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Analysis of bioactive compounds and antioxidant capacities in ‘Nainaiqingcai’ mustard

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Abstract. This experiment analyzed the bioactive compounds content and antioxidant capacities of different edible parts (the leaves, petioles and bolting stem) of ‘Nainaiqingcai’ mustard. The results showed that significant differences were found among different edible parts. The levels of chlorophylls (chlorophyll a, chlorophyll b, and total chlorophyll), carotenoids (neoxanthin, violaxanthin, lutein, β -carotene and total carotenoids), proanthocyanidins, flavonoids, total phenolic and antioxidant capacities were all followed as the trend of leaves> petioles>bolting stem, and the bioactive compound contents and antioxidant capacities of the leaves were far greater than other parts. Correlation analysis showed that there was a significant positive correlation between all indicators. The correlations between individuals of chlorophyll and carotenoids were extremely high, and the level even reached 1.000. Even the lowest correlation coefficient between ABTS and total phenolics was as high as 0.965. This study provides a theoretical basis and data reference for people's daily diet.

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 [2]. 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, which has a large amount of consumption at the local.

Vegetables are indispensable to the human diet and are valued for their nutritional properties [3]. A number of studies are available on various Brassica vegetables. However, there have limited studies on ‘Nainaiqingcai’ mustard, and even fewer regarding the nutritional composition of the individual edible parts. The objective of this study was to determine the contents of the main bioactive compounds and the antioxidant activities in individual edible parts of ‘Nainaiqingcai’ mustard. These findings will provide a guideline for the human diet.



2. Materials and methods

2.1. Plant materials

The 'Nainaiqingcai' mustard was sampled on December 15, 2017 at the vegetable base of Bijie Institute of Agricultural Science of Bijie City, Guizhou Province, China. 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. Total chlorophyll and total carotenoid content. Two hundred milligrams of the freeze-dried sample powder was added with 25 mL acetone. It was ultrasonic for 40 min. The supernatant was collected after centrifugation (10 min, 4500g). The sample supernatant was filtered into a small brown bottle with a nylon syringe filter of 2.2 μm . HPLC analysis of total chlorophyll and total carotenoid. Samples were separated at on a Waters Spherisorb C18 column (150 \times 3.9 mm i. d.; 3 μm particle size) using 80% acetonitrile and isopropyl alcohol at a flow rate of 1.0 mL min^{-1} . Absorbance was detected at 448 nm.

2.2.2. 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 centrifuged for 5 min at 8000 g. Subsequently, 2.1 mL p-dimethylaminocinnamaldehyde (DMACA) reagent was added to 700 μL of supernatant. The absorbance of the mixture was spectrophotometrically detected at 640 nm after 20 min, and the proanthocyanidin content was determined using a standard curve of procyanidin B2 [4].

2.2.3. Flavonoids content. Two hundred milligrams of sample powder was extracted in 50% ethanol. The suspension was centrifuged at 4500g for 10 min. The supernatant (6 mL) and 6 mL of 50% ethanol was combined with 600 μL of 2% aluminum chloride solution. Add the same volume (600 μL) of 1 mol L^{-1} potassium acetate, and 1.680 mL distilled water. Absorption was read at 415 nm after 40 min. The flavonoid content was determined using a standard calibration curve with quercetin as a reference standard and expressed as mg of quercetin equivalence per g dry weight [5].

2.2.4. Total phenolic content. Two hundred milligrams of sample powder was extracted in 50% ethanol. The suspension was then centrifuged at 4500g for 10 min. The supernatant (3 mL) was mixed with 15 mL 0.2 mol L^{-1} Folin–Ciocalteu reagent in a polypropylene tube, after 3 min, 12 mL saturated sodium carbonate was added to each polypropylene tube. The mixtures were allowed to stand for 20 min at room temperature and the absorbance was measured at 760 nm with the spectrophotometer [6].

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

2.2.6. 2,2-Azinobis (3-ethyl-benzothiazoline-6-sulfonic acid) (ABTS). Two hundred milligrams of sample powder was extracted in 50% ethanol. The suspension was then centrifuged at 4500g for 10 min. The extract (600 μL) was added to 2.4 mL of 50% ethanol solution. Add 30 ml of ABTS working solution for 2 h after cyclodimerizing, measure the absorbance at 734nm, and the percentage inhibition was calculated according to the formula [8].

