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Fruit Physiology and Sugar-Acid Profile of 24 Pomelo (*Citrus grandis* (L.) Osbeck) Cultivars Grown in Subtropical Region of China

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Abstract: In the present study, the fruit physiology and sugar-acid ratio of 24 pomelo cultivars grown in ten different locations of the subtropical region of China were measured. The contents of soluble sugars and organic acids were quantified using high-performance (HPLC-MS) and ultra-performance liquid chromatography-mass spectrometry (UPLC-MS), respectively. The results revealed that the physiological and basic quality attributes of 24 pomelo cultivars, including fruit weight, fruit width, fruit length, peel thickness, number of segments, pulp weight, pulp color, soluble solids, and Vitamin C, ranged between 264.63–1945.85 g, 8.60–19.56 g, 7.40–20.70 g, 0.46–3.33 mm, 11–18.66, 210.25–1351.66 g, 8.59–15.14 Brix°, and 34.79–84.58 mg/100 g, respectively. Soluble sugars, i.e., fructose, glucose, and sucrose, ranged between 16.25–24.25, 16.17–24.22, and 19.90–55.28 mg/g, respectively. Similarly, Organic acids, i.e., pyruvate, fumaric acids, succinic acid, tartaric acid, quinic acid, citric acid, malic acid, and cis-aconitic acid, in 24 pomelo cultivars ranged between 0.48–1.84, 0.02–0.45, 0–0.05, 0.01–0.1, 0–0.14, 3.01–11.85, 0.18–1.42, and 0.01–0.16 mg/g, respectively. The pomelo cultivars ‘Hongzuanmi’, ‘Minihong’, and ‘Hangwanmi’ exhibited maximum contents of citric acid and pyruvate and showed ultimately excessive organic acids. Overall, the ‘Guanximi’ and its budding cultivars, i.e., ‘Hongroumi’, ‘Huangjinmi’, and ‘Sanhongmi’, had the best quality fruits having maximum sugar-acid ratio. Correlation analysis showed that total soluble sugars had a significantly positive correlation with sucrose contents, while citric acids, malic acid, and pyruvate were positively correlated with total organic acids. The determined sugar-acid profile of pomelo cultivars provides the basis for future elucidation of key mechanisms regulating sugars and acids biosynthesis in pomelo.

Keywords: sucrose; citric acid; UPLC; pyruvate; latitude; vitamin C; citrus



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1. Introduction

Citrus juices are well known as complex mixtures of organic acids and sugars [1,2]. Sugar and organic acid are major soluble components in ripe fruits and have vital roles in fruit taste and flavor, which are key indicators of fruit quality [3–9]. Their nature and concentrations significantly contribute to the perception of citrus juices [10]. The fruit flavor is an important economic trait for evaluating fruit quality, and it is also one of the important sensory indicators that determine consumers’ choice [11]. Organic acids and soluble sugars are important flavor substances of fruits, and their components, contents, and composition ratios determine the sweetness and sourness/tartness of fruits, which are important indicators for the sensory (organoleptic) evaluation of the fruits [6,7]. Studies have shown that the consumption of fruits and vegetables is strongly influenced by their flavor [12,13]. As organic acids influence the fruit ripening, color, and shelf life of fruits, proper acidity can increase consumer acceptance of the fruits [14].

According to the accumulation characteristics of sugars in different fruits, the fruits are divided into three types, i.e., starch conversion type, sugar accumulation type, and intermediate conversion type [15]. Among them, citrus fruits are categorized into the sugar accumulation type [16]. The photosynthetic products produced by the leaves are converted into starch in the early stage of fruit development [12,17,18]. In the later stage of fruit development, the accumulated starch is converted into soluble sugars, which causes a sharp increase in sugar content [6]. Numerous studies showed that fructose, sucrose, and glucose were major sugars in ripe citrus fruits, but the ratio of these ingredients varied among different cultivars [19].

Organic acids usually accumulate during the early stages of fruit development and are used as respiratory substrates during fruit ripening [20,21]. The final organic acid concentration in ripe fruit is determined by the balance of organic acid biosynthesis, degradation, and vacuolar storage [22]. There are many organic acid components in the fruit; the common ones are aliphatic carboxylic acids (tartaric acid, oxalic acid, malic acid, citric acid, and ascorbic acid) and aromatic organic acids (quinic acid, salicylic acid, and caffeic acid), except for a few in the free state [23]. Fruits of different tree species have great differences in the composition and content of organic acids [24]. According to the main organic acids accumulated in mature fruits, the fruits can be roughly divided into three large types, i.e., malic acid type, citric acid type, and tartaric acid type [25]. For example, loquat, apple, peach, and plum use malic acid as the main organic acid type [26], while citrus and grapefruits are of mainly citric acid and tartaric acid types, respectively [27].

Pomelo (*Citrus grandis* (L.) Osbeck), also known as pummelo, shaddock, or Chinese grapefruit, is commonly classified as common (or white) or pigmented (or pink) [28]. It tastes sweet and is slightly acidic with a hint of bitterness [29]. Although some comparisons have been made to the flavor profiles of Nakon [30] and Chandler pummelo [31], the sugar-acid composition of pomelo fruit is still inexplicit. In this study, the fruit physiology and sugar-acid profile of 24 cultivars of pomelo fruits grown in a subtropical region of China were evaluated. The correlation analysis was performed between the contents of soluble sugars, e.g., fructose, glucose, and sucrose, and organic acids, e.g., pyruvate, fumaric acids, succinic acid, tartaric acid, quinic acid, citric acid, malic acid, and cis-aconitic acid. The relationship between 24 pomelo genotypes was also evaluated based on their physiological and quality traits. This study laid the foundation for revealing the mechanisms of sugars and organic acids accumulation in pomelo.

