Differences of sugar components in Different Mulberry Cultivars during its ripening

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Abstract. In this paper, seven mulberry varieties were used as experimental materials, and the composition and content of soluble sugar in different varieties of mulberry during its maturation process were analyzed by high performance liquid chromatography. The results showed that the changes of fructose and glucose content in different maturity of mulberry were basically the same during the ripening process, which reached the maximum in the color-changing period and slightly decreased in the mature period, among which the composition and content of soluble sugar in Taiwan long fruit mulberry were significantly different from other varieties; Fructose and glucose components were all detected in seven mulberry varieties, and only sucrose in Taiwan long fruit mulberry varieties. This finding provides a scientific basis for the breeding of mulberry varieties and the study of sugar accumulation patterns of different varieties.

1. Introduction

Sugar is the main component of fruit quality and flavor, and is also the basic raw material for the synthesis of other nutrients such as pigments, amino acids, vitamins and aromatic substances. Sugar in fruit is accumulating with the development of fruit. Different fruits have different accumulation patterns and different varieties have different components of sugar. The accumulation of sugar in the fruit is the result of the intrinsic genetic characteristics and external natural environmental factors, cultivation measures and other factors on sugar transport and metabolism, and is also an important factor for determining fruit quality [1]. Therefore, the accumulation and regulation of fruit sugar has been widely concerned by researchers at home and abroad. So far, researchers have used high performance liquid chromatography (HPLC) to analyze and evaluate the types and contents of soluble sugar in fruit trees such as peach [2], lychee [3], hazelnut [4] and jackfruit [5].

Mulberry (Fructusmori) is the mature fruit of mulberry, a deciduous tree. It is also called mulberry and mulberry jujube which is rich in nutrition and has many health functions. It is generally used for fresh food with abundant and juicy sugar [6]. China is a large country for mulberry cultivation. At present, more than a dozen provinces in China have extensively introduced and cultivated mulberry, and the cultivated area has reached 53,300 square hectares, including more than 60 species of germplasm resources [7]. Mulberry cultivation is simple, adaptable, strong in vitality, low in pests and...
diseases, high in yield, and almost no pesticides used in mulberry maturity. It is one of natural green fruits, combined with its nutrient-rich, and various health functions, and is also the best contemporary leisure and sightseeing tree species [8], which has a broad commercial prospect. The fruit quality directly determines the development and utilization prospects of mulberry resources. Fruit sugar is an important indicator of fruit quality and flavor formation. Qiao Yu [9] and Li Shengfeng [10] also compared the sugar and acid composition of different varieties of mulberry. The results showed that the main sugars of mulberry are fructose and glucose, and no sucrose component was detected. However, there are few reports on the study of soluble sugar components in different mulberry varieties during its maturation process. In this study, seven different varieties of mulberry mature fruit were used as experimental materials to study the composition and content of soluble sugar, which provided reference for the evaluation and utilization of mulberry resources, quality improvement and breeding of new varieties.

2. Materials and methods

2.1 Materials

2.1.1 Sample

The experiment was carried out in the leisure picking garden of South Asia Tropical Crop Research Institute (Zhanjiang, Guangdong), Chinese Academy of Tropical Agricultural Sciences.

Seven mulberry varieties were selected as follows: Da10, Guihuami, Taiwan Changguosang, Yueshen28, Yueshen74, Hongguo3, Yunguo1. The sampling time depends on the fruit physiological maturity of each mulberry variety (March to April 2019), which is divided into three development periods: green fruit period, color conversion period and maturity period (green fruit period refers to the fruit color of full green or micro red, color conversion period refers to the fruit color of 50% purple, and maturity period refers to the fruit color of full purple and black). In the experiment, 3 individual plants were selected for each variety, and 20 fruits distributed in different positions were taken from each individual plant. After picking, they were brought back immediately to the laboratory and stored at -20 °C for storage.

