



# Physicochemical properties, phenolic profiles, antioxidant capacities, and inhibitory effects on digestive enzymes of okra (*Abelmoschus esculentus*) fruit at different maturation stages

Dan-Dan Shen<sup>1</sup> · Xu Li<sup>1</sup> · Ya-Li Qin<sup>1</sup> · Mo-Ting Li<sup>1</sup> · Qiao-Hong Han<sup>1</sup> · Jie Zhou<sup>1</sup> · Shang Lin<sup>1</sup> · Li Zhao<sup>1</sup> · Qing Zhang<sup>1</sup> · Wen Qin<sup>1</sup> · Ding-Tao Wu<sup>1</sup>

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**Abstract** Phenolic compounds are considered the main bioactive components in okra fruits. In order to well understand the accumulation pattern of phenolic compounds in okra fruits during maturation, and to obtain okra fruits with high level of health-beneficial phenolic compounds, physicochemical properties, phenolic profiles, antioxidant capacities, and inhibitory effects on digestive enzymes of okra fruits at different maturation stages were investigated. Noticeable variations in physicochemical properties and phenolic profiles of okra were observed at different maturation stages. Phenolic compounds, including quercetin-3-O-gentiobioside, quercetin-3-O-glucoside (isoquercitrin), rutin, quercetin derivative, protocatechuic acid, and catechin derivative, were determined to be the major compounds in okra fruits, while quercetin-3-O-gentiobioside was the most abundant phenolic compound. Considering the accumulation patterns of fruit size, firmness, and total flavonoid content of okra fruits, the optimal harvest time of okra fruits with relatively high level of health-beneficial phenolic compounds was determined. Furthermore, okra fruits at different maturation stages exerted remarkable antioxidant capacities and inhibitory effects on the pancreatic lipase,  $\alpha$ -glucosidase, and  $\alpha$ -amylase. The Pearson's correlation showed that quercetin-3-O-gentiobioside was one of the major contributors to the antioxidant capacities and

inhibitory effects on digestive enzymes. Results are beneficial for understanding of the accumulation pattern of phenolic compounds in okra fruits during maturation, and can aid in the targeting of specific maturation stages with an optimal phenolic profile for the production of health-beneficial products.

**Keywords** Okra fruit · Phenolic compounds · HPLC analysis · Antioxidant capacity · Enzyme inhibition

## Introduction

The fruit of okra (*Abelmoschus esculentus*), known as lady's finger and gumbo, is an important vegetable crop (Xia et al. 2015). It is an annual plant native to Africa, and has been grown in different countries around the world, mainly in tropical, sub-tropical, and warm temperate regions (Xia et al. 2015). In recent years, okra has also been widely cultivated in the North and South China (Jiang et al. 2017). Okra fruits have a wide range of medicinal value, and have been used to control various diseases and disorders (Sabitha et al. 2011). The health-promoting effects of okra fruit are owing to its various biological activities. Pharmacological studies have demonstrated that okra fruit possesses various bioactivities, such as antioxidant, anti-diabetic, anti-hyperlipidemic, and anti-hyperglycemic effects (Graham et al. 2017; Lu et al. 2016; Sabitha et al. 2011; Zhang et al. 2018). Generally, phenolic compounds (Jiang et al. 2017; Liao et al. 2012; Lu et al. 2016; Xia et al. 2015; Zeng et al. 2015) are considered the main bioactive components in okra fruit, which are responsible for its various bioactivities. The content of phenolic compounds and their bioactivities of okra fruits are influenced by cultivars, growing conditions, and fruit

Dan-Dan Shen and Xu Li have contributed equally to this work.

✉ Wen Qin  
Qinwen@sicau.edu.cn

✉ Ding-Tao Wu  
DT\_Wu@sicau.edu.cn

<sup>1</sup> College of Food Science, Sichuan Agricultural University, Ya'an 625014, Sichuan, China

sizes (Olivera et al. 2012; Petropoulos et al. 2018). Nevertheless, studies on the changes of phenolic compounds, and their antioxidant capacities and inhibitory effects on digestive enzymes (pancreatic lipase,  $\alpha$ -glucosidase, and  $\alpha$ -amylase) of okra fruits at different maturation stages have seldom been investigated.

The ideal fruit harvesting time is an important factor affecting fruit quality (Petropoulos et al. 2018). Generally, the physicochemical properties, such as fruit weight, fruit sizes, firmness, free sugars, and organic acids are considered as the primary indicators for harvest (Olivera et al. 2012; Petropoulos et al. 2018). Especially, the fruit size has been used as a harvest index for okra harvest. Nowadays, with the improvement in quality of life, consumers are attaching increasing importance to health (Ma et al. 2017). Therefore, besides the physicochemical properties are necessary for okra fruit harvest, the changes of bioactive components and bioactivities of okra fruits during maturation are also important. Whether the accumulation pattern of health-beneficial phenolic compounds in okra fruits during development and maturation is consistent with that of fruit size remains unknown. Therefore, in order to well understand the accumulation pattern of phenolic compounds in okra fruits during maturation, and to obtain okra fruits with high level of health-beneficial phenolic compounds, physicochemical properties, phenolic profiles, antioxidant capacities, and inhibitory effects on digestive enzymes (pancreatic lipase,  $\alpha$ -glucosidase, and  $\alpha$ -amylase) of okra fruits at different maturation stages were systematically investigated.

## Materials and methods

### Samples and chemicals

“Wufu” (cultivar) okra fruits (*A. esculentus*) were harvested from 100 of marked plants (similar in vigor, size, and anthesis) at a commercial orchard (30°46′ 18.50″N, 104°02′ 20. 02″ E) located in Chengdu, Sichuan province, China. After 4 days post-anthesis (DPA), the fruits were harvested every day for six times during the period from 19th July to 24th July, 2017 (Fig. 1). At each time, forty of fruits were harvested and weighted, and then delivered to the laboratory. The whole okra fruits were washed with distilled water, and divided into two parts. The first part of fruits was used to measure the physicochemical properties. And the second part of fruits was frozen and freezing dried. Subsequently, the samples were ground to pass through a 60 mesh sieve, and stored at  $-20\text{ }^{\circ}\text{C}$  for further analysis.

