Comparative Studies on the Quality and Lycopene Content of Pomelo (*Citrus grandis* Osbeck) Cultivars

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**Abstract**

Three cultivars of pomelo “Guanximiyou” (GX) and its variants “Hongroumiyou” (HR) and “Sanhongmiyou” (SH) were selected as experimental materials to evaluate the quality and edible value of the fruit. In this study, the fruit quality and lycopene contents were investigated. The results show that among the 3 cultivars used, the contents of soluble solids, vitamin C, and total phenol in the juice sac of SH were the highest, followed by HR and GX. SH has the largest fruit shape index, soluble sugar content: GX > HR > SH, and titratable acid content: GX < HR < SH. From our findings, the content of lycopene was the highest in SH, followed by HR and GX. SH has the greatest potential for production due to its appreciable content of lycopene and fruit quality.

**Keywords**

pomelo, lycopene, fruit quality, total phenol, flavonoid

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Pomelo (*Citrus grandis* Osbeck), family Rutaceae, has a lot of health benefits when consumed (e.g., antiaging, antioxidant, anticancer, and hypoglycemic activities).¹ The nutritional value of pomelo is very high, for it contains essential trace elements and potassium, which is good for patients with hypertension, cardiovascular, cerebral vascular, and kidney diseases.²,³ According to pomelo consumers, it is of great importance to develop the selection and breeding criteria of the important varieties favored by the market in promoting the vigorous development of the pomelo industry of China and developing more new varieties with high quality, high yield, and high efficiency. The fruits, especially the juice sacs of red-fleshed pomelo, are light red in color, which is a feature of great interest to consumers and producers. One of the main production areas of pomelo is Zhangzhou Fujian province, which is located along the southeast coast of China, characterized by a subtropical marine monsoon climate zone, rich in resources, varieties, and types of many important crops. The cultivar Guanximiyou (GX) and its variants Hongroumiyou (HR), and Sanhongmiyou (SH) have no difference in biological characteristics during their growth and development, but the flesh color often changes at maturity. The flesh of pomelo is red because of lycopene,⁴ which can affect free radicals in human serum. Lycopene appears to promote an anabolic state of bone metabolism, stimulating osteoblastogenesis and inhibiting osteoclastogenesis, which may contribute to the promotion of proper health status of bone tissue.⁵ Pomelo peel contains flavonoids, natural pigments, dietary fiber, limonin, essential oil, and other active ingredients. These compounds contribute to the antioxidant, anticancer, bacteriostatic, hypoglycemic, and lipid-lowering activities.⁶,⁷ Therefore, the fruit quality and the variations in phytochemical profiles and antioxidant contents were explored in the 3 cultivars of pomelo to evaluate their nutritional value for growers, consumers, and industrial producers.

**Materials and Methods**

**Sourcing Plant Material and Preparation**

Pomelo cultivar GX and its variants HR and SH were randomly selected from Nanjing County in September 2018. Nanjing County is located in the northwest of Zhangzhou city, Fujian province, China. It is located at longitude 117°00′12″-117°36′36″ and latitude 24°26′20″-24°59′58″. The terrain slopes from northwest to southeast and the landform are successively divided into 4 geomorphologic types: middle and low
mountains, hills, platforms, and valley plains, among which hilly areas are the main ones, accounting for 44.1% of the land area, followed by middle and low mountains, accounting for 39.6%, and valley plains for 16.3%. Nanjing’s topography is very suitable for the production of GX. The pomelo cultivars were collected, transported to the laboratory, and the impurities on the fruit surface cleaned by washing with tap water before further analysis.

**Pretreatment of Test Samples**

The physical characteristics of the fruit were reflected by measuring the width, length, and mean weight of the fruit, and the flesh was crushed into juice for measuring the content of total soluble solid (TSS) using a digital refractometer (DR301-95, Krüss Optronic, Hamburg, Germany). The peel, albedo, segment membrane, and juice sac were separated from 10 pomelos, and then 3 replicate samples were collected from the mixture of each part. Samples were wrapped with tin foil and immediately treated with liquid nitrogen and refrigerated at −40 °C for use.

