Analysis of bioactive compounds and antioxidant capacities in different varieties of carrots

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Abstract. In this study, twelve varieties of carrots with five colors were evaluated for their levels of bioactive compounds and antioxidant capacities. Bioactive compound contents and antioxidant capacity levels were significantly different among distinct varieties. In detail, ascorbic acid contents ranged from 0.30 mg g\(^{-1}\)DW to 0.63 mg g\(^{-1}\)DW, carotenoid contents ranged from 0.06 mg g\(^{-1}\)DW to 0.54 mg g\(^{-1}\)DW, anthocyanin contents ranged from 0.03 mg g\(^{-1}\)DW to 6.18 mg g\(^{-1}\)DW, proanthocyanidin contents ranged from 0.36 mg g\(^{-1}\)DW to 1.34 mg g\(^{-1}\)DW, flavonoid contents ranged from 1.07 mg g\(^{-1}\)DW to 6.01 mg g\(^{-1}\)DW, total phenolics contents ranged from 7.25 mg g\(^{-1}\)DW to 33.25 mg g\(^{-1}\)DW, FRAP levels ranged from 0.02 mg g\(^{-1}\)DW to 0.16 mg g\(^{-1}\)DW, and ABTS levels ranged from 23.13% to 75.97%. Moreover, the highest contents of anthocyanin, proanthocyanidin, flavonoid, and total phenolics, as well as antioxidant capacity levels, all were in Tianzi (purple). While the highest contents of ascorbic acid and carotenoid were in Zishenghuang (yellow) and Zishengzi (purple with orange core), respectively. This information could be a valuable asset in the research and extension of carrots.

1. Introduction

Carrots (Daucus carota var. sativus), 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]. The roots of carrots are cone-shaped or columnar, and the flesh of carrots is purple, orange, yellow, white, and so on. Carrots are not strict in their choice of climate and can be cultivated everywhere, while they like coldness and plenty of sunlight [3].

Healthy eating guidelines have directed the general public to eat more fresh fruit and vegetables throughout the world. Among these, carrots are being increasingly consumed [4], mainly due to abundant health-promoting phytochemicals and high level of antioxidant capacity. In most of cases, the color of the carrots defines the type of compounds it may contain. Orange carrots contain large amounts of α- and β-carotene, whereas yellow and red carrots are rich in lutein and lycopene, respectively. Similarly, anthocyanins are mainly present in purple roots [5].

To understand bioactive compound contents and antioxidant capacity levels in carrots, this study used different varieties as the material and analyzed the difference of bioactive compounds and antioxidant capacities among different varieties, providing the basis and reference for the scientific consumption of carrots in the future.
2. Materials and methods

2.1. Plant materials
Twelve varieties of carrots, including five different colored carrots, purple (Tianzi), purple with orange core (Hongzishen, Yanzi, and Zishengzi), purple with yellow core (Zhongzidan Cs-z, Zs-h, Zs-z, and Caohaihong), orange (Chengzishen), and yellow (Hangzishen and Zishenghuang) were obtained from the vegetable base of Bijie Institute of Agriculture Science of Bijie City, Guizhou Province, China. The robust plants were selected at harvest stage. All samples were frozen at −80 °C, lyophilized, ground to a powder, and stored at −20 °C. Five fleshy roots were as a repeat, and four repeats per variety were used in this experiment.

2.2. Test methods

2.2.1. Ascorbic acid content. Two hundred mg of sample powder was extracted with 25 mL oxalic acid. The solution was stirred for 30 s, after which it was allowed to settle for 10 min. The solution was then centrifuged for 5 min at 8000 g and 5 mL transferred into an Erlenmeyer flask. Immediately titration with 2,6-dichloroindophenol solution to pink, no fading for 15 s. The volume of 2,6-dichloroindophenol solution consumed was recorded, and then calculate the content of ascorbic acid [6].

2.2.2. Carotenoid content. Two hundred milligrams of powder were extracted in 25 mL of ethanol solution for 20 min. The solution was stirred for 30 s, after which it was allowed to settle for 30 min. The solution was then centrifuged for 5 min at 8000 g and 1 mL transferred to a polypropylene tube. The absorbance of the reaction mixtures was measured at 665nm, 649nm and 470nm using a spectrophotometer. The content of carotenoids were calculated according to the formula [7].

2.2.3. Anthocyanin content. Anthocyanin content was determined according to the method described with slight modifications [8]. Twenty milligrams of the lyophilized powder was transferred to 5 mL of 1% methanol hydrochloride. The solution was stirred for 30 s using a vortex mixer, after which it was put in a 4 °C refrigerator for 24 h in dark. The solution was then centrifuged for 5 min at 8000 g. The liquid supernatant was measured at 534 nm, and then calculate the content of anthocyanin.