Protocatechuic acid, caffeic acid, catechin, quercetin-3-O-gentiobioside, quercetin, rutin, quercetin-3-O-glucoside (isoquercitrin), gallic acid, 6-hydroxy-2,5,7,8-tetramethyl



**Fig. 1** Morphological characteristic of “Wufu” okra fruits at different maturation stages. DPA days post-anthesis

chroman-2-carboxylic acid (Trolox), 2,2-diphenyl-1-picrylhydrazyl (DPPH), pancreatic lipase,  $\alpha$ -glucosidase,  $\alpha$ -amylase, starch, *p*-nitrophenyl- $\alpha$ -D-glucopyranoside (pNPG), *p*-nitrophenyl acetate, and Folin-Ciocalteu reagent were purchased from Sigma-Aldrich (St. Louis, Mo, U.S.A). All other reagents and chemicals used were of analytical grade.

### Determination of length, diameter, and firmness

Twenty of okra fruits were immediately measured for length and major diameter by using a digital vernier caliper with a sensibility of 0.01 mm. The firmness of okra fruits was measured by a penetration test using a TA.XT-plus Texture Analyser (Stable Micro System, United Kingdom) equipped with a 5-kg load cell. The penetrometer was fitted with the p/5 (5 mm diameter) needle probe. The probe was driven into the flesh okra fruit with a trigger force of 8.0 g at a pre-test speed of 5 mm/s, a test speed of 1.0 mm/s, and a post-test speed 10 mm/s. Firmness was measured five times at the equator of each okra fruit.

### Extraction of phenolic compounds

Phenolic compounds of okra fruits were extracted according to a previously reported method with minor modifications (Yang et al. 2015). Briefly, 30 mL of 70% acidified methanol (0.1% HCl, *v/v*) was added into 1.0 g of each sample. Then, the mixture was extracted twice with ultrasound (50 kHz, 480 W) for 60 min at room temperature. After centrifugation (4000  $\times$  g, 10 min), the supernatants were combined, and concentrated to dryness using a rotary evaporator at 45  $^{\circ}\text{C}$ . Finally, the dried residue was reconstituted in 70% of methanol, and stored at  $-20\text{ }^{\circ}\text{C}$  in dark for the further determination of total flavonoids, individual phenolic compounds, antioxidant capacities, and inhibitory effects on digestive enzymes. The extract was filtered

through a 0.22  $\mu\text{m}$  organic membrane prior to analysis using high performance liquid chromatography (HPLC).

### Determination of total flavonoid content

The total flavonoid content (TFC) of okra fruit extract was estimated according to a previously reported method with minor modifications (Lin et al. 2018). Briefly, 100  $\mu\text{L}$  of each okra extract or rutin standard solution was added into 30  $\mu\text{L}$  of 5% sodium nitrite solution (*w/v*). After 6 min, 30  $\mu\text{L}$  of 10% aluminum nitrate solution (*w/v*) was added. Subsequently, 400  $\mu\text{L}$  of 4% sodium hydroxide (*w/v*) was added, and incubated at room temperature for 25 min. Finally, the absorbance of the mixture was measured at 510 nm, and the TFC was expressed as milligram of rutin equivalents per gram of okra fruits dry weight (mg RE/g DW).

### HPLC analysis of individual phenolic compounds

Phenolic compounds of okra fruit extract were evaluated by an Agilent 1260 series LC system (Agilent Technologies, Santa Clara, CA, USA) equipped with a diode-array detector (DAD). Chromatographic separations were conducted at 25  $^{\circ}\text{C}$  on a ZORBAX Eclipse XDB-C18 column (250 mm  $\times$  4.6 mm, 5  $\mu\text{m}$ ). The chromatographic separation was achieved by gradient elution with 0.5% (*v/v*) acetic acid solution (A) and acetonitrile (B) according to a previous study with minor modifications (Ma et al. 2017). Briefly, samples were eluted as follows: 0 min, 5% B; 5 min, 5% B; 50 min, 5–20% B; 70 min, 20–70% B; 72 min, 70–5% B; 72–77 min, 5% B. The flow rate was 0.8 mL/min and the injection volume was 20  $\mu\text{L}$  for all samples. Detection was made at 280 nm, 320 nm, and 360 nm, respectively. Identification of phenolic compounds was carried out by comparing retention times and absorption spectra of commercial standards, and spiking of external reference standards, as well as comparing the information of the same type of samples previously reported (Arapitsas 2008). The identified compounds were quantified by external calibration method using calibration curves. Briefly, the contents of protocatechuic acid ( $y = 28.455x + 0.2127$ ,  $R^2 = 0.999$ ), catechin ( $y = 16.756x - 9.765$ ,  $R^2 = 0.999$ ), quercetin ( $y = 80.507x + 9.5089$ ,  $R^2 = 0.999$ ), quercetin-3-O-gentiobioside ( $y = 43.945x - 310.13$ ,  $R^2 = 0.998$ ), rutin ( $y = 30.337x - 6.2411$ ,  $R^2 = 0.998$ ), and isoquercitrin ( $y = 56.55x + 68.103$ ,  $R^2 = 0.999$ ) were measured by external calibration method using calibration curves. The contents of catechin derivative and quercetin derivative were expressed as catechin equivalents and quercetin equivalents, respectively. The content of each phenolic

compound was expressed as microgram per gram dry weight ( $\mu\text{g/g DW}$ ).

### Determination of antioxidant capacity of okra fruit extract

#### *The Folin–Ciocalteu reagent assay (FCR)*

The FCR assay, according to (Lin et al. 2018), measures sample reducing capacity, which does not reflect the total phenolic content; therefore, it is advised to use the FCR method as a reducing capacity assay (Górnas' et al. 2015). Briefly, 250  $\mu\text{L}$  of each okra fruit extract or gallic acid standard solution was added into 1.25 mL of Folin–Ciocalteu reagent. Then the mixture was incubated at room temperature for 3 min in dark, and 1.25 mL of sodium carbonate solution (20%, *w/v*) was added. After incubated at room temperature for 30 min in dark, the absorbance was measured at 765 nm using a Varioskan flash multi-mode reader (Thermo Fisher, Waltham, Mass., USA). The FCR reducing capacity was express as milligram of gallic acid equivalents per gram of okra fruits dry weight (mg GAE/g DW).

#### *DPPH radical scavenging capacity*

The DPPH radical scavenging antioxidant capacity of okra fruit extract was determined according to a previously reported method with minor modifications (Lin et al. 2018). Briefly, 25  $\mu\text{L}$  of each okra fruit extract or methanol as negative control was mixed with 200  $\mu\text{L}$  of DPPH solution (0.35 mM) in a 96-well microplate. Then the mixture was shaken, and incubated at 37  $^{\circ}\text{C}$  for 30 min in dark. The absorbance of the mixture was measured at 517 nm with a blank contain only DPPH solution and methanol. Trolox was used as the standard, and the DPPH radical scavenging capacity was expressed as  $\mu\text{mol}$  of Trolox equivalents per gram of okra fruits dry weight ( $\mu\text{mol TE/g DW}$ ). The  $\text{IC}_{50}$  value of DPPH radical scavenging capacity was expressed as mg of okra fruits dry weight/mL (mg DW/mL).