2.1.2 Main experimental instruments and reagents

High Performance Liquid Chromatograph (LC-20A), Shimadzu Corporation, Japan; Ethanol (chromatographic grade), Acetonitrile (chromatographic grade), Ultrapure water (deionized water).

2.2 Methods

2.2.1 Preparation of standard curve

Single standard: Weigh 1 g of glucose, fructose and sucrose respectively, and dilute them respectively into 100 mL volumetric flasks to obtain a single standard solution of 10 mg/mL. After mixing the single standard to get a mixed standard, and dilute to 8 mixed standard solutions with different concentration gradients, including 5mg/ml, 2.5mg/ml, 1.5mg/ml, 1.0mg/ml, 0.5mg/ml, 0.1mg/ml, 0.05mg/ml and 0.001 mg/ml.

2.2.2 Sample preparation [6]

After about 15g mulberry fruit is crushed by the pulverizer, accurately weigh 1.0g pulp, repeat three times, grind and extract with 4ml of 80% ethanol, centrifugate for 10min at 4 °C 10000 r / min, transfer the supernatant into 10ml plugged calibration tube, add 2ml of 80% ethanol into the residue twice respectively, and then continue centrifuging for 10min at 4 °C 10000 r / min, combine the supernatant into a graduated tube, and then steam them in a 90 °C water bath to evaporate dry, dilute to 10 ml with ultrapure water.
2.2.3 Analysis with the machine

Chromatographic conditions: Column NH2 column (250 mm × 4.6 mm × 5 μm); HPLC with RID detector; wavelength: 210 mm; mobile phase: acetonitrile-water (80:20, v/v); flow rate: 1.0 mL / min; column temperature: 30 °C.

Take 2 ml single standard samples of glucose, fructose and sucrose respectively, filter them with 0.22 μm membrane and analyze them with HPLC. The sample volume is 10 μL, and the retention time of each sugar is obtained. Take 2 ml of 8 different concentration gradients to analyze, then obtain the peak area of each sugar at different concentrations. The peak area and concentration are used as the horizontal and vertical coordinates of drawing a standard curve, and calculate the linear regression equation.

Take 2 ml of the prepared sample, filter it with a 0.22μm filter and analyze. The sample volume is 10 μL. And the concentration of each sugar is calculated by a linear regression equation according to the peak area value.

2.2.4 Data Processing

The SPSS software was used to analyze the variance of the original data of Mulberry.

3. Results and analysis

3.1 Analysis of sugar components in different varieties of mulberry

The composition and content of fructose, glucose and sucrose in the ripening process of 7 mulberry varieties were determined by experiments (see Table 1). The results showed that the soluble sugars in the six mulberry varieties mainly existed in the form of fructose and glucose. The proportion of fructose and glucose in different mulberry varieties was 0.8 ± 0.2, and only the sucrose in three development stages of Taiwan Changguosang was detected (see Figure 1 Fig. 2), which was not found in mulberry in the previous literature.

In the green fruit period, the fructose and glucose content of sweet-scented osmanthus were the lowest, which were 1.547mg/g and 1.799mg/g, respectively. The fructose, glucose and sucrose components of Hongguo 3 and Yunguo 1 were not detected in the green fruit period; the highest sugar content in maturity is Changguosang (140.09mg/g), and the lowest is Yunguo 1 (26.319mg/g).

![Fig.1 Liquid chromatogram of the sugar composition of Taiwan Changguosang in the color changing period](image-url)
Fig. 2 Liquid chromatogram of the sugar composition of Yueshen 28 in the color changing period