2.2.4. Proanthocyanidin content. Four hundred milligrams of the lyophilized powder was transferred to 40 mL of the extracting reagent (acetone: distilled water: acetic acid=150:49:1, v/v). The solution was stirred for 30 s, after which it was allowed to settle for 30 min, and the solution was centrifuged for 5 min at 8000 g. Subsequently, 2.1 mL p-dimethylaminocinnamaldehyde (DMACA) reagent was added to 700 μL of supernatant. The absorbance was spectrophotometrically detected at 640 nm after 30 min, and the proanthocyanidin content was determined using a standard curve of procyanidin B2 [9].

2.2.5. Flavonoid content. Flavonoid content was determined according to the method described by Sun et al [9]. Forty milligrams of sample powder was extracted in 50% ethanol and incubated at room temperature for 24 h in the dark. The suspension was then centrifuged at 4000 g for 5 min. The supernatant was mixed with aluminum trichloride, potassium acetate, and distilled water. Absorption was read at 415 nm after 40 min. The flavonoid content was determined using a standard calibration curve with quercetin in 50% ethanol as a reference standard.

2.2.6. Total phenolics content. Total phenolics were extracted with 50% ethanol and incubated at room temperature for 24 h in the dark. The suspension was centrifuged at 4000 g for 5 min. The supernatant was mixed with Folin-Ciocalteu reagent, after 3 min, saturated sodium carbonate was
added. The absorbance was measured at 760 nm [9]. Gallic acid was used as a standard and the results were expressed as mg garlic acid equivalent (GAE) g\(^{-1}\) dry weight.

2.2.7. Ferric reducing antioxidant power (FRAP). Two hundred milligrams of sample powder was extracted in 50% ethanol and incubated at room temperature for 24 h in the dark. The suspension was then centrifuged at 4500 g for 10 min. The supernatant (0.3 mL) was added to 2.7 mL of the FRAP working solution incubated at 37 °C. The absorbance was measured at 593 nm. FRAP values were calculated based on FeSO\(_4\)·7H\(_2\)O standard curves and expressed as mmol g\(^{-1}\) dry weight [9].

2.2.8. 2,2-Azinobis (3-ethyl-benzothiazoline-6-sulfonic acid) (ABTS). ABTS antioxidant activity was performed according to the method described by Sun et al [9]. An aliquot of 300 μL of each extracted sample was added to 3 ml of ABTS\(^+\) solution. The absorbance was measured spectrophotometrically at 734 nm after exactly 2 h. The percentage inhibition was calculated according to the formula: 

\[
\% \text{ inhibition} = \left( \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \right) \times 100\%.
\]

2.3. Data analysis
Microsoft Excel 2016 was adopted for data processing. Correlation analysis was performed using SPSS 18. Differential significance analysis was performed using DPS7.5 software. The results were subjected to one-way analysis of variance and differences between means were located using LSD test.

3. Results

3.1. Ascorbic acid and carotenoid
The highest ascorbic acid content was Zishengzi (0.63 mg g\(^{-1}\) DW), followed by Zhongzidan (0.59 mg g\(^{-1}\) DW) and Huangzishen (0.53 mg g\(^{-1}\) DW), and the lowest content was Tianzi (0.30 mg g\(^{-1}\) DW) (Figure 1A). The carotenoid content in twelve varieties ranged from 0.54 mg∙g\(^{-1}\) DW to 0.06 mg∙g\(^{-1}\) DW. The highest content is in Zishengzi, followed by Chengzishen and Yanzi. While the lowest content is in Caohaihong, and the content of Zishengzi was 9-fold than that of Caohaihong (Figure 1B).

![Figure 1. The content of ascorbic acid (A) and carotenoid (B) among different varieties of carrots](image)

3.2. Anthocyanins, proanthocyanidin, flavonoids, and total phenolics
Anthocyanins, proanthocyanidin, flavonoids, and total phenolics are important antioxidants in carrots. The anthocyanin content in twelve carrots varieties ranged from 0.03 mg g\(^{-1}\) DW to 6.18 mg g\(^{-1}\) DW, proanthocyanidin content ranged from 0.36 mg g\(^{-1}\) DW to 1.34 mg g\(^{-1}\) DW, flavonoid content ranged from 1.07 mg g\(^{-1}\) DW to 6.01 mg g\(^{-1}\) DW, and total phenolics content ranged from 7.25 mg g\(^{-1}\) DW to 33.25 mg g\(^{-1}\) DW. The highest contents of anthocyanin, proanthocyanidin, flavonoid, and total phenolics were in Tianzi, and its content is 6.18, 0.33, 6.01, and 33.25 mg g\(^{-1}\) DW, respectively. And the contents of anthocyanin, proanthocyanidin, flavonoid, and total phenolics in Zishengzi is also
relatively high. The lowest contents of anthocyanin, flavonoid and total phenolics were in Huangzishen, and the lowest proanthocyanidin content was in Chengzishe and Huangzishen (Figure 2).

