Variation in anthocyanins of different varieties of purple carrots

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Abstract. The composition and content of anthocyanins were investigated in nine varieties of purple carrots to reveal the diversity of the anthocyanins among different varieties of purple carrots. The results showed the significant differences of the composition and content of anthocyanins were found between nine varieties of purple carrots. Five anthocyanins were detected in purple carrots, including cyanidin 3-xylosyl(glucosyl), cyanidin 3-xylosylgalactoside, cyanidin 3-xylosyl(sinapoylglucosyl)galactoside, cyanidin 3-xylosyl(feruloylglucosyl)galactoside, and cyanidin 3-xylosyl(coumaroylglucosyl)galactoside. Only four of the five anthocyanins were detected in Caohaihong, Zhongzidan, and Yanzi, whereas 3-xylosyl(coumaroylglucosyl)galactoside were not detected. The highest content of total anthocyanins was found in Tianzi. In addition, the highest contents of individual anthocyanins except for cyanidin 3-xylosyl(sinapoylglucosyl)galactoside, were also in Tianzi, and the highest content of 3-xylosyl(sinapoylglucosyl)galactoside was found in Zishengzi. In summary, these findings provide a theoretical reference for the scientific consumption and a foundation for the further study of purple carrots in the future.

1. Introduction

Carrots (Daucus carota), belongs to umbelliferae, are an annual or biennial herb. Carrots originated in Europe and have been cultivated for more than 2000 years [1]. Carrots are one of the most economically important crops grown throughout the world [2]. Carrot is a good source of vitamins, minerals, and dietary fibre, which play important roles in the health of digestive systems [3,4]. In most cases, the color of the carrot defines the type of compounds that it may contain. The purple carrot is characterized by a high content of anthocyanins. Furthermore, purple carrot has attracted the attention of the scientific community due to the unique profile of anthocyanin compounds [3].

Anthocyanins are phenolic compounds which constitute the largest group of water-soluble pigments and they occur frequently in the plant kingdom [5]. Anthocyanins are mainly distributed among flowers, fruits and vegetables and are responsible for their bright colors such as purple, red and blue [6]. Interest in anthocyanins has increased because they are potential natural alternatives to artificial colorants in the food and pharmaceutical industries [4]. In addition, apart from their food
colorant properties, anthocyanins have many positive health benefits, such as reduction of risk of coronary heart disease, improved visual acuity, antioxidant activities and anticancer activities [4,5].

In this experiment, to understand the anthocyanin contents among different varieties of purple carrots, nine varieties of purple carrots were used as the material to analyze the difference of anthocyanins between different varieties, which provided the basis and reference for the scientific consumption of purple carrots in the future.

2. Materials and methods

2.1. Plant materials
The purple carrots were sampled in December 2019 at the vegetable base of Bijie Institute of Agricultural Science of Bijie City, Guizhou Province, China. The robust and free of external damages plants were selected at harvest stage. All samples were frozen at −80°C, lyophilized, ground to a powder, and stored at −20°C.

2.2. Sample preparation
The samples were extracted according to the method of Xu et al. [6]. The freeze-dried samples were soaked and incubated overnight in 50% (v/v) methanol, 49.9% (v/v) ddH2O, and 0.1% (v/v) HCl in dark at room temperature. The samples were collected after centrifugation.

2.3. High performance liquid chromatography (HPLC) analysis
The HPLC analysis of the anthocyanin was carried out using an Agilent 1260 HPLC instrument with a C18 column. The samples were eluted with a gradient of acetic 1% formic acid in water (A) and acetonitrile (B). The gradient elution conditions were from 90% of A and 10% of B to 82.5% of A and 17.5% of B in 30 min at 1.0 ml min⁻¹ at an absorbance of 530nm [6].

2.4. Data analysis
Four biological replicates were performed for each sample. Results are shown as mean ± standard deviation of four replicates. Microsoft Excel 2013 was adopted for data processing. Correlation analysis was performed using the SPSS 18.0 software.

