



## The flavonoid profiles in the pulp of different pomelo (*Citrus grandis* L. Osbeck) and grapefruit (*Citrus paradisi* Mcfad) cultivars and their *in vitro* bioactivity

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### ABSTRACT

Previous results indicated that the flavonoid profiles might have varietal differences in pomelo, but detailed information is unknown. We previously isolated 4 new flavonoids, cigranoside C, D, E, F, in *Citrus grandis* Shatianyu pulp. However, their distribution in different pomelo cultivars remains to be explored. Therefore, the flavonoid profiles and *in vitro* bioactivity of the pulp from 5 pomelo and 1 grapefruit cultivars commonly consumed in China were investigated. Fourteen flavonoids were identified, cigranoside C, D, E were detected in these pomelo and grapefruit. Naringin and cigranoside C were the major flavonoids in grapefruit, Guanximiyu-W, Guanximiyu-R and Liangpingyu, while melitidin and rhoifolin was the predominant flavonoid in Shatianyu and Yuhuanyu, respectively. Pomelo and grapefruit showed strong antioxidant activity, and were potent inhibitors of pancreatic lipase with IC<sub>50</sub> values of 11.4–72.6 mg fruit/mL except Shatianyu. Thus, pomelo and grapefruit are natural antioxidants and possess anti-obesity potential.

### 1. Introduction

Flavonoids are the most common group of polyphenols in fruits and can contribute to reducing the risk of many chronic diseases, such as metabolic syndrome, type 2 diabetes mellitus and cardiovascular diseases, possibly due to their antioxidant activity and digestive enzyme inhibitory activity (Liu, 2003; Sun, Warren and Gidley, 2019; Wu et al., 2017; Zhu et al., 2014). *Citrus* fruits, greatly popular in the world, are abundant in flavonoids, especially flavanones in aglycone or glycoside forms (Khan & Dangles, 2014; Lu et al., 2020). Pomelo (*Citrus grandis* (L.) Osbeck) is a kind of *Citrus* fruits and cultivated widely in southern China. *C. grandis* cvs. Guanximiyu, Shatianyu, Liangpingyu and Yuhuanyu are representative pomelo cultivars in China (Zhang et al., 2011). Grapefruit (*C. paradise* Mcfad), a hybrid of sweet orange (*C. sinensis*) and sweet pomelo (*C. maxima* Burm) mainly distributed in South Africa and the European Union, is also highly appreciated by Chinese consumers.

Earlier studies have preliminarily revealed the flavonoid profiles of the pulp of pomelo and grapefruit. Mäkyänen et al. (2013) detected 6 flavonoids, including naringin, naringenin, hesperidin, hesperetin,

dihydrochalcone and neohesperidin, in the pulp of pomelo cultivars from Thailand and found that naringin was their main flavonoid. Xi et al. (2014) revealed that naringin was the predominant flavonoid in pomelo, while naringin and neohesperidin were the predominant flavonoids in grapefruits. In our previous study, we isolated 4 new flavonoids (cigranoside C, D, E, F), together with 2 firstly reported flavonoids (neorierocitrin and bergamjuicin) in pomelo. Besides, the main flavonoid we isolated from Shatianyu pulp was melitidin (Deng et al., 2021). These different results from our lab and others indicated that the flavonoid profiles in pomelo pulp might have varietal difference. Furthermore, the distribution of the 6 newly isolated flavonoids from Shatianyu in different pomelo cultivars remains to be explored. Therefore, it is necessary to further analyze the compositions and contents of flavonoids among different pomelo cultivars.

Antioxidant activity and digestive enzymes (pancreatic lipase,  $\alpha$ -amylase and  $\alpha$ -glucosidase) inhibitory effects are the important effects of flavonoids, which account for their many health benefits. The bioactivities of flavonoids are closely related to their molecular structure (Liu et al., 2017; Salahuddin et al., 2020; Su et al., 2014). Previous

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studies showed that the flavonoid extracts of citrus peels (Huang et al., 2020) or the digesta of citrus fruits (Sun, Tao, Huang, Ye and Sun, 2019) exhibited CAA activity and pancreatic lipase inhibitory activity. The differences in antioxidant and enzyme inhibitory activity of flavonoids from different pomelo pulp are still unknown.

In order to clarify the varietal differences of pomelo pulp in flavonoid profiles and bioactivity, 5 representative pomelo cultivars together with a grapefruit cultivar commonly consumed in China were analyzed in the present study to determine their compositions and contents of flavonoids in the pulp; and to compare their differences in antioxidant activity and inhibitory activity to  $\alpha$ -amylase,  $\alpha$ -glucosidase and pancreatic lipase.

## 2. Materials and methods

### 2.1. Materials

Shatianyu was purchased from Meizhou county, Guangdong province, China, in December 2018. Liangpingyu was obtained from Liangping county, Chongqing province, China, in November 2018. Guanximiyu with red (Guanximiyu-R) and white (Guanximiyu-W) pulp were purchased from Pinghe county, Fujian province, China, in October 2018. Yuhuanpu was obtained from Yuhuan county, Zhejiang province, China, in November 2018. Grapefruits were collected from the local supermarket in Guangzhou in October 2018.

