Indian Journal of Agricultural Sciences **84** (10): 1178–81, October 2014/Article

Variation in chlorophyll and carotenoid contents in kale (*Brassica oleracea*) as influenced by cultivars and harvesting dates

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Received: 13 September 2012; Revised accepted: 8 August 2014

ABSTRACT

The levels of total chlorophyll and carotenoids were determined in kale (*Brassica oleracea* L.) leaves of three commercial cultivars, i.e. Siberian Kale, Khanyari and Japanese Green. The investigation was carried out at Central Institute of Temperate Horticulture, Srinagar, J & K in two successive years (2009-2010); in each year raw material was obtained in three harvests carried out 5, 7 and 9 weeks after planting young kale transplants in the field. During whole period of investigation the range of average content of chlorophyll in 100g fresh weight was 136.18 g in Siberian Kale to 172.10 g in Japanese kale. In all the cultivars chlorophyll content significantly increased from first to second harvest and maximum of 3.5% increase was in Japanese Green and subsequently decreased in third harvest. Similarly level of carotenoids in different cultivars varied significantly and maximum of 23.50 mg/100g as observed in Japanese Green and minimum of 18.99 mg/100g in Siberian Kale. Similarly significant difference was recorded with harvest dates and interaction of harvest date and cultivars. The increase of carotenoids from first to second harvest was recorded maximum (12.90%) in Siberian Kale and increase from second to third harvest was maximum in Khanyari (12.12%)

Key words: Chlorophyll, Carotenoids, Cultivars, Harvesting dates

Vegetables are an important part of the human diet. Awareness on nutritive value and significance of vegetable consumption can play an important role in maintaining health and the risk of illness. They are a low energy food containing small amounts of carbohydrates, high proportion for dietary fiber, minerals and vitamins (Giannakourou and Taoukis 2003). Leafy vegetables are also rich source of carotenoids (Prakash *et al.* 1995). Epidemiological studies have demonstrated that a diet rich in brassica vegetables can reduce the risk of cancer (Kohlemeier and Su 1997). Brassica vegetables belong to the cabbage family and comprise various species of cabbage, cauliflower, Brussels sprouts. Among, these kale (*Brassica oleracea* L.) is worthy of recommendation for composition and cultivation. This species provides a rich source of antioxidants (Davey *et al.* 2000 and Pfendt *et al.* 2003). Kale is most resistant to low temperatures and can even survive at -13°C (Altinok and Karakya 2003). Moreover, it is the most important vegetables in terms of its dry matter yields (Rosa and Heaney 1996).

In the selection of products for consumption consumers pay maximum attention sensory traits. Chlorophyll pigments are classed among the least stable natural pigments. The yellowing of green colour is associated with their degradation (Yamauchi and Watada 1991). Kale is one of the vegetables with a relatively high content of chlorophyll and similar to that in dill (Kmiecik and Lisiewska 1999) and parsely (Lisiewska *et al.* 2004) and exceeding that in spinach (Jaworska and Kmiecik 1991). According to Lisiewska *et al*. (2004), it is chiefly the level of chlorophyll pigments and ratio; that have a decisive bearing on the attraction of these. Tijskens *et al.* (2001) also stress that color is the most important trait used in consumer's evaluation of produce and playing a decisive role in the acceptability of the product. Chlorophyll pigments are accompanied by carotenoid ones, whose color, from yellow to orange, is frequented by green chlorophylls (Kidmose *et al.* 2001). Carotenoids are important nutritive and biological constituents common in them. Important source of carotenoids are green vegetables, with kale as a predominant species (Kalt 2005). Of the carotenes, beta carotene is the most valuable, showing the activity of pro-vitamin A (Nishino *et al*. 1999). Chlorophyll and carotenoids are the compounds characterized by anti oxidant properties (Kidmose *et al.* 2001). A serious issue in vegetable cultivation receiving increasing attention is crop yield and its quality (Jablonska-Coglarek and Rosa 2002). According to D'Antuono and Neri (2003), kale should be harvested on the optimal date to obtain good raw material yield of high

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quality. Babik (2003) also points the importance of cultivars and harvesting dates, climate, location and condition for vegetable yield and quality. The aim of the presented investigation was to determine the level of chlorophyll and carotenoid pigments depending on the cultivar and the harvesting date under temperate conditions.

