Food Research International 45 (2012) 342–350

Contents lists available at SciVerse ScienceDirect

Food Research International

journal homepage: www.elsevier.com/locate/foodres

Carotenoid content, its stability during drying and the antioxidant activity of commercial coriander (*Coriandrum sativum* L.) varieties☆

Peethambaran Divya, Bijesh Puthusseri, Bhagyalakshmi Neelwarne *

Plant Cell Biotechnology Department, Central Food Technological Research Institute, Mysore-570020, India

ARTICLE INFO

Article history:
Received 5 July 2011
Accepted 20 September 2011

Keywords:
β-carotene
Anti-oxidant
DNA protection
Microwave drying
HPLC
IC_{50}

ABSTRACT

Although populations of several tropical countries are under severe vitamin-A deficiency, traditional sources of pro-vitamin-A carotenoids have not been exploited due to the lack of relevant research data. In this study, ten commercial varieties of coriander (*Coriandrum sativum* L.), grown at identical conditions, were evaluated for carotenoids, their bio-efficacy and stability during drying, with the main emphasis on β-carotene (vitamin-A precursor) analysis by HPLC-MS. In all varieties, β-carotene content was higher in foliage at mature stage, than in seedlings and seeds. Variety GS4 Multicut produced highest biomass (6.18 ± 0.73 g/plant), total carotenoids (217.50 ± 5.6 mg/100 g DW) and β-carotene (73.64 ± 0.3 mg/100 g DW) at pre-flowering stage. Carotenoids extract showed a high antioxidant activity with IC_{50} value of 14.29 ± 1.68 μg/ml, scavenging hydroxyl radicals and rendering higher protection to DNA than by standard gallic acid (IC_{50} = 357.21 ± 4.29). Microwave drying of foliage was rapid with better retention of pigments, high intactness of *trans*-β-carotene and higher extractability of pigments when compared with oven drying.

1. Introduction

Coriander, also popular as Cilantro or Chinese Parsley, is an aromatic annual herb (of Apiaceae or Umbelliferae) cultivated for its seeds and foliage which are used all over the world as culinary spice, flavoring agent and for its various medicinal/aromatic applications. The foliage is a very good source of phytochemicals such as vitamin C (160 mg/100 g FW), vitamin A (β-carotene 12 mg/100 g FW) (Girenko, 1982) and Vitamin B12 (60 mg/100 g) (Prakash, 1990), polyphenols, and essential oils. Coriander, like many spices, contains antioxidants which can delay or prevent the spoilage of food seasoned with this spice and also provide such protections upon its ingestion. Most of the studies in coriander have been focused on its seeds (Dhanapakiam, Joseph, Ramaswamy, Moorthi, & Kumar, 2008; Srinivasan, 2005), and very little attention is paid to the constituents in leaves (Aruna & Baskaran, 2010), although foliage is popular for their versatile use in various types of foods.

Particularly the β-carotene, and other carotenoids present in green leafy vegetables are precursors for vitamin A in mammals, and the deficiency of vitamin A is prevalent in many tropical and under-developed temperate countries. An estimated 250,000 to 500,000 vitamin A-deficient children become blind every year globally, of which half die within 12 months of losing their sight (Micronutrient Initiative, 2009). Among populations under the high threat of vitamin- A deficiency, India occupies the red zone indicating serious clinical level deficiency. Vitamin A deficiency (VAD) and age-related macular degeneration (AMD) are recognized as serious public health problems among all-sections of Indian population, accounting to 25% of the 15 million blind world populations (Micronutrient Initiative, 2009). It is known that VAD and AMD are primarily due to inadequacy of provitamin A (mainly β-carotene) and other macular pigments in the diet. Another serious problem faced by developing countries is mortality and morbidity of neonates where supplemental vitamin A and β-carotene enhance the immune system in neonates (Nishiyama, Sugimoto, Ikeda, & Kume, 2010). Above all, β-carotene is generally regarded as the most commercially important and widely used carotenoid in food as a coloring agent and as an antioxidant both in the food and after its ingestion (Szpylka et al., 2005). Although many leafy vegetables, carrots and yellow fruits are rich sources of β-carotene, either such food items are not liked by many for routine consumption or inaccessible to poor people of developing countries, for various reasons. Certain green leafy vegetables grown and traditionally used routinely in some of these countries can form a good source to alleviate vitamin A deficiency, provided more organized research and product development is considered for their versatile applications (Raju, Varakumar, Lakshminarayana, Krishnakantha, & Baskaran, 2007). Coriander is one such green leafy vegetable used in various forms, liked by many, can be routinely consumed, could be an ingredient in various processed products, and is also a rich source of β-

☆ (A constituent laboratory of the Council of Scientific and Industrial Research, New Delhi).

* Corresponding author.
E-mail addresses: bneelwarne@cftri.res.in, blakshmi_1999@yahoo.com (B. Neelwarne).

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doi:10.1016/j.foodres.2011.09.021

carotene. Although a few preliminary studies have been conducted on the estimation of total carotenoids and β-carotene content (Aruna & Baskaran, 2010), no detailed study on carotenoids in different varieties of *C. sativum* has been done so far. Apart from the knowledge on high retinol equivalent value of coriander due to its combination of high β-carotene and lutein (Raju et al., 2007), there has been no report on the bio-efficacy of carotenoids from coriander, particularly in terms of DNA protection. For processing coriander leaves, cost-effective modern drying method such as microwave (MW) drying has not been screened. In case of olive oil, carotenoids were found to be lost in conventional heat-process whereas in MW-treated oil, the pigments were stable (El-Abassy, Donfack, & Materny, 2010). With this back ground, the present study was conducted to explore the efficacy of coriander as a source of important carotenoids such as β-carotene and lutein and as a source of natural efficient antioxidants in fresh and processed form.

2. Materials and methods

2.1. Samples

Certified seeds of ten commercially cultivated varieties of *C. sativum* were obtained from authorized seed distributers from different states (India). The germination and growth patterns of plants were monitored under controlled identical conditions in green house, using a mixture of soil containing equal proportions of red soil, garden compost and sand. The growth stages and biomass were periodically recorded by using 3 sets of plants; each set having 10 randomly picked plants, removed of the adherent soil, followed by washing and blotting off the adherent water. Each variety was grown in three different plots simultaneously to rule out variations due to changes in environmental effects (if any). In all the ten varieties, total carotenoids and β-carotene (separated as mentioned below) contents at different stages of plant growth were also determined, using 3 sets of plants, as mentioned above.

