Analysis of main nutrients among different edible parts of ‘Nainaiqingcai’ mustard under the alpine cold climate

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Abstract. Bijie City, Guizhou Province belongs to the alpine cold climate. In order to understand the nutritional values of ‘Nainaiqingcai’ mustard, the contents of main nutrients among different edible parts (leaves, petioles, and bolting stem) in ‘Nainaiqingcai’ mustard under the alpine cold climate were investigated. The results showed that significant differences were found among different edible parts. The levels of chlorophyll, carotenoids, ascorbic acid, soluble solids, and soluble proteins were followed as the trends of leaves > petioles > bolting stem. Whereas the sugar components showed different distributions, and the lowest content of sugar was detected in leaves. Moreover, significant negatively correlation were found between sugar and the other nutrients. Most of the extremely significant positive correlations were found between sugar components, and the correlation coefficient is high (except for the correlation between fructose and sucrose). The highest correlation coefficient was between chlorophyll and carotenoids, up to 1.000. In summary, the information in this study provides a theoretical reference for the study of nutritional quality of ‘Nainaiqingcai’ mustard under the alpine cold climate.

1. Introduction

Brassica juncea is an annual herb of cruciferae brassica, which originated from spontaneous hybridization of the ancestors of B. rapa (AA, n=10) and B. nigra (BB, n=8) [1]. The variety ‘Nainaiqingcai’ belongs to the mustard vegetables of the cruciferous family. It usually takes the leaves, petioles, and bolting stem as the edible parts. People usually pickle it to eat kimchi, but also eat it as fresh vegetable directly. ‘Nainaiqingcai’ mustard is one of the local winter-spring vegetables in Bijie City, Guizhou Province, and has a large amount of consumption at the local. Bijie City belongs to the alpine cold climate. Despite being a Brassica vegetable that is widely consumed in winter and spring in Southwest China, there is lack of information available on ‘Nainaiqingcai’ mustard.

In this experiment, in order to understand the nutritional value of ‘Nainaiqingcai’ mustard in a relatively systematic way, this study used ‘Nainaiqingcai’ mustard as the research material to analyze the difference between the main nutrients between different edible parts, which provided the basis and reference for the scientific consumption of ‘Nainaiqingcai’ mustard in the future.
2. Materials and methods

2.1. Plant materials

The ‘Nainaiqingcai’ mustard were sampled on December 15, 2017 at the vegetable base of Bijie Institute of Agricultural Science of Bijie City, Guizhou Province, China (27°18′34.36″-N105°19′20.70″-E). The robust, free of pest and mechanically damaged plants were selected at harvest stage. The samples were divided into three parts according to the leaves, petioles and bolting stem, and then all samples were frozen at −80°C, lyophilized, ground to a powder, and stored at −20°C.

2.2. Test methods

2.2.1. Chlorophyll and carotenoid content. Two hundred milligrams of powder were extracted in 25 mL of ethanol solution for 20 min. The solution was stirred for 15 s using a vortex mixer, 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 and concentration of chlorophyll and carotenoids were calculated according to the formula [2, 3].

2.2.2. Ascorbic acid content. Ascorbic acid content was determined using the methods of 2,6-dichloroindophenoltitration. Two hundred mg of sample powder was extracted with 25 mL oxalic acid. The solution was stirred for 30 s using a vortex mixer, 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 [4].

2.2.3. Soluble sugar content and sugar component. The determination of soluble sugar content was performed using the method proposed by Morris [5, 6]. Two hundred milligrams of powder were extracted in 25 mL of distilled water for 20 min at 90°C, following which the homogenates were centrifuged at 8000 g for 5 min. A combination of 1 mL of diluted sample, 0.25 mL anthrone-ethyl acetate reagent, and 2.5 mL concentrated sulfuric acid was homogenized and boiled for 5 min, and then cooled rapidly using ice water. The absorbance of the reaction mixtures was measured at 630 nm using a spectrophotometer, and the soluble sugar content was determined using a standard curve of sucrose [5, 6].

Sugar component: Two hundred milligrams of powder was extracted in 25 mL of distilled water for 20 min at 90°C. The solution was stirred for 15 s using a vortex mixer, following which the homogenates were centrifuged at 8000 g for 5 min. The supernatant was drawn 2 ml into a new 10 mL centrifuge tube and then 2 ml of distilled water was added again in the residue The solution was then centrifuged for 5 min at 8000 g. The supernatant of 2 mL was absorbed, combined with the supernatant extracted for the first time, and the mixture was uniform. HPLC analysis of sugar component carried out using a Model Agilent 1260 [7]. The total sugar content is determined by the sum of the contents of fructose, glucose and sucrose.