2. Materials and Methods

2.1. Plant Material

Ripe fruit samples from 24 different cultivars of pomelo were harvested from different orchards located at 10 different locations of Fujian, Sichuan, Guangdong, Chongqing, and Guangxi provinces (Figure 1, Table 1). Pomelo plants used in this study were young, healthy, diseases free, and grown in open fields. The experiment contained 3 biological replicates, where each replicate had 9 harvested fruits. Comprehensive pest control was routinely carried out for the fruit orchards. Fruits with the same growth and maturity were picked, and the samples were put into the sampling box and immediately brought back to the laboratory (Institute of Subtropical Fruits, FAFU). After measuring basic fruit quality attributes, the fruit samples (pulp) were stored at $-80\text{ }^{\circ}\text{C}$ for lately measuring sugar and acid contents.

2.2. Measurement of Fresh Fruit Weight, Length, Diameter, and Fruit Shape Index

Fresh Fruit weight, length (from maximum vertical point), diameter (from maximum horizontal point) was calculated by taking an average of 9 fruits from each replication of each cultivar. Fruit weight was measured with digital weighing balance (MJ-W176P, Panasonic, Japan), whereas length and diameter were measured with digital Vernier calipers (DR-MV0100NG, Ningbo Dongrun Imp. & Exp. Co., Ltd., Ningbo, China). The

length was divided by the diameter of each fruit to calculate length-to-width ratio, hereafter called as fruit shape index.



Figure 1. Pictorial view of pomelo fruits of 24 different cultivars harvested from 10 different locations of a subtropical region of China.

Table 1. Details about sampling location and harvesting time of pummelo.

Cultivars	Location	Harvesting Time
Liuyuezao	Pinghe, Fujian (24°16'57" N 117°15'52" E 200 m)	Early August
Hongzuanmi	Pinghe, Fujian (24°17'25" N 117°16'35" E 400 m)	Early December
Zhangyimi	Pinghe, Fujian (24°17'25" N 117°16'35" E 400 m)	Early December
Minihong	Pinghe, Fujian (24°16'57" N 117°15'52" E 200 m)	Early November
Guanximi1	Pinghe, Fujian (24°16'57" N 117°15'52" E 200 m)	Mid October
Hongroumi1	Pinghe, Fujian (24°16'57" N 117°15'52" E 200 m)	Mid October
Hongmianmi1	Pinghe, Fujian (24°16'57" N 117°15'52" E 200 m)	Mid October
Sanhongmi1	Pinghe, Fujian (24°16'57" N 117°15'52" E 200 m)	Mid October
Jinjumi	Pinghe, Fujian (24°16'57" N 117°15'52" E 200 m)	Mid October
Huangjinmi1	Pinghe, Fujian (24°16'57" N 117°15'52" E 200 m)	Mid October
Hongmianmi2	Yongchun, Fujian (25°20'18" N 118°15'54" E 200 m)	Early October
Sanhongmi2	Yongchun, Fujian (25°20'18" N 118°15'54" E 150 m)	Early October
Guanximi2	Pujiang, Sichuan (25°20'18" N 118°15'54" E 500 m)	Late October
Hongroumi2	Pujiang, Sichuan (25°20'18" N 118°15'54" E 500 m)	Late October
Hongmianmi3	Pujiang, Sichuan (25°20'18" N 118°15'54" E 500 m)	Late October
Sanhongmi3	Pujiang, Sichuan (25°20'18" N 118°15'54" E 500 m)	Mid November
Huangjinmi2	Pujiang, Sichuan (25°20'18" N 118°15'54" E 500 m)	Mid November
Sanhongmi4	Qingyuan, Guangdong (23°45'04" N 113°04'02" E 100 m)	Early November
Wendan	Xianyou, Fujian (25°24'23" N 118°44'06" E 100 m)	Late October
Siji	Fuding, Fujian (27°18'13" N 120°19'18" E 200 m)	Mid November
Shuijing	Fuqing, Fujian (25°44'50" N 119°12'00" E 100 m)	Late November
Wubu	Banan, Chongqing (29°20'54" N 106°30'47" E 250 m)	Early December
Hangwanmi	Shanghang, Fujian (24°56'45" N 116°31'17" E 200 m)	Early December
Shatian	Rong, Guangxi (22°46'45" N 110°36'37" E 400 m)	Mid December

2.3. Fruit Peel Thickness, Number of Segments, Pulp Weight, Pulp Color, Soluble Solids, and Vitamin C

After cutting the fruits horizontally, peel thickness was measured with digital Vernier calipers (DR-MV0100NG, Ningbo Dongrun Imp. & Exp. Co., Ltd., Ningbo, China) from randomly selected 4 symmetrical points on the equatorial plane. The number of segments was counted in each fruit, and the average was calculated after that. After dissecting the fruit segments, seeds were separated from the pulp, and pulp weight was measured with a digital weighing balance (MJ-W176P, Panasonic, Osaka, Japan). Pulp color was observed visually. Soluble solid contents were determined with a handheld digital refractometer (Atago, Hybrid PAL-BXIACID F5, Tokyo, Japan). For Vitamin C determination, 10 mL of fruit juice (extracted from pulp) was added in 0.4% oxalic acid solution (90 mL) and filtered, 5 mL aliquot titrated against 2,6-dichlorophenolindophen until the appearance of a light pink color [5].

2.4. Soluble Sugars Extraction through HPLC

Fruit samples (pulp stored at $-80\text{ }^{\circ}\text{C}$) were crushed and pulverized in liquid nitrogen, and 2 g of fine powder was blended in 10 mL 95% methanol with a moderate amount of polyvinylpyrrolidone. After ultra-sonification at $40\text{ }^{\circ}\text{C}$ for 30 min and centrifugation at 1000 rpm for 10 min, the supernatant fluid was separated into a 20 mL distilling flask. The process was repeated using residues with 8 mL ultrapure water. Then, the clear liquid was filtered through a $0.22\text{ }\mu\text{m}$ syringe filter (ANPEL, China). High-Performance Liquid Chromatography (HPLC) analysis was performed using a Waters 2695 autosampler system. The Ellistat Supersil NH₂ column ($4.6\text{ mm} \times 250\text{ mm}$, $5\text{ }\mu\text{m}$ particle size) (Waters Inc, Zellik, Belgium) was used to separate soluble sugars, operated at $40\text{ }^{\circ}\text{C}$. The mobile phases were 82% acetonitrile and 18% ultrapure water solution. The flow rate was 1.2 mL per minute, and the injection volume was 20 μL . Finally, the concentration of every individual soluble sugar was calculated according to the calibration curve of the corresponding standard.

