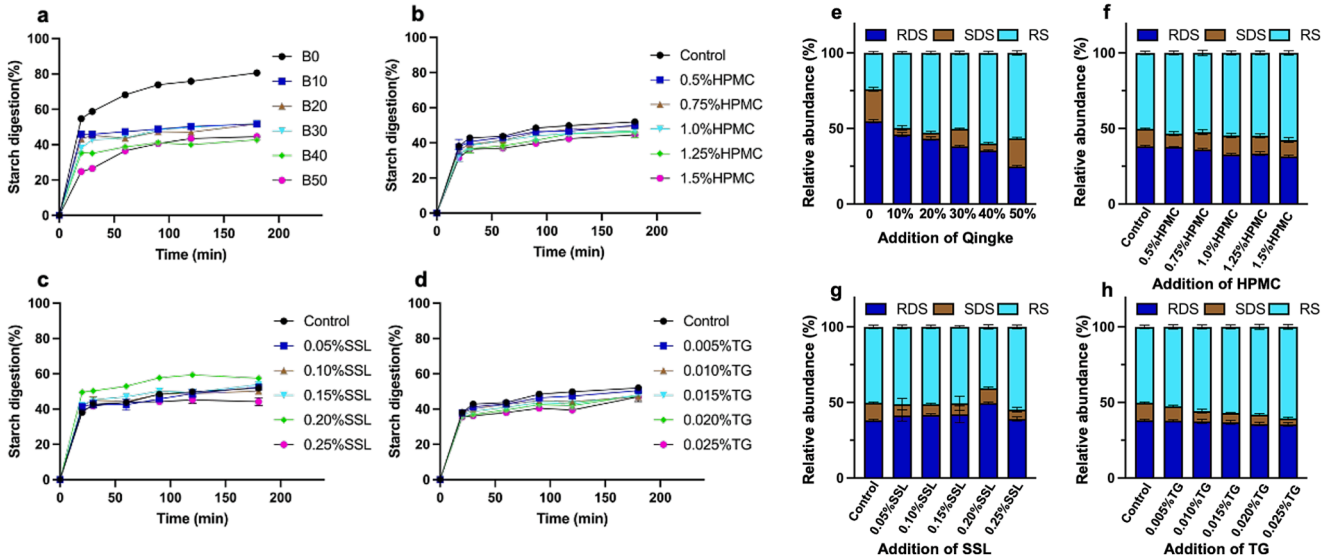

Fig. 2. *In vitro* starch digestion of wheat bread supplemented with different addition of Qingke flour (from 0% to 50%) during an enzymatic incubation with α-amylase up to 180 min (a). *In vitro* starch digestion of Qingke-supplemented bread improved by different addition of HPMC, SSL and TG (b-d); Relative abundance of rapidly digested starch (RDS), slowly digested starch (SDS) and resistance starch (RS) of each wheat bread supplemented different addition of Qingke flour (from 0% to 50%) during the *in vitro* starch digestion (e); Relative abundance of RDS, SDS and RS of each Qingke-supplemented bread improved by different addition of HPMC, SSL and TG (f-h).