2.2. Chemicals

HPLC grade standard β-Carotene (95%), gallic acid, calf thymus DNA, thiobarbituric acid (TBA), ferric chloride, dimethyl sulfoxide (DMSO), trichloro acetic acid (TCA), ethylene diaminetetraacetic acid free salt (EDTA) and 2,2-Diphenyl-1-picrylhydrazyl (DPPH) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Methanol, acetonitrile, ethyl acetate were of HPLC grade, whereas acetone, petroleum ether were of analytical grade and purchased from Qualigens (India). All other chemicals and reagents used were of analytical grade and purchased from Merck (Darmstadt, Germany).

2.3. Extraction of carotenoids for HPLC and MS analysis

All extractions were done under low light and protecting samples using black wrappers to avoid photo-oxidation of samples. Extraction and HPLC analysis of carotenoids from coriander leaves were done according to (Rodriguez-Amaya, 2001). A known quantity of different samples, such as fresh and dried seeds and foliage, was ground using mortar and pestle in the presence of 0.01% BHT as antioxidant. Total carotenoids were extracted in to ice-cold acetone; extraction was repeated thrice until the sample become colorless. The extract containing excess water was removed by partitioning against petroleum ether (PE) and measured for carotenoids, by measuring OD after centrifugation at 4 °C. The PE extract was subjected to saponification with methanolic KOH (10% w/v). Saponification was performed not only to remove chlorophylls and other lipids, but it also helps to release carotenes from bound forms, resulting in clean preparation of β-carotene for analysis, because such bound forms, fatty acids and lipids make the chromatographic separation difficult and complicated. Saponification is also an effective method for hydrolysing carotenoid-esterified forms (Rodriguez-Amaya and Kimura (2004). The crude extract was washed with water for removing alkali and concentrated at 40 °C in vacuo using rotavapour. Five milliliter of the concentrated sample was passed through silica (110 Å) column (15 cm long and 1 cm dia), eluted with acetone in PE (in various ratios) and the eluent was used for quantification of carotenoids. Whenever, necessary, the extract was concentrated under nitrogen gas and stored in freezer (−20 °C) for not more than one week and used for quantification and characterization.

The sample was re-dissolved in 1 ml of mobile phase (acetonitrile: methanol:ethyl acetate in proportion of 80:10:10 v/v) and injected in to the HPLC system (LC-10A; Shimadzu, Kyoto, Japan), equipped with a Shimadzu Photo Diode Array detector (PDA) for analysis of major carotenoids. All the carotenoids were separated on a Phenomenex Gemini C_{18} reverse phase stainless steel column (250×4.6 mm) isocratically with a flow rate of 1 ml min^{-1}. Chromatogram was acquired at 450 nm. Peak identities were done by their retention time and respective spectra recorded with the PDA detector. While the identification was done by using standards and by spiking individual ones, the quantification of β-carotene was done by considering peak area in relation to the concentration of standard (Schierle, Pietsch, Ceresa, Fizet, & Waysek, 2004).

Total carotenoid content in the extract was estimated spectrophotometrically according to Rodriguez-Amaya and Kimura (2004) which has been widely used including coriander (Guerra, Melo, & Filho, 2005), upon improvements made over earlier methods (Britton, 1991; Rodriguez-Amaya, 1999).

$$\frac{A \times \text{volume(ml)} \times 10^4}{A^{1\%}_{1cm} \times \text{sample weight(g)}}$$

Where A = absorbance (which was maximum at 450 nm); volume = total volume of the extract; $A^{1\%}_{1cm}$ = absorption coefficient of β-carotene in PE (2592). Here we used β-carotene for comparison, which is a common practice to measure such pigments, similar to the use of cyanidin-3-glycoside for the estimation of anthocyanins.

Routine quantification of β-carotene was done by the method of AOAC International (1993). Briefly, a known quantity of sample was blended for 5 min with acetone:hexane (4:6) containing 0.1 g of magnesium carbonate and centrifuged at 8000 rpm for 10 min. The residue was washed with two 25 ml portions of acetone followed by one 25 ml portion of *n*-hexane. The extracts were combined and washed with water to remove acetone. The upper layer was placed in a 100.0 ml volumetric flask containing 9 ml acetone and the volume was adjusted with hexane. The optical density of the suitably diluted solution was measured at 436 nm using UV–visible spectrophotometer (Shimadzu 160A, Japan).

Concentration of β-carotene was calculated from the formula:

$$C = (\text{OD at } 436 \text{ nm} \times 454)/(196 \times L \times W)$$

Where, C represents concentration of β-carotene (mg lb^{-1}), L represents the path length (cm) and W represents the weight of the sample in g ml^{-1} of final dilution. Concentration in mg kg^{-1} was calculated as C×2.2.

For simultaneous analysis of both carotenoids and chlorophylls, total carotenoid content in the extract was estimated by spectrophotometric reading by using the following equation Lichtenthaler (1987).

$$\text{Chlorophyll a, } Ca = 11.24\ A_{661.5} - 2.04\ A_{645},$$

$$\text{Chlorophyll b, } Cb = 20.13\ A_{645} - 4.19 A_{661.5},$$

$$\text{Total carotenoids, } Cx + c = (1000A_{470}) - [(1.9Ca) + (63.14Cb)]V \times DF/214$$

where

V = Total volume of the extract, DF = Dilution factor.

HPLC-MS analysis of β-carotene was done using a Phenomenex Gemini 5 μm C_{18} 110 reverse phase column, 250 × 4.6 mm i.d. Conditions of resolving total carotenoids using APCI + ve mode were Corona (μA): 1.7, Cone: 100 V, Source Temp: 120 °C, APCI Probe Temp: 500 °C, Cone Gas Flow: 100 L/h, Desolvation gas flow: 250 L/h.

The variety of coriander GS4 Multicut which exhibited highest biomass accumulation in shortest time as well as highest total carotenoids and β-carotene contents was selected for further studies.

2.4. Stability of pigments during microwave drying

To standardize the drying conditions for best retention of pigments, first the moisture content in fresh coriander leaves of var. GS4 Multicut was determined by drying foliage (wrapped in blotter sheets) in hot air oven at 70 °C overnight (Therdthai & Zhou, 2009). In case of drying in hot-air oven, 5 to 10 g fresh samples were wrapped in single layered blotters, placed in a perforated steel tray and incubated in an oven at 45 ± 2 °C until a constant weight was noted, which generally occurred within 24 h. For microwave (MW) drying, the samples were similarly wrapped and drying was done at five power levels (850 W, 600 W, 450 W, 300 W & 180 W) for five different periods (30 s, 60 s, 90 s, 120 s and 150 s). Dry weight, chlorophyll a, chlorophyll b and total carotenoids contents were estimated after acetone extraction followed by spectrophotometric reading (Lichtenthaler, 1987), as explained earlier. Mean gravimetric values of 3 sets of samples, each having 4–5 shoots were recorded. The quantity of pigments in samples after MW drying were compared with those of fresh sample and those obtained after oven drying. The MW-dried samples were also subjected to HPLC analysis where, sets of three similar leaves of fresh and after MW-drying were used, and total extract was quantitatively analyzed.