2.5. Organic Acids Determination through UPLC

Organic acids were extracted as described by Nour et al. [32], with some modifications. Fruit samples (pulp stored at $-80\text{ }^{\circ}\text{C}$) were crushed and pulverized in liquid nitrogen, and 2 g of fine powder was blended in 10 mL 95% methanol. After centrifugation at 4000 rpm for 15 min, the supernatant was diluted 25 times and filtered through a MF-Millipore™ Membrane Filter (Cat. No. GSWP04700, 0.22 μm pore size). Organic acids were analyzed by Ultra-Performance Liquid Chromatography (UPLC). A 10 μL elute sample was injected into an Acquity UPLC HSS T3 column (1.8 μm particle size, 2.1 mm \times 100 mm). The flow rate was 0.2 mL min^{-1} using 0.025% H_3PO_4 solution as the solvent. Organic acids were detected at 210 nm, while column temperature was 30 $^{\circ}\text{C}$. A Waters 2996 diode array detector (Waters Corporation, Milford, MA, USA) was used to detect the eluted peaks. The contents of individual organic acids were calculated using the calibration curve of the corresponding standard.

2.6. Statistical Analysis

Collected data were subjected to ANOVA using statistical software “Statistix 8.1” (<https://www.statistix.com/>, accessed on 15 August 2021). Means of replicated data from each cultivar were compared using the Fisher’s least significant difference (LSD) method when $p \leq 0.05$. Correlation coefficient values were determined through the Pearson (r) technique using ‘Statistix 8.1’ (<https://www.statistix.com/>, accessed on 15 August 2021) and visualized through a heat-map using “TBtools ver. 0.6655” (<https://github.com/CJ-Chen/TBtools>, accessed on 15 August 2021). Agglomerative hierarchical clustering (AHC) of tested cultivars was done through complete linkage using “XLSTAT ver. 2019” (<https://www.xlstat.com/en/>, accessed on 16 August 2021).

3. Results

3.1. Fresh Fruit Weight, Length, Diameter, and Fruit Shape Index

Among all studied cultivars, ‘Hongmianmi3’ and ‘Sanhongmi3’ exhibited maximum fruit weight and size, while ‘Minihong’ was found to have the lowest fruit weight and size. The fresh fruit weight of pomelo cultivars ranged between 264.63 and 1945.85 g, while fruit width was recorded from 8.60 to 19.57 mm. Since 7.40 to 20.70 mm was the range measured for the fruit length of pomelo cultivars, the fruit shape index was recorded between 0.80 and 1.10. The fruits of the majority of the cultivars had a weight of more than 1 Kg, except some, i.e., Minihong, Hongmianmi1, Huangjinmi1, Wendan, Siji, and Shatian (Table 2).

3.2. Fruit Peel Thickness, Number of Segments, Pulp Weight, Pulp Color, Soluble Solids, and Vitamin C

The fruit peel thickness of pomelo cultivars ranged between 0.46 and 3.33 mm. The maximum peel thickness was recorded in the fruits of ‘Hongroumi2’, which was seven times higher than those of ‘Minihong’. The number of segments ranged from 11 to 19, while pulp weight was recorded between 210.25 and 1351.66 g. The maximum pulp weight was recorded in the fruits of ‘Guanximi2’, harvested from Pujiang, Sichuan, followed by ‘Sanhongmi3’ and ‘Hongmianmi3’, harvested from the same location. The majority of fruits had a pulp of white color, while some cultivars showed red and orange pulp. The pulp color of ‘Zhangyimi’ was light yellow, which was unique among all cultivars. The soluble solid content (SSC) ranged between 8.59 and 15.14 Brix $^{\circ}$, corresponding to ‘Shuijing’ and ‘Shatian’ cultivars, respectively. The majority of pomelo cultivars exhibited more than 10 Brix $^{\circ}$ SSC, while ‘Guanximi2’, ‘Siji’, ‘Shuijing’, and ‘Wubu’ exhibited 9.94, 9.4, 8.59, and 9.82 Brix $^{\circ}$ SSC. The maximum vitamin C (84.58 mg/100 g) was observed in the fruits of the ‘Shatian’ cultivar, harvested from Rong, Guangxi, followed by ‘Wubu’ having 60.89 mg/100 g, which was 38.90% less than of ‘Shatian’ (Table 3).

Table 2. Comparison between fruit weight, length, width, and fruit shape index of 24 pummelo cultivars.