2.3. Data analysis

All assays were performed in quadruplicate. The results are shown as the mean \pm 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.

3. Results

3.1. Chlorophylls and carotenoids

There are significant differences in the chlorophylls and carotenoids between the different edible parts of the 'Nainaiqingcai' mustard. As shown in Table 1, chlorophyll content (chlorophyll a, chlorophyll b, and total chlorophyll) showed the trend of leaves > petioles > bolting stem. The content of chlorophyll in the leaves part was much higher than those of other parts, which was more than 8 times of that of the petioles, and was 32 ~ 42 times of that of the bolting stem. The content of chlorophyll a was more than 60% of total chlorophyll in leaves and petioles, whereas there was no significant difference of the contents of chlorophyll a and b in bolting stem.

According to Table 2, the contents of carotenoids (neoxanthin, violaxanthin, lutein, β -carotene and total carotenoids) generally showed the same trend as chlorophyll. The content of carotenoids in the leaves was much higher than those of other parts. The most significant difference among edible parts was lutein, which is 29 times more abundant in the leaves than the bolting stem. The smallest difference was found in the violaxanthin, which is only 1.4 times higher in the petioles than bolting stem. There were significant differences among neoxanthin, violaxanthin and lutein between each edible parts, while there was no significant difference between β -carotene and total carotenoid in petiole and bolting stem. In a word, the content of lutein is the highest, which accounted for more than half of the total carotenoids, followed by the neoxanthin, and the lowest was violaxanthin and β -carotene.

Table 1. The chlorophyll contents of 'Nainaiqingcai' mustard/ (mg·g⁻¹DW)

Edible parts	Chlorophyll a	Chlorophyll b	Total chlorophyll
Leaves	108.34 \pm 2.53 a	75.8 \pm 2.07 a	184.14 \pm 4.6 a
Petioles	12.42 \pm 0.13 b	9.67 \pm 0.13 b	22.08 \pm 0.26 b
Bolting stem	2.56 \pm 0.2 c	2.33 \pm 0.2 c	4.88 \pm 0.41 c

Note: Different letters indicate significant difference at 0.05 level.

Table 2. The of Carotenoid contents of 'Nainaiqingcai' mustard/ (mg·g⁻¹DW)

Edible parts	Neoxanthin	Violaxanthin	Lutein	β -carotene	Total carotenoids
Leaves	10.01 \pm 0.36 a	2.41 \pm 0.05 a	19.7 \pm 0.92 a	4.12 \pm 0.54 a	36.24 \pm 1.86 a
Petioles	1.52 \pm 0.04 b	0.4 \pm 0.01 b	2.44 \pm 0.02 b	0.47 \pm 0.01 b	4.84 \pm 0.05 b
Bolting stem	0.76 \pm 0.07 c	0.29 \pm 0.01 c	0.68 \pm 0.03 c	0.2 \pm 0.01 b	1.94 \pm 0.11 b

Note: Different letters indicate significant difference at 0.05 level.

3.2. Proanthocyanidins, flavonoids and total phenolics

As shown in Fig. 1 that the distribution of proanthocyanidins, flavonoids and total phenolics in the three edible parts of 'Nainaiqingcai' mustud showed a trend of leaves>petioles>bolting stem, and the difference between them was obvious. The content of total phenolics of leaves was significantly different from other parts, but the difference between petioles and bolting stem was minimal and not significant. The difference in the content of different parts was also different, and the content of flavonoids in leaves was 13 times than that of bolting stem, the difference was the largest. The total phenolics content in the petioles was just 1.1 times than that of bolting stem, which is the minimal difference.

