

Accumulation characteristics of total flavonoids in Sanhongmiyou and Huangjinmiyou

Jiaqi Wang¹, Wenxin Xu¹, Xiaoyu Tang¹, Yuan Yao¹, Bozhi Wang¹, Xinyao Jiang¹, Yifei Gao¹, Yan Han¹, Bo Xiong^{1*}

¹ College of Horticulture, Sichuan Agricultural University, Chengdu, Sichuan province, 611130, China

Abstract. The experiment was conducted to study the tendency of the change of flavonoids content in pomelo during its growth and development and the characteristics of flavonoids accumulation in different cultivars Guanximiyou. The dried samples of the exocarp, mesocarp, and pulp of Sanhongmiyou and Huangjinmiyou were used for measuring the content of total flavonoids. The results showed that the content of total flavonoids in exocarp decreased first and then increased during growth and development, while the content in mesocarp did not change obviously. Furthermore, the content in pulp showed a decreasing trend. The content in exocarp was the highest at the early stage, while in mesocarp was the highest at the mature stage of the fruit. The content of total flavonoids in exocarp and pulp of Sanhongmiyou was higher than that of Huangjinmiyou, total flavonoids content in the exocarp of Sanhongmiyou was similar to Huangjinmiyou within 150 days after anthesis. In addition, the variation trend of the two cultivars was consistent throughout the whole growth and development.

1. Introduction

Pomelo is an evergreen fruit tree of Rutaceae, also known as Xiangluan, Huluan, Citrus Maxima and so on, which is mainly distributed in Southeast Asia and other tropical areas [1]. The mesocarp is the main source of dietary fiber and pectin [2-3]. Therefore, the nature of exocarp has an important influence on fruit quality. Guanximiyou is known as medium-level grapefruit or even citrus fruit [4]. Sanhongmiyou and Huangjinmiyou are the bud mutation varieties of Guanximiyou, which have beautiful fruit shape and rich nutrition. Therefore, they have a good comprehensive performance [5]. Flavonoid is an important polyphenol secondary metabolites in plant [6], generally divided into flavonoids, flavonols, isoflavones, flavanones, flavanols, and anthocyanins [7]. Total flavonoids extracted from citrus fruits may improve the risk of nonalcoholic fatty liver disease [8]. Therefore, in recent years, flavonoids have been more and more regarded. Citrus germplasm such as tangerine, grapefruit, sweet orange and lemon are rich in flavonoid [9].

As the bud mutation varieties of Guanximiyou, the study in Sanhongmiyou (short in SH) and Huangjinmiyou (short in HJ) mainly focused on the external quality, such as the color of exocarp, fruit type, storage quality and so on. In this experiment, we investigated the distribution of total flavonoids in Guanximiyou varieties and the content characteristics in different fruit structures. The results provided a basis for selecting high-quality cultivars and the accumulated characteristics of flavonoids.

2. Materials and methods

2.1. Materials preparation

SH and HJ were grown in Pujiang, Sichuan Province, and were used in this study, of which the growth was strong and the tree shape and vigor was basically consistent. During the fruit development, five fruits were selected randomly from the periphery of the canopy. After the whole fruit was brought back to the laboratory, it was divided into exocarp, mesocarp, and pulp, which were then separated and dried. The experiment set 270 treatments, including two Guanximiyou varieties of exocarp, mesocarp and pulp, and extracted 5 times during anthesis and set 3 replicates.

2.2. Extraction of phenolic compounds

The pomelo fruit was immediately divided into three parts: exocarp, mesocarp, and pulp. The fruit was cut into small pieces and placed in an oven at 40 °C until the moisture content was less than 5%. The dried sample was sealed after being crushed through 60 mesh sieve, stored in a dryer for later use. 0.4 g of pomelo sample was extracted with 8 ml methanol ultrasonic extraction at 50 °C for 30 min, then centrifuged at 5000 r · min⁻¹ for 15 min. The residue was reextracted with 8ml methanol and mixed with the supernatant. The volume was set to 25 ml.

2.3. Determination of total flavonoids

*Corresponding author's e-mail: xiongbo1221@sicau.edu.cn.

Each part of the 0.5 mL extract was placed in a 10ml centrifuge tube, and 0.7ml of distilled water was added, mixed with 0.2ml 5% NaNO₂, and was set for 5 min. After mixing, 0.2 ml 10% Al(NO₃)₃ was added and shaken, then stood for 6 min. Then, 2mL 1mol · L⁻¹ NaOH was added. After shaking, 1.4ml distilled water was added and the volume was fixed to 5mL. The standard curve was made with rutin as the standard sample, and the total flavonoids content was expressed by rutin equivalent RE.

2.4. Data analysis

The test data was analysed with IBM SPSS19.0 software with P < 0.05 as significant level.