3. Results

3.1. Anthocyanin composition in purple carrots
Five anthocyanins were detected in varieties of purple carrots, including cyanidin 3-xylosyl(glucosyl) (Cy 3-xyl(gl)G), cyanidin 3-xylosylgalactoside (Cy 3-xylGal), cyanidin 3-xylosyl (sinapoylglucosyl)galactoside (Cy 3-xyl(sin)G), cyanidin 3-xylosyl(feruloylglucosyl)galactoside (Cy 3-xyl(fer)G), and cyanidin 3-xylosyl(coumaroylglucosyl)galactoside (Cy 3-xyl(cou)G). Only four of the five anthocyanins were detected in Caohaihong, Zhongzidan, and Yanzi, whereas Cy 3-xyl(cou)G were not detected in them. Cy 3-xyl(fer)G was the predominant anthocyanins in most varieties of purple carrots except for Zishengzi, whose content ranging from 55.71% in Cs-z to 69.22% that in Caohaihong. Cy 3-xyl(sin)G was the predominant anthocyanins in Zishengzi and accounted for 43.44% of anthocyanins detected in Zishengzi.

3.2. Total anthocyanins
The total anthocyanins distribution was absolutely different between the nine varieties of purple carrots (Figure 1). Tianzi has the highest total anthocyanins content, and was significantly higher than other varieties, followed by Zishengzi and Cs-z, whose contents were significantly higher than that of Hongzishen, Caohaihong, Zhongzidan, and Yanzi. However, no significant differences were observed
between other varieties. The total anthocyanins contents ranged from 103.35 μg g⁻¹ in Yanzi to 2923.61 μg g⁻¹ that in Tianzi.

![Figure 1](image)

**Figure 1.** The content of total anthocyanins in purple carrots

### 3.3. Individual anthocyanins

The individual anthocyanin contents and distribution in different varieties of purple carrots are presented in Table 1. The Cy 3-xy(l)G content in nine purple carrots varieties ranged from 2.26 μg g⁻¹ DW to 124.37 μg g⁻¹ DW, Cy 3-xy(l)Gal content ranged from 4.07 μg g⁻¹ DW to 413.89 μg g⁻¹ DW, Cy 3-xy(l)(sin)G content ranged from 10.47 μg g⁻¹ DW to 386.48 μg g⁻¹ DW, and Cy 3-xy(l)(fer)G content ranged from 58.37 μg g⁻¹ DW to 1521.19 μg g⁻¹ DW. However, Cy 3-xy(l)(cou)G was not detected in Caohaihong, Zhongzidan, and Yanzi, and its content ranged from 3.74 μg g⁻¹ DW to 131.56 μg g⁻¹ DW. The highest contents of individual anthocyanins except for Cy 3-xy(l)(sin)G, were in Tianzi, and its content is 124.37, 413.89, 1521.19, and 131.56 μg g⁻¹ DW, respectively. The highest contents of Cy 3-xy(l)(sin)G was in Zishengzi, and its content is 386.48 μg g⁻¹ DW.

| Varieties | Cy 3-xy(l)G       | Cy 3-xy(l)Gal        | Cy 3-xy(l)(sin)G    | Cy 3-xy(l)(fer)G   | Cy 3-xy(l)(cou)G      |
|-----------|------------------|---------------------|-------------------|-------------------|----------------------|
| Tianzi    | 124.37±26.82 a   | 413.89±101.98 a     | 138.61±16.10 b    | 1521.19±352.78 a  | 131.56±94.56 a       |
| Zishengzi | 34.08±12.52 d    | 29.28±1.31 d        | 386.48±384.72 a   | 371.77±93.88 b    | 68.11±57.86 b        |
| Cs-z      | 69.21±29.54 c    | 157.28±134.64 b     | 58.00±18.48 b     | 369.93±37.18 b    | 9.67±0.48 c          |
| Zs-z      | 97.69±29.33 b    | 123.83±37.74 bc     | 34.56±5.42 b      | 360.48±50.31 b    | 8.85±0.21 c          |
| Zs-h      | 26.67±1.53 de    | 52.86±36.99 cd      | 29.02±0.77 b      | 165.52±85.32 c    | 3.74±0.17 c          |
| Hongzishen| 6.26±2.94 e      | 11.45±0.79 d        | 57.09±10.88 b     | 147.51±53.87 c    | 4.60±2.80 c          |
| Caohaihong | 17.33±8.35 de    | 32.84±0.64 d        | 10.47±5.64 b      | 136.40±67.83 c    | ND                   |
| Zhongzidan| 11.81±2.41 de    | 25.07±1.21 d        | 34.44±7.52 b      | 120.89±25.20 c    | ND                   |
| Yanzi     | 2.26±0.87 e      | 4.07±1.84 d         | 38.66±10.75 b     | 58.37±19.49 c     | ND                   |
Note: Each value represents the mean (n = 4). Different letters indicate significant difference at 0.05 level. ND: Not detected.