2.5. Antioxidant activity of carotenoids extract

2.5.1. DPPH• assay

Free radical scavenging activity was carried out by following the reaction with stable free radical DPPH• (Blois, 1958). Briefly, an aliquot of extract (which had been extracted in acetone) was dissolved in methanol and mixed with 0.5 ml of 0.15 mM DPPH solution in methanol. Although the solubility of carotenoids is high in solvents such as acetone, the use of latter has been found to result in lower values (Guo, Lee, Chiang, Lin, & Chang, 2001; Molyneux, 2004). While the presence of phenolic groups in carotenes, particularly in β-carotene makes it partially soluble in methanol, other carotenoids such as lutein and xanthophylls are more hydrophilic and hence the procedure was adopted without any change. The solution was mixed well and allowed to stand for 30 min at room temperature. Absorbance was read at 517 nm. Gallic acid was used as positive control. The concentration required to scavenge 50% DPPH free radicals (IC_{50}) was calculated.

DPPH• scavenging activity was calculated using the equation

$$\text{DPPH}^{\bullet}\text{scavenging effect}\ (\%) = [(A0 - A1/A0) \times 100]$$

where'A0' is the absorbance of the control and'A1' is the absorbance of the sample.

2.5.2. DNA degradation assay by spectrophotometric method

Hydroxyl radical induced DNA damage was evaluated by the competition between deoxyribose and carotenoid fraction for hydroxyl radical generated by Fenton's reagents (Fe^{3+}-ascorbate-EDTA-H_2O_2 system) according to Cao, Chen, Zheng, and Zheng (2008) with slight modification. The reaction mixture (1 ml) contained calf thymus DNA (8 mg/ml), Fe^{3+}chloride (10 mM), EDTA (10 mM), and H_2O_2 (2 mM), without and with the test extract of varying concentrations in sodium phosphate buffer (pH 7.4). Ascorbic acid (10 mM) was added to trigger reaction, which reduces Fe^{3+} to Fe^{2+}, and the reaction mixture was kept at 37 °C for 30 min. Fenton's assay reagents were prepared just prior to use. To 1 ml of the above mixture, TBA in 25 mM NaOH (1 ml, 0.5%) and TCA (1 ml, 10% w/v aqueous solution) were added. The mixture was incubated in a boiling water bath at 80 °C for 90 min. After centrifugation at 3000 rpm for 10 min, pink supernatant chromogen produced was spectrophotometrically measured at 532 nm. Hydroxyl radical scavenging activity was calculated by the following equation:

$$\%\ \text{hydroxyl radical scavenging activity} = (1 - As/Ac) \times 100,$$

Where 'As' is the absorbance of the sample and 'Ac' is the absorbance of the control.

2.5.3. DNA degradation assay by gel electrophoresis

The extent of DNA protection offered by carotenoids extract of coriander var. GS4 Multicut against the attack by hydroxyl radicals was studied according to Lee, Kim, Kim, and Jang (2002) with minor modifications. The reaction mixture contained 5 μg of calf thymus DNA, Fenton's reagent (H_2O_2 30 mM, Ascorbic acid 50 μM, $FeCl_3$ 80 μM) in TE buffer, and extracts in DMSO in a final volume of 20 μl. The mixture was incubated for 30 min at 37 °C and intactness DNA was analyzed by loading in to 1% agarose gel and electrophoresed, which was followed by staining with ethidium bromide and recording results with gel documentation system (Hero Lab Documentation unit, Hero Lab 442 K, E.A.S.Y., Germany). Appropriate controls used were gallic acid (50 μg and 100 μg in DMSO) and all-*trans*-β-carotene (50 μg in DMSO).

Table 1
Time required for reaching different growth phases and foliage biomass accumulation in different varieties of coriander.

Variety	Seed germination period (d)	Days required to reach lag phase	Foliage biomass at lag phase (35 d) F.W.(g)	Initiation of profuse growth (d)	Foliage biomass at mature stage F.W. (g)	Floral leaf induction (d)
GS4 Multicut*	4–7	20–25	0.90 ± 0.16^{a}	37–42	6.18 ± 0.73^{a}	57–62
GC Gold 99*	4–7	20–25	0.71 ± 0.15^{ab}	37–42	4.92 ± 0.67^{ab}	57–62
KalmiGutchedar*	4–7	20–25	0.62 ± 0.12^{ab}	37–42	5.28 ± 0.71^{ab}	57–62
Amar*	4–7	20–25	0.48 ± 0.06^{b}	33–37	3.62 ± 0.56^{b}	45–52
Mahak**	4–8	20–25	0.54 ± 0.94^{ab}	40–45	5.92 ± 0.70^{a}	85–92
Ever Green**	5–9	25–30	0.66 ± 0.13^{ab}	40–45	4.62 ± 0.68^{ab}	85–92
Commander**	6–10	25–30	0.49 ± 0.08^{b}	45–50	6.14 ± 0.69^{a}	90–96
Surabhi**	6–10	25–30	0.45 ± 0.10^{b}	45–50	5.42 ± 0.58^{ab}	90–96
Super5**	6–10	25–30	0.70 ± 0.08^{ab}	45–50	5.85 ± 0.66^{a}	90–96
Nutan**	6–10	25–30	0.65 ± 0.07^{ab}	45–50	5.80 ± 0.68^{a}	90–96

Fresh weight data are mean ± S.D. of 10 plants from three batches. Means with different superscripts are significantly different from each other at $P \leq 0.05$ as analyzed by Duncan's Multiple Range Test. Varieties with single (*) and double asterisks (**) showed maturity on 55th and 75th day respectively.

2.6. Statistical analysis

The mean weights of samples were determined, and the significance difference ($p \leq 0.05$) was tested by one way ANOVA followed by Duncan's Multiple Range Test (DMRT) using SPSS 17 software (SPSS Inc., Chicago, IL, USA). The values obtained from analyses of total carotenoids, chlorophyll a, chlorophyll b and β-carotene were also compared by DMRT and are expressed as mean ± S.D. for three samples per assay.

3. Results and discussion

3.1. Growth pattern and carotenoids accumulation in different coriander varieties

Different coriander varieties screened in this study showed marked differences in growth pattern and biomass yield (Table 1), which are known to occur because of inherent genetic variations. While most of the varieties of coriander showed a short germination period of 4–10 days, there was a variable initial growth phase of 20 to 25 days, a lag phase and a profuse growth phase of variable periods before the onset of flowering stage. Three varieties namely, GS4 Multicut, GC Gold 99 and Kalmi Gutchedar required shorter time to produce higher amount of foliage than the rest, with var. GS4 Multicut being the best performer, attaining the initial lag phase between 20 and 25 days after germination with an average biomass of 0.90 ± 0.16 g/plant at this stage. Plants of this variety reached the mature stage between 37th and 42nd days after germination with an average biomass of 6.18 ± 0.73 g/plant.

