Study on the accumulation of Phenols during fruit development of two Pomelos

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Abstract: This experiment used Huangjinmiyou and Sanhongmiyou as materials to study the accumulation of phenolic substances during fruit development in different parts of pomelo fruit (exocarp, mesocarp, and pulp) via Folin-Ciocalteu assay. The results showed that the total phenolic content (TPC) of Huangjinmiyou was slightly higher than that of Sanhongmiyou. The sequence of TPC in different parts of the fruit was mesocarp > exocarp > pulp. TPC of the three parts reached maximum on the 60th day. The experiment aimed at two popular varieties showed their normal rules of phenols changing during fruit development and their differences, which consummated relevant theories.

1. Introduction
Pomelo, one of the important species of Citrus, is famous for rich nutrients and high medicinal value[1]. Studies have found that different types of citrus show unique accumulation of phenolics characteristics[2]. The antioxidant activity was relevant to the total phenolic content (TPC) in orange peel extracts[3]. In addition, most polyphenols (PPs) can be better absorbed, which may also influence the composition and function of the gut microbiota[7]. Besides, phenolics also have cosmetic applications[8]. Nowadays, concern to replace these synthetic antioxidants by natural such as phenolics is growing[9]. However, only half of pomelos go to the fresh market. Apart from pulp and juice, the non-edible part is nearly 50% by weight[10]. These by-products, such as peels, may pollute the environment[8]. Belonging to citrus of Rutaceae, pomelo peel may contain bioactive substances as well. On the other hand, the development of citrus industry tends to slow down[11]. How to get out of the predicament of pomelo industry has become a widely-concerned topic[12].

Pomelo cv. Huangjinmiyou (HJ in short) is a bud variety of Guanxi honey pomelo, with orange pulp, fleshy residue, sweet and sour taste, and high quality.[13]. It has early bearing and high output[14]. Pomelo cv. Sanhongmiyou (SH in short) is also the same strain as Guanxi honey pomelo[15]. It is named SH because of its three red characteristics of red pulp, soft red peel and light red skin after bagging[16]. The peel and capsule are easy to peel off and the pulphas light tomato flavor. Both of pomelo cv. HJ and pomelo cv. SH have great potential for promotion[11].

The aim of the present investigation was to find rules of TPC changes in fruit of two new varieties. Moreover, the experiment provides theoretical basis for saving the exocarp and mesocarp in producing phenolic production, which may improve the utilization rate of pomelos and protect the environment.
2. Materials and Methods

2.1 Plant Material and Sample Preparation
The experimental materials, HJ and SH fruits, were collected from an orchard in Pujiang county, chengdu city, sichuan province. Three plants of each variety were a small area with four repetitions and row spacing of 3×4m. The plants with strong vigor, basically consistent growth, no diseases were selected. Two fruits were randomly picked from the top of the crown periphery of four directions. The picking activity lasted from June to October 2019, one time per 30 days. Once fruits were picked, cleaned and separated them into three parts, exocarp, mesocarp and pulp. Then they were cut into small pieces, dried, crushed, sealed and stored. After these, 0.4g of the prepared sample and 8ml of methanol were mixed. After ultrasound extraction and centrifugation, the supernatant was taken and the remaining vegetal material was re-extracted twice. After combination, the volume was fixed to 25mL and the sample was stored at -20℃ for use.

2.2 Chemicals
All chemicals were of analytical grade. Allic acid was purchased from Sigma-Aldrich (St. Louis, MO, USA). Methanol was purchased from Fisher Scientific Chemicals (Leics, UK). Folin-Ciocalteu was purchased from Sigma Aldrich (Germany). Anhydrous sodium carbonate was acquired from Shanghai Chemical Reagent Company (Shanghai, China). Ultrapure water was supplied by a super pure water system from EPED Co., Ltd. (Nanjing, China). Double-distilled water (ddH₂O) was used.

