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, Guanximiuyu-W, Guanximiuyu-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 IC50 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. Guanximiuyu, 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. Mäkynen 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 (neoerioicitrin 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, α-amylase and α-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...
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. Yuhuanyu 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, bergamujucin, neoeuficrin, 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-\( \alpha \)-glucopyranoside (pNPG) were obtained from Sigma Aldrich Co. (St. Louis, MO, USA). HBSS, new bovine calf serum and DMEM (H) medium were purchased from Thermo Fisher Scientific (Suwanee, GA, 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 aceton (1:2, v/v) were further homogenized at 5000 rpm for 5 min in an ice bath using an ISTRH-300 homogenizer (Shanghai Sotin Intelligent Equipment Co., Ltd., Shanghai, China). The homogenates were centrifuged at 4000 g for 10 min at 4 \( ^\circ \)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 \( ^\circ \)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 \(-20 ^\circ \)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/chloramin-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 \( ^\circ \)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 \( ^\circ \)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). PBS (20 \( \mu \)L), respectively, and incubated at 37 \( ^\circ \)C for 3 min. Then, soluble starch solution (1 mg/mL, 20 \( \mu \)L) was added, and the microplate was incubated at 37 \( ^\circ \)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: \[ 1- (A_2-A_1)/A_4 \] *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 \( ^\circ \)C for 15 min. Then, 20 \( \mu \)L of 5 mmol/L pNPG was added, and the microplate was incubated at 37 \( ^\circ \)C for another 6 min. After the enzyme reaction was stopped by adding 100 \( \mu \)L of 200 mmol/L Na\(_2\)CO\(_3\), the absorbance was taken at 405 nm. The \( \alpha \)-glucosidase inhibition (%) was determined as follows: \[ A_1 - (A_2-A_3) \] /\( A_1 \) *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 both the samples and enzyme were replaced by PBS.
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 mM Tris, 1.3 mM CaCl₂ and 150 mM NaCl was used to prepare 4-MUO and lipase. Briefly, 25 μL of samples and 50 μL of 4-MUO (0.1 mM/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: \[
\text{Inhibition} = \left(1 - \frac{A_2 - A_3}{A_1 - A_3}\right) \times 100%,
\]
where \(A_2\) is the fluorescence of the samples in the above measurement; \(A_1\) is the fluorescence of the measurement in which the enzyme was replaced by buffer; \(A_3\) is the absorbance of the measurement in which both the samples and enzyme were replaced by buffer; \(A_4\) 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 (Guanximiyu-R) to 170.9 (Liangpingyu) mg CE/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 Yuhuanyu (\(p < 0.05\)). Guanximiyu-W and Guanximiyu-R had the lowest TPC (\(p > 0.05\)). In previous studies, the TPC 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 TPC in grapefruit and pomelo was 64.3 mg CE/100 g FW, which was higher than most of the fruits mentioned above.

#### 3.2. Total flavonoid contents

The TFC of the grapefruit and pomelo cultivars are presented in Fig. 1. The TFC ranged from 13.4 (Guanximiyu-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 Yuhuanyu (\(p < 0.05\)). Guanximiyu-W and Guanximiyu-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 Guanximiyu-W and Guanximiyu-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 Guanximiyu-W and Guanximiyu-R, respectively. Melitidin was the largest amount of flavonoid in Shatianyu, followed by bergamujicin, 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 Yuhuanyu, followed by naringin, and the 2 compounds took up 84% of the detected flavonoids in Yuhuanyu. 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

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**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**