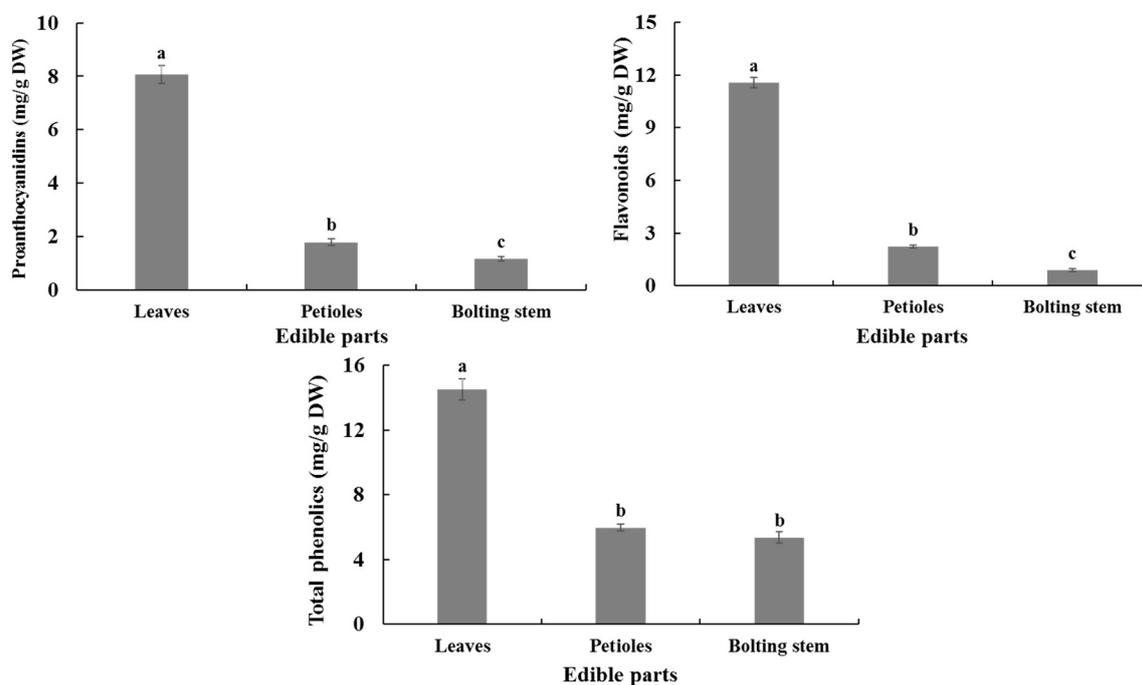


Figure 1. Distribution of proanthocyanidins, flavonoids and total phenolics of ‘Nainaiqingcai’ mustard.

3.3. Antioxidant capacities

The antioxidant capacities of 'Nainaiqingcai' mustard was determined by FRAP and ABTS methods. The results showed that there were significant differences in antioxidant capacities between different parts of 'Nainaiqingcai' mustard (Fig. 2). The FRAP results showed that the leaves had the highest antioxidant capacities, reaching 106.51mmol/kg DW, and the petioles had the least antioxidant capacities, only 44.24 mmol/kg DW. The leaves antioxidant capacities was 2.4 times that of the petioles. The ABTS⁺ clearance rate of leaves was also the highest determined by the ABTS⁺ method, and the level was 27.7%.

There was no significant difference between the petioles and the bolting stem no matter which method was used.

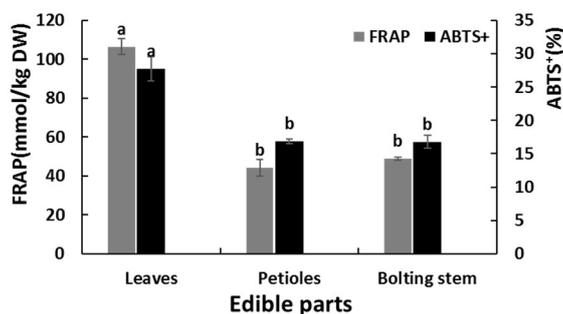


Figure 2. Distribution of antioxidant capacities of ‘Nainaiqingcai’ mustard

3.4. Correlation analysis

The correlation analysis between the bioactive compounds and antioxidant capacities of ‘Nainaiqingcai’ mustard was shown in Table 3. All correlation coefficients were positive and highly significant. The correlation coefficient among chlorophyll a, chlorophyll b, total chlorophyll, neoxanthin, lutein and total carotenoid was very high, even reaching 1.000. The correlation coefficient related to proanthocyanidins

was greater than 0.995. Even the lowest correlation coefficient between ATBS⁺ and total phenolics was as high as 0.965.