Carotenoids are of ubiquitous occurrence in all plants with higher concentrations in reproductive organs. In green leafy vegetables, carotenoids, particularly β-carotene is deposited mainly in leaves (Speek, Saichua, & Schreurs, 1988) where, in addition to other roles, they chiefly function as scavengers of the free radicals produced by chlorophylls during photo-oxidation. In all the coriander varieties, contents of total carotenoids were higher in foliage than in seeds (Table 2). Among coriander varieties, foliage of GS4 Multicut was found to contain highest total carotenoids (217.50 ± 5.57 mg/100 g DW) and β-carotene content (73.64 ± 0.26 mg/100 g DW) at mature stage, whereas in case of seeds, Kalmi Gutchedar (112.66 mg/100 g DW) showed highest carotenoids (Table 2). Dry seeds of all varieties contained very low level of total carotenoids and hence the β-carotene. Total carotenoids in leaves of other varieties at mature stage were (all in mg/100 g DW) Amar (216), Nutan (215) Kalmi Gutchedar (213) and GC Gold 99 (212), of which approximately 30% of was β-carotene. The field-grown samples of coriander also showed nearly similar contents of carotenoids and β-carotene (Aruna & Baskaran, 2010; Raju et al., 2007).

HPLC (Fig. 1) and HPLC-MS (Fig. 2) analyses results of major carotenes and chlorophylls showed the abundance of nutritionally important carotenoids such as β-carotene and lutein, and this profile was similar in all varieties of coriander, although profiles of high carotenoid-containing varieties have been shown (Fig. 1). Saponification is a well-accepted first- step chemical process for the separation of β-carotene (Rodriguez-Amaya, 2001 and Rodriguez-Amaya & Kimura, 2004). Thus, the carotenoid fraction obtained after saponification showed the dominant β-carotene peak, with loss in the peak areas of neoxanthin, violaxanthin and lutein, and complete absence of chlorophylls and β-cryptoxanthin (Fig. 1c). Since β-carotene is the most preferred pro-vitamin A carotenoid, and particularly high content in coriander (Raju et al., 2007), this experiment focused mainly on tracking the levels of β-carotene in coriander foliage. When the concentration of β-carotene before and after saponification was compared (Fig. 1), from 20 to 30% of β-carotene and 50% of other carotenes were found lost (see Table 2) during the process of saponification. The higher peak area of β-carotene in Fig. 1c is due to the injection of same amount of sample containing high β-carotene level,

Table 2
Total carotenoids (mg/100 g D.W.) and β-carotene (mg/100 g D.W.) contents in leaves and seeds of commercially important varieties of coriander.

Name of variety	In leaves at young plant stage			In leaves at mature plant stage			In fresh seeds		In dry seeds	
	Total carotenoids	β-carotene (BS)	β-carotene (AS)	Total carotenoids	β-carotene (BS)	β-carotene (AS)	Total carotenoids	β-carotene	Total carotenoids	β-carotene
GS4 Multicut	169.15 ± 4.73^{a}	58.61 ± 2.87^{a}	45.58 ± 2.20^{a}	217.50 ± 5.57^{a}	82.28 ± 2.39^{a}	73.64 ± 0.26^{a}	102.46 ± 4.99^{a}	32.89 ± 3.93^{abc}	1.85 ± 0.021^{bc}	0.29 ± 0.011^{e}
GC Gold 99	167.06 ± 3.13^{ab}	53.86 ± 1.97^{abc}	40.36 ± 2.07^{bc}	212.94 ± 5.24^{ab}	78.48 ± 2.11^{ab}	70.43 ± 1.48^{ab}	106.20 ± 5.03^{a}	30.51 ± 4.15^{abc}	2.11 ± 0.042^{a}	0.52 ± 0.023^{bc}
Kalmi Gutchedar	158.19 ± 5.38^{b}	48.34 ± 2.15^{c}	38.55 ± 2.01^{bc}	213.38 ± 4.33^{ab}	78.35 ± 2.78^{ab}	68.44 ± 2.05^{bc}	112.66 ± 5.92^{a}	41.50 ± 6.70^{a}	2.15 ± 0.049^{a}	0.60 ± 0.014^{a}
Surabhi	157.62 ± 4.17^{b}	42.44 ± 2.28^{d}	35.51 ± 1.97^{c}	205.26 ± 5.10^{abc}	63.79 ± 2.36^{c}	57.78 ± 1.42^{d}	104.28 ± 5.73^{a}	40.60 ± 4.33^{a}	1.86 ± 0.042^{bc}	0.42 ± 0.014^{d}
Super 5	160.44 ± 3.99^{ab}	51.54 ± 2.65^{bc}	37.40 ± 2.36^{bc}	210.40 ± 4.46^{ab}	75.60 ± 2.32^{b}	69.72 ± 2.13^{abc}	83.90 ± 4.20^{b}	25.37 ± 2.90^{bc}	1.84 ± 0.035^{c}	0.39 ± 0.013^{d}
Mahak	152.79 ± 5.06^{c}	32.26 ± 1.72^{e}	22.55 ± 1.86^{e}	197.73 ± 5.10^{c}	50.70 ± 2.26^{d}	39.04 ± 1.68^{e}	106.09 ± 4.59^{a}	37.11 ± 3.30^{a}	1.76 ± 0.042^{c}	0.41 ± 0.021^{d}
Evergreen	159.57 ± 4.23^{ab}	40.55 ± 2.94^{d}	30.53 ± 1.63^{d}	207.79 ± 5.02^{abc}	74.82 ± 1.50^{b}	68.01 ± 1.61^{bc}	101.33 ± 4.54^{a}	32.46 ± 4.20^{abc}	1.95 ± 0.049^{b}	0.55 ± 0.021^{b}
Commander	159.11 ± 3.90^{ab}	38.33 ± 2.89^{d}	27.14 ± 1.82^{de}	203.55 ± 4.56^{bc}	67.88 ± 1.44^{c}	58.57 ± 1.57^{d}	105.58 ± 3.74^{a}	35.72 ± 3.98^{ab}	1.84 ± 0.035^{c}	0.51 ± 0.012^{c}
Nutan	162.55 ± 4.68^{ab}	50.67 ± 2.39^{bc}	40.75 ± 2.70^{b}	215.53 ± 4.83^{ab}	76.73 ± 1.54^{b}	65.54 ± 1.88^{c}	75.91 ± 4.89^{b}	24.47 ± 4.74^{c}	1.85 ± 0.035^{bc}	0.32 ± 0.010^{e}
Amar	165.09 ± 3.18^{ab}	56.52 ± 2.62^{ab}	47.51 ± 1.96^{a}	216.87 ± 6.73^{a}	79.01 ± 2.63^{ab}	68.69 ± 2.46^{bc}	109.96 ± 5.08^{a}	36.35 ± 4.97^{a}	2.14 ± 0.070^{a}	0.54 ± 0.021^{bc}

Values are presented in mg/100 g DW as mean ± S.D. for 3 sets, each having 10 plants. Significance was tested by Duncan Multiple Range Test at $P \leq 0.05$. Means with different superscripts are significantly different from each other. BS: Before saponification, AS: After saponification.