### 2.2. Chemicals and reagents

Cigranoside A, B, C, D, E, F, bergamjuicin, neoeriocitrin, melitidin, rhoifolin, and naringin were prepared in our laboratory (Deng et al., 2021). Hesperidin, neohesperidin, narirutin, isoquercitrin, (+)-catechin, quercetin, gallic acid, Folin-Ciocalteu reagent, DCFH-DA, Trolox, AAPH, fluorescein sodium, 4-methylumbelliferyl oleate (4-MUO),  $\alpha$ -glucosidase,  $\alpha$ -amylase and pancreatic lipase, and 4-nitrophenyl- $\alpha$ -D-glucopyranoside (pNPG) were obtained from Sigma Aldrich Co. (St. Louis, MO, USA). HBSS, new bovine calf serum and DMEM (H) medium were obtained from Gibco Life Technologies (Grand Island, NY, USA). HepG2 human liver cancer cells were purchased from the ATCC (Rockville, MD, USA). Acetonitrile and glacial acetic acid in HPLC-grade were purchased from Thermo Fisher Scientific (Suwanee, GA, USA).

### 2.3. Extraction of phenolics

Phenolics were extracted following the method of Zhang et al (2013). The pulp of grapefruit and pomelo obtained by peeling were homogenized using a blender (WBL2521H, Midea Group Co., Ltd., Foshan, Guangdong, China). Subsequently, each pulp sample (100 g) with 80% aqueous acetone (1:2, w/v) were further homogenized at 5000 rpm for 5 min in an ice bath using an STSRH-300 homogenizer (Shanghai Sotint Intelligent Equipment Co., Ltd., Shanghai, China). The homogenates were centrifuged at 4000g for 10 min at 4 °C (TG16, Shanghai Lu Xiangyi Centrifuge Instrument Co. Ltd., China). Then, the residue was repeated the above extraction steps and the pooled supernatants were condensed to dry at 45 °C using a rotary evaporator (N-1300V, Tokyo Rika Machinery Co., Ltd.). Finally, the condensed phenolics were dissolved with 25 mL distilled water and stored at -20 °C for further analysis.

### 2.4. Measurement of total phenolic contents (TPC)

The Folin-Ciocalteu colorimetric method (Dewanto et al., 2002) was used to determine the TPC and the results were presented as mg gallic acid equivalents (GAE)/100 g fresh weight (FW) of the pulp sample.

### 2.5. Measurement of total flavonoid contents (TFC)

The TFC was measured according to the sodium borohydride/chloranil-based (SBC) assay (He et al., 2008) and the results were presented as mg catechin equivalents (CE)/100 g FW of the pulp sample.

### 2.6. Analysis of flavonoid compositions

The separation of phenolic extracts of different pomelo and grapefruit cultivars was conducted on a Thermo Scientific Dionex UltiMate 3000 UHPLC (Tempe, Arizona, USA) with a Waters HSS C18 column (1.8  $\mu$ m, 2.1  $\times$  100 mm, MA, USA). Acetonitrile (solvent A) and 0.4% aqueous acetic acid (v/v, solvent B) were used as the mobile phase. The elution of flavonoid compounds using the following conditions: 0–10 min, 5–8% A; 10–20 min, 8–12% A, 20–22 min, 12–14% A, 22–52 min, 14% A. Other analysis conditions were as follows: injection volume, 2  $\mu$ L; flow rate, 1 mL/min; column temperature, 30 °C; detection wavelength, 280 nm.

ESI-MS analysis was performed on a Thermo Scientific TSQ Endura Triple Quad LC/MS/MS (Suwanee, GA, USA) equipped with an ion trap mass spectrometer and a diode array detector. The negative mode was chosen to conduct electrospray ionization using the following conditions: spray needle voltage, 4000 V; capillary temperature, 350 °C; dry gas, 10 L/min; collision energy, 10–30 V; mass spectra,  $m/z$  100–1000.

### 2.7. Analysis of antioxidant activity

The oxygen radical absorbance capacity (ORAC) was measured according to the method of Huang et al. (2002) and the results were shown as  $\mu$ mol Trolox equivalents (TE)/100 g FW of the pulp sample. The cellular antioxidant activity (CAA) was determined using the method reported by Wolfe and Liu (2007) and the results were shown as  $\mu$ mol quercetin equivalents (QE)/100 g FW of the pulp sample.

### 2.8. In vitro enzymes inhibition assays

#### 2.8.1. $\alpha$ -Amylase inhibition

The  $\alpha$ -amylase inhibitory activity was measured according to the method reported by Salahuddin et al. (2020). A 96-well microplate was seeded with diluted samples (40  $\mu$ L),  $\alpha$ -amylase (0.25 U/mL, 40  $\mu$ L) and PBS (20  $\mu$ L), respectively, and incubated at 37 °C for 3 min. Then, soluble starch solution (1 mg/mL, 20  $\mu$ L) was added, and the microplate was incubated at 37 °C for another 4 min. After the enzyme reaction was stopped by adding HCL (1 mol/L, 20  $\mu$ L), 60  $\mu$ L of iodine reagent containing 5 mmol/L potassium iodine and 5 mmol/L iodine was added to the microplate. Then, the absorbance was taken at 650 nm. The  $\alpha$ -amylase inhibition (%) was determined as follows:  $[1-(A_2-A_1)/(A_4-A_3)] \times 100\%$ , where  $A_1$  is the absorbance of the samples in the above measurement;  $A_2$  is the absorbance of the measurement in which the enzyme was replaced by PBS;  $A_3$  is the absorbance of the measurement in which the samples were replaced by PBS;  $A_4$  is the absorbance of the measurement in which both the samples and enzyme were replaced by PBS.