Cultivar	Fruit Weight (g)	Fruit Width (mm)	Fruit Length (mm)	Fruit Shape Index
Liuyueza	1243.27 ^{eh}	14 ^{efg}	14.87 ^{def}	1.06 ^{a-d}
Hongzuanmi	1553.17 ^{bcd}	16.63 ^{bc}	15 ^{b-f}	0.9 ^{ghi}
Zhangyimi	1608.28 ^{bc}	16.56 ^{bc}	16.88 ^{bc}	1.01 ^{a-f}
Minihong	264.63 ^k	8.6 ^h	7.4 ⁱ	0.86 ^{ij}
Guanximi1	1268.9 ^{d-g}	14.27 ^{d-g}	14.33 ^{e-h}	1 ^{b-f}
Hongroumi1	1109.99 ^{fi}	13.64 ^{efg}	13.6 ^{fgh}	0.99 ^{b-g}
Hongmianmi1	961.92 ^{hij}	12.92 ^g	12.82 ^{gh}	0.99 ^{b-g}
Sanhongmi1	1442.06 ^{cde}	16.73 ^{bc}	16.99 ^b	1.01 ^{a-f}
Jinjumi	1036.04 ^{f-j}	14.52 ^{def}	16.02 ^{b-e}	1.1 ^a
Huangjinmi1	899.05 ^{ij}	14.42 ^{def}	14.81 ^{efg}	1.02 ^{a-f}
Hongmianmi2	1105.52 ^{fi}	13.97 ^{efg}	15.25 ^{b-f}	1.09 ^{ab}
Sanhongmi2	1223.4 ^{e-h}	14.55 ^{de}	15.68 ^{b-e}	1.07 ^{abc}
Guanximi2	1852.92 ^{ab}	17.03 ^b	16.83 ^{bcd}	0.98 ^{c-h}
Hongroumi2	1730.98 ^{abc}	18.49 ^a	19.4 ^a	1.05 ^{a-e}
Hongmianmi3	1936.14 ^a	18.7 ^a	20.7 ^a	1.1 ^a
Sanhongmi3	1945.85 ^a	19.56 ^a	19.63 ^a	1 ^{b-f}
Huangjinmi2	1290.44 ^{def}	14.36 ^{def}	15.56 ^{b-f}	1.08 ^{ab}
Sanhongmi4	1103.97 ^{f-i}	14.63 ^{de}	15.53 ^{b-f}	1.06 ^{a-d}
Wendan	827.66 ^{ij}	14.86 ^{de}	14.05 ^{eh}	0.94 ^{fi}
Siji	765.09 ^j	13.13 ^{fg}	12.76 ^h	0.97 ^{d-h}
Shuijing	1304.11 ^{def}	16.3 ^{bc}	14.43 ^{e-h}	0.88 ^{hij}
Wubu	1496.53 ^{cde}	16.96 ^{bc}	13.6 ^{fgh}	0.8 ^j
Hangwanmi	1669.23 ^{abc}	15.6 ^{cd}	14.93 ^{c-f}	0.95 ^{e-i}
Shatian	968.11 ^{g-j}	14.66 ^{de}	14.9 ^{c-f}	1.01 ^{a-f}
LSD ($p \leq 0.05$)	301.640	1.392	2.003	0.099

Different letters indicate a significant difference ($p \leq 0.05$) among pomelo cultivars, according to Fisher's least significant difference (LSD) technique ($n = 3$, 9 fruits per replicate).

Table 3. Comparison between fruit peel thickness, number of segments, pulp weight, pulp color, soluble solids, and vitamin C content of 24 pummelo cultivars.

Cultivar	Peel Thickness	Segments (No.)	Pulp Weight (g)	Pulp Color	Soluble Solids (Brix ^o)	Vitamin C (mg/100 g)
Liuyueza	1.1 ^k	13.88 ^{def}	880.58 ^{e-h}	White	10.27 ^{h-k}	41.93 ^{d-g}
Hongzuanmi	1.51 ^{g-k}	15 ^{cde}	1154.41 ^{a-d}	Light yellow	13.51 ^b	51.86 ^{bc}
Zhangyimi	2.05 ^{c-f}	13.88 ^{def}	1078.61 ^{b-e}	Yellow	11.03 ^{e-j}	48.71 ^{cde}
Minihong	0.46 ^l	11 ^g	210.25 ^m	Red	12.42 ^{bcd}	36.53 ^{fg}
Guanximi1	1.51 ^{g-k}	14 ^{def}	981.67 ^{def}	White	10.62 ^{f-k}	35.95 ^{fg}
Hongroumi1	1.37 ^{jk}	14.66 ^{cde}	869.66 ^{fgh}	Red	13.02 ^{bc}	34.79 ^g
Hongmianmi1	1.26 ^{jk}	15.22 ^{cd}	699.05 ^{hij}	White	11.3 ^{d-h}	41 ^{d-g}
Sanhongmi1	1.42 ^{h-k}	12 ^{fg}	1009.34 ^{c-f}	Red	11.73 ^{c-g}	41.31 ^{d-g}
Jinjumi	1.63 ^{f-k}	15 ^{cde}	708.83 ^{hij}	Orange	11.18 ^{d-i}	47.55 ^{cde}
Huangjinmi1	1.42 ^{ijk}	15.22 ^{cd}	628.88 ^{ijk}	Orange	12.07 ^{cde}	48.44 ^{cde}
Hongmianmi2	2.19 ^{b-e}	14.22 ^{cde}	701.57 ^{hij}	White	10.91 ^{e-j}	46.57 ^{cde}
Sanhongmi2	2.66 ^b	14.44 ^{cde}	738.22 ^{hij}	Red	10.84 ^{e-j}	44.2 ^{c-g}
Guanximi2	1.94 ^{c-i}	14.66 ^{cde}	1351.66 ^a	White	9.94 ^{ijk}	42.08 ^{d-g}
Hongroumi2	3.33 ^a	15 ^{cde}	1042.95 ^{c-f}	Red	10.43 ^{h-k}	39.39 ^{efg}
Hongmianmi3	2.3 ^{bcd}	13 ^{efg}	1192.52 ^{abc}	White	10.25 ^{h-k}	48.53 ^{cde}
Sanhongmi3	2.43 ^{bc}	16.33 ^{bc}	1257.27 ^{ab}	Red	10.54 ^{g-k}	47.62 ^{cde}
Huangjinmi2	1.95 ^{c-h}	14 ^{def}	868.08 ^{fgh}	Orange	11.18 ^{d-i}	49.16 ^{cd}
Sanhongmi4	1.76 ^{d-j}	15.66 ^{cd}	776.07 ^{ghi}	Red	10.9 ^{e-j}	45.3 ^{c-f}
Wendan	2.05 ^{c-f}	15 ^{cde}	403.55 ^{lm}	White	11.83 ^{c-f}	42.36 ^{d-g}
Siji	1.97 ^{c-g}	14 ^{def}	438.43 ^{kl}	White	9.4 ^{kl}	34.99 ^g
Shuijing	1.74 ^{e-j}	13 ^{efg}	878.48 ^{e-h}	White	8.59 ^l	37.12 ^{fg}
Wubu	1.99 ^{c-g}	18.66 ^a	957.86 ^{d-g}	White	9.82 ^{kl}	60.89 ^b
Hangwanmi	2.3 ^{bc}	18 ^{ab}	995.75 ^{c-f}	White	11.76 ^{c-g}	48.56 ^{cde}
Shatian	1.73 ^{e-j}	11.66 ^g	559.54 ^{ijkl}	White	15.14 ^a	84.58 ^a
LSD ($p \leq 0.05$)	0.538	2.189	203.69		1.285	9.441

Different letters indicate a significant difference ($p \leq 0.05$) among pomelo cultivars, according to Fisher's least significant difference (LSD) technique ($n = 3$, 9 fruits per replicate).