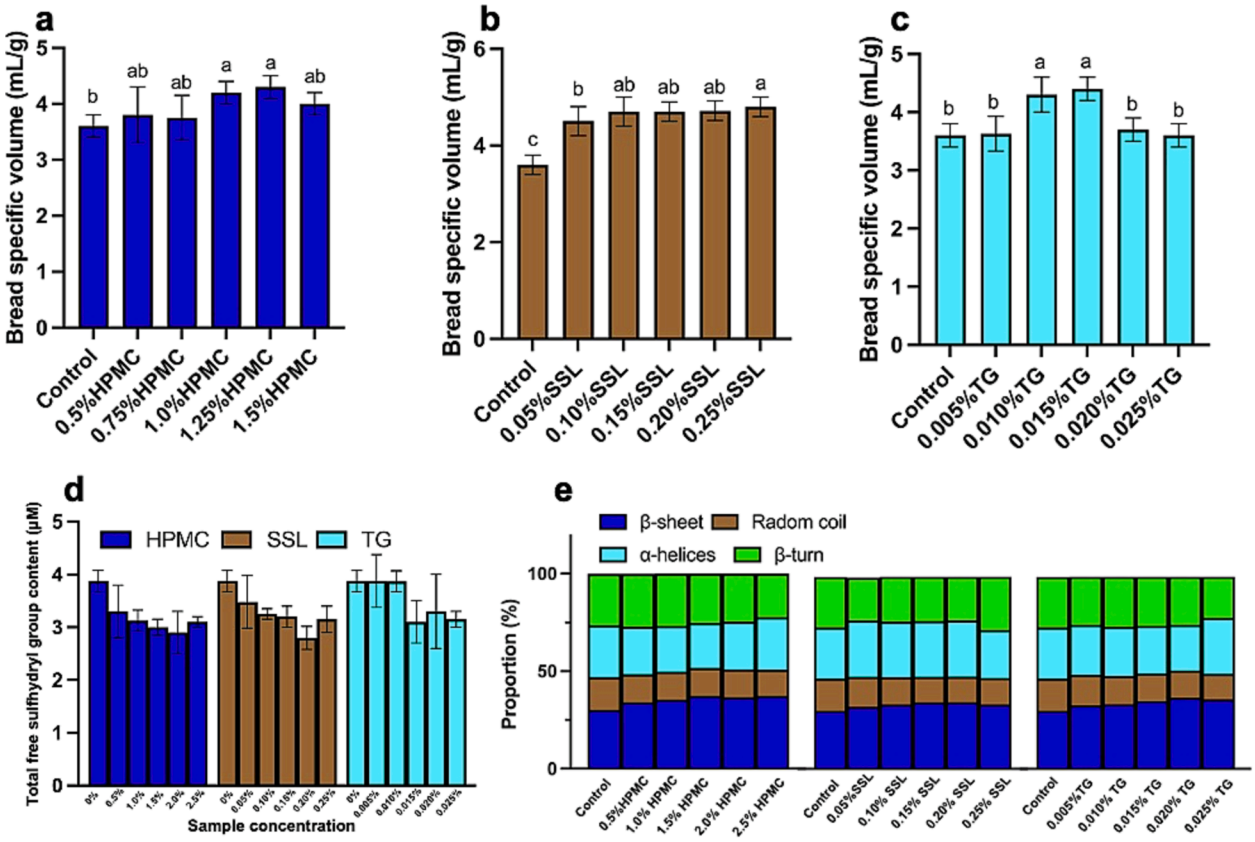

Fig. 3. Bread specific volume of each Qingke-supplemented wheat bread improved by different addition of HPMC, SSL and TG (a-c). Total free sulfhydryl content of each Qingke-supplemented wheat bread improved by different addition of HPMC, SSL and TG (d); Secondary protein structure abundance of each Qingke-supplemented wheat bread improved by different addition of HPMC, SSL and TG (e). a-c significantly ($p < 0.05$) differs among different Qingke-supplemented wheat bread samples.

was also observed in bread with 0.15% SSL and 015% TG, respectively. Meanwhile, the higher addition of HPMC, SSL and TG improved the bread texture and structure. The gas cell of bread was more evenly distributed and less compact compared to the control, which agreed with the lower hardness and chewiness as discussed above (Fig. 1B-D and Table 1). The results indicated that HPMC, SSL and TG were all good

quality improvers for Qingke-supplemented wheat bread. This could be supported by some previously reported literatures that HPMC and SSL are food emulsion stabilizer, and could strengthen the dough, increase the bread specific volume and decrease the bread hardness (Gomes-Ruffi et al., 2012; Kim & Yokoyama, 2010). Meanwhile, the bread with addition of TG also exhibited better specific volume, crumb structure, hardness, elasticity and water-holding capacity (Boukid et al., 2018). However, an extreme high addition of quality improvers is not recommended, as either type of them did not contribute a significantly greater enhancement on bread quality.