Table 1. Composition and content of mulberry sugar in different varieties and periods (mg/g)

| Period | Varieties | Fructose | Glucose | Sucrose | Total Sugar | Fructose / Glucose |
|-------|-----------|----------|---------|---------|-------------|-------------------|
| 1     | 1         | 3.92     | 5.95    | ND      | 9.87        | 0.66              |
| 2     | 1.55      | 1.80     | ND      |         | 3.35        | 0.86              |
| 3     | 44.07     | 50.68    | 11.46   |         | 106.21      | 0.87              |
| A     | 4         | 4.10     | 6.71    | ND      | 10.82       | 0.61              |
| 5     | 2.73      | 5.34     | ND      |         | 8.07        | 0.51              |
| 6     | ND        | ND       | ND      |         | ND          | ND                |
| 7     | ND        | ND       | ND      |         | ND          | ND                |
|       | 1         | 28.15    | 33.61   | ND      | 61.76       | 0.84              |
|       | 2         | 15.76    | 16.89   | ND      | 32.64       | 0.93              |
|       | 3         | 42.82    | 48.20   | 45.51   | 136.53      | 0.89              |
| B     | 4         | 29.20    | 37.72   | ND      | 66.91       | 0.77              |
| 5     | 26.35     | 34.26    | ND      |         | 60.61       | 0.77              |
| 6     | 20.17     | 18.34    | ND      |         | 38.51       | 1.10              |
| 7     | 27.82     | 30.41    | ND      |         | 58.24       | 0.91              |
|       | 1         | 27.64    | 33.23   | ND      | 60.87       | 0.83              |
|       | 2         | 22.36    | 23.73   | ND      | 46.10       | 0.94              |
|       | 3         | 57.79    | 66.72   | 15.38   | 140.09      | 0.87              |
| C     | 4         | 29.97    | 38.02   | ND      | 67.99       | 0.79              |
| 5     | 18.61     | 26.93    | ND      |         | 45.54       | 0.69              |
| 6     | 20.99     | 24.75    | ND      |         | 45.74       | 0.85              |
| 7     | 12.84     | 13.47    | ND      |         | 26.31       | 0.95              |

Note:  ¹The green fruit period, ²The color conversion period, ³The maturity period;
⁴Da10, ⁵Guihuami, ⁶Taiwan Changguosang, ⁷Yueshen 28, ⁸Yueshen74, ⁹Hongguo3, ¹⁰Yunguo1
ND: not detected

3.2 Composition and content analysis of sugar in different mulberry varieties during fruit ripening

After analysing Duncan variance of SPSS, the data of 7 different varieties in three periods were analyzed and the results were obtained.
During the green fruit stage, the fructose content and glucose content of Taiwan Changguosang were significantly different from those of Da10, Guihuami, Yueshen 28 and Yueshen 74 (P < 0.05) (see Figure 3).

During the color change period, the fructose content and glucose content of Taiwan Changguosang were significantly different from those of the other six varieties (P<0.05); the fructose content of no seed Da10, Yueshen 28, Yueshen 74 was significantly higher than that of Guihuami and Hongguo3 (P<0.05). The glucose content of Da10, Yueshen 28 and Yueshen74 was significantly higher than that of Guihuami and Hongguo3 (P < 0.05) (see Figure 4).

At the mature stage, the fructose content and glucose content of Taiwan Changguosang were significantly different from those of other six varieties (P < 0.05); the fructose content of Yueshen 28 was significantly higher than that of Yueshen 74 and Yunguo 1 (P < 0.05); the glucose content of Yueshen 28 was significantly higher than that of Guihuami, Yueshen 74, Hongguo 3 and Yunguo 1 (P < 0.05) (see Figure 5).
4. Summary

This study found that no sucrose was detected in 7 varieties of mulberry except Taiwan long fruit mulberry, which was different from the previous research results, and the content of fructose and glucose in Taiwan long fruit mulberry was the highest in different periods, and the content of fructose and glucose was significantly different from other 6 varieties. Most of the mulberry contains mainly glucose and fructose of soluble sugars, and the mass concentration of the two is different between the same variety. The results may be slightly different due to different varieties and different determination methods, and more varieties need to be collected for the future research. The results of this study will provide new ideas for the breeding of mulberry varieties and the study of sugar accumulation patterns of different varieties.

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