#### 2.8.2. $\alpha$ -Glucosidase inhibition

The  $\alpha$ -glucosidase inhibitory activity was determined following the method of Lin et al. (2015). A 96-well microplate was seeded with 20  $\mu$ L of samples, 40  $\mu$ L of PBS and 10  $\mu$ L of  $\alpha$ -glucosidase (0.2 U/mL), respectively, and incubated at 37 °C for 15 min. Then, 20  $\mu$ L of 5 mmol/L pNPG was added, and the microplate was incubated at 37 °C for another 6 min. After the enzyme reaction was stopped by adding 100  $\mu$ L of 200 mmol/L  $\text{Na}_2\text{CO}_3$ , the absorbance was taken at 405 nm. The  $\alpha$ -glucosidase inhibition (%) was determined as follows:  $[A_1-(A_2-A_3)]/A_1 \times 100\%$ , where  $A_2$  is the absorbance of the samples in the above measurement ;  $A_1$  is the absorbance of the measurement in which the samples were replaced by PBS ;  $A_3$  is the absorbance of the measurement in which the

enzyme and pNPG were replaced by PBS.

### 2.8.3. Pancreatic lipase inhibition

The pancreatic lipase inhibitory activity was measured according to the method of Zhu et al. (2014). A buffer containing 13 mmol/L Tris, 1.3 mmol/L CaCl<sub>2</sub> and 150 mmol/L NaCl was used to prepare 4-MUO and lipase. Briefly, 25 µL of samples and 50 µL of 4-MUO (0.1 mmol/L) were added to a 96-well microplate, respectively. Then, the lipase (50 U/mL, 25 µL) was added to initiate the enzyme reaction. After the 96-well microplate was incubated at 25 °C for 30 min, the enzyme reaction was stopped by adding sodium citrate (0.1 mol/L, pH 4.2, 100 µL). Finally, the fluorescence intensity was measured (excitation, 355 nm; emission, 460 nm). The pancreatic lipase inhibition (%) was calculated as follows:  $[1 - (A_2 - A_1) / (A_4 - A_3)] * 100\%$ , where A<sub>2</sub> is the fluorescence of the samples in the above measurement; A<sub>1</sub> is the fluorescence of the measurement in which the enzyme was replaced by buffer; A<sub>3</sub> is the absorbance of the measurement in which both the samples and enzyme were replaced by buffer; A<sub>4</sub> is the absorbance of the measurement in which the samples were replaced by buffer.

## 2.9. Statistical analysis

All measurements were conducted in triplicates and the results were presented as the mean ± SD. Statistical analysis were conducted by one-way ANOVA of SPSS 19.0 software, and  $p < 0.05$  indicated statistical significance. Pearson correlation was used to analyze the correlation between variables.

## 3. Results and discussion

### 3.1. Total phenolic contents

The TPC of the grapefruit and pomelo cultivars are presented in Fig. 1. The TPC ranged from 91.8 (Guanximiyou-R) to 170.9 (Liangpingyu) mg GAE/100 g FW with the coefficient of variation (CV) of 24.9% in the determined pomelo. Liangpingyu had the highest TPC, followed by Shatianyu, grapefruit and Yuhuanu ( $p < 0.05$ ). Guanximiyou-W and Guanximiyou-R had the lowest TPC ( $p > 0.05$ ). The pomelo and grapefruit cultivars in the present study showed higher TPC than pomelo cultivars in Thailand (51.9–94.9 mg GAE/100 g FW) (Mäkynen et al., 2013). This is partly due to different methods used for phenolics extraction, together with the different cultivars tested. The TPC of Liangpingyu was higher than those of cherry (Wolfe et al., 2008) and the majority of litchi cultivars (Zhang et al., 2013) (101.5–170.5 mg GAE/100 g FW). Furthermore, the determined pomelo and grapefruit

cultivars showed higher TPC than pineapple, banana, peach, lemon, pear and orange (70.6–94.3 mg GAE/100 g FW) (Wolfe et al., 2008). Therefore, pomelo, especially cultivars like Liangpingyu and Shatianyu, are excellent dietary sources of phenolics.

### 3.2. Total flavonoid contents

The TFC of the grapefruit and pomelo cultivars are presented in Fig. 1. The TFC ranged from 13.4 (Guanximiyou-R) to 193.3 (Liangpingyu) mg CE/100 g FW with the CV of 90.1%, indicating significant genotype differences in TFC among pomelo and grapefruit cultivars. Liangpingyu had the highest TFC, followed by grapefruit, Shatianyu and Yuhuanu ( $p < 0.05$ ). Guanximiyou-W and Guanximiyou-R had the lowest TFC ( $p > 0.05$ ). In previous studies, the TFC of the pulp from different litchi, apple and strawberry varied from 39.4 to 129.8 (averaging 67 mg CE/100 g FW), 35.7–46.8, and 46.2–70.5 mg CE/100 g FW, respectively (Meyers et al., 2003; Wolfe et al., 2003; Zhang et al., 2013). The average content of the TFC in grapefruit and pomelo was 64.3 mg CE/100 g FW, which was higher than most of the fruits mentioned above.