| Compound          | Retention time (min) | [M-H] (m/z) | MS² ions (m/z) |
|-------------------|----------------------|-------------|----------------|
| Cigranoside C     | 10.97                | 595         | 567, 259, 577  |
| Isoquercetin      | 19.71                | 463         | 300, 271       |
| Neohesperidin     | 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  |
| Bergamujicin      | 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 (μg/100 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 | Yuhuanyu | Liangpingyu | Grapefruit |
|-----------------|---------------|--------------|-----------|----------|-------------|------------|
| Cigarsoside A   | 55.1 ± 1.7 cd | 49.7 ± 4.2 cd | 1145 ± 85 a | 17.2 ± 0.9 d | 172 ± 2 b | 99.9 ± 3.1 c |
| Cigarsoside B   | 93.1 ± 2.4 d | 84.8 ± 3.3 d | 1773 ± 40 a | 19.4 ± 1 e | 321 ± 12 b | 168 ± 5 c |
| Cigarsoside C   | 800 ± 36 c | 544 ± 21 d | 188 ± 11 f | 404 ± 7 e | 3375 ± 113 a | 3165 ± 132 b |
| Cigarsoside D   | 83.8 ± 16 c | 75.7 ± 3.2 cd | 380 ± 12 a | 101 ± 6 b | 69.3 ± 3.9 d | 6.25 ± 0.11 e |
| Cigarsoside E   | 106 ± 2 b | 98.8 ± 4.7 c | 203 ± 5 a | 81.9 ± 1.9 d | 22.0 ± 0.8 e | 1.06 ± 0.09 f |
| Bergamjuicin    | 191 ± 3 bc | 227 ± 6 b | 5148 ± 91 a | 10.2 ± 0.6 e | 6.98 ± 3.8 d | 142 ± 2 c |
| Neotericisitrin | 185 ± 3 d | 82.9 ± 1.6 f | 104 ± 6 e | 481 ± 9 b | 1473 ± 15 a | 380 ± 10 c |
| Melitidin       | 478 ± 19 c | 330 ± 17 c | 23338 ± 874 a | 194 ± 7 c | 1745 ± 100 b | 1520 ± 17 b |
| Rhiiformin      | 4023 ± 46 b | 1768 ± 37 e | 164 ± 6 f | 4135 ± 91 a | 1025 ± 37 d | 569 ± 29 e |
| Naringin        | 6875 ± 72 c | 3613 ± 111 d | 2448 ± 100 d | 3245 ± 129 d | 30100 ± 885 b | 40430 ± 770 a |
| Hesperidin      | 1.7 ± 0.2 b | 1.1 ± 0.1 b | 1.35 ± 0.05 b | 0.38 ± 0.01 b | 2.8 ± 0.2 b | 1670 ± 43 a |
| Neohesperidin   | Tr            | Tr            | Tr        | Tr       | Tr           | Tr         |
| Narirutin       | nd            | nd            | nd        | nd       | nd           | nd         |
| Isoquercitrin   | 15.9 ± 0.4 d | 12.1 ± 1 de | 9.88 ± 0.83 e | 109 ± 7 a | 23.6 ± 1.2 c | 35.5 ± 2.1 b |
| Sum             | 12607 ± 82 d | 6887 ± 191 f | 34902 ± 1175 c | 8796 ± 105 e | 38398 ± 1672 b | 72325 ± 1554 a |

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 μmol TE/100 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 Yuhuanyu (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 correlation 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 μmol TE/100 g FW, respectively) (Wolfe et al., 2008), they are comparable to those of pear, nectarine, watermelon, avocado, kiwifruit, mango, pineapple and banana (565–1759 μmol TE/100 g FW) (Wolfe et al., 2008).

The CAA values of these pomelo and grapefruits ranged from 12.6 to 48.4 μmol QE/100 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 Yuhuanyu (p < 0.05). In comparison, the CAA value of the former was approximately twice that of the latter two. The CAA activity of grapefruit was lower than that of other...
determined cultivars except for Guanximiuy-W, which also showed similar activity to Guanximiuy-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 (Wolle and Liu, 2007). Our previous study found that naringin contributed the least to the CAA activity of phenolic compounds ($r = 0.89$, $p < 0.05$). In the tested grapefruit and pomelo, strong correlations were observed between the CAA values of pancreatic lipase inhibitory activity and the content of naringin ($r = 0.91$, $p < 0.05$), cigranoside D ($r = 0.97$, $p < 0.05$), cigranoside E ($r = 0.89$, $p < 0.05$), bergamujicin ($r = 0.95$, $p < 0.01$) and melittin ($r = 0.94$, $p < 0.01$), respectively. These above-mentioned flavonoids all had a 3-hydroxy-3-methylbutyluran (HM) substitution at 7-0-neohesperosidose of the A ring, indicating that the presence of HM 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 Guanximiuy-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 mandarine (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.