MATERIALS AND METHODS

Experimental material consisted of fresh kale leaves obtained from 3 terms of harvest, i e 5, 7, and 9 weeks (15 October, 1 November and 15 November) after planting seedlings in the field. Three traditional and commercial cultivars of kale, i e Siberian kale, Khanyari and Japanese green were used for experiments. Kale was cultivated in a 2 yrs cycle (2009, 2010) in an experimental field of Central Institute of Temperate Horticulture, Srinagar, J & K, India. The results of soil analysis prepared for kale cultivation site are presented in Table 1. The mineral fertilization was given according to the recommendations of package of practices of vegetables for Kashmir valley (Anonymous 2004) which were as: FYM: 25-30 tonnes/ha, nitrogen (N) 90 kg/ha, phosphorus (P) 60 kg/ha, potash (K) 60 kg/ha.

Kale seeds were sown in first week of September (2008 and 2009) in the seed beds. The transplants were planted out when the plants had formed 3-4 leaves, which occurred in last week of September. The experiment was set up using a randomized block method: Cultivars × harvest date having four replications. An area of 10 sq m was used for each cultivar and harvest date at a row spacing of 50 cm×50 cm. Cultivation procedure were identical for all the cultivars and included mechanical weed removal, nitrogen top dressing and protection against diseases and pests, carried out according to commercial recommendations. Each year kale leaves were harvested at three dates, i e first at 5 weeks after transplanting when the number of marketable leaves was 25-30 per plant and second harvest after 2 weeks and third further after 2 weeks. Harvesting involved cutting the whole plants and removing the unmarketable leaves including yellow leaves (below 10 cm of length from plant top bud).

Table 1 Initial soil properties of the experimental soil

Soil properties	Inceptisol
pH	7.1
EC (ds/m)	0.48
OC (%)	0.71
Available nitrogen (kg/ha)	420
Available phosphorus (kg/ha)	28.2
Available potassium (kg/ha)	346
Zinc (kg/ha)	1.2
Iron (kg/ha)	5.7
Copper (kg/ha)	0.8
Manganese (kg/ha)	6.8

pH (1:2.5), EC (1:2.5), electrical conductivity (dS/m)

From market quality leaves, i e leaves of good colour undamaged by diseases and pests, the main rib was removed and the material for analysis of chemical composition was sampled in four replications of 1000 g each. The method of Anderson and Boardman (1964) was followed for the estimation of chlorophylls. Fresh leaf sample (100 mg) from each treatment was crushed in 5 ml of 80 per cent acetone and centrifuged at 3×1000 rpm (revolutions per minute) for ten minutes. The supernatant was retained and the residue was crushed again in 3 ml of 80 per cent acetone. The two supernatants were pooled and final volume was made to 10 ml with 80 per cent acetone. The absorbance was recorded at 645 nm and 663 nm for chlorophylls and at 420 nm for carotenoids on a spectrophotometer. The acetone (80%) was used as blank. The following formulae were used for calculation of chlorophylls,

$$\text{Chlorophyll a} = [12.7\ (A_{663}) - 2.69\ (A_{645})] \frac{v}{100 \times w}$$

$$\text{Chlorophyll b} = [22.9\ (A_{645}) - 4.86\ (A_{663})] \frac{v}{100 \times w}$$

$$\text{Total chlorophyll} = [20.2\ (A_{645}) + 8.02\ (A_{663})] \frac{v}{100 \times w}$$

Where, V, Total volume of solution made (ml); w, Fresh weight of sample (g); A_{645}, Absorbance at 645 nm = Absorbance at 663 nm.

The chlorophyll content was expressed as mg chl per g FW (mg chl/g Fw)

The carotenoids were determined according to the procedure of Jensen and Jensen (1972)

$$\text{Carotenoids} = A_{420} \frac{0.0054\ \text{Chl a} + 0.00101\ \text{Chl b}}{0.2185}$$

Where, A_{420}, Absorbance at 420 nm; Chl a, Chlorophyll a; Chl b, Chlorophyll b.