Microstructural analysis: The improvement of bread quality may be explained by the change of microstructure of Qingke-supplemented wheat dough. When analyzing the dough with addition of each quality improvers, it was clearly displayed that HPMC, SSL and TG significantly reduced the free sulfhydryl group in dough (Fig. 3d). It should be noted that HPMC and SSL were able to reduce the free sulfhydryl group in dough even at very low concentration, whereas TG appeared only to be efficient at the concentration higher than 0.01% (Fig. 3d). Nevertheless, all three quality improvers could reduce the sulfhydryl content in dough from 3.9 μM to around 3.0 μM, and this result indicated more disulfide bonds were formed in dough. It is believed that the formation of gluten protein network in dough is associated with the non-covalent or/and covalent interactions between glutenin molecules, and the disulfide bond is considered as one of the most dominant and vital covalent bonds to link the glutenin subunits (Veraverbeke & Delcour, 2002). The formation of more disulfide bonds in dough could enhance the polymerization of gluten network, and thereby resulting in a better gas retention property of dough (Beghin et al., 2022). It is reported that a good gas retention of dough may be important to produce a light and aerated bread during the dough fermentation and initial breadmaking (Beghin et al., 2022), and this is also believed to affect the bread specific volume. Meanwhile, the secondary structure of gluten protein in dough was analyzed by FT-IR. HPMC, SSL and TG increased the proportion of α-helices and β-sheets compared to control (Fig. 3e). The result suggested HPMC, SSL and TG were able to improve the gluten protein network according to a previous study that the amount of α-helices and β-sheets is positively correlated to the protein stability (Xiong, Zhang, Niu, & Zhao, 2017). SEM analysis also revealed the impact of three quality improvers in the Qingke-supplemented wheat dough. In the control sample, the multi-size starch granules were unevenly dispersed in the gluten protein network. Additionally, many starch granules were exposed in the surface of gluten network, which indicate the starch was not well-covered by the gluten network (Fig. 4a). Gluten network was significantly changed with addition of HPMC. The gluten structure of dough with 0.50% and 0.75% HPMC were more compact compared to control sample, and the gluten network interactions were more significant. However, there were still some large starch granules exposed in the surface of gluten network in dough (Fig. 4a). When the addition of HPMC was further increased, more gluten was formed, and the gluten network was more well-structured. Similarly, SSL was also able to improve the gluten network structure of Qingke-supplemented dough. But interestingly, only the dough with 0.15% and 0.20% addition of SSL showed the obviously improved gluten network structure, whereas the structure of gluten network in dough with 0.25% addition of SSL appeared to be damaged, and the starch granules were again exposed (Fig. 4b). We have observed the similar phenomenon in the Qingke-supplemented dough with addition of TG. As expected, TG enhanced the gluten network interactions and improved the structure stability, but an extremely high addition of TG i.e. 0.025% appeared to be harmful for the gluten network structure (Fig. 4c).

Sensory evaluation: As expected, in general, three quality improvers successfully enhanced all the sensorial parameters of Qingke-supplemented wheat bread samples compared to the control sample (Fig. 1 b-d). Except for the bread sample with 0.005% of TG, the texture score was significantly ($p < 0.05$) increased by HPMC, SSL and TG, and this agreed with the better bread specific volume and dough microstructures (Fig. 3a-c and Fig. 4). The significantly ($p < 0.05$) highest sensory scores were observed in the bread sample with addition of 1.25% HPMC (crumb color: 17.5 ± 0.5, aroma: 15.8 ± 0.2, taste: 16.5 ± 0.3, texture: 17.5 ± 0.1, overall acceptability: 17.2 ± 0.5), followed by addition of 1.0% and 1.5% HPMC. The same was observed in SSL and TG, where the significantly highest sensory score was found in 0.20% addition of SSL and 0.020% TG, respectively (Fig. 2b-d).

In vitro starch digestion: Interestingly, as indicated by digestion curve and eGI values (Fig. 2b-d and Fig. S4), addition of any concentration of HPMC, SSL and TG appeared not to further decease the *in vitro* starch digestibility of Qingke-supplemented bread. Despite we observed a clear change on the microstructure of dough sample caused by addition of HPMC, SSL and TG, it seems not to contribute to the inhibition on the *in vitro* starch digestion.

Lastly, through the response surface method, the optimal condition of each bread quality improvers is 1.15% of HPMC, 0.20% SSL and 0.015% TG (Fig. S5 and Table S3), and the optimal Qingke-supplemented bread showed 62% lower hardness and 46% lower chewiness, and 23% higher bread specific volume (data not shown) compared to the non-improved Qingke-supplemented bread.

