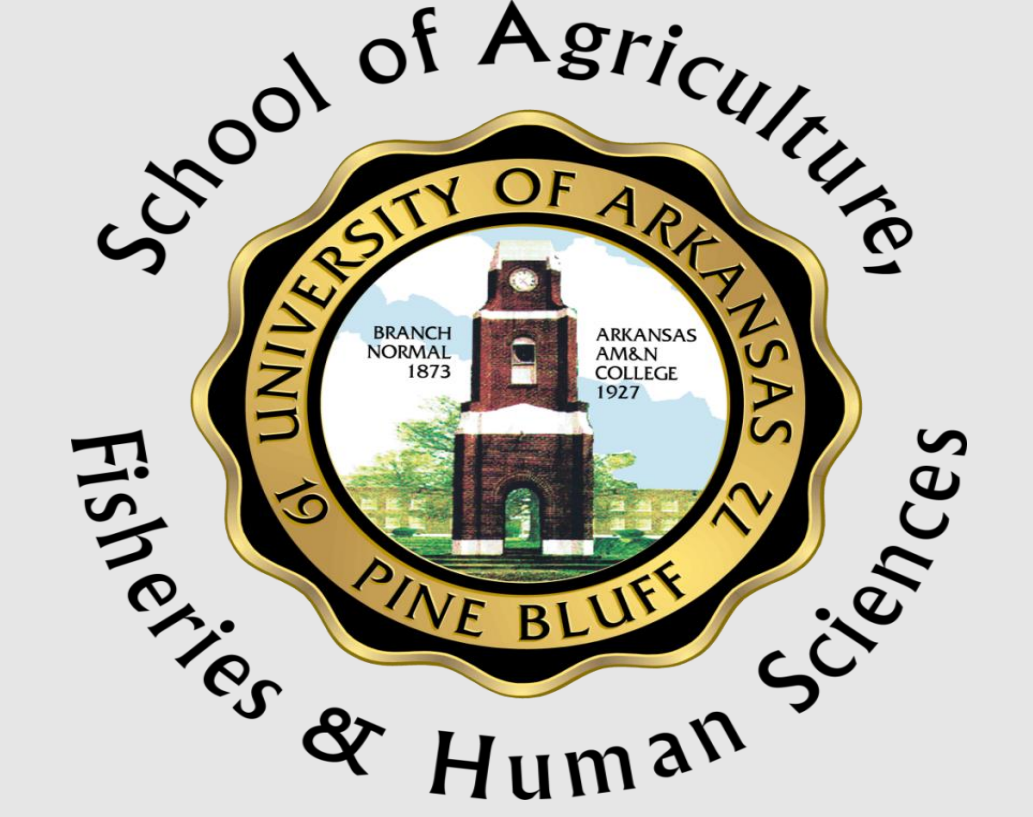


Determination of carotenoids in Chinese Cabbage by HPLC

Chromatogram



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Abstract

Nine varieties of Chinese cabbages were investigated for B-carotene, lutein, zeaxanthin and violaxanthin, the most common carotenoids in leafy vegetables. Separation and identification were conducted using HPLC and spectroscopic properties of each carotenoid. Seven varieties happen to contain varying concentrations of one or more of the four carotenoids. B-carotene and lutein appear to be more frequent, while zeaxanthin occurs in only one variety.

Introductions

Carotenoids are indispensable to plants and play a critical role in human nutrition and health as well. They have long been recognized as essential nutrients and important health beneficial compounds. As humans are unable to synthesize carotenoids, they have to depend on plants for these essential products. β -carotene, largely known as a "Pro-vitamin A" carotenoid, serves as the primary dietary source of vitamin A. The deficiency of vitamin A is one of the most noticeable nutritional problems in many parts of the world. Over recent years there has been considerable increase of interest in the study of dietary carotenoids with respect to their potential in alleviating age-related diseases in humans. Food biofortification with enhanced pro-vitamin A carotenoids offers a sustainable way to combat vitamin A deficiency in developing countries. It has been known that food sources of carotenoids include carrots, sweet potatoes, spinach, kale, collard greens, and tomatoes.