**Extraction of Total Phenolics and Flavonoids**

The pomelo peel, albedo, segment membrane, and juice sac samples were separately ground in liquid nitrogen using a mortar and pestle. Each ground sample (3 g) was homogenized in 6 mL of 2% methanol hydrochloride. The extracts were centrifuged at 12,000 rpm at 4 °C for 20 minutes and used to measure the total phenolics and flavonoid content. The total phenol extractions were performed with a protocol similar to that of Huang, where 0.5 mL aliquots and gallic acid at different concentrations (20, 40, 60, 80, 100 µg/mL) were added to a 10 mL tube, and then 4 mL of distilled water and 0.5 mL of Folin-Ciocalteu’s reagent were added and the tubes gently shaken. After 5 minutes, 1 mL of 70% sodium carbonate was added and mixed thoroughly. The mixture was kept in the dark at room temperature (25 °C) for 120 minutes. After the solution in the tube changed color, the absorbance was measured against a blank at 765 nm using a spectrophotometer (UV5100H METASH, China). Measurements were repeated 3 times. The content of total flavonoid was determined by the rutin method assay according to Zheng, with minor modifications. All reagents used were bought from Shanghai Macklin Biochemical Co, Ltd. China.

**Extraction and HPLC Analysis**

The juice sac samples were ground in a mortar in liquid nitrogen. Powdered sample (1 g) was homogenized in 10 mL of acetone and subjected to ultrasonic treatment for 15 minutes at 50 °C. The extracts were centrifuged at 5000 rpm at 4 °C for 10 minutes to obtain the supernatants. The remaining residue was added to 5 mL of acetone, extracted using the same procedure, mixed thoroughly with the supernatant, and stored in a refrigerator at −20 °C. The analysis of lycopene was achieved using a high-performance liquid chromatography (HPLC) system (LC-100; Wufeng series, Shanghai, China) equipped with an LC-P100 pump and operated by LC-WS100 software, according to Lui et al and Cui et al, with slight modification. Pomelo flesh was squeezed, and the juice was filtered through a 0.45 µm membrane filter (MillexHV; Millipore, Bedford, MA, USA); 40 µL of the solution was injected directly into the HPLC system. The eluting solvent was acetonitrile/methanol at a ratio of 95:5 (v/v), the flow rate 1.0 mL/min at 30 °C, and detection was at 475 nm. The peak detected in the ultraviolet-visible spectrum matched that of lycopene. The content of lycopene was quantified by the external standard peak area method; standard curves were constructed using peak area versus concentration.

**Conventional Fruit Quality Index**

The soluble sugar content was determined by anthrone colorimetry, determination of organic acids by sodium hydroxide neutralization titration according to Hesheng, and the vitamin C (VC) content by the 2,6-dichlorophenol indophenol method according to Yuan et al. The edible rate was the weight of the juice sac divided by the weight of the whole fruit.

**Statistical Analysis**

Data obtained were subjected to a one-way analysis of variance using SPSS v22.0 (SPSS Inc., Chicago, IL, USA). Results are

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**Table 1. The Physical Properties and Soluble Solids of the 3 Cultivars of Pomelo.**

| Cultivar | Mean weight (kg) | Width (cm) | Length (cm) | Fruit shape index | TSS (°Brix) | Edible rate |
|----------|-----------------|------------|-------------|-------------------|-------------|-------------|
| GX       | 1.19 ± 0.15c    | 14.7 ± 1.1b| 13.7 ± 1.0c | 0.93              | 11.4 ± 0.7c | 79.0 ± 0.01a|
| HR       | 1.51 ± 0.15a    | 16.1 ± 0.5a| 16.6 ± 1.3b | 1.03              | 11.6 ± 0.4b | 70.4 ± 0.02b|
| SH       | 1.45 ± 0.16b    | 16.1 ± 0.7a| 16.9 ± 0.8a | 1.05              | 11.8 ± 0.2a | 69.8 ± 0.04b|

Abbreviations: GX, Guanximiyou; HR, Hongroumiyou; SH, Sanhongmiyou; TSS, total soluble solid. Different lower case letters in the same column indicate significant differences at P < 0.05 level of probability.
provided as the mean ± SD. The mean separation was made via a Tukey's test with 0.05 taken as significant differences.

