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 yellow varieties, *Brassica oleracea var. capitata* L. f. *alba*, Cabbage green and purple varieties, *Zaleya decandra* L. green and purple varieties, *Trianthema portulacostrum* 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 *Zaleya decandra* (309.28 ppm green variety). Lowest content of lutein was recorded in *Zaleya decandra* (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. capitata* (147.38 ppm green variety). Lowest concentration was reported in *Cucurbita pepo var. cylindrica* (0.90 ppm red variety), and (0.70 ppm 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. Lutein and zeaxanthin have a potential role in the prevention and treatment of eye diseases like age-related macular degeneration, cataract and other 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 zeaxanthin from 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 (C₉H₁₂O₂), and bete-cryptoxanthin (C₁₀H₁₆O) and carotenes (α-carotene, β carotene and lycopene (C₉H₁₈O₂)).

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. The presence of a hydroxyl group at both ends of the molecules distinguishes lutein and zeaxanthin from 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 (C₉H₁₂O₂), and bete-cryptoxanthin (C₁₀H₁₆O) and carotenes (α-carotene, β carotene and lycopene (C₉H₁₈O₂))

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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. Most recent reports indicate that the mean dietary intake of lutein and zeaxanthin in are 0.8 mg to 2.4 mg per day approximately.

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.

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

MATERIAL AND METHOD

Experimental plant materials

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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 oleracea var. capitata L., were obtained from local market whereas, Zaleya decandra L. Trianthema portulacastrum Linn were collected from wild. Later they were washed with water, sun dried, pulvarized 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 HPLC analysis. About 10 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 1 h 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.

RESULTS AND DISCUSSION

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 decandra (309.28 ppm green variety). Lowest content of lutein was recorded in Zaleya decondra (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. capitata (147.38 ppm green variety), lowest concentration was reported in Cucurbita pepo var. cylindrica (0.90 ppm purple variety) and in Zaleya decandra (0.14 ppm red variety). The content of zeaxanthin was recorded high in purple, green, varieties of all plants.

Similar studies where done on the content of lutein and zeaxanthin in several leafy vegetable by many persons (expressed as mg/100g D.W.), 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 five green and colored plants investigated, the *Basella alba* (purple) and *Brassica oleracea* var. *capitata* (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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