### 3.3. Flavonoid compositions and contents

The compositions and contents of flavonoids in grapefruit and pomelo varieties were analyzed by UHPLC-ESI MS/MS. Fourteen flavonoids were detected in the determined grapefruit and pomelo. The MS, MS-MS data and retention time of the 14 flavonoid standards are presented in Table 1. The flavonoid compositions and contents are presented in Table 2. All the 14 flavonoids were detected in grapefruit, but narirutin and neohesperidin were not detected or below the detection limit in the 5 pomelo cultivars. In Guanximiyou-W and Guanximiyou-R, naringin was the most abundant flavonoid, followed by rhoifolin and cigranoside C, and the 3 compounds accounted for 91% and 86% of the detected flavonoids in Guanximiyou-W and Guanximiyou-R, respectively. Melitidin was the largest amount of flavonoid in Shatianyu, followed by bergamjuicin, naringin, cigranoside B and cigranoside A, and the 5 compounds possessed 97% of the detected flavonoids in Shatianyu. Nevertheless, Rhoifolin was the most abundant flavonoid in Yuhuanu, followed by naringin, and the 2 compounds took up 84% of the detected flavonoids in Yuhuanu. Naringin was the most abundant flavonoid in Liangpingyu, followed by cigranoside C, melitidin, neoeriocitrin and rhoifolin, and the 5 compounds accounted for 98% of the detected flavonoids in Liangpingyu. Naringin was the largest amount of flavonoid in grapefruit, followed by narirutin, cigranoside C, neohesperidin, hesperidin and melitidin, and the 6 compounds possessed 98% of the detected flavonoids in grapefruit.

As firstly separated compounds from Shatianyu with new structures in our previous work (Deng et al., 2021), cigranoside C, D and E had never been reported before in pomelo and grapefruit. Their contents

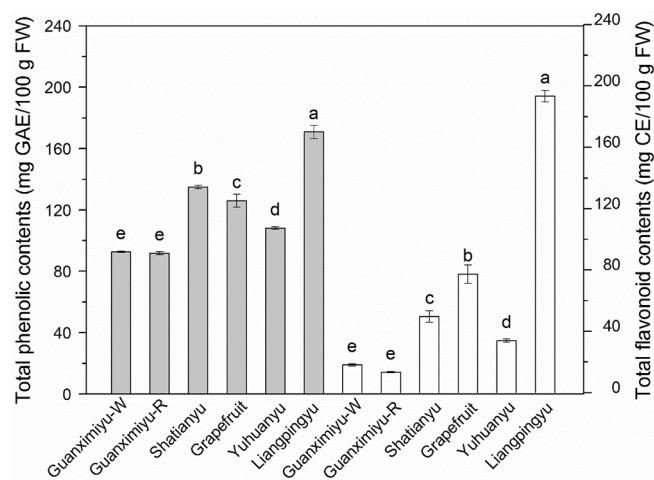


Fig. 1. TPC and TFC of different pomelo and grapefruit cultivars. Bars with no letters in common are significantly different ( $p < 0.05$ ).

Table 1  
The retention time, MS and MS-MS data of flavonoid standards observed by UHPLC-ESI-MS/MS analysis.

| Compound      | Retention time (min) | [M-H] <sup>-</sup> (m/z) | MS <sup>2</sup> ions (m/z) |
|---------------|----------------------|--------------------------|----------------------------|
| Cigranoside C | 10.97                | 595                      | 567, 259, 577              |
| Isoquercitrin | 19.71                | 463                      | 300, 271                   |
| Neoeriocitrin | 21.53                | 595                      | 459, 151, 576              |
| Narirutin     | 24.58                | 579                      | 271, 151, 295, 313         |
| Naringin      | 26.47                | 579                      | 459, 271, 235              |
| Cigranoside E | 27.65                | 883                      | 619, 577, 659              |
| Bergamjuicin  | 27.96                | 885                      | 579, 621                   |
| Rhoifolin     | 28.13                | 577                      | 269, 413                   |
| Hesperidin    | 28.53                | 609                      | 301, 325, 242              |
| Neohesperidin | 30.48                | 609                      | 301, 286, 343, 242         |
| Cigranoside A | 32.38                | 723                      | 677, 659, 580, 621         |
| Cigranoside B | 34.87                | 723                      | 677, 659, 580, 451         |
| Melitidin     | 36.76                | 723                      | 677, 579, 621, 661         |
| Cigranoside D | 37.28                | 721                      | 268, 577, 619, 659         |

**Table 2**

Flavonoid compositions of pomelo and grapefruit cultivars ( $\mu\text{g}/100\text{ g FW}$ ). Values with no letters in common in each row are significantly different ( $p < 0.05$ ). Tr: trace; nd: not detected.