#### *Reducing power*

The reducing power of okra fruit extract was determined according to a previously reported method with minor modifications (Lin et al. 2018). Briefly, an aliquot of 100  $\mu\text{L}$  okra fruit extract or Trolox solution was mixed with 100  $\mu\text{L}$  of potassium ferricyanide (1%, *w/v*) in PBS (0.2 M, pH 6.8). Then the mixture was incubated at 50  $^{\circ}\text{C}$  for 20 min, and followed by the addition of 100  $\mu\text{L}$  of trichloroacetic acid (10%, *w/v*). After centrifugation (3000  $\times$  g, 10 min), 100  $\mu\text{L}$  of supernatant was mixed

with 100  $\mu\text{L}$  of distilled water and 20  $\mu\text{L}$  of ferric chloride (0.1%, w/v). Finally, the absorbance of the mixture was measured at 700 nm after 30 min incubation. The reducing power was expressed as  $\mu\text{mol}$  of Trolox equivalents per gram of dry weight ( $\mu\text{mol TE/g DW}$ ).

### Inhibitory effects of okra fruit extract on pancreatic lipase, $\alpha$ -glucosidase, and $\alpha$ -amylase

#### *In vitro* pancreatic lipase inhibition assay

The *in vitro* pancreatic lipase inhibition assay was conducted according to a previously reported method with minor modifications (Tan et al. 2017). In brief, the *p*-nitrophenyl acetate (10 mM, dissolved in DMSO) stock solution was diluted with distilled water to reach a final concentration of 2 mM. 100  $\mu\text{L}$  of each okra extract at different concentrations was mixed with 200  $\mu\text{L}$  of Tris buffer (50 mM, pH 7.4) and 100  $\mu\text{L}$  of pancreatic lipase solution (5 mg/mL, dissolved in 50 mM, pH 7.4 Tris buffer). After incubated at 37 °C for 10 min, 100  $\mu\text{L}$  of *p*-nitrophenyl acetate solution (2 mM) was added, and then incubated at 37 °C for 15 min. A portion of 200  $\mu\text{L}$  of reaction mixture was taken and added into a 96-well microplate, and the absorbance was measured at 410 nm. The commercial capsule of orlistat was used as a positive control. Results were expressed as inhibition (%) of pancreatic lipase activity according to the following equation below. Pancreatic lipase inhibitory effect was measured at five different concentrations, and a logarithmic regression curve was established to calculate  $\text{IC}_{50}$  values (mg of okra fruit dry weight/mL).

$$\text{Pancreatic lipase inhibition}\% = \left[ 1 - \frac{A_{\text{sample}} - A_{\text{blank}}}{A_{\text{control}}} \right] \times 100\%$$

where  $A_{\text{sample}}$  is the absorbance of the mixture of okra fruit extract, Tris buffer, pancreatic lipase solution, and *p*-nitrophenyl acetate solution;  $A_{\text{blank}}$  is the absorbance of the mixture of okra fruit extract, Tris buffer, Tris buffer (instead of pancreatic lipase solution), and *p*-nitrophenyl acetate solution;  $A_{\text{control}}$  is the absorbance of the mixture of 70% methanol (instead of okra fruit extract), Tris buffer, pancreatic lipase solution, and *p*-nitrophenyl acetate solution.

#### *In vitro* $\alpha$ -glucosidase inhibition assay

The *in vitro*  $\alpha$ -glucosidase inhibitory activity was conducted according to the previously described method with slight modifications (Tan et al. 2017). In brief, 100  $\mu\text{L}$  of okra fruit extract at different concentrations was mixed with 100  $\mu\text{L}$  of  $\alpha$ -glucosidase (0.5 U/mL, dissolved in

0.1 M, pH 6.8 phosphate buffer), and incubated at 37 °C for 15 min. Then, 25  $\mu\text{L}$  of 4 mM *p*-nitrophenyl- $\alpha$ -D-glucopyranoside (pNPG) solution (dissolved in 0.1 M, pH 6.8 phosphate buffer) was added to initiate the reaction at 37 °C for 20 min. The absorbance of the mixture was measured at 405 nm. Acarbose standard was used as a positive control. Results were expressed as inhibition (%) of  $\alpha$ -glucosidase activity according to the following equation below.  $\alpha$ -Glucosidase inhibitory effect was measured at five different concentrations, and a logarithmic regression curve was established to calculate  $\text{IC}_{50}$  values (mg of okra fruit dry weight/mL).

$$\alpha\text{-Glucosidase inhibition}\% = \left[ 1 - \frac{A_{\text{sample}} - A_{\text{blank}}}{A_{\text{control}}} \right] \times 100\%$$

where  $A_{\text{sample}}$  is the absorbance of the mixture of okra fruit extract,  $\alpha$ -glucosidase solution, and pNPG solution;  $A_{\text{blank}}$  is the absorbance of the mixture of okra fruit extract, buffer, and  $\alpha$ -glucosidase solution;  $A_{\text{control}}$  is the absorbance of the mixture of  $\alpha$ -glucosidase solution, buffer, and pNPG solution.

#### *In vitro* $\alpha$ -amylase inhibition assay

The *in vitro*  $\alpha$ -amylase inhibition assay was carried out by a previously described method with minor modifications (Tan et al. 2017). Briefly, 100  $\mu\text{L}$  of okra fruit extract was mixed with 100  $\mu\text{L}$  of  $\alpha$ -amylase solution (50 U/mL, dissolved in 0.1 M, pH 6.8 phosphate buffer), and incubated at 37 °C for 30 min with continuous shaking. Then 200  $\mu\text{L}$  of soluble starch (0.5%, w/v) was added into the mixture, and incubated at 37 °C for 10 min. Subsequently, 1.6 mL of 3, 5-dinitrosalicylic acid (DNS) reagent was added into the mixture, and incubated at a boiling water bath for 5 min. Finally, the absorbance of the mixture was measured at 540 nm. Acarbose standard was used as a positive control. Results were expressed as inhibition (%) of  $\alpha$ -amylase activity according to the following equation below.  $\alpha$ -Amylase inhibitory activity was measured at five different concentrations, and a logarithmic regression curve was established to calculate  $\text{IC}_{50}$  values (mg of okra fruit dry weight/mL).

$$\alpha\text{-Amylase inhibitory}\% = \left[ 1 - \frac{A_{\text{sample}} - A_{\text{control}}}{A_{\text{test}} - A_{\text{blank}}} \right] \times 100\%$$

where  $A_{\text{sample}}$  is the absorbance of the mixture of okra fruit extract,  $\alpha$ -amylase solution, soluble starch, and DNS reagent;  $A_{\text{control}}$  is the absorbance of the mixture of okra fruit extract, buffer (instead of  $\alpha$ -amylase), soluble starch, and DNS reagent;  $A_{\text{test}}$  is the absorbance of the mixture of 70% methanol (instead of okra fruit extract),  $\alpha$ -amylase

solution, soluble starch, and DNS reagent;  $A_{\text{blank}}$  is the absorbance of the mixture of 70% methanol (instead of okra fruit extract), soluble starch, buffer (instead of  $\alpha$ -amylase), and DNS reagent.