![Figure 2](image)

**Figure 2.** The contents of ascorbic acid (A), proanthocyanidin (B), flavonoids (C), and total phenolics (D) among different varieties of carrots

### 3.3. Antioxidant capacity

The antioxidant capacity was investigated using by both FRAP and ABTS in this study (Figure 3). The highest levels of FRAP and ABTS were in Tianzi, and the levels are 0.161 mg g\(^{-1}\) DW and 75.97%, respectively. The levels of FRAP and ABTS in Zishengzi is also relatively high. The lowest FRAP levels was in Huangzishen, and the lowest ABTS levels was in Zishenghuang.

![Figure 3](image)

**Figure 3.** The content of FRAP (A) and ABTS (B) among different carrots varieties

### 3.4. Correlation analysis

A correlation analysis was performed to investigate the correlations among the nutritional qualities in carrots (Table 1). There were significantly positive correlation between anthocyanin, proanthocyanidin, flavonoids, total phenolics, FRAP and ABTS. Whereas ascorbic acid was negatively correlated with anthocyanin, Proanthocyanidins, and ABTS, respectively.
Table 1. Correlation coefficients of the different nutritional qualities in carrots

|                     | Ascorbic acid | Carotenoids | Anthocyanin | Proanthocyanidins | Flavonoids | Total phenolics | FRAP    |
|---------------------|---------------|-------------|-------------|-------------------|------------|----------------|---------|
| Ascorbic acid       | 1             | 0.049       | -0.671*     | -0.595*           | 0.866      | 0.923         | 1       |
| Carotenoids         |               | 1           | 0.144       | 0.062             | 0.934**    | 1              |         |
| Anthocyanin         |               |             | 1           |                   |            |                |         |
| Proanthocyanidins   |               |             |             |                   |            |                |         |
| Flavonoids          |               |             |             |                   |            |                |         |
| Total phenolics     |               |             |             |                   |            |                |         |
| FRAP                |               |             |             |                   |            |                |         |
| ABTS                | -0.615*       | 0.350       | 0.972**     | 0.898**           | 0.958**    | 0.957**        | 0.956*  |

4. Discussion

This experiment determined the health-promoting phytochemicals and antioxidant capacity in different varieties of carrots. There were significant differences between the health-promoting phytochemicals and antioxidant capacity in different varieties of carrots. This discrepancy may be caused by differences of varieties. Similar results were reports on rice [10], tomato [11], mango [12], grape [13], and so on.

Ascorbic acid is an important vitamin widely found in fresh fruits and vegetables. As a highly active substance, it is involved in many metabolic processes [14]. In the study, ascorbic acid content ranged from 0.30 mg g\(^{-1}\)DW to 0.63 mg g\(^{-1}\)DW. The highest ascorbic acid content was in Zishengzi, while the lowest ascorbic acid content was in Tianzi. In previous study, Alasalvar [4] found that ascorbic acid content varied between 0.0125 and 0.0533 mg g\(^{-1}\)FW, and Favell [15] found 0.028-0.045 mg g\(^{-1}\)FW in fresh carrots. The discrepancy of ascorbic acid content in carrots are due to the difference of variety and agronomic conditions. Carotenoids are a natural pigment and has important positive effects on human health. In the experiment, The content of Zishengzi (purple with orange core) is the highest, followed by Chengzishen (orange) and Yanzi (purple), and the content of Caohaihong (purple with yellow core) is the lowest. However, Alasalvar [4] found that purple carrot contained more carotenoid content than that of orange carrot. The discrepancy of carotenoids content in carrots are also due to the difference of variety and agronomic conditions.

In current survey, the contents of anthocyanins, proanthocyanidins, flavonoids, and total phenolics, as well as antioxidant capacity in different varieties of carrots were investigated. The highest contents of anthocyanins proanthocyanidin, flavonoid, and total phenolics were in Tianzi (purple), and the highest levels of FRAP and ABTS were also in Tianzi. While the contents of anthocyanins, proanthocyanidins, flavonoids, and total phenolics, as well as antioxidant capacity of Huangzishen (yellow) and Zishenghuang (yellow) were the lowest or relatively low compared with other varieties. This is consistent with the study of Alasalvar [4], which found that purple carrots had higher content of phenolics than that of orange, yellow, and white carrots. Cefola [16] also found purple Polignano carrots had rich antioxidants, with a mean content about 4-fold higher than that of orange carrots. In the study, there were significantly positive correlation between anthocyanin, proanthocyanidin, flavonoid, total phenolics, FRAP and ABTS. This result may be due to the fact that anthocyanins, proanthocyanidins, and flavonoids are all polyphenols, and these substances contribute significantly to antioxidant capacity.

In conclusion, among the 12 varieties of carrots, Tianzi has the highest contents of anthocyanins, proanthocyanidins, flavonoids, and total polyphenols, and the highest levels of FRAP and ABTS. Therefore, purple carrot variety Tianzi could be used in place of other carrots varieties in order to take advantage of its health-promoting phytochemicals and antioxidant capacity.
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