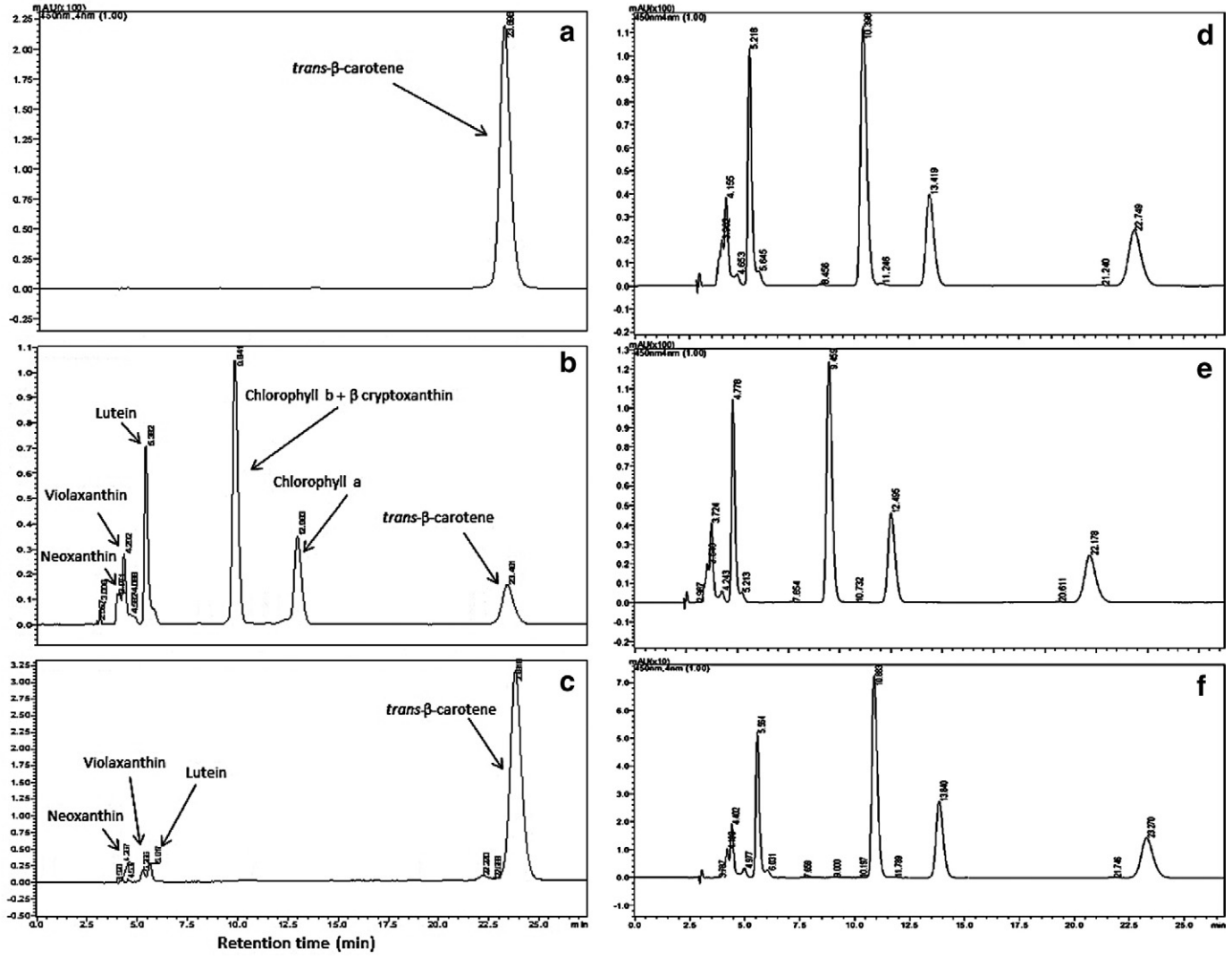

Fig. 1. HPLC chromatogram* of carotenoids: a. Standard *trans*-β-carotene, Total carotenoids from coriander var. GS4 Multicut foliage before (b) and after (c) saponification, d) Kalmi Gutchedar, e) GC-Gold 99 and f) Mahak. Peak assignments and their retention times for d, e and f are as in b. *Injected samples were not quantitatively similar.

and the absence of chlorophylls and some xanthins. Other studies have also recorded that prolonging the saponification process significantly reduced the recovery of β-carotenes (Hart & Scott, 1995; Inbaraj et al., 2008). Therefore, shortening saponification time, or avoiding saponification wherever possible has been suggested for curtailing such pigment losses (Rodriguez-Amaya & Kimura, 2004). β-Carotene content was found to increase with foliage maturation in all varieties. Among the factors affecting carotene content, varietal differences which is mainly because of variation in genetic composition between any two varieties is a prominent factor (Kiss, Kiss, Milotay, Kerek, & Markus, 2005). Wide variations in carotenoids can occur within the same variety at varying growth conditions (Hart & Scott, 1995). However, the present study conducted at identical growth conditions indicate that the differences were purely due to varietal differences resulting from inherent genetic make-up, which grossly remain significantly different from each other.

In view of the wide prevalence of vitamin A deficiencies among nutritionally deficient populations, the foliage of coriander holds great promise due to the high content of carotenoids, particularly the β-carotene — an immediate precursor of vitamin A. The next important carotenoid holding high promise for nutrition and bioefficacy is lutein (Aruna & Baskaran, 2010), which is also a major component in coriander (Fig. 1b). Similar studies conducted by (Singh, Kawatra, & Sehgal, 2001) also revealed that coriander leaves possess higher concentration of β-carotene compared to spinach and *Amaranthus* — the vastly recommended green vegetables as sources of vitamin A, and 3-folds more than that in carrots. A large number of leafy vegetables of India systematically analyzed by Raju et al. (2007) indicated high total carotenoids (mg/100 g DW) in coriander (166) which was lesser only than in *Chenopodium album* (449), *Commelina benghalensis* (416) and *Rumes acetosella* (224). In these latter species β-carotene was about 10% of the total carotenoids, whereas in coriander it was about 25% (Raju et al., 2007), somewhat similar to the present study showing 30% of β-carotene. Since coriander can be processed variously for application in a large number of routinely used food and savory preparations, the information in the present study forms a basis for preferring this materials for alleviating vitamin A deficiencies.