3.3. Volatile compound

The addition of Qingke flour and any kinds of quality improvers is believed to contribute to a change of aroma of Qingke-supplemented wheat bread based on the different sensory score on aroma as discussed above (Fig. 1 a-d). A total of 21 volatile compounds belonging to 6 chemical classes comprising alcohols, aldehydes, alkenes, esters, alkanes and benzenes were detected in all three bread samples i.e. wheat bread, Qingke-supplemented wheat bread and Optimal Qingke-supplemented bread (Fig. 5). It was clearly demonstrated that addition of Qingke flour could significantly change the volatile compounds of bread sample compared to wheat bread. Meanwhile, the volatile compounds could also be changed by addition of HPMC, SSL and TG. Phenylethyl alcohol is associated with a honey and sweet aroma (Xu et al., 2022), and the addition of Qingke flour did not affect the production of phenylethyl alcohol, but the addition of quality improvers appeared to slightly reduce the relative abundance of phenylethyl alcohol (Fig. 5). The relative abundance of 1-Pentanol, 3-Hexen-1-ol, Methyl *p-tert*-butylphenylacetate, 2,4-Hexadiene and 2-Pentylfuran were lower in Qingke-supplemented wheat bread sample, and this was not increased by addition of HPMC, SSL and TG (Fig. 5). Compared to original wheat bread, Qingke-supplemented wheat bread produced 3,3-Dimethyl-1,2-epoxybutane, Heptane, Hexadecane, Dodecane and m-Xylene. Interestingly, the relative abundance of these compounds was reduced to be a minor level with addition of quality improvers (Fig. 5). Interestingly, the optimal Qingke-supplemented bread produced some new volatile compounds i.e. Cyclopropane, Ethylbenzene, p-Xylene, Styrene, D-Limonene, Myrcene and Terpinolene compared to wheat bread and Qingke-supplemented wheat bread (Fig. 5). These newly produced volatile compounds are considered to contribute to the new aroma in optimal Qingke-supplemented wheat bread. For instance, myrcene is believed to be associated with flower aroma and Terpinolene is associated with pine and sweet aroma.

4. Conclusion

In the current work, Qingke flour was used to partially replace the wheat flour in order to upgrade the wheat bread formulation. The supplement of Qingke flour significantly affected the physicochemical properties of wheat bread e.g. bread specific volume and texture, and it was in principle negatively correlated to the addition of Qingke flours. Hence, a worse sensory evaluation results were found in Qingke-supplemented wheat bread compared to the original bread sample. As expected, the addition of Qingke flours significantly reduced the *in vitro* starch digestibility of wheat bread, as indicated by lower content of