### Statistical analysis

All experiments were conducted in triplicate, and data were expressed in means  $\pm$  standard deviations ( $n \geq 3$ ). Statistical analysis was performed using SPSS 21.0 software, and the differences among mean values were tested by one-way ANOVA, taking a level of  $p < 0.05$  as significant to Duncan's multiple range test. Pearson's correlation coefficients were determined by Origin 2017 software.

## Results and discussion

### Weight, size, and firmness of okra fruits at different maturation stages

The fresh weight of per fruit, length, major diameter, and firmness are considered the primary indicators for okra fruit harvest (Olivera et al. 2012). The changes of physicochemical properties of okra fruits at different maturation stages are shown in Table 1. The fresh weight, length, and major diameter increased significantly ( $p < 0.05$ ) during maturation, and reached the maximum values at 9 DPA, respectively. The okra fruit grown rapidly during the stages from 5 to 8 DPA, and the growth rate of weight of per fruit was more than 5 g/day. In addition, the length/major diameter increased rapidly at the first three stages, and then reached fairly stable values during the next stages. The firmness of okra fruits increased significantly during maturation, and reached the fairly stable values at the stages from 8 to 9 DPA. The differences in firmness of okra fruit harvested at 8 DPA and 9 DPA were not statistically significant. As shown in Table 1, the lengths of okra fruits increased from  $9.72 \pm 0.55$  to  $17.08 \pm 0.64$  cm during maturation stages from 4 DPA to 9 DPA. Generally, the length of okra fruit has been considered the most important harvest index for okra fruits (Olivera et al. 2012; Petropoulos et al. 2018). Both small and big sizes of fruits are harvested and consumed (Petropoulos et al. 2018). Therefore, considering both firmness and length of okra fruits during maturation, the harvest time of "Wufu" okra fruits could be the stages from 4 DPA to 9 DPA.

### Phenolic profiles of okra fruits at different maturation stages

Phenolic compounds are considered the main bioactive components in okra fruits (Liao et al. 2012; Xia et al.

2015). Nevertheless, the accumulation pattern of phenolic compounds in "Wufu" okra fruit has seldom been investigated. Table 1 summarized the changes of TFC in okra fruits at different maturation stages. The TFC of okra fruits changed significantly during fruit maturation. Briefly, the TFC of okra fruits increased from  $1.88 \pm 0.03$  to  $2.95 \pm 0.06$  mg RE/g DW at the stages from 4 DPA to 6 DPA, and then decreased slowly from  $2.86 \pm 0.04$  to  $2.54 \pm 0.08$  mg RE/g DW at the stages from 7 to 9 DPA, respectively. The possible reason for this observation could be due to the stoppage or slow rate of new biosynthesis of phenolic compounds during fruit maturation (a dilution effect as fruits increase in size) (Anand and Aradhya 2005). Considering the accumulation patterns of TFC, fruit size, and firmness of okra fruits during maturation, the optimal harvest time of "Wufu" okra fruits with higher level of health-beneficial phenolics could be the stages from 6 to 9 DPA.

Furthermore, in order to well understand the change patterns of individual phenolic compounds in okra fruits during maturation, the HPLC–DAD analysis was performed. Previous studies have shown that quercetin, isoquercitrin, rutin, quercetin-3-O-gentiobioside, hydroxycinnamic derivatives, and catechin derivatives have been found in okra seeds and skins (Arapitsas 2008; Lin et al. 2014; Xia et al. 2015). Therefore, a total of 7 phenolic compounds, including quercetin, isoquercitrin, rutin, quercetin-3-O-gentiobioside, catechin, caffeic acid, and protocatechuic acid, were selected and investigated in okra fruit extract. Figure 2 showed the chromatograms of mixed standards and representative phenolic profiles of "Wufu" okra fruit. As shown in Fig. 2, five phenolic compounds, including protocatechuic acid, quercetin-3-O-gentiobioside, quercetin, rutin, and isoquercitrin, were identified based on their HPLC retention time and UV spectral information. Moreover, one catechin derivative and one quercetin derivative were also determined based on their UV spectral information. Briefly, the catechin derivative had the same characteristic UV spectrum of catechin (UV  $\lambda_{\text{max}}$ , 225 nm and 280 nm). The quercetin derivative also had the same characteristic UV spectrum of quercetin (UV  $\lambda_{\text{max}}$ , 255 nm and 365 nm). The changes of these phenolic compounds in okra fruits during maturation were presented in Table 2.