3.2. Antioxidant activity

3.2.1. DPPH radical scavenging activity

Previous studies have shown that both leaves and seeds contain antioxidants, but the leaves were found to have stronger efficacies than seeds (Wangensteen, Samuelsen, & Materud, 2004), which can be attributed to their high content of pigments, particularly carotenoids. The IC_{50} value of DPPH radical scavenging activity of coriander carotenoids fraction was 303.36 ± 6.8 μg/ml, whereas standard gallic acid showed much higher efficiency (1.46 ± 0.22 μg/ml). An earlier

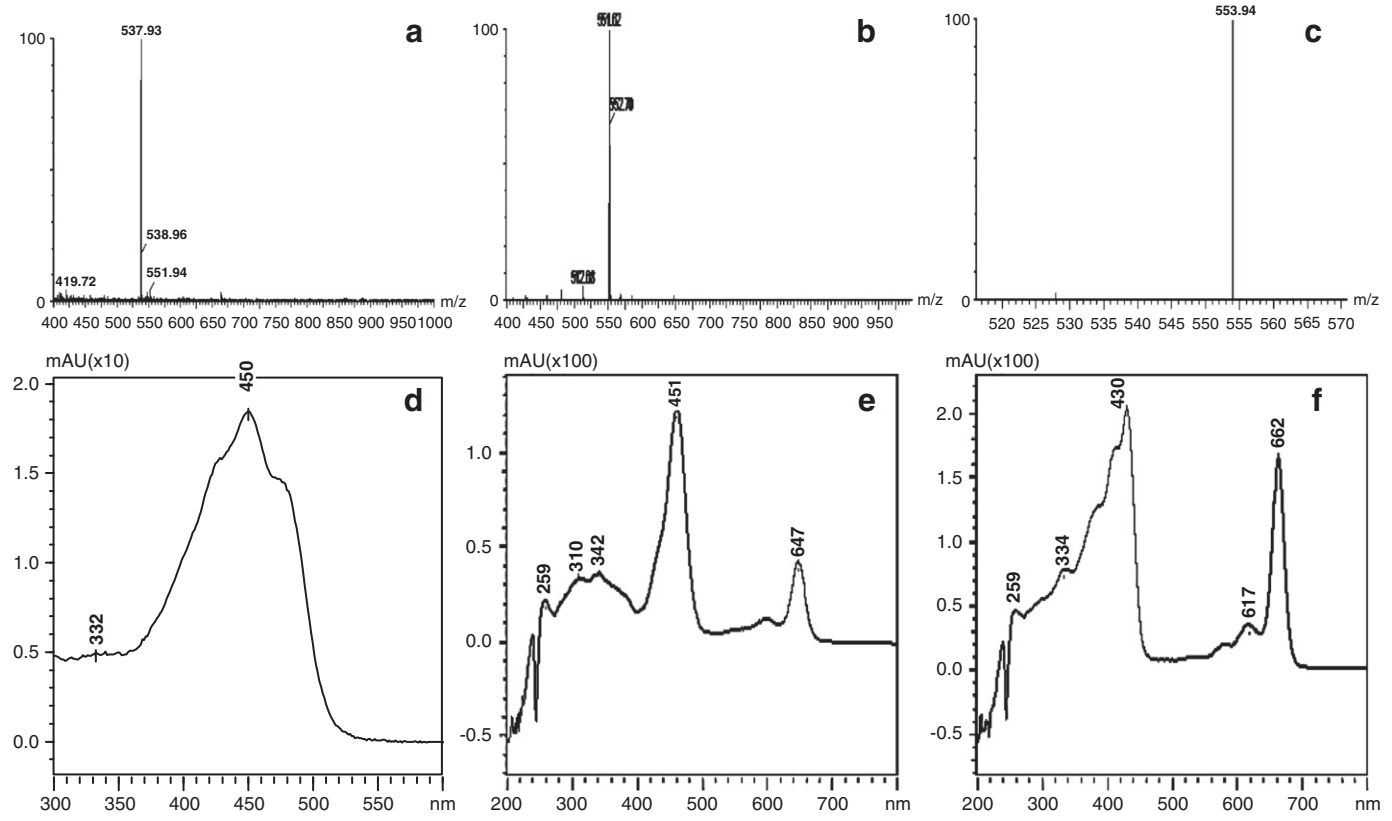

Fig. 2. Mass spectrum of a. β-Carotene, b. Lutein and c. β-cryptoxanthin, and PDA spectrum obtained through HPLC: d. β-carotene (λ_{max} 450), e. Chlorophyll b (λ_{max} 647) and β-Cryptoxanthin (λ_{max} 461), and f. Chlorophyll a (λ_{max} 662).

study stated that lipophilic extracts of coriander leaves are inactive in this assay as the reaction medium is more hydrophilic (Wangensteen et al., 2004). It is worth mentioning here that we used higher volume of methanol to solubilize β-carotene (in which it is sparingly soluble), and methanol being most suited for DPPH assay than other solvents such as acetone (Molyneux, 2004), we assume that the above argument of hydrophobicity appears inapplicable here. This is supported by the fact that even ethanolic extract of coriander showed a higher DPPH IC_{50} value of 389 ± 5 μg/ml (Wangensteen et al., 2004), showing lesser efficacy of a hydrophilic extract than the lipophilic extract of the present study.

3.2.2. DNA degradation assay by spectrophotometric method

Carotenoids of coriander extract was found to show higher hydroxyl radicals scavenging potential (IC_{50}, 14.29 ± 1.68) than standard gallic acid (IC_{50} 357.21 ± 4.29), protecting DNA from degradation. β-carotene, lutein and other carotenoids exhibit antioxidant functions by quenching hydroxyl and other free radicals (Sies & Stahl, 1995). Among the free radicals, hydroxyl radicals are the most deleterious ones, since they directly react with DNA and probably with similar such molecules such as RNA which are rampantly distributed in the cytoplasm. While each carotenoid has potential health benefits, a combination of all carotenoids is probably more effective than a single compound, as is shown by the total carotenoids. Similarly, antioxidant activity of ether extract of coriander analyzed by β-carotene/linoleic acid model showed that individual carotenoids were inferior to the functionalities of whole carotenoids (Guerra et al., 2005).

3.2.3. DNA degradation assay by gel electrophoresis

DNA protective effect of coriander carotenoids against hydroxyl radicals generated by the Fenton's reaction was further confirmed by the gel electrophoresis pattern of the DNA. Coriander carotenoids fraction was found to inhibit the degradation induced by Fenton's reagent more effectively than the standard gallic acid and all-trans- β-carotene (Fig. 3.).