| Compounds     | Guanximiyu-W      | Guanximiyu-R      | Shatianyu          | Yuhuanu           | Liangpingyu        | Grapefruit         |
|---------------|-------------------|-------------------|--------------------|-------------------|--------------------|--------------------|
| Cigranoside A | 55.1 $\pm$ 1.7 cd | 49.7 $\pm$ 4.2 cd | 1145 $\pm$ 85 a    | 17.2 $\pm$ 0.9 d  | 172 $\pm$ 2 b      | 99.9 $\pm$ 3.1 c   |
| Cigranoside B | 93.1 $\pm$ 2.4 d  | 84.8 $\pm$ 3.3 d  | 1773 $\pm$ 40 a    | 19.4 $\pm$ 1 e    | 321 $\pm$ 12 b     | 168 $\pm$ 5 c      |
| Cigranoside C | 800 $\pm$ 36 c    | 544 $\pm$ 21 d    | 188 $\pm$ 11 f     | 404 $\pm$ 7 e     | 3375 $\pm$ 113 a   | 3165 $\pm$ 132 b   |
| Cigranoside D | 83.8 $\pm$ 1.6 c  | 75.7 $\pm$ 3.2 cd | 380 $\pm$ 12 a     | 101 $\pm$ 6 b     | 69.3 $\pm$ 3.9 d   | 6.25 $\pm$ 0.11 e  |
| Cigranoside E | 106 $\pm$ 2 b     | 98.8 $\pm$ 4.7 c  | 203 $\pm$ 5 a      | 81.9 $\pm$ 1.9 d  | 22.0 $\pm$ 0.8 e   | 10.6 $\pm$ 0.9 f   |
| Bergamjuicin  | 191 $\pm$ 3 bc    | 227 $\pm$ 6 b     | 5148 $\pm$ 91 a    | 10.2 $\pm$ 0.6 e  | 69.8 $\pm$ 3.8 d   | 142 $\pm$ 2 c      |
| Neoeriocitrin | 185 $\pm$ 3 d     | 82.9 $\pm$ 1.6 f  | 104 $\pm$ 6 e      | 481 $\pm$ 9 b     | 1473 $\pm$ 15 a    | 380 $\pm$ 10 c     |
| Melitidin     | 478 $\pm$ 19 c    | 330 $\pm$ 17c     | 23338 $\pm$ 874 a  | 194 $\pm$ 7 c     | 1745 $\pm$ 100 b   | 1320 $\pm$ 17 b    |
| Rhoifolin     | 4023 $\pm$ 46 b   | 1768 $\pm$ 37 c   | 164 $\pm$ 6 f      | 4135 $\pm$ 91 a   | 1025 $\pm$ 37 d    | 569 $\pm$ 29 e     |
| Naringin      | 6875 $\pm$ 72 c   | 3613 $\pm$ 111 d  | 2448 $\pm$ 100 d   | 3245 $\pm$ 129 d  | 30100 $\pm$ 885 b  | 40430 $\pm$ 770 a  |
| Hesperidin    | 1.7 $\pm$ 0.2 b   | 1.1 $\pm$ 0.1 b   | 1.35 $\pm$ 0.05 b  | 0.38 $\pm$ 0.01 b | 2.8 $\pm$ 0.2 b    | 1670 $\pm$ 43 a    |
| Neohesperidin | Tr                | Tr                | Tr                 | Tr                | Tr                 | 2356 $\pm$ 107     |
| Narirutin     | nd                | nd                | nd                 | nd                | nd                 | 21973 $\pm$ 664    |
| Isoquercitrin | 15.9 $\pm$ 0.4 d  | 12.1 $\pm$ 1 de   | 9.88 $\pm$ 0.83 e  | 109 $\pm$ 7 a     | 23.6 $\pm$ 1.2 c   | 35.5 $\pm$ 2.1 b   |
| Sum           | 12907 $\pm$ 82 d  | 6887 $\pm$ 191 f  | 34902 $\pm$ 1175 c | 8798 $\pm$ 105 e  | 38398 $\pm$ 1672 b | 72325 $\pm$ 1554 a |