3.3. Soluble Sugars

Three soluble sugars (i.e., fructose, glucose, and sucrose) in the fruit pulp of 24 pomelo cultivars were detected by HPLC (Table 4). The results indicated that sucrose was the most abundant soluble sugars, followed by glucose and fructose. According to an average estimate, the percentage abundance of sucrose, glucose, and fructose was 48, 26, and 25%, respectively, in fruit pulp. Briefly, ‘Minihong’ exhibited maximum fructose and glucose (24.24 and 24.22 mg/g, respectively), while lowest fructose and glucose (16.25 and 16.17 mg/g, respectively) were observed in the fruit pulp of ‘Shuijing’. The sucrose concentration ranged between 19.90 and 55.28 mg/g among all tested cultivars. ‘Minihong’ exhibited the minimum sucrose level (19.9 mg/g), while the maximum (55.28 mg/g) was recorded in the fruit pulp of ‘Hongroumi1’, which was 2.77-fold higher than that of ‘Minihong’. Overall, different fruit cultivars from different regions exhibited a great variation in sugar contents.

Table 4. Comparison between soluble sugar content of 24 pummelo cultivars.

Cultivar	Fructose (mg/g)	Glucose (mg/g)	Sucrose (mg/g)	Total Sugars (mg/g)
Liuyuezao	20.68 ^{bc}	21 ^b	21.16 ^k	62.84 ^{mn}
Hongzuanmi	21.62 ^b	21.19 ^b	38.91 ^{b-e}	81.72 ^c
Zhangyimi	17.08 ^{jk}	16.86 ^{ij}	34.92 ^{fgh}	68.87 ^{i-l}
Minihong	24.24 ^a	24.22 ^a	19.9 ^k	68.37 ^{kl}
Guanximi1	18.16 ^{g-j}	17.9 ^{f-i}	39.49 ^{bcd}	75.57 ^{efg}
Hongroumi1	19.88 ^{cde}	19.3 ^{cde}	55.28 ^a	94.47 ^a
Hongmianmi1	20.44 ^{bcd}	20.17 ^{bc}	40.48 ^{bc}	81.1 ^{cd}
Sanhongmi1	18.71 ^{e-i}	18.33 ^{d-h}	40.91 ^b	77.96 ^{cde}
Jinjumi	18.56 ^{f-i}	18.39 ^{d-h}	35.95 ^{efg}	72.91 ^{ghi}
Huangjinmi1	19.6 ^{c-f}	19.39 ^{cde}	37.26 ^{def}	76.26 ^{efg}
Hongmianmi2	17.8 ^{ij}	17.65 ^{ghi}	29.19 ^j	64.66 ^{lmn}
Sanhongmi2	17.99 ^{hij}	17.2 ^{hij}	37.85 ^{b-f}	73.06 ^{f-i}
Guanximi2	18.53 ^{f-i}	18.13 ^{e-i}	29.95 ^j	66.62 ^{klm}
Hongroumi2	18.49 ^{f-i}	18.28 ^{d-h}	38.01 ^{b-f}	74.79 ^{e-h}
Hongmianmi3	19.11 ^{e-h}	18.65 ^{d-g}	31.35 ^{ij}	69.12 ^{ijk}
Sanhongmi3	18.43 ^{f-i}	18.14 ^{e-i}	31.87 ^{hij}	68.45 ^{kl}
Huangjinmi2	19.02 ^{e-i}	18.59 ^{d-g}	33.51 ^{ghi}	71.13 ^{hij}
Sanhongmi4	19.3 ^{d-g}	18.76 ^{d-g}	37.6 ^{c-f}	75.67 ^{efg}
Wendan	18.16 ^{g-j}	18.04 ^{e-i}	40.31 ^{bcd}	76.51 ^{efg}
Siji	18.59 ^{f-i}	18.49 ^{d-h}	29.81 ^j	66.9 ^{j-m}
Shuijing	16.25 ^k	16.17 ^j	28.96 ^j	61.39 ⁿ
Wubu	19.33 ^{d-g}	19.26 ^{c-f}	38.75 ^{b-e}	77.35 ^{def}
Hangwanmi	19.23 ^{d-h}	19.61 ^{cd}	37.21 ^{def}	76.05 ^{efg}
Shatian	17.85 ^{ij}	17.73 ^{ghi}	54.04 ^a	89.62 ^b
LSD ($p \leq 0.05$)	1.249	1.365	3.117	4.292

Different letters indicate a significant difference ($p \leq 0.05$) among pomelo cultivars, according to Fisher’s least significant difference (LSD) technique ($n = 3$).

3.4. Organic Acids

Eight organic acids (i.e., pyruvate, fumaric acid, succinic acid, tartaric acid, quinic acid, citric acid, malic acid, and cis-aconitic acid) in fruit pulp of 24 pomelo cultivars were quantified by Ultra-High Performance Liquid Chromatography (UPLC) (Table 5). The results revealed that citric acid was the most abundant organic acid, followed by malic acid and pyruvate. The smaller concentrations of fumaric acid, succinic acid, tartaric acid, quinic acid, and cis-aconitic acid were also detected in the fruit pulp of pomelo cultivars. Briefly, the ‘Hongzuanmi’, ‘Minihong’ and ‘Hangwanmi’ having maximum citric acid (11–12 mg/g) and pyruvate (1.7–1.9 mg/g) content were the most acidic genotypes among all tested cultivars. The minimum organic acid contents (4.62 mg/g) were recorded in the fruits of ‘Shuijing’, harvested from Fuqing, Fujian. Fumaric and malic acid (0.45

and 1.42 mg/g, respectively) were also found in ‘Hangwanmi’ at their maximum level, while ‘Minihong’ exhibited the highest concentration of cis-aconitic acid (0.16 mg/g). The maximum concentrations of succinic acid (0.05 mg/g), tartaric acid (0.1 mg/g), and quinic acid (0.14 mg/g) were recorded in the fruit pulp of ‘Huangjinmi1’, ‘Huangroumi2’, and ‘Liuyuezao’, respectively.