3.4. Correlation analysis

A correlation analysis was performed to investigate the correlations among the different anthocyanins (Table 2). There was a significantly positive correlation between Cy 3-xyl(gl)G, Cy 3-xylGal, Cy 3-xyl(fer)G and Cy 3-xyl(cou)G. However, there was no significant correlation between Cy 3-xyl(sin)G and other individual anthocyanins. Moreover, Total anthocyanins was positively correlated with all individual anthocyanins except for Cy 3-xyl(sin)G.

|                   | Cy 3-xyl(gl)G | Cy 3-xylGal | Cy 3-xyl(sin)G | Cy 3-xyl(fer)G | Cy 3-xyl(cou)G |
|-------------------|--------------|-------------|----------------|----------------|---------------|
| Cy 3-xyl(gl)G     | 1            |             |                |                |               |
| Cy 3-xylGal      | 0.897**      | 1           |                |                |               |
| Cy 3-xyl(sin)G   | 0.134        | 0.091       | 1              |                |               |
| Cy 3-xyl(fer)G   | 0.831**      | 0.963**     | 0.294          | 1              |               |
| Cy 3-xyl(cou)G   | 0.664        | 0.800**     | 0.608          | 0.926**        | 1             |
| Total             | 0.834*       | 0.933**     | 0.424          | 0.989**        | 0.958**       |

Note: *and** indicate significant and extremely remarkable difference at 0.05 and 0.01 level, respectively.

4. Discussion

As reported by many studies, there were obvious differences in the contents of anthocyanins among different varieties [6,8,9]. Our study also showed the diversity of the anthocyanin contents in the different varieties of purple carrots. The discrepancy of anthocyanins content in purple carrots may be caused by the difference of variety and agronomic conditions.

Anthocyanins are responsible for the unlimited diversity of colors from orange and red through purple and blue hues of several fruits, vegetables, and plants. The most commonly known anthocyanins are based on six anthocyanidins: cyanidin, delphinidin, malvidin, pelargonidin, peonidin and petunidin, but there are almost 600 anthocyanins reported to be isolated from plants [6]. In the study, five anthocyanins were detected in varieties of purple carrots, including Cy 3-xyl(gl)G, Cy 3-xylGal, Cy 3-xyl(sin)G, Cy 3-xyl(fer)G, and Cy 3-xyl(cou)G. Only four of the five anthocyanins were detected in Caohaihong, Zhongzidan, and Yanzi, whereas Cy 3-xyl(cou)G were not detected. Cy 3-xyl(fer)G was the predominant anthocyanins in most varieties of purple carrots except for Zishengzi. Cy 3-xyl(sin)G was the predominant anthocyanins in Zishengzi. Assous et al. [6] also found purple carrots contained five anthocyanins. However, anthocyanin composition of purple carrots is inconsistent with our results, and the major anthocyanin pigments extracted from purple carrots were Cyanidin-3-xyllosyl-glucosyl-galactoside acylated.

The total anthocyanins contents ranged from 103.35 μg g⁻¹ in Yanzi to 2923.61 μg g⁻¹ that in Tianzi. Alasalvar et al. [4] found total anthocyanins of purple carrots was 51000 μg g⁻¹. Assous et al. [6] found purple carrots contained about 1.69 mg g⁻¹ on fresh weight of anthocyanins pigments. Lazcano et al. [8] also found a wide range of total anthocyanins in purple carrot. These results indicate that there were obvious differences in the contents of total anthocyanins among different varieties of purple carrots. That maybe they have different genetic backgrounds.

In this experiment, the distribution of anthocyanins in nine varieties of purple carrots was analyzed. These findings provide a theoretical reference for human dietary nutrition and a foundation for the further study of purple carrots.
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