Table 3. Correlation coefficients of bioactive compounds and antioxidant capacities of 'Nainaiqingcai' mustard

Item	Chlorophyll a	Chlorophyll b	Total Chlorophyll	Neoxanthin	Violaxanthin	Lutein	β -carotene	Total carotenoids	Proanthocyanidins	Flavonoids	Total phenolics	FRAP
Chlorophyll b	1.000**											
Total Chlorophyll	1.000**	1.000**										
Neoxanthin	1.000**	1.000**	1.000**									
Violaxanthin	0.999**	0.999**	0.999**	0.999**								
Lutein	1.000**	1.000**	1.000**	0.999**	0.999**							
β -carotene	0.995**	0.995**	0.995**	0.996**	0.995**	0.997**						
Total Carotenoids	1.000**	1.000**	1.000**	0.999**	0.999**	1.000**	0.997**					
Proanthocyanidins	0.999**	0.999**	0.999**	0.999**	0.998**	0.999**	0.997**	0.999**				
Flavonoids	0.998**	0.998**	0.998**	0.997**	0.996**	0.997**	0.987**	0.996**	0.995**			
Total phenolics	0.994**	0.993**	0.994**	0.993**	0.994**	0.992**	0.980**	0.991**	0.990**	0.995**		
FRAP	0.982**	0.981**	0.982**	0.982**	0.987**	0.980**	0.974**	0.981**	0.980**	0.978**	0.982**	
ABTS	0.980**	0.980**	0.980**	0.983**	0.982**	0.983**	0.992**	0.984**	0.982**	0.967**	0.965**	0.969**

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

4. Discussion

This experiment determined the biological activity and antioxidant capacity of individual edible parts of 'Nianaiqingcai' mustard. The results showed that there were significant differences between the antioxidant activities and biological compounds of individual edible parts of 'Nianaiqingcai' mustard.

In this experiment, eight indexes of chlorophyll and carotenoid were determined. They showed a trend of leaves > petioles > bolting stem between the different edible parts of 'Nianaiqingcai' mustard, and the content of the leaves was much larger than other parts. This is consistent with the study of Sun et al. in Chinese kale [9]. The chlorophyll a content in this test was greater than chlorophyll b whatever part was, which may be because the photosynthetic pigment used in photosynthesis is mainly chlorophyll a. Chlorophyll a can not only absorb light energy, but also collect it, and the conversion of light energy into chemical energy meets the needs of plants themselves. Among the chloroplasts, the carotenoids containing more content include β -carotene, lutein, violaxanthin and neoxanthin, which account for 25% to 30%, 40% to 50%, and 15% of the total carotenoids, respectively [10]. In this study, the lutein content was accounting for 35.05%~54.35% of the total carotenoids. The violaxanthin content accounted for 6.14%~14.95% of total carotenoids. There was a general phenomenon that the average leaves content in the eight pigment indicators was 6-8 times than those of petioles, but the difference between leaves and bolting stem were different. This may be the physiological distance between the leaves and the petioles, so there was a stable difference between the pigment content. Leaves are the main organ of photosynthesis in plants. Chlorophylls and carotenoids are the material basis of plant photosynthesis. They play light energy capture in plant photosynthesis, maintain the stability of thylakoid membrane, and energy transduction [11].

The results of three bioactive substances of proanthocyanidins, flavonoids and total phenolics showed that the difference in the content of different edible parts was also different. The content of flavonoids in leaves was 13 times than that of bolting stem, with the largest difference. The total phenolics content in the petioles was just 1.1 times that of the bolting stem, the difference was minimal. In the study of *Houttuynia cordata*, the three indicators are also the most abundant in the leaves [12]. The distribution of total phenolics and antioxidant capacity in different parts is much larger than that of stalks and alfalfa, which is the same as that of broccoli and Chinese kale [9, 13].

Antioxidant capacities is an indicator of the overall effect of all antioxidants in the reaction sample. Two methods, FRAP and ABTS, are usually carried out for measuring antioxidant capacities. Antioxidant capacities between different plant organs generally differs significantly. In this study, the results of chlorophyll, carotenoids, proanthocyanidins, and flavonoids showed a high degree of agreement that was leaves > petioles > bolting stem. Correlation analysis showed that all indicators measured in this study had a very high correlation, indicating that there was a strong correlation between these bioactive compounds and antioxidant capacities. This is consistent with the results of previous studies [12, 14-16].

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