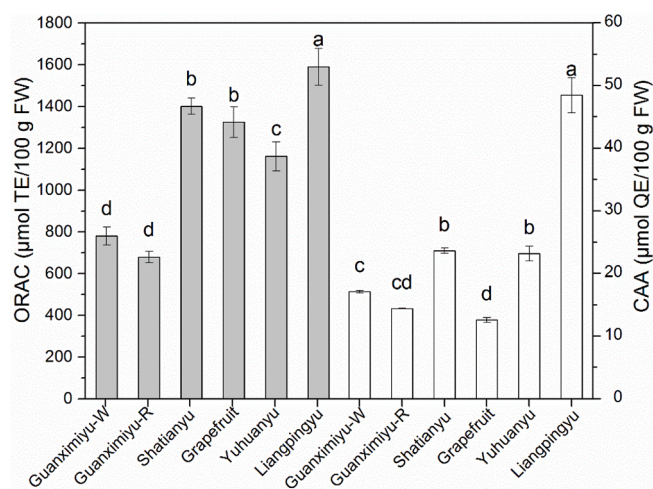
ranged from 188 (Shatianyu) to 3375 (Liangpingyu), 6.25 (grapefruit) to 380 (Shatianyu), 10.6 (grapefruit) to 203 (Shatianyu)  $\mu\text{g}/100\text{ g FW}$ , respectively. The contents of cigranoside C ranked second or third place in Liangpingyu, Guanximiyu-W, Guanximiyu-R and grapefruit among all the detected flavonoids. Although cigranoside F was also a new flavonoid isolated from Shatianyu, it was not detected in the pomelo and grapefruit cultivars. These might be attributed to the fact that cigranoside F was below the detection limit in Shatianyu or did not exist in other pomelo cultivars. Cigranoside A, cigranoside B, bergamjuicin and neoeriocitrin were previously found in traditional Chinese medicine, such as the pericarp of *C. grandis* huajuhong (cigranoside A and B) (Ma et al., 2018), the juice of *C. bergamia* bergamot (bergamjuicin) (Formisano et al., 2019) and the rhizomes of *Drynaria fortunei* (Kunze et Mett.) J. Sm (neoeriocitrin) (Yang et al., 2015). However, the 4 flavonoids had not been detected in pomelo pulp before, and their contents ranged from 17.2 (Yuhuanu) to 1145 (Shatianyu), 19.4 (Yuhuanu) to 1773 (Shatianyu), 10.2 (Yuhuanu) to 5148 (Shatianyu) and 82.9 (Guanximiyu-R) to 1473 (Liangpingyu)  $\mu\text{g}/100\text{ g FW}$ , respectively. Melitidin and rhoifolin were previously detected in the juice of pomelo and grapefruit, but their contents were calculated based on naringin equivalent (Zhang et al., 2011). In the present study, melitidin and rhoifolin, with the contents ranging from 194 (Yuhuanu) to 23,338 (Shatianyu) and 164 (Shatianyu) to 4135 (Yuhuanu)  $\mu\text{g}/100\text{ g FW}$ , respectively, were determined by absolute quantification. Naringin was the predominant flavonoid in citrus fruits including sour orange, grapefruit and pomelo (Gattuso et al., 2007). Its contents ranged from 2448 (Shatianyu) to 40,430 (grapefruit)  $\mu\text{g}/100\text{ g FW}$  among the tested pomelo and grapefruit. This was comparable to the pomelo cultivars in Thailand (25–39 mg/100 g FW) (Chaiwong & Theppakorn, 2010). Hesperidin, neohesperidin and narirutin were mainly reported in grapefruit, sweet oranges, lemons, and limes (Gattuso et al., 2007; Khan & Dangles, 2014; Tocmo et al., 2020). These 3 flavonoids were mainly detected in grapefruit in the present study, with the contents of 1670, 2356 and 21,973  $\mu\text{g}/100\text{ g FW}$ , respectively. These was comparable to the levels in grapefruit reported previously (Igual et al., 2013).

TFC of the tested pomelo and grapefruit determined by calculating the monomer flavonoid contents was quite different from those measured by an SBC assay. Using the former method, grapefruit showed the highest TFC among these pomelo and grapefruit, almost twice that of Shatianyu and Liangpingyu. However, using the SBC assay, Liangpingyu showed the highest TFC, which was 3.8 and 2.5 times that of Shatianyu and grapefruit, respectively. The reason for the inconsistency between the two results might be that there were other undetected flavonoids among pomelo and grapefruit cultivars besides the 14 flavonoids detected in the present study, which could be inferred from the liquid chromatograms of these pomelo and grapefruit cultivars (supplementary Fig. S1).

### 3.4. Antioxidant activity

The antioxidant activity of the pomelo and grapefruit cultivars evaluated by ORAC and CAA are presented in Fig. 2. The ORAC values of these pomelo and grapefruit ranged from 678.9 to 1590.2  $\mu\text{mol TE}/100\text{ g FW}$  with a CV of 31.1%, indicating significant genotype differences in the ORAC activity among pomelo and grapefruit cultivars. Liangpingyu showed the highest ORAC activity ( $p < 0.05$ ), followed by Shatianyu and grapefruit. The ORAC values of the latter two were higher than that of Yuhuanu ( $p < 0.05$ ). Guanximiyu-W and Guanximiyu-R presented the lowest ORAC activity ( $p > 0.05$ ) among the determined cultivars. Highly significant correlations were observed between the ORAC activity and phenolic contents of the tested pomelo and grapefruits, and the relative coefficient  $r$  was as high as 0.93 ( $p < 0.01$ ). Although the ORAC activities of pomelo and grapefruit are lower than that of some citrus fruits, such as orange and lemon (2887 and 1848  $\mu\text{mol TE}/100\text{ g FW}$ , respectively) (Wolfe et al., 2008), they are comparable to those of pear, nectarine, watermelon, avocado, kiwifruit, mango, pineapple and banana (565–1759  $\mu\text{mol TE}/100\text{ g FW}$ ) (Wolfe et al., 2008).

The CAA values of these pomelo and grapefruits ranged from 12.6 to 48.4  $\mu\text{mol QE}/100\text{ g FW}$  with a CV of 56.7%, indicating that the CAA activity showed higher genotype differences than the ORAC activity among pomelo and grapefruit. Liangpingyu presented the highest CAA activity followed by Shatianyu and Yuhuanu ( $p < 0.05$ ). In comparison, the CAA value of the former was approximate twice that of the latter two. The CAA activity of grapefruit was lower than those of other



**Fig. 2.** ORAC and CAA values of different pomelo and grapefruit cultivars. Bars with no letters in common are significantly different ( $p < 0.05$ ).