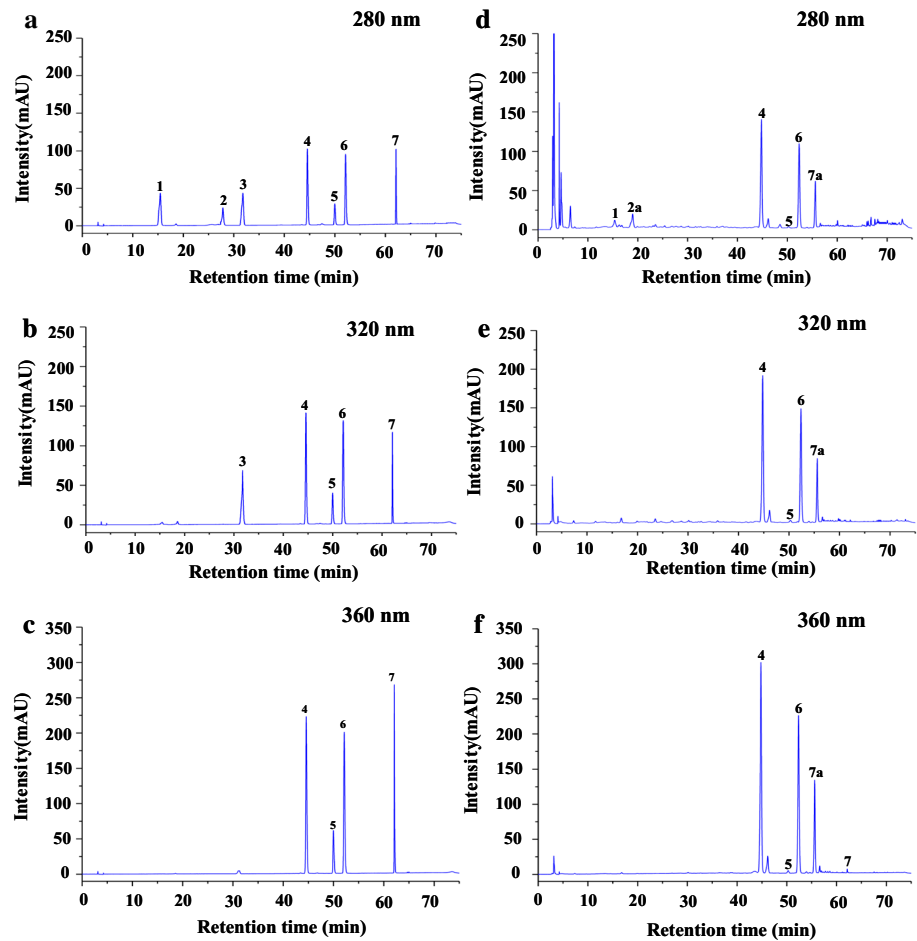
As shown in Table 2, one phenolic acid, protocatechuic acid, was detected. The contents of protocatechuic acid in okra fruits increased from  $107.67 \pm 0.37$  to  $114.77 \pm 0.35$   $\mu\text{g/g}$  DW at the stages from 4 DPA to 5 DPA, and then decreased significantly from  $114.77 \pm 0.35$  to  $64.13 \pm 0.34$   $\mu\text{g/g}$  DW at the stages from 5 to 9 DPA. One catechin derivative was also detected. Its contents increased from  $157.64 \pm 0.34$  to  $284.92 \pm 0.44$   $\mu\text{g/g}$  DW at the stages from 4 to 6 DPA, and then decreased slowly

**Table 1** Fruit weight, length, diameter, firmness, total flavonoid content (TFC), Folin–Ciocalteu reagent assay (FCR), DPPH radical scavenging capacities, and reducing power of okra fruits at different maturation stages

Stages	Fruit weight (g)	Length (cm)	Diameter (cm)	Length/diameter	Firmness (kg/cm <sup>2</sup> )	TFC (mg RE/g DW)	FCR (mg GAE/g DW)	DPPH (μmol TE/g DW)	Reducing power (μmol TE/g DW)
4 DPA	8.90 ± 0.38 <sup>f</sup>	9.72 ± 0.55 <sup>f</sup>	1.32 ± 0.09 <sup>e</sup>	6.70 ± 0.45 <sup>c</sup>	7.42 ± 1.61 <sup>c</sup>	1.88 ± 0.03 <sup>e</sup>	5.19 ± 0.14 <sup>d</sup>	25.77 ± 0.64 <sup>d</sup>	42.37 ± 0.55 <sup>e</sup>
5 DPA	10.66 ± 0.36 <sup>e</sup>	10.50 ± 0.47 <sup>e</sup>	1.45 ± 0.07 <sup>e</sup>	7.28 ± 0.31 <sup>b</sup>	7.99 ± 1.85 <sup>bc</sup>	2.72 ± 0.04 <sup>bc</sup>	7.84 ± 0.17 <sup>c</sup>	31.70 ± 0.91 <sup>c</sup>	61.26 ± 0.56 <sup>d</sup>
6 DPA	15.72 ± 0.69 <sup>d</sup>	12.78 ± 0.43 <sup>d</sup>	1.63 ± 0.10 <sup>d</sup>	7.72 ± 0.31 <sup>a</sup>	8.16 ± 1.18 <sup>bc</sup>	2.95 ± 0.06 <sup>a</sup>	9.43 ± 0.14 <sup>a</sup>	36.32 ± 1.05 <sup>a</sup>	73.53 ± 0.60 <sup>a</sup>
7 DPA	21.35 ± 0.97 <sup>c</sup>	14.05 ± 0.65 <sup>c</sup>	1.83 ± 0.07 <sup>c</sup>	7.61 ± 0.41 <sup>ab</sup>	8.24 ± 1.17 <sup>bc</sup>	2.86 ± 0.04 <sup>ab</sup>	8.51 ± 0.13 <sup>b</sup>	35.74 ± 0.12 <sup>a</sup>	70.25 ± 1.48 <sup>b</sup>
8 DPA	27.33 ± 1.61 <sup>b</sup>	15.64 ± 0.25 <sup>b</sup>	2.02 ± 0.12 <sup>b</sup>	7.73 ± 0.26 <sup>a</sup>	8.75 ± 0.92 <sup>ab</sup>	2.64 ± 0.08 <sup>cd</sup>	8.34 ± 0.09 <sup>b</sup>	33.76 ± 0.74 <sup>b</sup>	70.20 ± 1.83 <sup>b</sup>
9 DPA	30.79 ± 1.01 <sup>a</sup>	17.08 ± 0.64 <sup>a</sup>	2.21 ± 0.06 <sup>a</sup>	7.80 ± 0.37 <sup>a</sup>	9.41 ± 0.78 <sup>a</sup>	2.54 ± 0.08 <sup>d</sup>	8.15 ± 0.21 <sup>bc</sup>	32.64 ± 0.89 <sup>bc</sup>	64.29 ± 0.71 <sup>c</sup>

Each value represents the mean ± standard deviation. Different letters in the same column indicate significant differences at  $p < 0.05$

**Fig. 2** HPLC chromatograms of mixed standards (a–c) and representative phenolic profiles of okra fruits (d–f). **1** protocatechuic acid; **2** catechin; **3** caffeic acid; **4** quercetin-3-O-gentiobioside; **5** rutin; **6** isoquercitrin; **7** quercetin; **2a** catechin derivative; **7a** quercetin derivative