2.2.4. Soluble solids content. The content of soluble solids was determined by portable sugar meter [8].

2.2.5. Soluble proteins content. Two hundred milligrams of powder were extracted in 25 mL, after which it was allowed to settle for 40 min. The solution was then centrifuged for 5 min at 8000 g and 0.5 mL transferred to a polypropylene tube. Subsequently, 0.5 mL of distilled water and 5 mL of Coomassie brilliant blue G- 250 was combined with 0.5 mL of supernatant. The absorbance was measured at 595 nm within 20 min after the reaction. Soluble proteins in the samples were calculated based on a standard curve of bovine serum albumin [9].
2.3. Data analysis

All assays were performed in quadruplicate. The results are shown as the mean ± standard deviation (SD). Microsoft Excel 2016 was adopted for data processing. Correlation analysis was performed using the PASWStatistics18 version. Differential significance analysis was performed using DPSSOFT 7.5 software. The results were subjected to one-way analysis of variance (ANOVA) and differences between means were located using Duncan test.

3. Results

3.1. Chlorophyll, carotenoids and ascorbic acid.

There are significant differences in the chlorophyll, carotenoids and ascorbic acid between the different edible parts of the ‘Nainaiqingcai’ mustard. The above three nutrients showed the same trend, the leaves had the highest content, followed by the petioles and the lowest content in the bolting stem. In addition, the fold differences of different nutrients among different edible parts were also significantly different. The chlorophyll content in leaves was 53 times that of bolting stem, and the difference was the largest. The ascorbic acid content in leaves was 1.32 times that of bolting stem, and the difference was the smallest.

Table 1. The contents of main nutritional components among different edible parts of ‘Nainaiqingcai’ mustard / (mg∙g⁻¹DW)

| Edible parts     | Chlorophyll    | Carotenoids | Ascorbic acid |
|------------------|----------------|-------------|---------------|
| Leaves           | 16.17±0.84 a   | 2.47±0.11 a | 5.41±0.19 a   |
| Petioles         | 1.76±0.05 b    | 0.25±0.01 b | 4.08±0.16 b   |
| Bolting stem     | 0.30±0.01 c    | 0.06±0.01 c | 3.52±0.12 c   |

Note: Different letters indicate significant difference at 0.05 level.

3.2. Soluble proteins and soluble solids

The content of soluble proteins and soluble solids in ‘Nainaiqingcai’ mustard were shown in Table 2. The soluble proteins content in the three edible parts ranged from 25.82 mg∙g⁻¹DW to 121.94 mg∙g⁻¹DW, which was expressed as leaves>bolting stem>petioles. The soluble proteins content of leaves was nearly five-fold of that in other edible parts. The trend of soluble solids in edible parts was concordant with soluble proteins. The soluble solids content in leaves was 2 times of that of petioles, and 1.2 times of that of bolting stem. (Figure 1).

![Figure 1](image_url)
3.3. Soluble sugar and sugar components

The soluble sugar content was lowest in the leaves, significantly lower than the contents of the petioles and bolting stem. The contents of three soluble sugars of sucrose, fructose and glucose were determined by HPLC. The distributions of three soluble sugars were similar with that of soluble sugar content, and the contents in the leaves significantly lower than those in the petioles and bolting stem. However, the distributions of three soluble sugars between petioles and bolting stem were remarkably distinct. Moreover, the content of glucose was also significantly more abundant than those of sucrose and fructose. Especially in the petioles, the glucose content was as high as 347.96 mg·g^{-1}DW, which was 3 times more than that of fructose and 21 times more than that of sucrose.

Table 2. The contents of sugar among different edible parts of ‘Nainaiqingcai’ mustard /(mg·g^{-1}DW)

| Edible parts     | Soluble sugar | Fructose | Glucose | Sucrose | Total sugar |
|------------------|---------------|----------|---------|---------|-------------|
| Leaves           | 110.17±10.0 b | 35.63±2.93 b | 95.32±1.46 c | 2.47±0.07 c | 133.42±4.33 c |
| Petioles         | 302.95±19.67 a| 112.93±1.77 a | 347.96±5.31 a | 16.19±0.81 b | 477.09±5.68 a |
| Bolting stem     | 310.47±73.94 a| 126.72±9.92 a | 229.11±9.55 b | 26.32±3.53 a | 382.15±22.99 b |

Note: Different letters indicate significant difference at 0.05 level.

3.4. Correlation analysis

The correlation analysis between the main nutrients of Nainaiqingcai mustard was shown in Table 3. The positive correlation coefficient between chlorophyll and carotenoids was highest, the value came up to 1.000. While the greatest negative correlation coefficient was observed between fructose and chlorophyll, and the level was -0.992. In addition, all the five sugars index (soluble sugar, fructose, glucose, sucrose, total sugar) showed highly negative correlation with other nutrients measured. However, the correlations between sugars were extremely significant positive, except for that between fructose and sucrose. The correlation analysis indicated that Soluble proteins and soluble solids was significantly positively associated with chlorophyll, carotenoids and ascorbic acid (except that soluble solids and ascorbic acid were significantly higher at 0.05 level and the correlation coefficient was lower). The difference was that soluble solids and these three were extremely significantly lower than soluble proteins. Soluble proteins and soluble solids were significantly positively associated with chlorophyll, carotenoids and ascorbic acid. However, the correlation coefficients between soluble solids and these three above latter all were notably lower than those of soluble proteins. The correlation coefficients between chlorophyll, carotenoids and ascorbic acid were very high, and these values were all above 0.96 (Table 3).

