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Comparative study on the levels of carotenoids lutein, zeaxanthin and β-carotene in Indian spices of nutritional and medicinal importance

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ABSTRACT

The carotenoid levels in Indian spices of nutritional and medicinal importance were determined using high performance liquid chromatography. Lutein (L) and zeaxanthin (Z) levels (mg/100 g dry wt) in curry leaves (27.34), spearmint (18.0), green chilli (13.74), coriander leaves (9.92) and mustard seeds (1.2) were higher (2–22-fold) than mace, anise seeds, onion, fenugreek seeds and carum seeds (0.62 and 0.85) whilst their levels in cumin seeds, black pepper, green cardamom and coriander seeds were in the range of 0.32–0.47. β-Carotene (mg/100 g dry wt) was higher in coriander leaves (67.5), green chilli (9.06), curry leaves (8.95) and spearmint (7.5) than black cardamom (0.22) and coriander seeds (0.22), respectively. Neoxanthin, violaxanthin and α-carotene levels were also discussed. Spices analysed are a better source of L + Z than β-carotene (except for coriander leaves). Usage of spices as an adjuvant in food preparations also provides L + Z as antioxidants.

1. Introduction

Consumer interest in natural products instead of synthetically produced commodities has grown considerably in recent years. Health-conscious nutritionists have long recommended natural products including spices and herbs in dishes to add flavour. Spices are strongly flavoured and used in small quantities as either a preservative or flavouring in cooking. Spices are also widely used in pharmaceuticals, neutraceuticals, perfumery, toiletry and cosmetics industries. In India, spices and herbs have been used for centuries in traditional medicinal systems like ayurveda, unani and siddha. These have been used to cure a wide range of diseases from common cold to diabetes and from cough to cancerous tumours. Medicinal properties of spices used in this study are given in Table 1.

In recent years, the use of spices and their ingredients as potential chemo-preventive agents remains a topic of intense research (Surh, 2002). Spices have a diverse array of natural phytochemicals that have antioxidative effect, modulation of detoxification enzymes, stimulation of immune system and reduction of inflammation, antibacterial and antiviral effects (Lampe, 2003). Ozaki, Kawahara, and Harada (1991) investigated the anti-inflammatory effect of ginger and its active principles. Abdulldev (2002) studied the cancer chemo-preventive and tumouricidal properties of saffron. The active components in spices – phthalides, polyacetylones, phenolic acids, flavonoids, coumarins and terpenes are reported to be powerful antioxidants (Uhl, 2000). Studies from India and other parts of the world demonstrate various health benefits of spices. They evaluated the effect of spice active compounds like eugenol, capsaicin, piperine, quercetin, curcumin, cinnamaldehyde and allyl sulphide on human platelet aggregation (Raghavendra & Naidu, 2009), diabetes in mice (Grover, Yadav, & Vats, 2002), arteriosclerosis (Kinsella, Lokesh, & Stone, 1990) and anti-inflammatory effect (Burger, Warren, Lawson, & Hughes, 1993).

Literature survey on health benefits of spices is limited to their active principles, but not on carotenoids, which are reported as powerful antioxidants. Epidemiological studies have demonstrated that the consumption of carotenoid rich fruits and vegetables is associated with a lower incidence of cancer, cardiovascular disease, age related macular degeneration (ARMD) and cataract formation (Landrum & Bone, 2001). Barlett and Eperjesi (2003) have reviewed the influence of lutein supplementation in prevention of ARMD. The protective roles of lutein and zeaxanthin have recently been added to the list of potentially beneficial nutrients for ARMD and cataract (Wisniewska & Subczynski, 2006). Blindness due to retinol and lutein deficiency is reported as serious public health problem among children and adults in India. It is reported that 25% of the 15 million blind in the world are from India (Bhaskarachary, Ananthan, & Longvah, 2008). From the literature, it is evident that not many studies are available on carotenoid composition in spices. Hence, the present study was aimed at evaluating the levels of major carotenoids neoxanthin, violaxanthin, L, Z, α- and β-carotenes in selected Indian spices.

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Table 1
Botanical name and medicinal properties of spices used in this study.