### 3.6. Inhibitory activity to pancreatic lipase

The pancreatic lipase inhibitory activity of different pomelo and grapefruit cultivars are presented in Table 3. The IC$_{50}$ values of pancreatic lipase inhibitory activity varied from 11.4 to 240 mg fruit/mL. Grapefruit, Liangpingyu and Guanximiuy-W presented the highest pancreatic lipase inhibitory activity, followed by Guanximiuy-R and Yuhuanyu ($p < 0.05$). Shatianyu showed the lowest inhibitory activity to pancreatic lipase, and its 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 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 ($r = 0.97$, $p < 0.05$), cigranoside E ($r = 0.89$, $p < 0.05$), bergamujicin ($r = 0.95$, $p < 0.01$) and melittin ($r = 0.94$, $p < 0.01$), respectively. These above-mentioned flavonoids all had a 3-hydroxy-3-methylbutyluran (HM) substitution at 7-O-neohesperosidose of the A ring, indicating that the presence of HM 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 Guanximiuy-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 mandarine (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 grapefruit. Naringin was the major flavonoid in grapefruit, Guanximiuy-W, Guanximiuy-R, and Liangpingyu, while melittin and rhoifolin was the predominant flavonoid in Shatianyu and Yuhuanyu, 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 major flavonoid compounds in Liangpingyu, Guanximiuy-W, Guanximiuy-R and grapefruit, ranking second or third place among all the detected flavonoids. Cigranoside A, cigranoside B, bergamujicin and neoeoriocitrin were also firstly quantified in pomelo and grapefruit pulp. The total contents of the 7 flavonoids ranged from 1141.7 (Yuhuanyu) to 8941 (Shatianyu) μg/100 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 α-amylase and α-glucosidase inhibitory activity, the determined pomelo and grapefruit cultivars presented strong inhibition of pancreatic lipase, especially grapefruit, Liangpingyu and Guanximiuy-W.

### Table 3

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

| Cultivars       | Enzyme inhibition | pancreatic lipase inhibition |
|-----------------|-------------------|-----------------------------|
|                 | IC$_{50}$ (mg of fresh fruit/mL) | IC$_{50}$ (mg of fresh fruit/mL) |
| Guanximiuy-W    | 1558 ± 33b        | 1274 ± 18b                  |
| Guanximiuy-R    | 1788 ± 208b       | 2514 ± 72 d                 |
| Shatianyu       | 707 ± 11.2 a      | 1988 ± 10c                  |
| Yuhuanyu        | 1693 ± 99.3b      | 1058 ± 62 a                 |
| Liangpingyu     | 798.3 ± 39.6 a    | 1053 ± 73 a                 |
| Grapefruit      | 877.8 ± 49.9 a    | 1255 ± 37b                  |

$\alpha$-amylase

| Cultivars       | IC$_{50}$ (mg of fresh fruit/mL) | IC$_{50}$ (mg of fresh fruit/mL) |
|-----------------|----------------------------------|----------------------------------|
| Guanximiuy-W    | 1558 ± 33b                      | 1274 ± 18b                      |
| Guanximiuy-R    | 1788 ± 208b                     | 2514 ± 72 d                     |
| Shatianyu       | 707 ± 11.2 a                    | 1988 ± 10c                      |
| Yuhuanyu        | 1693 ± 99.3b                    | 1058 ± 62 a                     |
| Liangpingyu     | 798.3 ± 39.6 a                  | 1053 ± 73 a                     |
| Grapefruit      | 877.8 ± 49.9 a                  | 1255 ± 37b                      |

$\alpha$-glucosidase

$\alpha$-glucosidase inhibition

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 grapefruit. Naringin was the major flavonoid in grapefruit, Guanximiuy-W, Guanximiuy-R, and Liangpingyu, while melittin and rhoifolin was the predominant flavonoid in Shatianyu and Yuhuanyu, 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 major flavonoid compounds in Liangpingyu, Guanximiuy-W, Guanximiuy-R and grapefruit, ranking second or third place among all the detected flavonoids. Cigranoside A, cigranoside B, bergamujicin and neoeoriocitrin were also firstly quantified in pomelo and grapefruit pulp. The total contents of the 7 flavonoids ranged from 1141.7 (Yuhuanyu) to 8941 (Shatianyu) μg/100 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 α-amylase and α-glucosidase inhibitory activity, the determined pomelo and grapefruit cultivars presented strong inhibition of pancreatic lipase, especially grapefruit, Liangpingyu and Guanximiuy-W.
W, which showed lower IC₅₀ 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.foodchem.2022.100368.

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