3. Results and analysis

The content of flavonoids in the exocarp, mesocarp, and pulp of SH and HJ varied from 200 mg · L⁻¹ to 400 mg · L⁻¹ in the whole progress, the content of flavonoids in the exocarp was the highest at the mature period of fruit, which were 281 mg · L⁻¹ and 257 mg · L⁻¹ respectively. As shown in Fig 1, the content of total flavonoids in exocarp of SH and HJ was fluctuant. The

content of total flavonoids in exocarp of SH was the highest on the 90th day after anthesis, and it was nearly 100 mg · L⁻¹ higher than that of the lowest on the 120th day after anthesis. The content of total flavonoids in HJ was the highest (332 mg · L⁻¹) on the 60th day after anthesis and the lowest (181 mg · L⁻¹) on the 120th day after anthesis. As shown in Fig 2, the content of total flavonoids in the mesocarp of SH and HJ fluctuated from 250 mg · L⁻¹ to 350 mg · L⁻¹, and reached the highest value on the 90th day of anthesis, which was 235 mg · L⁻¹ and 253 mg · L⁻¹ respectively. As shown in Fig 3, the contents of total flavonoids in the pulp of SH and HJ decreased gradually. The contents of total flavonoids on the 60th day after anthesis were 711 mg · L⁻¹, 611 mg · L⁻¹ respectively. And the contents of total flavonoids on the 180th day after anthesis were the lowest, both were 166 mg · L⁻¹.

The results showed that the content of total flavonoids in exocarp decreased at first and then increased during the growth and development, but the content of total flavonoids in mesocarp did not change obviously. The content of total flavonoids in pomelo pulp showed a decreasing trend. The SH's tendency of the change in the content of total flavonoids is the same as HJ's.

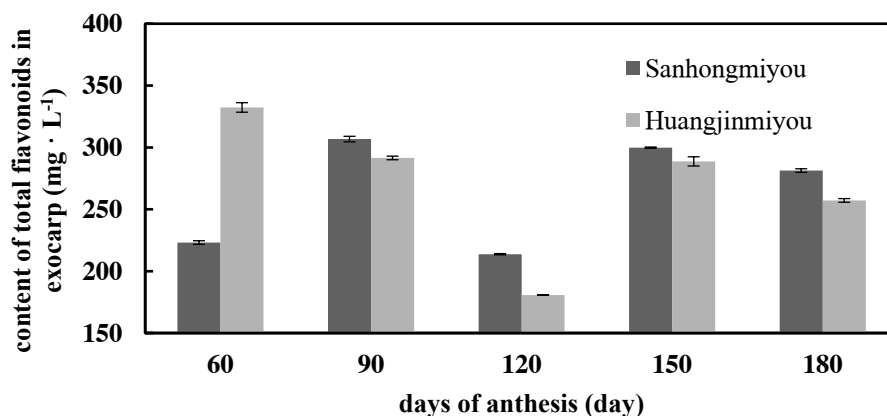


Fig.1 changes of total flavonoids content in exocarp during the growth of SH and HJ