Table 5. The organic acid content of 24 pummelo cultivars grown in the subtropical region of China.

Cultivar	Pyruvate (mg/g)	Fumaric Acid (mg/g)	Succinic Acid (mg/g)	Tartaric Acid (mg/g)	Quinic Acid (mg/g)	Citric Acid (mg/g)	Malic Acid (mg/g)	Cis-Aconitic Acid (mg/g)	Total Acids (mg/g)
Liuyuezao	1.12 ^{cde}	0.04 ^{de}	0.03 ^{b-e}	0.02 ^{bcd}	0.14 ^a	9.08 ^{bc}	1.41 ^a	0.13 ^{ab}	12 ^b
Hongzuanmi	1.76 ^a	0.02 ^e	0.02 ^{c-f}	0.02 ^{cd}	0.01 ^{efg}	11.65 ^a	1.29 ^{ab}	0.03 ^{gh}	14.83 ^a
Zhangyimi	1.23 ^{bcd}	0.03 ^{de}	0.02 ^{b-f}	0.02 ^{bcd}	0.05 ^{cd}	7.91 ^{cde}	1.02 ^{bc}	0.03 ^{gh}	10.36 ^{cd}
Minihong	1.84 ^a	0.04 ^{de}	0.02 ^{c-f}	0.02 ^{cd}	0.05 ^{cd}	11.85 ^a	0.51 ^{e-i}	0.16 ^a	14.52 ^a
Guanximi1	0.74 ^{g-j}	0.15 ^{cde}	0.01 ^{c-f}	0.01 ^d	0.01 ^{efg}	6.57 ^{efg}	0.3 ^{hi}	0.07 ^{cde}	7.89 ^{ghi}
Hongroumi1	1.35 ^{bc}	0.04 ^{de}	0 ^f	0.01 ^d	0.06 ^c	6.59 ^{d-g}	0.37 ^{f-i}	0.09 ^{cd}	8.55 ^{fgh}
Hongmianmi1	1.45 ^b	0.03 ^{de}	0.02 ^{c-f}	0.02 ^{cd}	0.03 ^{d-g}	6.76 ^{d-g}	0.29 ⁱ	0.1 ^{bc}	8.72 ^{e-h}
Sanhongmi1	0.61 ^{i-l}	0.15 ^{cde}	0.01 ^{c-f}	0.01 ^d	0.02 ^{efg}	5.72 ^{fgh}	0.33 ^{ghi}	0.06 ^{d-g}	6.95 ^{ijk}
Jinjumi	0.74 ^{g-j}	0.18 ^{bcd}	0.04 ^{ab}	0.04 ^{bc}	0.06 ^{cd}	6.41 ^{fg}	0.48 ^{f-i}	0.08 ^{cde}	8.06 ^{ghi}
Huangjinmi1	0.67 ^{h-l}	0.19 ^{bcd}	0.05 ^a	0.04 ^b	0.06 ^{cd}	5.94 ^{fgh}	0.53 ^{d-i}	0.07 ^{c-f}	7.59 ^{hij}
Hongmianmi2	0.9 ^{e-h}	0.15 ^{cde}	0.02 ^{b-f}	0.02 ^{cd}	0.04 ^{cde}	7.79 ^{cde}	0.3 ^{hi}	0.07 ^{c-g}	9.32 ^{d-g}
Sanhongmi2	0.64 ^{i-l}	0.17 ^{b-e}	0.03 ^{abc}	0.02 ^{bcd}	0.05 ^{cd}	5.57 ^{f-i}	0.72 ^{c-f}	0.06 ^{d-g}	7.32 ^{h-k}
Guanximi2	1.01 ^{def}	0.03 ^{de}	0.02 ^{b-f}	0.01 ^d	0.02 ^{efg}	6.43 ^{fg}	0.46 ^{f-i}	0.13 ^b	8.15 ^{ghi}
Hongroumi2	0.96 ^{efg}	0.15 ^{b-e}	0.02 ^{b-f}	0.1 ^a	0.03 ^{c-g}	8.22 ^c	0.51 ^{e-i}	0.02 ^h	10.06 ^{c-f}
Hongmianmi3	0.72 ^{h-k}	0.14 ^{cde}	0.02 ^{c-f}	0.01 ^d	0.01 ^g	6.79 ^{def}	0.18 ⁱ	0.03 ^{gh}	7.93 ^{ghi}
Sanhongmi3	1 ^{ef}	0.15 ^{b-e}	0.02 ^{b-f}	0.01 ^d	0.01 ^{efg}	8.65 ^{bc}	0.23 ⁱ	0.05 ^{e-h}	10.16 ^{cde}
Huangjinmi2	0.89 ^{e-h}	0.03 ^{de}	0.02 ^{c-f}	0.01 ^d	0.04 ^{c-f}	9.67 ^b	0.67 ^{c-h}	0.02 ^h	11.38 ^{bc}
Sanhongmi4	0.48 ^l	0.25 ^{bc}	0.01 ^{def}	0.01 ^d	0 ^g	4.31 ^{ij}	0.88 ^{cde}	0.02 ^h	5.99 ^{kl}
Wendan	1.01 ^{def}	0.15 ^{cde}	0.01 ^{ef}	0.01 ^d	0.05 ^{cd}	8.84 ^{bc}	0.89 ^{cd}	0.02 ^h	11.01 ^{bc}
Siji	0.52 ^{jkl}	0.17 ^{b-e}	0.02 ^{b-f}	0.02 ^{cd}	0.1 ^b	4.87 ^{hi}	0.41 ^{f-i}	0.03 ^{gh}	6.17 ^{jk}
Shuijing	0.5 ^{kl}	0.05 ^{de}	0.03 ^{abc}	0.02 ^d	0.01 ^{efg}	3.01 ^j	0.95 ^{bc}	0.01 ^h	4.62 ^l
Wubu	1.28 ^{bc}	0.04 ^{de}	0.03 ^{abc}	0.02 ^{cd}	0.02 ^{efg}	7.93 ^{cd}	0.69 ^{c-g}	0.04 ^{fgh}	10.08 ^{c-f}
Hangwanmi	1.77 ^a	0.45 ^a	0.03 ^{bcd}	0.02 ^{cd}	0.05 ^{cd}	11.08 ^a	1.42 ^a	0.03 ^{gh}	14.89 ^a
Shatian	0.83 ^{f-i}	0.31 ^{ab}	0.04 ^{ab}	0.02 ^{cd}	0.01 ^{fg}	5.43 ^{ghi}	0.94 ^{bc}	0.09 ^{cd}	7.7 ^{hij}
LSD ($p \leq 0.05$)	0.234	0.155	0.021	0.022	0.031	1.348	0.373	0.035	1.551