Spice	Botanical name	Medicinal importance[a]
Curry leaves	*Murraya koenigii*	Anti-diabetic, improves digestion and skin conditioning
Spearmint	*Mentha spicata*	Tonic, carminative and antiseptic
Green chilli	*Capsicum annuum*	Rheumatic disorders, improves digestion
Coriander leaves	*Coriander sativum*	Carminative and diuretic
Mustard	*Brassica nigra*	Anti-diabetic, treatment of scorpion stings and snake bites, epilepsy, tooth ache and rheumatism
Carum	*Tarchyspermum ammi*	Anti-spasmodic, treatment of atonic dyspepsia and diarrhoea
Fenugreek	*Trigonella foenum-graceum*	Carminative, galactogue and anti-diabetic
Onion	*Allium cepa*	Digestive stimulant, diuretic and expectorant
Anise seeds	*Pimpinella animsum*	Used to treat flatulence, colic, diaphoretic, in preparation of asthma medicine, increases lactation in nursing mothers
Mace	*Myrstica fragrens*	Used to treat urinary inflammation of bladder and urinary passage
Coriander seeds	*Coriander sativum*	Diuretic, digestive stimulant and carminative
Green cardamom	*Elattaria cardamomum*	Used for indigestion, flatulence, diuretic and aromatic stimulant
Black pepper	*Piper nigrum*	Anti-periodic in malarial fever, curing indigestion, constipation, joint pain
Cumin seeds	*Cuminum cyminum*	Digestive stimulant, carminative, anti microbial, astringent
Cinnamon	*Cinnamomum zeylanicum*	Carminative, antiseptic, antifungal, anti viral and blood purifier
Black cardamom	*Amomum subulatum*	No data available
Spillanthes	*Spilanthes mauritiana*	Used in scabies, psoriasis and diuretic
Ginger	*Zingiber officinale*	Cure diarrhoea, stomach cramping and flatulence
Cloves	*Synzyngium aromaticum*	Used in dyspepsia, gastric irritation, hernia, anti-gout medicine
Garlic	*Allium sativum*	Used in pulmonary phthisis, gangrene of lung, whooping cough, arteriosclerosis, high blood pressure, high cholesterol and improves immunity
Star anise	*Illicium verum*	Carminative and antispasmodic
White pepper	*Piper nigrum*	Essential oil used in aromatherapy
Poppy seeds	*Papaver somniferum*	Narcotic and sedative
Nutmeg	*Myrstica fragrens*	Used to treat inflammation of bladder and urinary passage
Tamarind	*Tamarindus indica*	Carminative and laxative
Turmeric	*Curcuma longa*	Prevents various sexual transmitted diseases, anti-tumoral and anti-diabetic

[a] National Institute of Science Communication (1999).

2. Materials and methods

2.1. Materials

2.1.1. Samples

Spices ($n = 26$) were obtained from local supermarkets (Mysore, India) and cleaned before they were used and analysed in duplicate for xanthophylls as well as α- and β-carotenes content by HPLC. The botanical names and medicinal properties of spices used in this study are given in Table 1.

2.1.2. Chemicals

Standard lutein (99%), β-carotene (95%) and butylated hydroxytoluene (BHT) were purchased from Sigma–Aldrich (St. Louis, MO). β-Apo-carotenal was purchased from Fluka chemicals, Sigma–Aldrich (St. Louis, MO). Zeaxanthin, violaxanthin and neoxanthin were kindly donated by Dr. A. Nagao, National Food Research Institute, Tsukuba, Japan. Methanol, acetonitrile and dichloromethane were of HPLC grade, whereas acetone, ethanol and ammonium acetate of analytical grade were purchased from Sisco Research Lab Ltd. (Mumbai, India). Sodium sulphate was obtained from Ranbaxy Chemicals (Mumbai, India).

2.2. Methods

2.2.1. Extraction of carotenoids from spices

Each spice (10 g) in duplicate was ground well (particle size, approximately, 60 mesh size) separately in a blender (Sumeet, Bombay, India) along with sodium sulphate (5 g) and 0.1% BHT (antioxidant) in ethanol. The homogenised mixture was transferred to a conical flask and total carotenoids were extracted into ice-cold acetone as per Raju, Varakumar, Lakshminarayana, Krishnakantha, and Baskaran (2007) with slight modification. Extraction was repeated thrice or otherwise until the samples became colourless (total volume 200 mL). The pooled acetone extract was dried over anhydrous sodium sulphate (20 g) and filtered through Whatman No. 1 filter paper. The filtered crude extract was concentrated using a rota evaporator (Buchi, Flawil, Switzerland) at 30–35 °C and made up to 50 mL. β-Apo-carotenal was used as an internal standard to quantify the carotenoids.

2.2.2. HPLC analysis

An aliquot of crude extract was evaporated to dryness using a stream of nitrogen, re-dissolved in 1 mL of acetonitrile/methanol/dichloromethane (60:20:20, v/v/v) (mobile phase) containing 0.1% ammonium acetate for analysis of neoxanthin, violaxanthin, lutein, zeaxanthin, α- and β-carotenes and injected into the HPLC system (LC-10Avp; Shimadzu, Kyoto, Japan) equipped with a Shimadzu photodiode array (PDA) detector (SPD-M20A).

All the carotenoids were separated on a Supelco, C-18 (ODS) column, 25 cm–4.6 mm i.d., 5 μm, 120 Å (Supelco, Bellefonte, PA, USA) isocratically with (1 ml/min) mobile phase. The carotenoids were monitored at 450 nm using Shimadzu Class-VP version 6.14SP1 software. The peak identities and λ_{max} (absorption maxima) values of carotenoids were confirmed by their retention times and characteristic spectra of standard chromatograms, recorded with a PDA detector. They were quantified from their peak areas in relation to the respective standards. Handling, homogenisation and extraction procedures were carried out at 4 °C under a dim yellow light to minimise photo-isomerization and oxidation of carotenoids.

3. Results and discussion

Twenty-six spices were evaluated for xanthophylls (neoxanthin, violaxanthin, lutein and zeaxanthin) and hydrocarbon carotenoids (α- and β-carotenes) levels (Table 2). Since the objective of this study was to quantify xanthophylls and hydrocarbon carotenoids levels, this paper does not report the level of chlorophylls. HPLC elution profile of carotenoids in green chilli, for example, their