Table 3. Correlation coefficients of main nutritional components in ‘Nainaiqingcai’ mustard

| Item            | Soluble solids | Soluble proteins | Soluble sugar | Fructose | Glucose | Sucrose | Total Sugar | Chlorophyll | Carotenoids |
|-----------------|----------------|------------------|---------------|----------|---------|---------|-------------|-------------|-------------|
| Soluble proteins| 0.820**        | -0.699**         | -0.719**      | -0.965** | -0.501 | -0.92** | 0.755**     | 0.764**     | 0.589**     |
| Soluble sugar   | -0.699**       | -0.978**         | 0.896**       | 0.794**  | 0.810** | 0.955** | 0.969**     | 0.986**     | 0.933**     |
| Fructose        | -0.719**       | -0.978**         | 0.896**       | 0.794**  | 0.810** | 0.955** | 0.969**     | 0.986**     | 0.933**     |
| Glucose         | -0.965**       | -0.894**         | 0.794**       | 0.807**  | 0.958** | 0.969** | 0.986**     | 0.986**     | 0.933**     |
| Sucrose         | -0.501         | -0.88**          | 0.810**       | 0.955**  | 0.958** | 0.969** | 0.986**     | 0.986**     | 0.933**     |
| Total Sugar     | -0.92**        | -0.967**         | 0.869**       | 0.916**  | 0.976** | 0.759** | 0.986**     | 0.986**     | 0.933**     |
| Chlorophyll     | 0.755**        | 0.986**          | -0.922**      | -0.992** | -0.839**| -0.926**| -0.935**    | 1.000**     | 0.967**     |
| Carotenoids     | 0.764**        | 0.988**          | -0.923**      | -0.991** | -0.846**| -0.922**| -0.94**     | 1.000**     | 0.967**     |
| Ascorbic acid   | 0.589**        | 0.933**          | -0.864**      | -0.976** | -0.702**| -0.968**| -0.836**    | 0.967**     | 0.963**     |

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

The present study was the first to assess the main nutrients of the different edible parts of ‘Nainaiqingcai’ mustard. Our results also indicated that there were significant differences in the contents of main nutrients among different parts of ‘Nianaiqingcai’ mustard. Many studies have indicated that significant differences exist among different plant organs/tissues in terms of nutritional composition and content. In our study, the content of major nutrients (except sugar) in the leaves was higher than other edibles parts. This phenomenon was consistent with the results of previous studies in other vegetables. For example, Liu et al. found that the contents of chlorophyll, carotenoids and ascorbic acid in the leaves were significantly higher than those in other edibles parts. Ma et al. found that the contents of chlorophyll, carotenoids and ascorbic acid in the leaves of peas were also significantly higher than those in other edible parts [10]. Sun et al. also found the same phenomena in the studies of broccoli and Chinese kale [3, 11]. It might be related to the fact that leaves were the main photosynthesis organs. The main pigments of chlorophyll and carotenoids in chloroplasts are the material basis for the absorption, transmission and conversion of light energy during plant photosynthesis [12]. Ascorbic acid was a small molecule antioxidant that plays an important role in resisting plant stress and can be rapidly oxidatively decomposed to form monodehydroascorbic acid and dehydroascorbic acid [13]. The contents soluble solids and soluble proteins in leaves were higher than those in other edibles parts, and showed the trend of leaves>bolting stem>petioles, which was consistent with Sun et al.’s research on six varieties of Chinese kale [11].

Sugar is an important class of energy substances that play an important role in the growth and development of plants. It not only provides energy for the daily metabolism of plants, but also is the main components of vegetable quality and flavor. In this experiment, the content and components of soluble sugar in different edibles parts of ‘Nainaiqingcai’ mustard was determined. Results showed that the components of soluble sugar did not differ between different edibles parts, but the content of soluble sugar was significantly different between different edibles parts. The contents of five sugar index (soluble sugar, fructose, glucose, sucrose, total sugar) were different. The distribution of soluble sugar, sucrose and fructose all were bolting stem>petioles>leaves, and the contents of glucose and total sugar both were petioles>bolting stem>leaves. In theory, soluble sugar should be consistent with the trends of fructose, glucose, and sucrose, and the same result as total sugar [14]. The reason may be that the different detection methods of soluble sugar and other sugars, the soluble sugar was detected by the fluorenone colorimetric method [6], and fructose, glucose and sucrose were detected by HPLC. Only three kinds of sugars were detected in the test by HPLC, but the soluble sugar also included maltose and so on. This was why there was a slight difference between the sugar index. It was worth noting that the trend of the content of petioles and bolting stem may be different in sugar index, no matter which sugar index, the sugar content of leaves was much lower than those of other edible parts. Compared with the research of An et al., the sugar components of some varieties of pear fruit were consistently distributed, and the distribution of sugar components varies depending on the plant species and varieties.

In this experiment, the distribution of several main nutrients in leaves, petioles and bolting stem of ‘Nainaiqingcai’ mustard was analyzed. The information in this study provides a theoretical reference for human dietary nutrition and a foundation for the further study of ‘Nainaiqingcai’ mustard.

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