2.3 Total Phenols Extraction
TPC of the plant samples was determined by the Folin-Ciocalteu assay[17]. 250 ul of extract was mixed with 750 ul of pure water and 1.0 mL Folin-Ciocalteu reagent. After 5 minutes of dark treatment, sample was mixed with sodium carbonate solution (5%, 10 mL) followed by incubation at room temperature for 60 minutes. Absorbance was measured at 765 nm using an enzyme mark instrument and compared with a calibration curve of gallic acid. Results were expressed as milligrams of gallic acid equivalents per gram (GAE) of sample.

3. Results

3.1 Changes of Total Phenols in the Exocarp
During fruit development, the TPC of HJ exocarp was always higher than that of SH (Figure 1). TPC of both decreased first and then increased. From the 60th day to the 120th day, TPC of both decreased gradually. From the 120th day to the 180th day, TPC of both increased gradually. But the change range of pomelo cv. SH was smaller. On the 60th day, TPC of HJ reached the highest 1527.35mg·kg⁻¹. SH reached the highest content of total phenols 927.86mg·kg⁻¹ on the 180th day. On the 120th day, TPC was the lowest, with 664.02mg·kg⁻¹ of HJ and 553.09mg·kg⁻¹ of SH.
3.2 Changes of Total Phenols in the Mesocarp

It can be seen from Figure 2 that, in the fruit period, TPC in the mesocarp of pomelo cv. HJ and SH fluctuated and decreased, changing steadily, but TPC of the former increased after the 150th day, and the latter increased on the 120th day and the 180th day. The highest content of TPC was on the 60th day. TPC of HJ was 1861.93 mg·kg⁻¹, and that of SH was 1714.57 mg·kg⁻¹. The lowest stage of TPC was the 150th day. The former was 1186.32 mg·kg⁻¹ while the latter was 1223.07 mg·kg⁻¹. In the first two stages, TPC of HJ was slightly higher than that of SH, but in the latter three stages, TPC of SH was higher.

3.3 Changes of Total Phenols in the Pulp

It can be seen from Figure 3 that, in the fruit period, TPC in the pulp of HJ and SH decreased with fluctuation and increased on the 120th day. After the 60th day, TPC decreased sharply, and then the change was gentler. The highest TPC appeared in the first stage. The former was 3060.02 mg·kg⁻¹ while the latter was 3428.92 mg·kg⁻¹. The lowest TPC occurred inconsistently. The former was on the 180th day 763.89 mg·kg⁻¹, the latter was on the 150th days 637.23 mg·kg⁻¹. In the first three stages, TPC of pomelo cv. SH was higher than HJ, while in the last two stages it was lower than HJ.
Fig. 3 Changes of total phenolic content in Pulp.

4. Discussion and Conclusion
In this experiment, the following conclusions are drawn from the study on the changes of TPC in pomelo cv. HJ and pomelo cv. SH in the fruit period. In general, TPC of pomelo cv. HJ was higher than that of SH. Thus, in the production concerning total phenols, pomelo cv. HJ may have more advantages. In harvest period, TPC was mesocarp > exocarp > pulp, which matched previous studies [18, 19]. In the whole fruit period, the change rule of TPC in three parts of two varieties was fluctuating and decreasing. Compared with the mesocarp, TPC of the exocarp and pulp changed more in the whole fruit process, and the exocarp decreased significantly in the third stage, showing the character of high on both sides and low in the middle in the column chart. However, TPC of the pulp decreased sharply after the first stage, and then the change tended to be gentle, which was roughly L-shaped in the column chart. Therefore, the exocarp and the mesocarp worth actual extraction and processing [10]. In addition, TPC of the three parts reached maximum on the 60th day, but the minimum appeared later and later from outside to inside. The lowest TPC in the exocarp appeared in the third stage, the mesocarp’s appeared in the fourth stage, and the pulp’s appeared in the fourth to the fifth stage or later.

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