Though seemingly new to Southern diets, Chinese cabbage has been cultivated in North America for more than a century and in China for almost 1,500 years. It is a member of the Cruciferae family, which also includes cabbage, broccoli, cauliflower and mustard. In fact, Chinese cabbage is more closely related to mustard than to cabbage (Palada 1984) and is known as an excellent source of provitamin A carotenoids. This vegetable contains about 25 times of the provitamin A carotenoids contained in ordinary cabbage, and is also a very good source of vitamins C and K (Kirschmann. 1984; Kreutner. 1980). However, less research has reported the distribution of carotenoids in Chinese cabbage. Leafy vegetables are known to contain essential carotenoids like B-carotene, violaxanthin, lutein and zeaxanthin (Kimura et. al. 2002). It is, therefore, the objective of this work to investigate the distribution of these essential carotenoids in different varieties of the Chinese cabbage.

Materials and Methods

Nine varieties of Chinese cabbage were planted in green house of the University of Arkansas at Pine Bluff, in the Spring and Fall seasons of 2012. Samples were taken at the harvest time and freeze dried. The carotenoids from the cabbages were extracted and analyzed according to Norris et. al. (1995), with slight modifications. Briefly, 0.1g of the dried samples were ground with mortar and pestle in 0.8ml of 80% acetone. The homogenates were transferred into a 2.2 ml centrifuge tube, extracted with 0.5ml ethyl acetate by vortexing for 30 seconds. This was followed by centrifuging at high speed for 5 minutes, after adding 0.5 ml of water. The carotenoid containing upper phase was transferred into a new tube and dried under nitrogen gas.

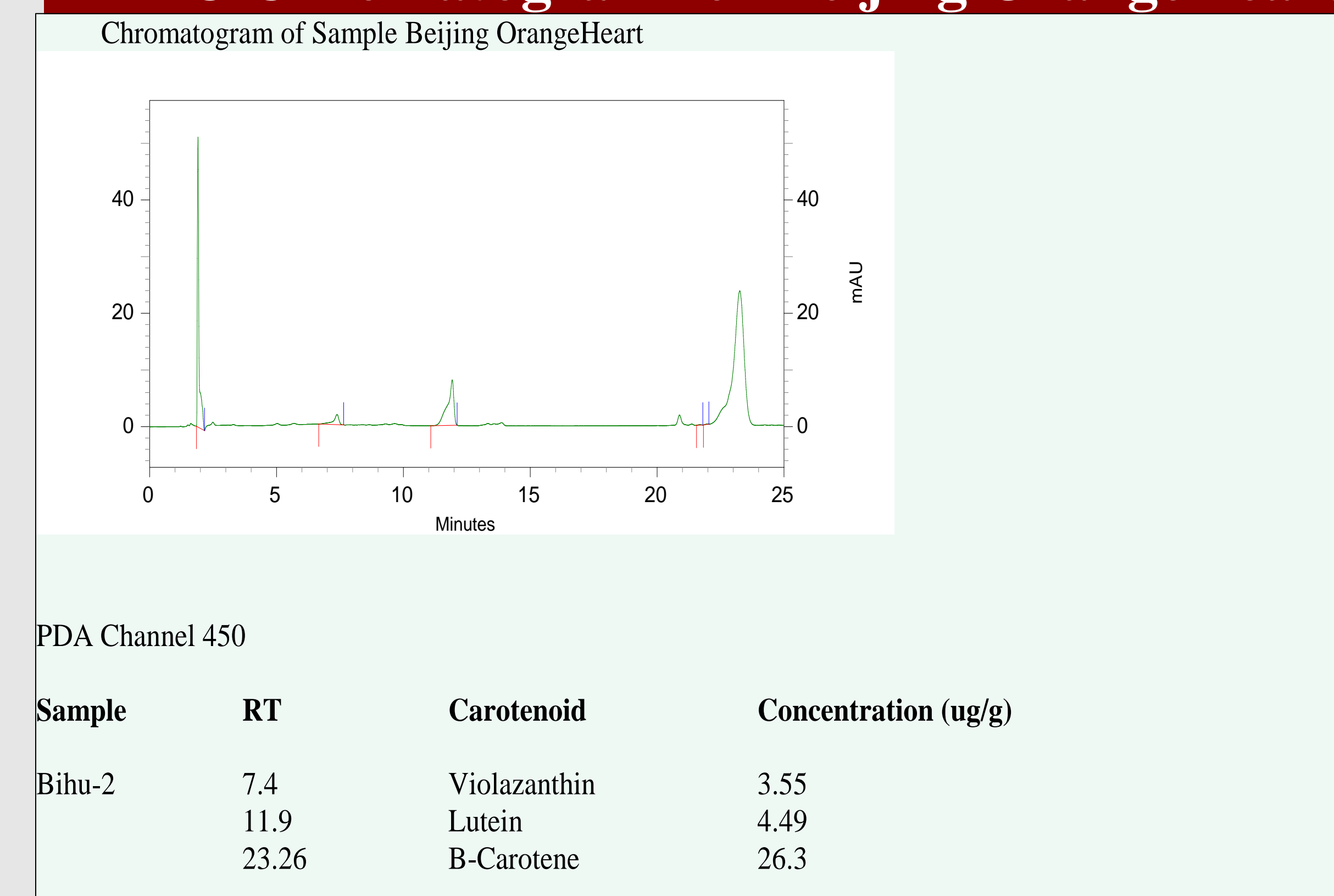
The dried samples were re-suspended in 200 μ l of ethyl acetate. Separation and identification of carotenoids were carried out on a Hitachi Lachrom Ultra C18 column (5 μ m particle size, 4.6mm I.D X 150mm) using a Hitachi Elite LaChrom High Performance Liquid Chromatograph, equipped with a diode array detector, Model (Hitachi L-2455), monitored at 450nm. The pigments were separated by a linear gradient between solvent A(made of 90% solution of acetonitrile:H₂O:triethylamine at 9:1:0.01, 10% ethyl acetate) and solvent B(80% ethyl acetate, 20% solution of acetonitrile: H₂O: triethylamine at 9:1:0.01) over 30 minutes at a flow rate of 1.0 ml per minute. The elution program used was: 0 minutes at 100% A, 0% B; 1-25 minutes with linear gradient to 0%A and 100% B; 25-30 minutes at 0% A, 100% B. Identification and quantification of each component was based on the comparison of the retention times and spectroscopic properties of the unknown with that of the standards.