**Table 2** Contents (µg/g DW) of individual phenolic compounds in okra fruits at different maturation stages

Peak	Phenolic compounds (µg/g DW)	Stages					
		4 DPA	5 DPA	6 DPA	7 DPA	8 DPA	9 DPA
1	Protocatechuic acid	107.67 ± 0.37 <sup>b</sup>	114.77 ± 0.35 <sup>a</sup>	97.36 ± 0.44 <sup>c</sup>	89.74 ± 0.28 <sup>d</sup>	83.13 ± 0.33 <sup>c</sup>	64.13 ± 0.34 <sup>f</sup>
2	Catechin	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
2a	Catechin derivative	157.64 ± 0.34 <sup>c</sup>	277.85 ± 0.40 <sup>b</sup>	284.92 ± 0.44 <sup>a</sup>	226.00 ± 0.42 <sup>c</sup>	226.22 ± 0.43 <sup>c</sup>	216.63 ± 0.30 <sup>d</sup>
3	Caffeic acid	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
4	Quercetin-3-O-gentiobioside	625.09 ± 0.44 <sup>f</sup>	1028.27 ± 0.37 <sup>e</sup>	1359.04 ± 0.36 <sup>a</sup>	1178.42 ± 0.45 <sup>b</sup>	1167.71 ± 0.45 <sup>c</sup>	1044.40 ± 0.23 <sup>d</sup>
5	Rutin	23.19 ± 0.25 <sup>f</sup>	38.40 ± 0.37 <sup>e</sup>	52.86 ± 0.33 <sup>a</sup>	44.81 ± 0.39 <sup>b</sup>	43.66 ± 0.33 <sup>c</sup>	40.01 ± 0.38 <sup>d</sup>
6	Isoquercitrin	647.05 ± 0.33 <sup>c</sup>	905.45 ± 0.46 <sup>a</sup>	812.35 ± 0.37 <sup>b</sup>	806.55 ± 0.42 <sup>c</sup>	721.72 ± 0.62 <sup>e</sup>	551.23 ± 0.42 <sup>f</sup>
7	Quercetin	N.Q.	N.Q.	N.Q.	N.Q.	N.Q.	N.Q.
7a	Quercetin derivative	181.45 ± 0.59 <sup>e</sup>	278.84 ± 0.42 <sup>a</sup>	255.03 ± 0.35 <sup>b</sup>	252.46 ± 0.53 <sup>c</sup>	226.33 ± 0.15 <sup>d</sup>	176.59 ± 0.37 <sup>f</sup>
	Total	1743.55	2646.37	2864.79	2600.23	2471.00	2093.76

N.D.: the compound can not be detected; N.Q.: the amount is not available for quantification

Each value represents the mean ± standard deviation. Content of catechin derivative was expressed as catechin equivalents, and content of quercetin derivative was expressed as quercetin equivalents

Significant ( $p < 0.05$ ) differences are shown by data bearing different letters (a–f)

The peaks were the same as in Fig. 2

from  $284.92 \pm 0.44$  to  $216.63 \pm 0.30$   $\mu\text{g/g}$  DW at the stages from 6 to 9 DPA. In addition, five flavonols, including quercetin-3-O-gentiobioside, isoquercitrin, rutin, quercetin, and quercetin derivative, were detected in okra fruits. As shown in Table 2 and Fig. 2, the quercetin-3-O-gentiobioside was not only the most abundant individual flavonols, but also the most abundant individual phenolic compounds in okra fruits, which is in accordance with previous studies (Lin et al. 2014; Xia et al. 2015). The contents of quercetin-3-O-gentiobioside increased significantly ( $p < 0.05$ ) from  $625.09 \pm 0.44$  to  $1359.04 \pm 0.36$   $\mu\text{g/g}$  DW at the stages from 4 to 6 DPA, and then decreased slightly from  $1359.04 \pm 0.36$  to  $1044.40 \pm 0.23$   $\mu\text{g/g}$  DW at the stage from 6 to 9 DPA. In addition, the contents of another abundant flavonol, isoquercitrin, increased from  $647.05 \pm 0.33$  to  $905.45 \pm 0.46$   $\mu\text{g/g}$  DW at the stages from 4 DPA to 5 DPA, and then decreased from  $905.45 \pm 0.46$  to  $551.23 \pm 0.42$   $\mu\text{g/g}$  DW at the stage from 5 to 9 DPA. The significant decrease in contents of isoquercitrin at the stages from 5 to 6 DPA might be attributed to the biosynthesis of quercetin-3-O-gentiobioside using isoquercitrin as a substrate (Cho et al. 2016), which results a significant increase in contents of quercetin-3-O-gentiobioside at the stages from 5 to 6 DPA. The accumulation pattern of rutin was similar with that of quercetin-3-O-gentiobioside during okra maturation. Besides, the contents of quercetin derivative increased from  $181.45 \pm 0.59$  to  $278.84 \pm 0.42$   $\mu\text{g/g}$  DW at the stages from 4 to 5 DPA, and then decreased slowly from  $255.03 \pm 0.35$  to  $176.59 \pm 0.37$   $\mu\text{g/g}$  DW at the stage from 6 DPA to 9 DPA.

#### Antioxidant capacities of okra fruits at different maturation stages

The contribution of okra fruits to health improvement has been partially attributed to their antioxidant capacities (Xia et al. 2015). Table 2 summarized the antioxidant capacities of okra fruits at different maturation stages, and the  $\text{IC}_{50}$  values of DPPH radical scavenging capacity was shown in Fig. 3a. The antioxidant capacities of okra fruits changed significantly during fruit maturation. Briefly, the DPPH radical scavenging capacities increased from  $25.77 \pm 0.64$  to  $36.32 \pm 1.05$   $\mu\text{mol TE/g}$  DW at the stages from 4 to 6 DPA, and then decreased slightly from  $35.74 \pm 1.05$  to  $32.64 \pm 0.89$   $\mu\text{mol TE/g}$  DW at the stages from 7 to 9 DPA. The DPPH radical scavenging capacities of “Wufu” okra fruit were similar with previous studies (Liao et al. 2012). The FCR assay was also used to assess the reducing capacity of the samples (Górnas' et al. 2015). The FCR values increased from  $5.19 \pm 0.14$  to  $9.43 \pm 0.14$  mg GAE/g DW at the stages from 4 DPA to 6 DPA, and then

decreased slightly from  $8.51 \pm 0.13$  to  $8.15 \pm 0.21$  mg GAE/g DW at the stages from 7 to 9 DPA. Furthermore, the reducing power also increased from  $42.37 \pm 0.55$  to  $73.53 \pm 0.60$   $\mu\text{mol TE/g}$  DW at the stages from 4 to 6 DPA, and then declined slightly from  $70.25 \pm 1.48$  to  $64.29 \pm 0.71$   $\mu\text{mol TE/g}$  DW at the stages from 7 to 9 DPA. The antioxidant capacities of okra fruits among FCR, DPPH and reducing power assays showed a similar change pattern during fruit maturation. The change pattern of antioxidant capacities was also similar with the accumulation pattern of TFC as abovementioned. Moreover, the  $\text{IC}_{50}$  values of DPPH assay decreased from  $15.94 \pm 1.41$  to  $10.25 \pm 0.17$  mg DW/mL, and then increased from  $10.25 \pm 0.17$  to  $12.73 \pm 0.73$  mg DW/mL during maturation (Fig. 3a), which is in accordance with the antioxidant capacities. The low  $\text{IC}_{50}$  values confirmed that okra fruits possessed high antioxidant capacity, which suggested that okra fruits could be potential resources of antioxidants for the production of health-beneficial products.