Results

Physical Properties and Soluble Solids of the 3 Cultivars of Pomelo

The physical properties and soluble solids of the 3 cultivars of pomelo are presented in Table 1. Significant differences were observed between GX, HR, and SH for mean weight. GX had a significantly higher edible rate than HR and SH, but no significant difference in the edible rate was found between HR and SH. The TSS value was significantly higher in SH than in GX and HR.

Fruit Quality of the 3 Cultivars of Pomelo

The differences in fruit quality of the pomelo varieties are presented in Table 2. In the juice sac, the highest total phenol and VC in SH were significantly higher than the others; the lowest content was observed in GX. The flavonoid content of HR was significantly higher than that of GX and SH. The soluble sugar content of GX was significantly higher in SH than in GX and HR.

Table 2. Analysis of Fruit Quality in the 3 Cultivars of Pomelo.

| Fruit quality indicator | Vitamin C (mg/100 g) | Total phenol (mg/g) | Flavonoid (mg/g) | Titratable acid (mmol/100 g) | Soluble sugar (mg/mL) |
|-------------------------|----------------------|---------------------|-----------------|-----------------------------|----------------------|
| Juice sac               |                      |                     |                 |                             |                      |
| GX                      | 34 ± 0.01b           | 0.36 ± 0.01c        | 0.04 ± 0.01b    | 2.90 ± 0.00b                | 0.58 ± 0.00a         |
| HR                      | 42 ± 0.02a           | 0.39 ± 0.01b        | 0.05 ± 0.01a    | 2.90 ± 0.02b                | 0.46 ± 0.03b         |
| SH                      | 45 ± 0.02a           | 0.41 ± 0.00a        | 0.04 ± 0.00b    | 4.35 ± 0.00a                | 0.41 ± 0.01c         |
| Albedo                  |                      |                     |                 |                             |                      |
| GX                      | 11 ± 0.00a           | 2.04 ± 0.08a        | 0.60 ± 0.02a    | 2.17 ± 0.00a                | 0.27 ± 0.06a         |
| HR                      | 10 ± 0.00a           | 1.68 ± 0.13b        | 0.48 ± 0.04b    | 1.45 ± 0.00b                | 0.29 ± 0.01a         |
| SH                      | 9 ± 0.00a            | 1.68 ± 0.07b        | 0.45 ± 0.04b    | 1.69 ± 0.21b                | 0.28 ± 0.00a         |
| Segment membrane        |                      |                     |                 |                             |                      |
| GX                      | 13 ± 0.01a           | 2.30 ± 0.06a        | 0.64 ± 0.02a    | 1.45 ± 0.00a                | 0.38 ± 0.01b         |
| HR                      | 13 ± 0.02a           | 1.87 ± 0.05b        | 0.35 ± 0.01b    | 1.45 ± 0.00a                | 0.43 ± 0.00a         |
| SH                      | 11 ± 0.01a           | 1.60 ± 0.13b        | 0.33 ± 0.00b    | 1.69 ± 0.21a                | 0.45 ± 0.02a         |
| Peel                    |                      |                     |                 |                             |                      |
| GX                      | 24 ± 0.04a           | 2.30 ± 0.10a        | 1.02 ± 0.01a    | 2.90 ± 0.01a                | 0.33 ± 0.01a         |
| HR                      | 13 ± 0.03b           | 2.03 ± 0.17b        | 0.68 ± 0.07b    | 2.17 ± 0.01b                | 0.27 ± 0.03b         |
| SH                      | 15 ± 0.04b           | 1.87 ± 0.06c        | 0.52 ± 0.00c    | 2.66 ± 0.23ab               | 0.23 ± 0.00c         |

Abbreviations: GX, Guanxiniyou; HR, Hongroumiyou; SH, Sanhongmiyou. Different lower case letters in the same column indicate significant differences at P < 0.05 level of probability.

