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Research Article

HPLC Analysis of Lutein and Zeaxanthin in Green and Colored Varieties of Vegetables

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ABSTRACTS
Lutein and zeaxanthin was estimated in green and colored varieties of vegetable.*Basella alba* L.(Indian spinach),purple and green varieties,.*Cucurbita pepo var.cylindrica* (Zucchini)green and yellowvarieties,*Brassicaoleracea*.*var. capitata* L. f. *alba*),Cabbage green and purple varieties,*Zaleyadecandra*.Lgreen and purple varieties,*Trianthemaportulacastrum* Linn, Green and Purple varieties,The concentration of lutein was maximum in all plant when compared to zeaxanthin.Maximum lutein concentration was reported in *Basella alba* (504 ppm purple variety), in *Brassica oleracea var. capitata* (314.9 ppm green variety)and in *Zaleyadecondra* (309.28 ppm green variety).Lowest content of lutein was recorded in *Zaleyadecondra*(60.91 ppm red variety).The content of lutein was reported to be high in purple, green colored varieties of all plants.Maximum content of zeaxanthin was reported in *Brassica oleracea var. capitate* (147.38 ppm green variety) ,lowest concentration was reported in *Cucurbita pepo var .cylindrica*(0.90 ppm red variety), and (0.70ppm in yellow variety).The concentration of zeaxanthin was recorded high in purple, green, varieties of all plants.

Keywords: lutein, zeaxanthin, xanthophyll carotenoids

INTRODUCTION

Xanthophyll carotenoids supplementation was associated with significant increase in macular pigment optical density (MPOD) in age related macular degeneration (AMD) patients[1,2,3]. Lutein and zeaxanthin have a potential role in the prevention and treatmentofeyediseaselikeage-relatedmaculardegeneration,cataract[4,5,6,7,8]. studies also indicated the role of lutein and zeaxanthin in eye diseases management. Therefore the content of lutein and zeaxanthin in some green and colored vegetable is taken up for the present research study.

The presence of a hydroxyl group at both ends of the molecules distinguishes lutein and zeaxanthinfrom other carotenoids and it is responsible for the high chemical reactivity with singlet oxygen. Carotenoids are divided into two sub classes depending on the presence of oxygen in the molecule: xanthophylls (lutein, zeaxanthin, isomer of lutein ($C_{40}H_{56}O_2$), and bete-cryptoxanthin ($C_{40}H_{56}O$) and carotenes (α-carotene,β carotene and lycopene ($C_{40}H_{56}$)

Humans being do not have the capacity to synthesis lutein and zeaxanthin, and therefore has to be taken as dietary source, whilemesoxanthin is rarely found in diet and is believed to be formed at the macula by metabolic transformation of integrated carotenoids

Location of lutein and zeaxanthin in the eye: These pigments are present in the eye are called macular pigments (MP). The macula lutea is a specialized part in the posterior pole of retina, since it mediates central vision provides the sharpest visual activity and facilitate component in the macular region, macula is uniquely concentrated in the inner central layer.

By absorbing blue-light the macular pigments protects the underlying photoreceptor cell layer due to powerful blue-light filtering activities and antioxidant properties,ascribed to lutein:(inhibition of membrane lipid peroxidation, particularly in photoreceptors, which have plenty of polyunsaturated fatty acids directs, antioxidant action and anti-inflammatory and immunomodulatory properties).

Risk of age-related macular degeneration was significantly higher in people with lower plasma concentrations of zeaxanthin.Recent papers reported that lutein is predominantly accumulated in the brain is positively associated with improved cognitive function in the elderly persons[9]. Most recent reports indicate that the mean dietary intake of lutein and zeaxanthin in are 0.8 mg to2.4 mg per day approximately[10].

The ability of zeaxanthin and lutein to protect ocular tissues against damage. Preventive and therapeutic effects of lutein and zeaxanthin and various ocular diseases was studied in various experimental animal models[11].

The content of beta carotene, lutein and zeaxanthin examined in thai vegetable by[12] HPLC analysis of lutein and zeaxanthin studied[13].

MATERIAL AND METHOD

Experimental plant materials

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Table 1: Content of Lutein in green and colored varieties of vegetables.

Plant name	Colour variety of plants	Lutein(ppm)
Basella alba	Green	167.39
	Purple	504.82
Brassica	Green	314.9
oleraceavar.capitata	Purple	195.69
Cucurbita pepo var.	Green	105.45
cylindrica	yellow	116.73
Trianthema	Green	162.28
portulacastrum	Red	296.49
Zaleyadecondra	Green	309.28
	Red	60.91

Table 2: Content of zeaxanthin ingreen and colored varieties of vegetables.