determined cultivars except for Guanximiyou-R, which also showed similar activity to Guanximiyou-W. Correlation analysis showed that the Pearson coefficient  $r$  between the ORAC and CAA values of the tested pomelos and grapefruit was 0.66 ( $p = 0.15$ ), indicating that the antioxidant activities reflected by these 2 methods were not completely consistent. Compared with ORAC, a chemical antioxidant determination method, CAA assay was conducted in a more physiological reaction system involving cells, which might give more indicative information for the *in vivo* activity of the tested samples. In addition to phenolic contents, the CAA activity of the samples is also influenced by their phenolic compositions since the uptake and metabolism of the antioxidants depended on their structures (Wolfe and Liu 2007). Our previous study found that naringin contributed the least to the CAA activity of phenolic extracts of Shatianyu pulp among the 11 isolated flavonoids (Deng et al., 2021). Coincidentally, the percentage of naringin in the TFC of grapefruit (52.3%) was higher than all the tested pomelo. These might explain why grapefruit showed the lowest CAA value despite its relatively high phenolic content. Liangpingyu showed higher CAA activity than cranberry, plum and cherry (27.4–47.9  $\mu\text{mol QE}/100 \text{ g FW}$ ) (Wolfe et al., 2008), which were considered as good sources of natural antioxidants. Although Shatianyu and Yuhuanu had much lower CAA values than Liangpingyu, they exhibited higher CAA activity than apple, red grape, kiwifruit, mango, pineapple and orange (13.7–21.9  $\mu\text{mol QE}/100 \text{ g FW}$ ) (Wolfe et al., 2008). Moreover, the CAA activity of all tested pomelo and grapefruit was higher than lemon, peach, pear, cantaloupe and banana (3.2–12.3  $\mu\text{mol QE}/100 \text{ g FW}$ ) (Wolfe et al., 2008). Therefore, pomelo, especially Liangpingyu and Shatianyu, are excellent sources of natural antioxidants.

### 3.5. Inhibitory activity to $\alpha$ -amylase, $\alpha$ -glucosidase

The inhibitory activity to  $\alpha$ -amylase and  $\alpha$ -glucosidase of different grapefruit and pomelo cultivars are presented in Table 3. The  $\text{IC}_{50}$  values of  $\alpha$ -amylase and  $\alpha$ -glucosidase inhibitory activity varied from 707.1 to 1788 and 1053 to 2514 mg fruit/mL, respectively. Shatianyu, grapefruit and Liangpingyu with proximate  $\text{IC}_{50}$  values showed higher  $\alpha$ -amylase inhibitory activity than Guanximiyou-W, Guanximiyou-R and Yuhuanu ( $p < 0.05$ ). However, Yuhuanu and Liangpingyu showed the highest inhibitory activity to  $\alpha$ -glucosidase, followed by grapefruit, Guanximiyou-W and Shatianyu ( $p < 0.05$ ), and Guanximiyou-R presented the lowest inhibitory activity to  $\alpha$ -glucosidase ( $p < 0.05$ ).  $\text{IC}_{50}$  values of commonly consumed fruits, such as sour cherry, strawberry, apple, banana, bilberry, peach and pomegranate, ranged from 18.18 to more than 200 mg fruit/mL against  $\alpha$ -amylase and from 156.4 to 399.1 mg fruit/mL against  $\alpha$ -glucosidase (Podsedek et al., 2014). Evidently, the tested pomelo and grapefruit presented much weaker inhibition to the saccharides hydrolyzing enzymes. Generally, flavonols and flavones showed stronger inhibition to these enzymes than flavanones and flavanols (Spínola et al., 2020; Sun, Warren and Gidley, 2019), in which the hydroxylated 2,3-double bond transformed the near-planar

**Table 3**

The inhibitory activities of different pomelo and grapefruit cultivars to  $\alpha$ -amylase,  $\alpha$ -glucosidase and pancreatic lipase. Values with no letters in common in each column are significantly different ( $p < 0.05$ ).

| Cultivars     | Enzyme inhibition $\text{IC}_{50}$ (mg of fresh fruit/mL) |                                  |                              |
|---------------|---|----------------------------------|------------------------------|
|               | $\alpha$ -amylase inhibition                              | $\alpha$ -glucosidase inhibition | pancreatic lipase inhibition |
| Guanximiyou-W | 1558 $\pm$ 33b  | 1274 $\pm$ 18b                   | 23.7 $\pm$ 2.1 a             |
| Guanximiyou-R | 1788 $\pm$ 208b   | 2514 $\pm$ 72 d                  | 58.1 $\pm$ 1.4b              |
| Shatianyu     | 707.1 $\pm$ 11.2 a  | 1988 $\pm$ 10c                   | 240 $\pm$ 15c                |
| Yuhuanu       | 1693 $\pm$ 99.3b  | 1058 $\pm$ 62 a                  | 72.6 $\pm$ 6.3b              |
| Liangpingyu   | 798.3 $\pm$ 39.6 a  | 1053 $\pm$ 73 a                  | 12.8 $\pm$ 0.3 a             |
| Grapefruit    | 877.8 $\pm$ 49.9 a  | 1255 $\pm$ 37b                   | 11.4 $\pm$ 1.1 a             |

molecular structure to a more non-planar and flexible stereochemical structure, forming steric hindrance to reduce the ability of flavonoids to bind with the enzymes (Sun, Warren and Gidley, 2019). The main flavonoids in pomelo and grapefruit analyzed in the present study were flavanones, while flavonols and flavonones were important components of many other fruits apart from flavanones (Balasuriya and Rupasinghe, 2012; Pérez-Navarro et al., 2021).