The Differences in Lycopene Content

The lycopene contents of the 3 cultivars were 2593 µg/g in SH, 2486 µg/g in HR, and 102 µg/g in GX. The lycopene content in SH is significantly higher than that in HR and GX (Figure 1).

Discussion

In this experiment, the quality (soluble sugar, titratable acidity, VC, total phenolic, flavonoids) and the lycopene content of 3 cultivars of pomelo were studied. The results showed that the soluble solid content of SH was the highest compared with the others. The titratable acid of SH was also higher than GX, which had a significant difference in the ratio of TSS to acid between its pedicle and navel parts.17 In the 3 cultivars studied, the content of total phenol and flavonoid was the lowest in the juice sac and highest in the peel. This was consistent with the findings of Lu et al, Ran et al, and Zhuang et al.18-20 Phenolics and flavonoids are broadly available in many plants and are very important secondary metabolites with various roles in the plants’ development. These types of compounds have been drawing increasing attention due to their potent antioxidant properties and their marked effects in the prevention of various oxidative stress associated diseases, such as cancer.21 It was found that the antioxidant and hypolipidemic effects of the pomelo juice sac and the total phenolics were significantly correlated with ferric-reducing antioxidant power and Trolox-equivalent antioxidant capacity.3 Previous studies showed that the segment membranes of GX have stronger antioxidant...
capacity than the juice sac.\textsuperscript{22} The flavonoid extract of pomelo peel has an inhibitory effect on bacteria and can be used to produce antibacterial agents to overcome the resistance of antibiotics.\textsuperscript{23} Actually, people only eat the juice sac of pomelo and discard the other parts of fruit, which is a waste of natural resources. What we are going to do next is how to make use of the peel.

In this study, the lycopene content of SH and HR were significantly higher than GX. Apart from weight, size, and TSS contents, color is one of the key parameters evaluated in the context of commercial quality and food acceptability. It was found in HR that the carotenoids are mainly β-carotene and lycopene, and the red color is due to high levels of lycopene,\textsuperscript{24} which can reduce the number of proliferating cancer cells in the tumor and metastatic tissues.\textsuperscript{25} HR and SH have high contents of lycopene, and so long-term consumption of pomelo could reduce the spread of cancer. Our study showed that both HR and SH have higher levels of lycopene than the fruits that are commonly eaten; the content of lycopene was much higher than that reported by previous researchers,\textsuperscript{26,27} and these 2 cultivars can provide better natural resources of lycopene.

Conclusions

In this study, conventional fruit quality indicators and lycopene content of mature GX and its varieties HR and SH were analyzed and evaluated. The highest content of lycopene was found in SH and the lowest in GX. The fruit quality parameters such as the contents of VC, TSS, total phenol, and flavonoid in HR and SH were higher than those in GX. These suggested that SH cultivar has the greatest potential for production and utilization as a source of important metabolites that could be consumed fresh or for industrial uses.

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Declaration of Conflicting Interests

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\textbf{Figure 1.} Transverse and longitudinal sections of the 3 pomelo cultivars. (A) Longitudinal section of the pulp flap. (B) The pulp flap of fruit from which the membrane is removed. (C) Pulp flap of fruit. (D) Cross-section of fruit. (E) Longitudinal section of fruit. (F) Whole fruit. GX, Guanximiyou; HR, Hongroumiyou; SH, Sanhongmiyou.
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