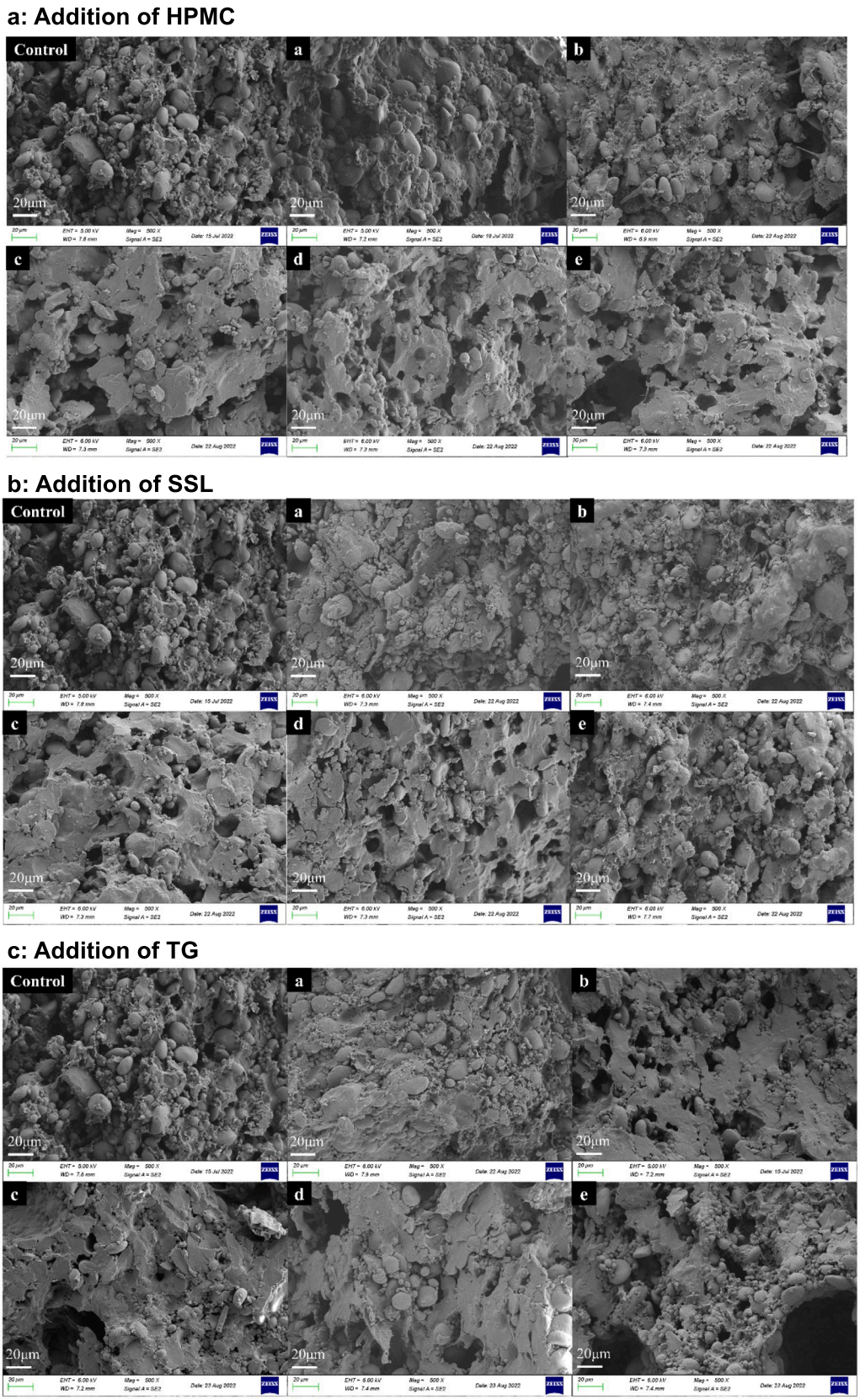

Fig. 4. Scanning electron microscopy images of Qingke-supplemented wheat dough improved by different addition of HPMC, SSL and TG (a-c).

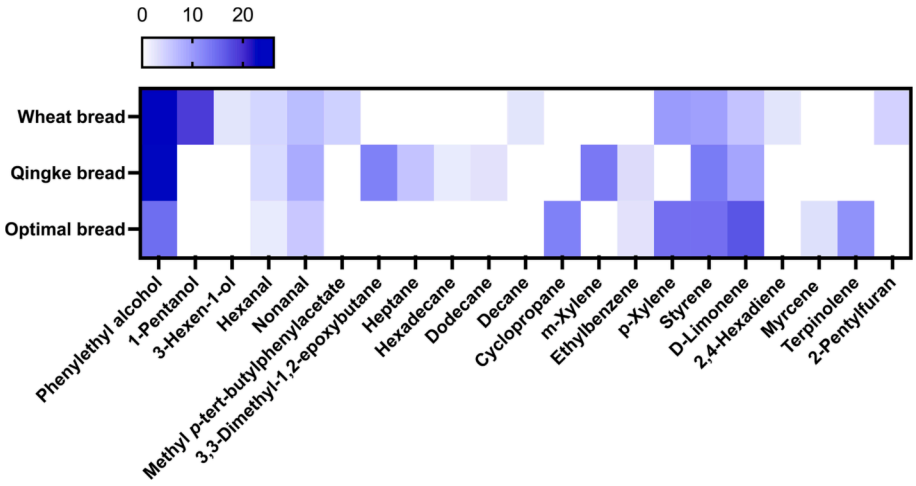

Fig. 5. Heatmap image showing the difference of each detected volatile compounds among original wheat bread sample (i.e. without any addition of Qingke flour), Qingke-supplemented wheat bread sample and Optimal Qingke-supplemented wheat bread sample (i.e. improved by an optimal addition of mixture of HPMC, SSL and TG).

rapidly digested starch and eGI values. The utilization of three widely-used bread quality improvers i.e. HPMC, SSL and TG contributed to the enhancement of quality and sensory evaluation of Qingke-supplemented wheat bread. However, the high level of addition of SSL and TG is not recommended because they did not show very positive results compared to lower addition of SSL and TG. Compared to original wheat bread the Qingke-supplemented wheat bread could generate several new volatile compounds, which may indicate this type of bread could show new aroma. Nevertheless, the addition of Qingke flour was aimed to upgrade the wheat bread formulation to produce the low-glucose intake wheat bread diet. This may be beneficial for the anti-diabetes and anti-obesity in the future.

CRediT authorship contribution statement

Shang Lin: Formal analysis, Validation, Writing – original draft. **Bingyu Huang:** Investigation, Formal analysis, Validation. **Shuxiang Liu:** Methodology. **Yaowen Liu:** Methodology. **Qing Zhang:** Formal analysis, Investigation. **Wen Qin:** Methodology, Supervision, Validation, Project administration, Funding acquisition, Writing – review & editing.

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.

Data availability

Data will be made available on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2023.100855.

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