Table 1. Carotenoids contents in Chinese Cabbage

Varieties	B-Carotene ug/gwt	Zeaxanthin ug/g wt	Lutein ug/g wt	Violaxanthin ug/g wt
FA 002	7.425	<0.1	<0.1	<0.1
FA 004	<0.1	69	<0.1	<0.1
FA 102	<0.1		1.8	<0.1
FA 104	<0.1	<0.1	<0.1	<0.1
FA 109	<0.1		7.84	0.15
Beijing OrangeHeart	26.3	4.49	3.55	
Beijing Yellow	4.2	<0.1	<0.1	<0.1
Jing (yellow)	<0.1	<0.1	<0.1	<0.1
Beijing Autumn	<0.1		1.15	<0.1

HPLC Chromatogram for Beijing OrangeHeart



Discussions and Conclusions

The carotenoid content of the nine varieties of the Chinese cabbages are presented in Table 1. As could be seen from the Table, the preliminary investigation of the varieties FA002 and Beijing Orange appear to have a B-Carotene content of 7.43 and 26.30 μ g per gram tissues, respectively. In addition, Beijing Orange contains 4.49 μ g Lutein and 3.55 μ g Violaxanthin per gram tissue. FA004 contains 69 μ g Zeaxanthin only, while FA102 and FA109 have 1.8 μ g and 7.84 μ g Lutein per gram tissue respectively. Beijing Autumn appears to have 1.15 μ g Lutein per gram tissue. Carotenoids have long been recognized as essential nutrients and important health beneficial compounds. "Pro-vitamin A" carotenoids, such as β -carotene, provide the primary dietary sources of vitamin A. The deficiency of vitamin A is one of the most noticeable nutritional problems in many parts of the world. In fact, the high economic value of carotenoids arises from their ability as a source of vitamin A, and other health-promoting compounds taken as food components. The indispensable role of carotenoids in plants and the increasing interest in their health benefits to humans have prompted a significant effort to gain a better understanding of carotenoid biosynthesis in plants. Chinese cabbage is low in calories and low in sodium, but high in vitamin A, and is an excellent source of provitamin A carotenoids. Chinese cabbage contains about 25 times that of the provitamin A carotenoids contained in ordinary cabbage and is believed by some to play a positive role in preventing stomach cancer and colon cancer.

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