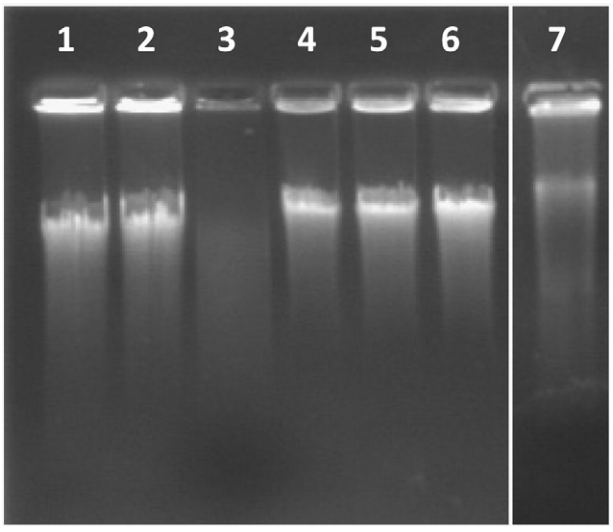

Fig. 3. Gel showing the protective activity of coriander carotenoid fraction on hydroxyl radical induced DNA damage. Lanes: 1. DNA + Standard Gallic acid (50 μg in DMSO), 2. DNA + Standard (Gallic acid 100 μg in DMSO), 3. DNA treated with Fenton's reagent, 4. DNA + Sample (50 μg in DMSO), 5. DNA + Sample (100 μg in DMSO), 6. DNA in Buffer, 7. DNA + Standard *trans*-β- carotene (50 μg in DMSO). Except lane 6, all other tests were done by treating DNA with Fenton's reagent (see Section 2.5.3).

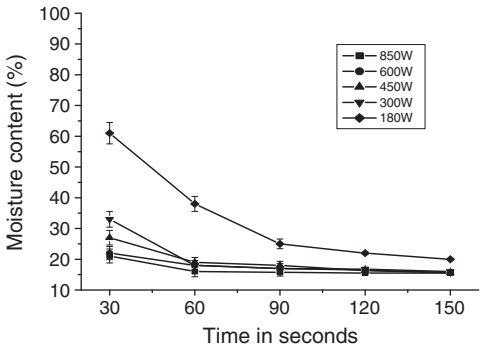

Fig. 4. Loss of moisture (gravimetric%) from coriander leaves var. GS4 Multicut during microwave drying at different power levels. The mean values of 3 sets of samples are significantly different at $p \leq 0.05$. Vertical bars represent ± SD.

3.3. Stability of pigments during microwave drying

Processing of plant materials by thermal treatments generally results in the loss of biologically active compounds. However, it is well-known that brief treatment such as blanching enhances pigments by freeing them from bound forms. Oven drying at 70 °C overnight was found to remove the moisture content in leaves, and it was found to be 87%. Microwave drying at different power levels for different periods indicated that drying at 850 W for 90 s was found ideal for drying coriander foliage to match with the moisture content equal to that of oven-dried (Fig. 4). Although the main emphasis of the study was on β-carotene, in view of the involvement of other carotenoids like lutein and zeaxanthin in the protection of eyes from macular degeneration, acting as shields against the damaging blue and near-ultraviolet light, reversal of oxidative DNA damage by β-cryptoxanthin (Lorenzo et al., 2009) and chlorophylls serving as antioxidants, all these pigments were considered while performing drying experiments. Moreover, each of these pigments function as a protectant to another, as has been recently found in *Momordica cochinchinensis* (Cao-Hoang, Phan-Thi, Osorio-Puentes, & Wache, 2011). The oven drying of coriander foliage, even at a very low temperature of 45 °C resulted in substantial loss of both chlorophylls (65%) and carotenoids (35%) (Fig. 5). Further experiments with various power levels and drying time indicated that in case of microwave drying, there was an increase in the yield of chlorophylls a and b, as well as total carotenoids at all power levels up to 90 s. A similar study in pumpkin also showed an increase in β-carotene content (Azizah, Wee, Azizah, & Azizah, 2009), and comparable effects were observed during heat processing of fruits (Jatunov, Quesad, Diaz, & Murillo, 2010). Another study conducted on the effects of cooking and processing on fruits and vegetables revealed about 8–10% destruction of total carotenoids (Mazzeo et al., 2011) and α-and β-carotene as a result of heat treatment (Khachik & Beecher, 1987), with significant variations in response to treatment conditions. Even in case of carotenoids in olive oil, where oil is expected to offer better protection to carotenoids, conventional heating rapidly and completely degraded carotenoids at high temperature, which was not the case in microwave-heated oil (El-Abassy et al., 2010). Thus, there is a need to screen and evaluate the specific processing conditions to preserve the bio-active pigments in different plant materials and products. Our comparative analysis of fresh and microwave samples showed that the carotenoid yield, upon microwave drying, increased to an extent of 35 to 40% (Fig. 5), probably due to higher extractability and liberation of carotenoid molecules that are bound to membranes and other pigments such as chlorophylls. Such increases have also been observed during steaming (Mazzeo et al., 2011). For such reasons, the bioavailability of carotenoids from heat-processed food products has been found to be higher than in fresh fruits and vegetables (Fernandez-Garcia et al., in press). Similarly, even in the case of saponification, nearly 20% was lost in fresh samples indicating that more loss occurs in fresh samples probably due to carotenoid bondage with chlorophylls, and the latter are completely removed during saponification (Fig. 5).

Microwave treatment time was found to affect the total chlorophylls and carotenoids contents. As the treatment time increased, carotenoids content decreased, particularly when exposed to lower power levels for longer periods. However, chlorophyll a decreased with the increase in treatment time (Fig. 5).

HPLC analysis of total carotenoids in microwave-dried foliage indicated that *trans*-β-carotene was found to be more stable when compared to other carotenoids, but partial degradation of lutein and other carotenoids were observed (Fig. 6). HPLC profile of carotenoids and quantification of *trans*-β-carotene before and after microwave drying showed no *trans* to *cis* isomerization of β-carotene, which is evident from the absence of a peak immediately after that of *trans*-β-carotene (Rodriguez-Amaya & Kimura, 2004). Since studies in human subjects have revealed the presence of higher levels of all-*trans* β-carotene in plasma and serum than *cis*-β carotene (Deming, Teixeira, & Erdman, 2002), the dry coriander foliage