The carotenoid content was expressed as mg/g Fw. The data of results were subjected to ANOVA and the smallest

Table 2 Mean daily air temperature and total atmospheric precipitation during the growth of kale

Year of investigation	Month									
	August		September		October		November		December	
	Tempt. °C	Rainfall mm	Tempt. °C	Rainfall mm	Tempt. °C	Rainfall mm	Tempt. °C	Rainfall mm	Rainfall °C	Rainfall mm
2009	24.00	35.80	19.47	2.70	13.13	02.00	7.53	45.2	4.44	14.1
2010	23.06	47.90	19.85	7.20	14.63	34.00	9.88	00.0	3.14	58.0
Mean	23.52	41.85	19.65	4.95	13.88	18	11.7	22.6	6.69	36.05

significant differences were computed for the error probability level of P = 0.05.

RESULTS AND DISCUSSIONS

Chlorophyll content significantly differed among cultivars, harvesting dates, interaction of cultivars and harvesting dates (P = 0.05). During whole period of investigation, the average content of chlorophyll in 100 g of fresh matter of kale varied from 136.18 mg in Siberian Kale to 172.10 mg in Japanese Kale (Table 3). In all the cultivars the chlorophyll content significantly increased from first to second harvest and consequently decreased in the third harvest. At second harvest stage maximum increase was found in Japanese green (3.55%) followed by Siberian Kale (2.14%) and least in Khanyari (1.32%). Similarly decrease rate of chlorophyll with advent of third harvest was recorded maximum in Japanese green (3.56%) followed by Khanyari (1.81%) whereas minimum was recorded in Siberian Kale (1.45%). Similar results showing decreasing trend of chlorophyll towards harvesting dates was reported by Kopsell *et al.* 2004 and Khachik *et al.* 1986 at the maturity stages of broccoli floret and observed that chlorophyll content decreasing with ripening. Similarly Lefsrud *et al.* (2004) also reported that chlorophyll pigment in kale leaf tissue increased and then decreased in response to leaf age.

The recorded quantities of carotenoids exceed the level of 12.7-18.7 mg/100g as reported by Azevedo *et al.* 2005 but were much lower than the content of 77 mg/100 g given by Kachick *et al.* (1986). Compared with other cabbages and vegetables the trend of increase of carotenoids in kale exceeds than that found in Brussels sprouts, green lettuce or spinach (Muller 1997) but was similar to that of parsley (Kmiecik and Lisiewska 1999) and in dill (Lisiewska *et al.* 2004). Saric *et al.* 1990 also found greatest content of carotenoids in oldest leaf buds of cabbage. However, Drews *et al.* (1997) noted lower β carotene with the course of maturation of both head and Ice burg lettuce. In the present experiment it was also observed a tendency for the carotenoid contents to increase with the growth of the plant. A mean of the 10.22% increase in the carotenoid content was recorded in first to third harvest. The level of carotenoid varied significantly with cultivars (P = 0.05) and maximum of 23.50 mg/100 g carotenoid content was recorded in Japanese green followed by Khanyari (22.01 mg/100 g) whereas it was least in Siberian kale (18.99 mg/100 g) (Table 4). Similarly significant difference was recorded with the harvest date and interaction that of cultivars and harvesting dates (P = 0.05). The increase from first harvest to second harvest was recorded maximum (12.20%) in Siberian Kale followed by 10.04% in Khanyari, whereas, least was recorded in Japanese Green (5.59%). Increase from second to third harvest was maximum in Khanyari (12.12%), whereas, Siberian kale and Japanese Green recorded minimum increase (7.41% and 6.31% respectively). Korus and Kmiecik (2007); Farham and Kopsel (2009) also reported variation in chlorophyll and carotenoids in response to cultivars and harvesting dates. Similar trend of increase in carotenoids with harvesting dates is reported by Baskarachary *et al.* (1995).

Table 3 Chlorophyll content (mg/100g fresh weight) of kale varieties at different harvesting stages

Harvest	Varieties		
	Siberian Kale	Khanyari	Japanese Green
I	134.92	157.01	169.89
II	137.82	159.10	175.90
III	135.81	156.21	170.51
Mean	136.18	157.44	172.10
LSD (P = 0.05)			
Harvesting		0.4023	
Varieties		0.4023	
Harvesting × Varieties		0.697	

Table 4 Carotenoid content (mg/100g fresh weight) of kale varieties at different harvesting stages

Harvest	Varieties		
	Siberian Kale	Khanyari	Japanese Green
I	18.03	21.00	23.25
II	20.23	23.11	24.55
III	18.73	21.92	22.71
Mean	18.99	22.01	23.50
LSD (P = 0.05)			
Harvesting		0.1581	
Varieties		0.1581	
Harvesting × Varieties		0.274	

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