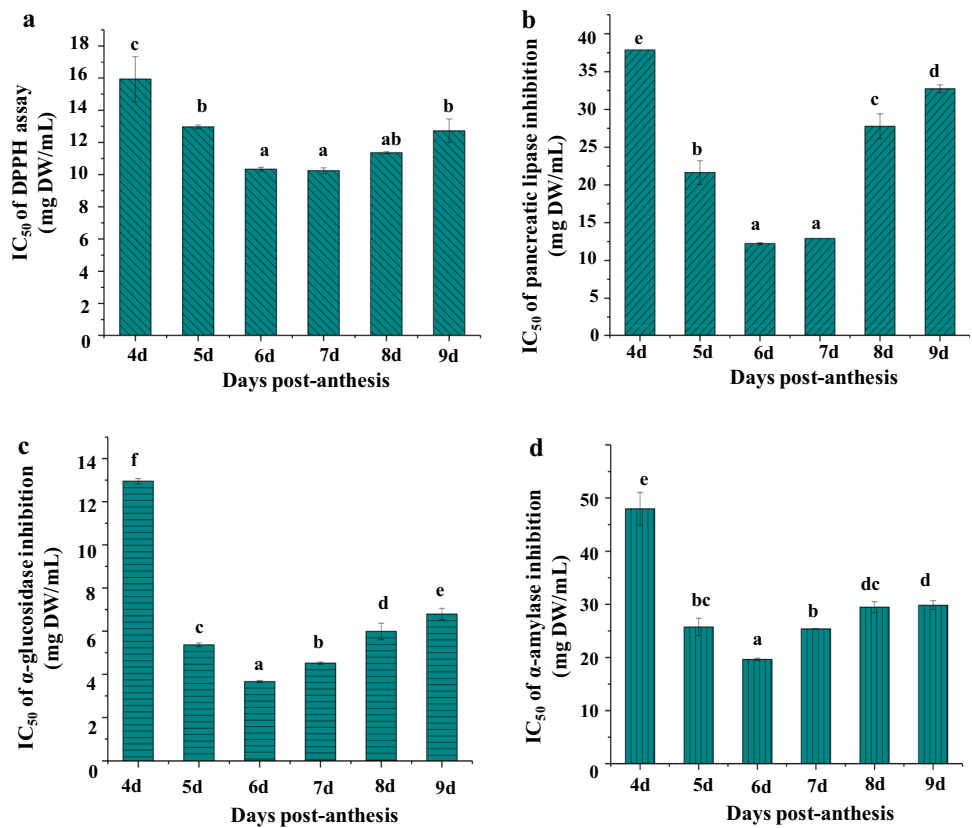
#### Inhibitory effects on digestive enzymes of okra fruits at different maturation stages

Pancreatic lipase is a key enzyme involved in triglyceride digestion. The inhibition of pancreatic lipase is a key approach to the control of hyperlipidaemia and obesity (Zhang et al. 2015). Figure 3b showed that the inhibitory activities of okra fruit extract on pancreatic lipase changed significantly at different maturation stages. Briefly, the  $\text{IC}_{50}$  values significantly decreased from  $37.88 \pm 0.02$  to  $12.23 \pm 0.11$  mg DW/mL at the stages from 4 DAP to 6 DPA, and then significantly increased from  $12.23 \pm 0.11$  to  $32.74 \pm 0.52$  mg DW/mL at stages from 6 to 9 DPA. The change pattern of inhibitory effects on pancreatic lipase was also similar with the accumulation pattern of TFC. Furthermore, compared with the commercial orlistat drug ( $\text{IC}_{50} = 6.34$  mg/mL), the inhibitory effects on pancreatic lipase of okra fruit with the highest level of flavonols also presented moderate  $\text{IC}_{50}$  value ( $12.23 \pm 0.11$  mg DW/mL). Therefore, okra fruits could be explored as functional food ingredients for the prevention of hyperlipidaemia and obesity.

$\alpha$ -Glucosidase and  $\alpha$ -amylase are key enzymes responsible for the breakdown of oligosaccharides and disaccharides into monosaccharides suitable for absorption. Therefore, the inhibition of  $\alpha$ -glucosidase and  $\alpha$ -amylase is one of the main strategies to counteract metabolic alterations related to hyperglycaemia and type 2 diabetes (Nowicka et al. 2016). Previous studies have shown that the okra extract exhibits strongly inhibitory effect on  $\alpha$ -glucosidase and  $\alpha$ -amylase (Cahyana et al. 2017; Karim et al. 2014). However, the changes of inhibitory effects on  $\alpha$ -glucosidase and  $\alpha$ -amylase of okra fruit at different



**Fig. 3** DPPH radical scavenging capacity (a), and inhibitory activities on pancreatic lipase (b),  $\alpha$ -glucosidase (c), and  $\alpha$ -amylase (d) of okra fruits at different maturation stages. Significant ( $p < 0.05$ ) differences are shown by data bearing different letters (a–f)



maturation stages have seldom been investigated. Figure 3c, d showed that the IC<sub>50</sub> values of inhibitory effects on  $\alpha$ -glucosidase and  $\alpha$ -amylase varied from  $3.66 \pm 0.05$  to  $12.95 \pm 0.12$  mg DW/mL, and from  $19.64 \pm 0.21$  to  $47.97 \pm 3.13$  mg DW/mL, respectively. The change patterns of inhibitory effects on  $\alpha$ -glucosidase and  $\alpha$ -amylase of okra fruit during maturation were also similar with the accumulation pattern of TFC. Furthermore, compared with the acarbose standard (IC<sub>50</sub> = 4.63 mg/mL), the okra fruit with the highest level of flavonols exerted remarkable inhibitory effects on  $\alpha$ -glucosidase (IC<sub>50</sub> =  $3.66 \pm 0.05$  mg DW/mL). Therefore, okra fruits could be potential anti-hyperglycemic agents.