Different letters indicate a significant difference ($p \leq 0.05$) among pomelo cultivars, according to Fisher’s least significant difference (LSD) technique ($n = 3$).

3.5. Correlation among Organic Acid and Soluble Sugar Contents

The correlation analysis between organic acid and soluble sugar contents of 24 pomelo cultivars was done (Figure 2). Pyruvate exhibited negative correlations with fumaric acid, succinic acid, tartaric acid, and sucrose content, and significantly ($p \leq 0.01$) positive association with citric acid, fructose, and glucose contents.

Fumaric acid was positively correlated with succinic acid, tartaric acid, malic acid, and sucrose, while negatively correlated with quinic acid, citric acid, cis-aconitic acid, fructose, and sucrose. A strong negative correlation was found between citric acid, fructose, glucose, and succinic acid, while succinic acid was positively associated with tartaric acid, quinic acid, malic acid, and cis-aconitic acid. Tartaric acid showed a positive association with quinic acid, citric acid, and sucrose, while quinic acid was negatively correlated with sucrose content. Citric acid showed a significantly positive correlation with fructose and glucose ($p \leq 0.05$ and $p \leq 0.01$, respectively). Fructose and glucose showed a highly significant ($p \leq 0.01$) association among each other. Overall, total organic acids were significantly and positively correlated with pyruvate, citric acid, malic acid, fructose, and glucose. Whereas total soluble sugars were significantly associated with sucrose content (Figure 2).

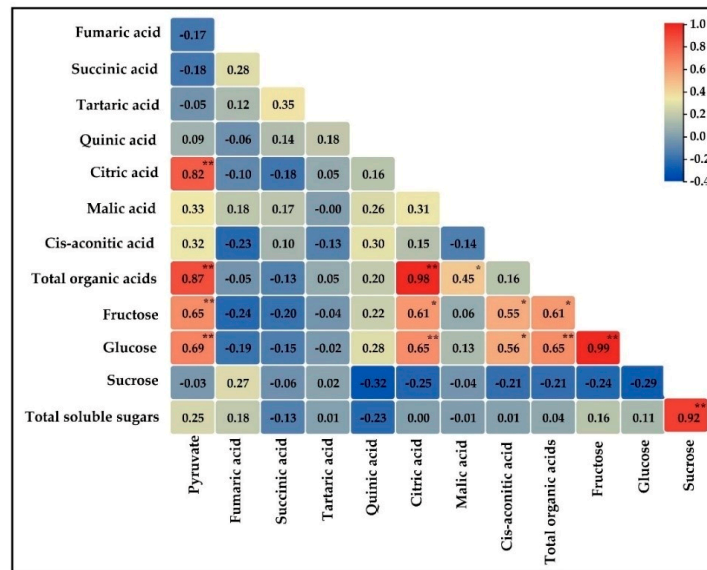


Figure 2. The heat-map showing the correlation of organic acid and soluble sugar contents of 24 pomelo cultivars. The * and ** represent significance at $p \leq 0.05$ and $p \leq 0.01$, respectively, following Pearson (n) method.

3.6. AHC Analysis of 24 Pomelo Cultivars Based on Their Physiology and Sugar-Acid Profiles

Based on physiological traits and sugar-acid profiles, 24 pomelo cultivars (harvested from the subtropical region of China) were divided into three main classes Class-1, Class-2, and Class-3 (Figure 3). Class-1 having a within-class variance of 60,779.64, showed clustering of 14 cultivars. This class was further divided into two subclasses, i.e., 1A and 1B. Subclass 1A showed clustering of Sanhongmi2, Hongroumi1, Hongmianmi2, Jinjumi, Sanhongmi4, Shuijing, Huangjinmi2, Liuyueza, and Guanximi1, while subclass 1B exhibited grouping of Hongmianmi1, Huangjinmi1, Wendan, Siji, and Shatian. Class-2 having a within-class variance of 52,560.64, showed clustering of 9 pomelo genotypes, i.e., Hongzuanmi, Zhangyimi, Sanhongmi1, Guanximi2, Hongroumi2, Hongmianmi3, Sanhongmi3, Wubu, and Hangwanmi. The Minihong cultivar was categorized in Class-3.

The clustering of different pomelo cultivars indicated their similarity to each other with respect to their physiology and sugar-acid profiles.

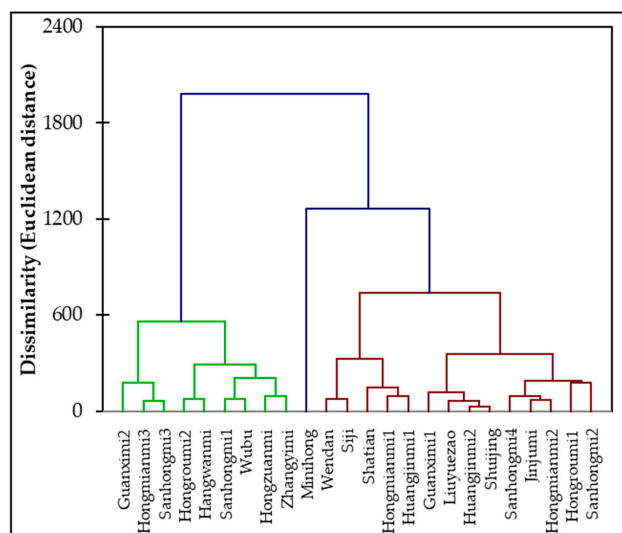


Figure 3. A dendrogram representing the relationship of 24 pomelo genotypes based on their physiology and sugar-acid profiles.