Plant name	Colour variety of plants	Zeaxanthin (ppm)
Basella alba	Green	12.48
	Purple	40.96
Brassica	Green	147.38
oleraceavar.capitata	Purple	8.87
Cucurbita pepo var.	Green	0.90
cylindrica	yellow	0.70
Trianthema	Green	12.12
portulacastrum	Red	36.3
Zaleyadecondra	Green	40.82
	Red	6.98

The following plants were used for the estimation of lutein and zeaxanthin content *Basella alba* L.(Indian spinach),purple and green varieties, *Cucurbita pepo var.* cylindrica (Zucchini) green and yellow varieties, *Brassica oleracea*.*var. capitata* L.,(Cabbage) green and purple varieties, *Zaleya decandra* .L green and purple varieties, *Trianthema portulacastrum* Linn, green and purple varieties,

Fresh vegetables, *Basella alba* L, *Cucurbita pepo var. cylindrica*,*Brassica oleraceavar. capitata* L, were obtained from localmarket whereas, *Zaleya decandra* L,*Trianthema portulacastrum* Linnwere collected from wild.Later they were washed with water, sun dried, pulverized in mill and sieved and stored in an airtight container for further use.

HPLC Analysis

Extraction of lutein, zeaxanthin

Carotenoids were saponified, prior to their HPLCanalysis[14]. About10 mg of plant extracts, diluted in 10 ml of the mobile phase, was saponified with an equal volume of 10 % potassium hydroxide in methanol (under nitrogen in the dark with stirring) for 1h at room temperature. The carotenoids were extracted from the KOH/ methanolic phase by careful shaking with 20 ml petroleum ether (containing 0.1 % BHT), and 20 ml 10% sodium chloride in a separating funnel. The lower KOH/MeOH/aqueous phase was removed to another separating funnel and extracted two more times with 20 ml of petroleum ether. The petroleum ether phases were combined in a separating funnel and washed with water until the washings were neutral to pH paper and transferred to a 100 ml round bottom flask, the solvent was evaporated on a rotary evaporator at 35°C. The residue was redissolved in 10 ml of the mobile phase and diluted with the mobile phase to a suitable concentration and later filtered through a 0.4 mm syringe filter. This extract was used directly for HPLC analysis of, lutein and zeaxanthin.

HPLC system and conditions

HPLC, instrumentation, chromatographic Column type, conditions and solvent systems were followed as described by[15].

An HPLC system consisting of two LC-20AT pumps, SPD-M20A diode array detector, DGU-20A3 degasser and CBM- 20A system controller (all from Shimadzu, Kyoto, Japan) was used. The chromatographic data were recorded using an HP-Vectra (Hewlett Packard, Waldron, Germany) computer system with LC solution data acquisition software (Shimadzu, Kyoto, Japan). A vortex shaker, sample tubes, repeater (Tarsons, Chennai, India) and centrifuge (model 2–16P, supplied by Sigma, Zurich, Switzerland) were used.After several trials, chromatographic separation was accomplished on water symmetry C_{18} column (250×4.6 mm; 5 μm; Quadrex, Woodbridge, USA) under isocratic mode of elution.

The mobile phase was a mixture of Acetonitrile: Methanol (85:15, v/v). Degassed continuously by an on-line degasser. Separation was performed at room temperature using a 0.7 mL/min flow-rate and 20 min run time. The injection volume was 20 μL and the detection wavelengths were set at 447 nm. The chromatographic and the integrated data were recorded using an HP-Vectra (Hewlett Packard, Waldron, Germany) computer system using LC-Solution data acquiring software (Shimadzu, Kyoto, Japan). Lutein and zeaxanthin standards were purchased from sigma Aldrich company.

RESULTS AND DICUSSION

The concentration of lutein was maximum in all plant when compared to zeaxanthin.

Maximum lutein concentration was reported in *Basella alba* (504 ppm purple variety), in *Brassica oleracea var.capitata* (314.9 ppm green variety)and in *Zaleya decondra* (309.28 ppm green variety).Lowest content of lutein was recorded) in *Zaleya decondra* (60.91ppm red variety).The content of lutein was reported to be high in purple, green colored varieties of all plants.

Maximum content of zeaxanthin was reported in *Brassica oleracea var.capitate* (147.38 ppm green variety),lowest concentration was reportedin*Cucurbita pepo var. cylindrical* (0.90 ppmred variety), and (0.70ppm in yellowvariety).The concentration of zeaxanthin was recorded high in purple, green,varieties of all plants.

Similar studies where done on the content of lutein and zeaxanthin inseveral leafy vegetable by many persons (expressed as mg/100g D.W).[16] has reported the content of lutein and zeaxanthin (113.87), (1.76) mg/100g D.W.,),respectively in *Basella alba.*, (33.97), (0.14) mg/100g D.W) in *Brassica oleracea var. botrytis*, (90.43), (1.04) mg/100g D.W) in *Cucurbita maxima*, (181.30), (2.06) mg/100g D.W.), in *Trianthema Portulacastrum*, (32.47), (0.26) mg/100g D.W., in *Amaranthus sessilis* has recorded the content of zeaxanthin (331 µg/100g F.W.) in spinach, (187 µg/100g F.W.) in Lettuce,(23 µg/100g F.W.) in carrots, (3 µg/100g F.W.) in celery.Majority of our observations are similar to the results of the other workers

CONCLUSION

Out of the of five green and colored plants investigated, the *Basella alba* (purple) and *Brassica oleracea* var. *capitate* (green) has recorded maximum content of lutein and zeaxanthin.In view of this, consuming these plants in their diets of people having diagnosed macular degeneration(MD) ,can delay the process of MD ,and also prevent MD in many people.

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