### 3.6. Inhibitory activity to pancreatic lipase

The pancreatic lipase inhibitory activity of different pomelo and grapefruit cultivars are presented in Table 3. The  $\text{IC}_{50}$  values of pancreatic lipase inhibitory activity varied from 11.4 to 240 mg fruit/mL. Grapefruit, Liangpingyu and Guanximiyou-W presented the highest pancreatic lipase inhibitory activity, followed by Guanximiyou-R and Yuhuanu ( $p < 0.05$ ). Shatianyu showed the lowest inhibitory activity to pancreatic lipase, and its  $\text{IC}_{50}$  value was 10 to 21 times that of the former 3 cultivars ( $p < 0.05$ ). In the tested grapefruit and pomelo, strong correlations were observed between the  $\text{IC}_{50}$  values of pancreatic lipase inhibitory activity and the content of cigranoside A ( $r = 0.92$ ,  $p < 0.01$ ), cigranoside B ( $r = 0.91$ ,  $p < 0.05$ ), cigranoside D ( $r = 0.97$ ,  $p < 0.01$ ), cigranoside E ( $r = 0.89$ ,  $p < 0.05$ ), bergamjuicin ( $r = 0.95$ ,  $p < 0.01$ ) and melitidin ( $r = 0.94$ ,  $p < 0.01$ ), respectively. These above-mentioned flavonoids all had a 3-hydroxy-3-methylglutaryl (HMG) substitution at 7-O-neohesperidose of the A ring, indicating that the presence of HMG moiety in the structure of flavonoids might weaken their inhibitory activity to pancreatic lipase. The lowest inhibitory activity to pancreatic lipase of Shatianyu might be attributed to its highest content of HMG substituting flavonoids, which was 13 to 75 times higher than those of other pomelo and grapefruit cultivars. Huang et al. (2020) revealed that hesperidin could interact with pancreatic lipase through hydrogen bonds and van der Waals forces to change the secondary structure of pancreatic lipase, making itself the key pancreatic lipase inhibitor in citrus peel extracts. The highest content of hesperidin in grapefruit might explain its strongest pancreatic lipase inhibitory activity among the tested pomelo and grapefruit cultivars. Grapefruit, Liangpingyu and Guanximiyou-W showed comparable pancreatic lipase inhibitory activity with blackberry, strawberry, cherry, plum and apple (5.7–14 mg fruit/mL) and stronger inhibition of pancreatic lipase than pear, peach, banana and mandarin (30–135 mg fruit/mL) (Podsedek et al., 2014). Therefore, grapefruit and some pomelo cultivars were effective inhibitors of pancreatic lipase and had anti-obesity potential.

## 4. Conclusion

Significant varietal differences were observed in flavonoid profiles and *in vitro* bioactivity among different pomelo and grapefruit cultivars. Liangpingyu, Shatianyu and grapefruit had higher phenolic and flavonoid contents than other 3 pomelo cultivars. Fourteen flavonoid compounds were identified in pomelo and grapefruits. Naringin was the major flavonoid in grapefruit, Guanximiyou-W, Guanximiyou-R, and Liangpingyu, while melitidin and rhoifolin was the predominant flavonoid in Shatianyu and Yuhuanu, respectively. Cigranoside C, D and E were firstly quantified in pomelo and grapefruit since they were isolated from Shatianyu as new compounds. Furthermore, cigranoside C was one of the main flavonoid compounds in Liangpingyu, Guanximiyou-W, Guanximiyou-R and grapefruit, ranking second or third place among all the detected flavonoids. Cigranoside A, cigranoside B, bergamjuicin and neoeriocitrin were also firstly quantified in pomelo and grapefruit pulp. The total contents of the 7 flavonoids ranged from 1114.7 (Yuhuanu) to 8941 (Shatianyu)  $\mu\text{g}/100 \text{ g FW}$ , accounting for 5.5% to 25.6% of the total contents of 14 detected flavonoids. Pomelo and grapefruit possessed strong antioxidant activity, especially CAA activity. Despite their weak  $\alpha$ -amylase and  $\alpha$ -glucosidase inhibitory activity, the determined pomelo and grapefruit cultivars presented strong inhibition of pancreatic lipase, especially grapefruit, Liangpingyu and Guanximiyou-

W, which showed lower IC<sub>50</sub> values than many commonly consumed fruits. Therefore, pomelo and grapefruit are good daily sources of flavonoids and possess anti-obesity potential.

#### CRedit authorship contribution statement

**Mei Deng:** Conceptualization, Methodology, Investigation, Data curation, Formal analysis, Writing – original draft, Visualization. **Lihong Dong:** Methodology, Investigation, Data curation, Formal analysis. **Xuchao Jia:** Resources, Validation. **Fei Huang:** Methodology, Visualization. **Jianwei Chi:** Methodology, Formal analysis. **Zafarullah Muhammad:** Writing – review & editing. **Qin Ma:** Formal analysis. **Dong Zhao:** Formal analysis. **Mingwei Zhang:** Supervision, Project administration, Funding acquisition, Writing – review & editing. **Ruifen Zhang:** Conceptualization, Supervision, 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.

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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.2022.100368>.

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