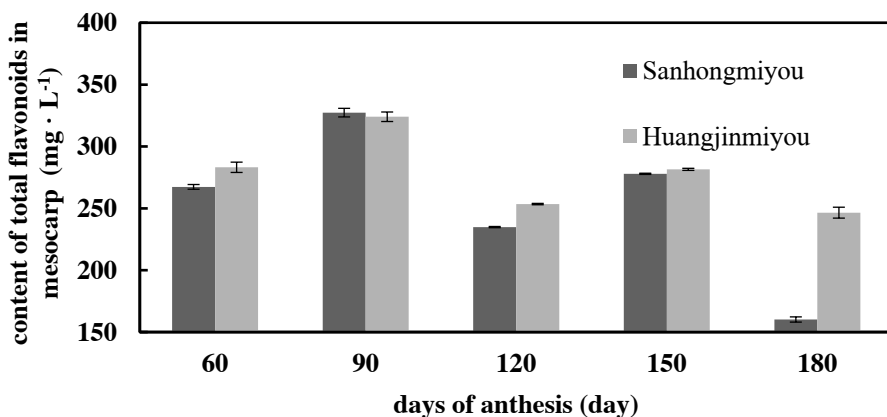


Fig.2 changes of total flavonoids content in mesocarp during growth of SH and HJ

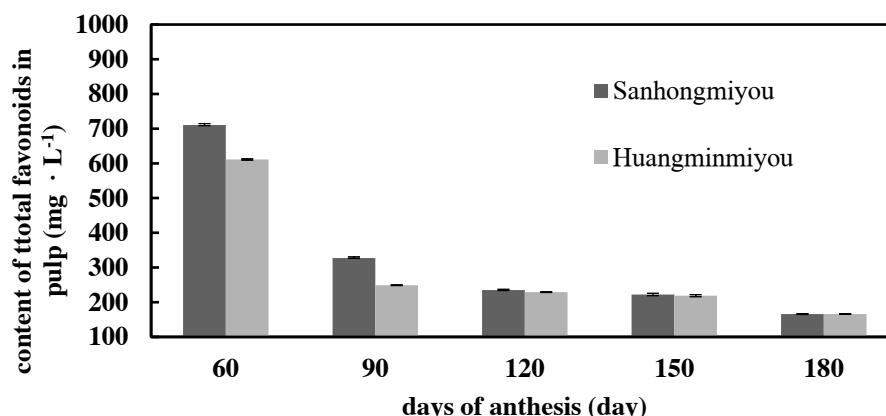


Fig.3 changes of total flavonoids in pulp of SH and HJ

4. Discussion

Pomelo is rich in natural chemical components with high nutritional value [10]. And pomelo fruit is rich in flavonoids, which has high values. Because of some affinities among the pomelo varieties [11], the trend of the changes in total flavonoid contents in SH and HJ were similar in the growth process. And by comparison, total flavonoids content in a different part of the fruit in SH was higher than HJ. The cause of this phenomenon may be because during bud mutation the expression and variation of the chalcone synthase gene, which control flavonoid synthesis, are different between SH and HJ. Concurrently, different fruit bagging time and different rootstock-scion combinations at the seedling stage also had great influence [5,11]. The content of total flavonoids in pomelo pulp decreased gradually, and in exocarp decreased at first and then increased, but did not change obviously in mesocarp, which was consistent with Fang's conclusion [12]. The pomelo exocarp was often discarded and caused environmental pollution. Therefore, the recovery and utilization of pomelo exocarp should be taken seriously. In practice, the content of total flavonoids in the pulp was the highest at the early growth stage and was the highest at the mature stage in the pomelo exocarp, which was the critical period to extract and utilize the total flavonoids.

References

[1] Methacanon, P, Krongsin, J., Gamonpilas, C. (2014) Pomelo (*Citrus maxima*) pectin: Effects of extraction parameters and its properties. *Food Hydrocolloids*, 35: 383-391.

[2] Huang, R, Cao, M, Guo, H, et al. (2014) Enhanced ethanol production from pomelo peel waste by integrated hydrothermal treatment, multienzyme formulation, and fed-batch operation. *Journal of Agricultural and Food Chemistry*, 62(20): 4643-4651.

[3] Liew, S. Q , Ngoh, G. C, Yusoff, R, et al. (2016) Sequential ultrasound-microwave assisted acid extraction (UMAE) of pectin from pomelo peels. *International Journal of Biological Macromolecules*, 93(Pt A): 426-435.

[4] Zhi-fa HE. (2014) Status and Development Idea of 'Guanximiyou' Pomelo Industry in Pinghe County. *Southeast Horticulture*, 2(5): 58-62.

[5] Yanqing Gong. (2016) Graft Compatibility and Photosynthetic Characteristics Reaserch of Different Rootstock-scion Combinations of Honey Pomelo at Seedling Stage[D]. Sichuan Agricultural University.

[6] B. Barfi, A. Asghari, M. Rajabi, et al. (2013) Simplified miniaturized ultrasound-assisted matrix solid phase dispersion extraction and high performance liquid chromatographic determination of seven flavonoids in citrus fruit juice and human fluid samples: hesperetin and naringenin as biomarkers. *Chromatography A*, 1311: 30-40.

[7] M. Paniaguaa,b, J. Crespoc, A. Bach, et al. (2018) Effects of flavonoids extracted from *Citrus aurantium* on performance, eating and animal behavior, rumen health, and carcass quality in Holstein bulls fed high-concentrate diets. *Animal Feed Science and Technology*, 246: 114-126.

[8] Liyan Wu, Maoxiang Yan, Jianping Jiang, et al. (2017) Pure total flavonoids from citrus improve non-alcoholic fatty liver disease by regulating TLR/CCL signaling pathway: A preliminary high-throughput 'omics' study. *Biomedicine & Pharmacotherapy*, 93: 316-326.

[9] CHEN Jia-jing, PENG Zhao-xin, SHI Mei-yan, et al. (2016) Advances in on Flavonoid Composition and Meta bolism in Citrus. *Acta Horticulturae Sinica*, 43 (2): 384-400.

[10] Chen, Q., Hu, Z., Yao, F. Y.-D., et al. (2016) Study of two-stage microwave ex-traction of essential oil and pectin from pomelo peels. *Lebensmittel-Wissenschaft und-Technologie-Food Science and Technology*, 66: 538-545.

[11] Qi you-heng.(2016) Cultivation and Genetic Evaluation guanximiyou mutant of varieties in Sichuan Puijiang. Chengdu, Sichuan Agricultural University.

[12] FANG Bo, ZHAO Qi-yang, XI Wan-peng, et al. (2013) Determination of Flavonoids in 10 Pummelo and Pummelo Hybrid Fruits by Ultra Performance Liquid Chromatography. *Scientia Agricultura Sinica*, 46 (9): 1892-1902.