4. Discussion

Fruit morphology is the external manifestation of genetic traits, and it is also the most intuitive basis for population classification and species identification [33]. The traditional classification of pomelo varieties is mainly based on the description results of one or several biological characteristics of pomelo to classify groups and distinguish germplasm [34]. Due to the metabolite competition between vegetative and reproductive growth, fruit size is closely related to tree metabolite supply and tree age [35,36]. Moreira et al. [37] reported that the younger the 'ponkan' tree, the larger the fruit and the lower the yield. In the present study, the Guanximi2 pomelo cultivar harvested from Sichuan had larger fruits, but the pulp weight was not high (Table 2), which is related to the younger tree age. Guanximi is the most popular commercial cultivar in China. It is extensively grown in Pinghe County, Zhangzhou City, Fujian Province, the largest producer of this cultivar with the production of 1.2 metric tons representing 40% of total Chinese production [38] together with some production in Sichuan Province [39]. Guanxi pomelo fruit was pyriform with golden-orange-colored, delicate, thin, and smooth rind. The albedo and segment walls were white, and the flesh was pale yellow. The pulp was tender, crisp, and juicy. The taste was sweet to moderately sour with 9.17–11.6% soluble solids, 0.73–1.01% titratable acidity, and 48.9–52.0 mg·(100 mL)⁻¹ vitamin C [40].

Guanxi has produced a series of mutant cultivars with different colors of either fruit flesh or peels through the selection of natural mutations in the field. Hongroumi, a red-fleshed pomelo, is a high-quality spontaneous bud mutant of cv. Guanxi. This cultivar matures from late September to mid-October which is 20–25 days earlier than its maternal cultivar. Huangjinmi, an orange-fleshed pomelo, was also one of the bud mutants of cv. Guanxi. It had a unique orange pulp containing high β -carotene, and its texture was fine, tender, crisp, and juicy. Sanhongmi was another bud mutant of cv. Guanxi. It was different from its sister mutants in that its albedo and segment walls were pink. The flesh was red, tender, crisp, and juicy. These three mutant cultivars consisted of 10.6–11.7% soluble solids, 0.54–0.87% titratable acids, and 34.3–40.5 mg·(100 mL)⁻¹ Vitamin C (Table 3) [40,41].

It is believed that the content of soluble sugars accumulated in pomelo fruits from different producing areas is different [42,43]. Regional and climatic conditions have effects on sugar and acid levels in fruits [44–46]. Zheng et al. [44] reported that the contents of fructose, glucose, and total sugar in *Hippophae rhamnoides* ssp. decreased with latitude. In this study, the soluble sugars, i.e., fructose, glucose, and sucrose, of 24 pomelo cultivars from ten different producing areas were quantified through HPLC (Table 4). The results showed that there were differences in the sugar content of different varieties from different locations to different degrees, indicating that the climate of different producing areas can affect the sugar quality of the fruit, thereby affecting the fruit flavor. For example, in Sanhongmi cultivars harvested from Qingyuan, Pinghe, Yongchun, and Pujiang, the total sugar content gradually decreased from south to north as the latitude increased, and the sucrose content changed more obviously with the latitude.

Organic acid in the fruit is one of the important factors that determine the flavor of the fruit [7]. Fruit organic acids can be divided into aliphatic carboxylic acids, sugar-derived organic acids, and phenolic acids [47]. Most of the organic acids in citrus fruits are aliphatic carboxylic acids, such as citric acid, fumaric acid, malic acid, etc. [25,48,49], and also contain a small amount of lactic acid and oxalic acid [50,51], and oxaloacetic acid, etc. [52]. In the present study, the optimized UPLC-MS method was used to detect citric acid, pyruvate, fumaric acid, and other organic acids from different varieties of pomelo fruits. Among them, the content of citric acid accounted for about 70–80% of the total acid. The composition and content of organic acids in citrus fruits have genetic variation [53,54], and the differences are not only manifested between different citrus types [55], also manifested in different varieties of the same species. It has been reported that there are significant differences in the content of citric acid and malic acid in the fruits of the four budding varieties of Guanximi [39]. In the present study, in addition to the difference between citric acid and malic acid, organic acids, such as pyruvate and fumaric acid, also had significant

differences between Guanximi pomelo and its bud varieties, i.e., Hongroumi, Huangjinmi, and Sanhongmi, etc. (Table 5).

The correlation analysis showed that citric acid significantly contributed to the accumulation of organic acids in pomelo fruits, followed by pyruvate and malic acid (Figure 2). Recent studies have shown that citric acid is the main organic acid of citrus fruits, including lemon, lime, mandarin, sweet orange, pomelo, and grapefruit [52,56,57]. Similarly, sucrose was the most abundant sugar among all tested soluble sugars in pomelo fruits (Figure 2). Sucrose has been reported as a major soluble sugar in citrus fruits [58], melons [59], and kiwifruit [60]. Hence, citric acid and sucrose were the main acid and sugar recorded in pomelo fruits, respectively.

5. Conclusions

In the present study, the physiological and basic quality attributes especially, soluble sugars and organic acids, were measured in 24 pomelo cultivars grown in a subtropical region of China. The results suggested that the Guanximi and its budding cultivars, i.e., Hongroumi, Huangjinmi, and Sanhongmi, had the best quality fruits having maximum fruit weight, size, soluble solids, Vitamin C, and sugar–acid ratio. In pomelo fruits, sucrose was found in abundance as compared to fructose and glucose, contributing 48% in total soluble sugars. Among measured organic acids, citric acid was the most abundant organic acid, followed by malic acid and pyruvate. The Minihong pomelo cultivar ranked lowest among all cultivars with respect to fruit weight and size, although the fruits of this cultivar had high acid and low sugar contents. This study provided basic information about fruit quality profiles of some local pomelo cultivars and provided the basis for future studies about sugar and acid mechanism in pomelo fruits.

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