### Correlations between bioactivities and phenolic compounds

The correlations among phenolic compounds, antioxidant capacities, and inhibitory effects on digestive enzymes (pancreatic lipase,  $\alpha$ -glucosidase, and  $\alpha$ -amylase) were summarized in Table 3. The TFC ( $r \geq 0.944$ ) showed significantly ( $p < 0.01$ ) positive correlations with antioxidant capacities measured by FCR, DPPH, and reducing power assays, respectively. The total flavonoids might be the main contributors toward the antioxidant capacities of okra fruits. Previous studies have also noticed good

correlations between phenolic compounds and antioxidant capacities of okra fruits (Ahmed and Kumar 2016; Chao et al. 2014). Furthermore, highly positive correlations among quercetin-3-O-gentiobioside ( $r \geq 0.979$ ), rutin ( $r \geq 0.974$ ) and antioxidant capacities were measured, which suggested that quercetin-3-O-gentiobioside and rutin might play significant roles in antioxidant capacities of okra fruits. Similar studies have shown that quercetin-3-O-gentiobioside exhibits remarkable antioxidant activity in vitro (Xia et al. 2015; Fan et al. 2014). Moreover, significant correlations were also observed between phenolic compounds and inhibitory effects on digestive enzymes. Briefly, the strong correlations were observed between IC<sub>50</sub> values of pancreatic lipase inhibition and TFC ( $r = -0.882$ ), which is in accordance with previous studies that phenolic compounds exerted strongly inhibitory activity on pancreatic lipase (Podsedeck et al. 2014; Zhang et al. 2015). In addition, the IC<sub>50</sub> values of pancreatic lipase inhibition were significantly ( $p < 0.05$ ) correlated with quercetin-3-O-gentiobioside ( $r = -0.813$ ), quercetin derivative ( $r = -0.827$ ), and rutin ( $r = -0.814$ ), respectively. The IC<sub>50</sub> values of pancreatic lipase inhibition were also correlated with isoquercitrin ( $r = -0.745$ ). Previous studies have shown that quercetin and its derivatives exert inhibition effect on the pancreatic lipase (Sergent et al. 2012; Sakulnarmrat and Konczak 2012). Therefore,

**Table 3** Pearson's correlation coefficients among phenolic compounds, antioxidant capacities, and inhibitory activities on digestive enzymes of okra fruits at different maturation stages

	TFC	PA	CD	QOG	RU	IS	QUID	FCR	DPPH	RP	PL	$\alpha$ -Glu	$\alpha$ -Amy
TFC	1												
PA	-0.171	1											
CD	0.867*	0.156	1										
QOG	0.955**	-0.301	0.797	1									
RU	0.947**	-0.309	0.792	0.998**	1								
IS	0.574	0.687	0.707	0.416	0.393	1							
QUID	0.733	0.518	0.814*	0.586	0.563	0.976**	1						
FCR	0.964**	-0.367	0.810*	0.988**	0.987**	0.368	0.553	1					
DPPH	0.960**	-0.365	0.723	0.979**	0.977**	0.384	0.560	0.976**	1				
RP	0.944**	-0.413	0.726	0.982**	0.974**	0.344	0.529	0.982**	0.985**	1			
PL	-0.882*	-0.160	-0.726	-0.813*	-0.814*	-0.745	-0.827*	-0.775	-0.834*	-0.755	1		
$\alpha$ -Glu	-0.947**	0.233	-0.873**	-0.950**	-0.934**	-0.493	-0.662	-0.958**	-0.909*	-0.949**	0.725	1	
$\alpha$ -Amy	-0.941**	0.202	-0.912**	-0.946**	-0.936**	-0.491	-0.657	-0.958**	-0.891*	-0.926**	0.726	0.992**	1

TFC total flavonoid content, PA protocatechuic acid, CD catechin derivative, QOG quercetin-3-O-gentiobioside, RU rutin, IS isoquercitrin, QUD quercetin derivative, FCR Folin-Ciocalteu reagent assay, DPPH DPPH radical scavenging capacity, RP reducing power, PL pancreatic lipase inhibition activity,  $\alpha$ -Glu  $\alpha$ -glucosidase inhibition activity,  $\alpha$ -Amy  $\alpha$ -amylase inhibition activity; Correlation is significant at \*  $p < 0.05$ , \*\*  $p < 0.01$  level (two-tailed)

quercetin-3-O-gentiobioside and isoquercitrin could be the major contributors toward the pancreatic lipase inhibition effects of okra fruits due to their high contents. There were also significant ( $p < 0.01$ ) correlations among the  $IC_{50}$  values of  $\alpha$ -glucosidase and  $\alpha$ -amylase inhibition and TFC ( $r \leq -0.941$ ). The  $IC_{50}$  values of  $\alpha$ -glucosidase and  $\alpha$ -amylase inhibition were significantly ( $p < 0.01$ ) correlated with catechin derivative ( $r \leq -0.873$ ), quercetin-3-O-gentiobioside ( $r \leq -0.946$ ), and rutin ( $r \leq -0.934$ ), respectively. As a matter of fact, proanthocyanidins from okra fruits, which are composed of (epi)gallocatechins and (epi)catechins, exhibit remarkable  $\alpha$ -amylase and  $\alpha$ -glucosidase inhibitory activities (Lu et al. 2016). Previous studies have also indicated that phenolic compounds, such as quercetin derivatives, are major contributors to the inhibitory activities on digestive enzymes of okra fruit (Karim et al. 2014; Zeng et al. 2015). Therefore, quercetin-3-O-gentiobioside and catechin derivative might play important roles in  $\alpha$ -amylase and  $\alpha$ -glucosidase inhibition effects of okra fruits due to their high contents. However, for further understanding of the structure–function relationships of phenolics from okra, the chemical structures of the catechin derivative and quercetin derivative are required to be elucidated, and individual phenolic compounds with well known chemical structure are also required for the investigation of bioactivities in future study.

## Conclusion

In the present study, noticeable variations in okra fruit physicochemical properties and phenolic profiles were observed at different maturation stages. Considering the accumulation patterns of fruit size, firmness, and phenolic compounds in okra fruit during maturation, the optimum harvest time of okra fruits with relatively high level of health-beneficial phenolic compounds was determined. Results are beneficial for better understanding of the accumulation pattern of phenolic compounds, and can aid in the targeting of specific maturation stages with an optimal phenolic profile for the production of health-beneficial products. Furthermore, the okra fruits exerted remarkable antioxidant capacities and inhibitory effects on digestive enzymes, which suggested that okra fruits could be explored further as function food ingredients for industrial applications.

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## Compliance with ethical standards

**Conflict of interest** The authors declare that there are no conflicts of interest.

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