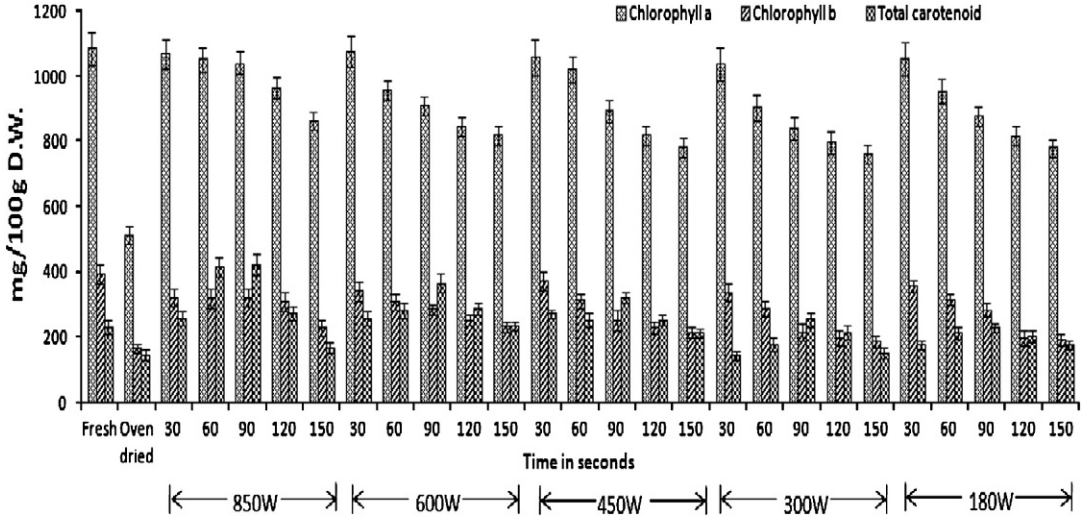

Fig. 5. Effect of microwave drying on chlorophyll a, chlorophyll b and total carotenoids in foliage of coriander var. GS4 Multicut. The mean values (on dry weight basis) of three sets of samples are significantly different at $p \leq 0.05$. Vertical bars represent ± SD.

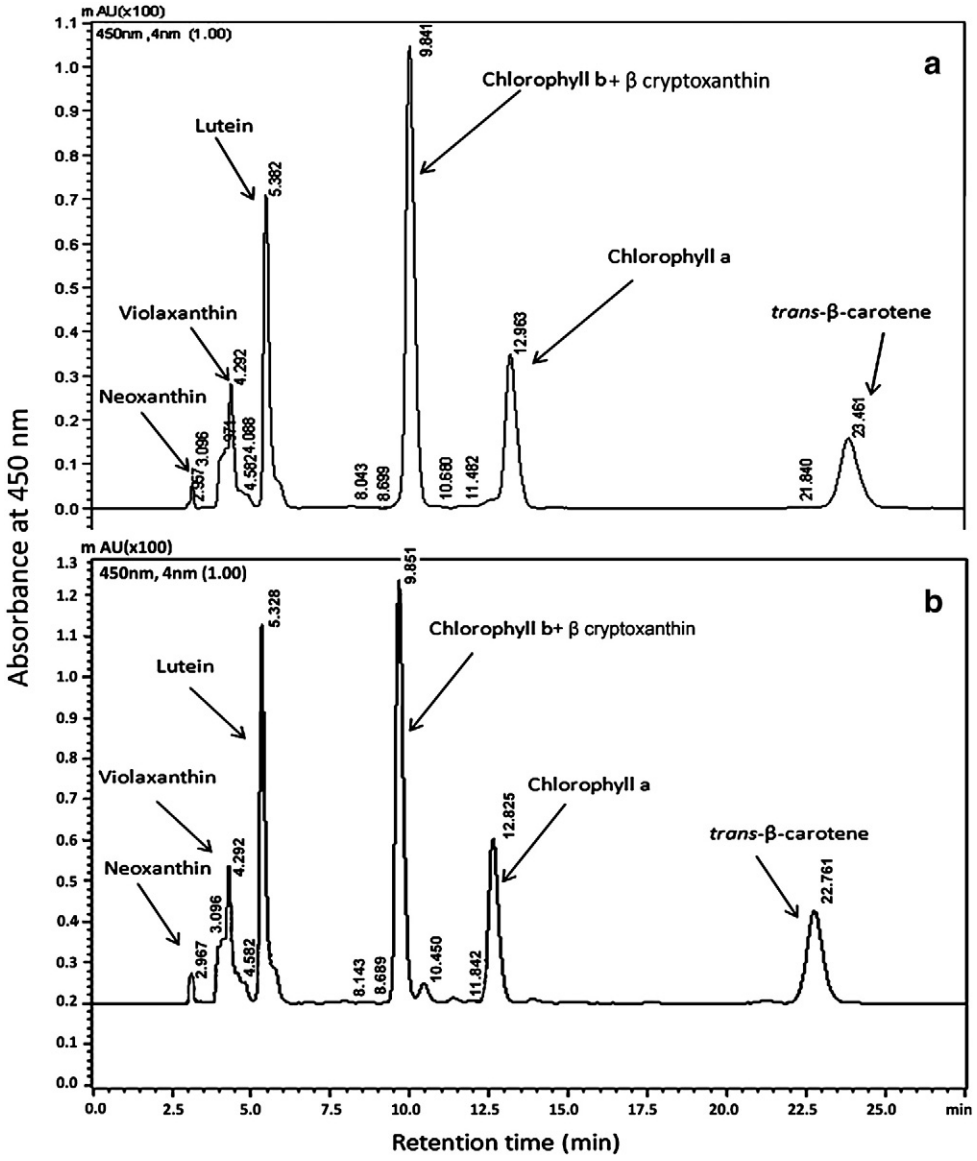

Fig. 6. HPLC chromatogram of total carotenoids of mature foliage of coriander var. GS4-Multicut: a. Fresh foliage and b. after microwave drying at 850 W for 90 s. Sample preparation and dilutions were quantitatively similar.

obtained after microwave treatment would be an ideal material for human consumption. The present study suggests microwave treatment as an efficient cost-effective method for drying of *trans*-β-carotene-rich leafy materials. In case of coriander such dried foliage can be easily crushed into powder for further use in various food products.

4. Conclusion

The present comprehensive study in commercial coriander varieties reveals the presence of wide variations in growth patterns as well as in the accumulation of total carotenoids and β-carotene in both foliage and seeds. By evaluating the changes in growth phases, it has been found that highest foliage biomass was recorded before flowering stage in all varieties of coriander, which also coincided with high β-carotene content and other carotenoids. Carotenoids extract prepared from coriander foliage was highly efficient in scavenging the reactive hydroxyl radicals and protecting DNA. Microwaving for short time at high power was effective in drying foliage without affecting *cis*/*trans*-isomerisation of β-carotene with negligible loss of pigments, allowing biologically active *trans*-β-carotene-rich dried-foliage for direct use in processed foods and in culinary preparations. The data also suggests that more detailed analyses of carotenogenic biosynthetic pathway is needed for further improvement in the cultivation and processing conditions to make this crop an important routine source of provitamin-A with additional benefits offered as anti-oxidants.

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