

## 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, *Zaleyadecondra*. L green 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. *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<sup>1,2,3</sup>. Lutein and zeaxanthin have a potential role in the prevention and treatment of eye disease like age-related macular degeneration, cataract<sup>4,5,6,7,8</sup>. 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 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 (C<sub>40</sub>H<sub>56</sub>O<sub>2</sub>), and beta-cryptoxanthin (C<sub>40</sub>H<sub>56</sub>O) and carotenes ( $\alpha$ -carotene,  $\beta$  carotene and lycopene (C<sub>40</sub>H<sub>56</sub>)).

Humans being do not have the capacity to synthesis lutein and zeaxanthin, and therefore has to be taken as dietary source, while mesoxanthin 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<sup>9</sup>. 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<sup>10</sup>.

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<sup>11</sup>.

The content of beta carotene, lutein and zeaxanthin examined in thai vegetable by<sup>12</sup> HPLC analysis of lutein and zeaxanthin studied<sup>13</sup>.

### MATERIAL AND METHOD

*Experimental plant materials*

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

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

Table 2: Content of zeaxanthin in green and colored varieties of vegetables.

Plant name	Colour variety of plants	Zeaxanthin (ppm)
<i>Basella alba</i>	Green	12.48
	Purple	40.96
<i>Brassica oleraceavar.capitata</i>	Green	147.38
<i>Cucurbita pepo var. cylindrica</i>	Purple	8.87
	Green	0.90
<i>Trianthema</i>	yellow	0.70
<i>portulacastrum</i>	Green	12.12
<i>Zaleyadecondra</i>	Red	36.3
	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 HPLC analysis<sup>14</sup>. 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 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<sup>15</sup>.

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<sub>18</sub> 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 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 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. cylindrical* (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.

Similar studies were done on the content of lutein and zeaxanthin in several leafy vegetable by many persons (expressed as mg/100g D.W).<sup>16</sup> 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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## REFERENCE

- Ozawa Y, Nagai N, Suzuki M, Kurihara T, Shinoda H, Watanabe M, Tsubota K. Effect of Content intake of lutein rich spinach on macular pigment optical density : a pilot study. *Nippon Ganka Gakkai Zashi* (2016); JAN ;120(1):41-8.
- Le Ma, Rong Liu, Jun Hui Du, Tao Liu, Shan Shan Wu, and Xiao Hong Liu. Lutein, Zeaxanthin And Meso-zeaxanthin Supplementation Associated with Macular Pigment Optical Density. *Nutrients* (2016); 8,426; doi:10.3390/nu8070426.
- Maria Ramirez. Why lutein is important for the eye and the brain. *OCL* (2016); 23(1) D107 , DOI: 10.1051/ocl/2015027.
- Huang LL, Coleman HR, Kim J, de Monasterio F, Wong WT, Schleicher RL, Oral supplementation of lutein/zeaxanthin and omega-3 long chain polyunsaturated fatty acids in persons aged 60 years or older, with or without AMD. *Invest Ophthalmol Vis Sci* (2008); 49:3864–3869.
- Trieschmann M, Beatty S, Nolan JM, Hense HW, Heimes B, Austermann U, Changes in macular pigment optical density and serum concentrations of its constituent carotenoids following supplemental lutein and zeaxanthin: the LUNA study. *Exp Eye Res* (2007); 84:718–728.
- Catharine R. Gale, Nigel F. Hall, David I. W. Phillips, and Christopher N. Martyn Lutein and Zeaxanthin Status and Risk of Age-Related Macular Degeneration. *Investigative Ophthalmology & Visual Science*, June (2003); Vol. 44, No. 6.
- AREDS-1 (age related eye disease study), national eye institute 2001.
- AREDS-2 (age related eye disease study), national eye institute 2006.
- E. J. Johnson, R. Vishwanathan, M. A. Johnson., "Relationship between serum and brain carotenoids,  $\alpha$ -tocopherol, and retinol concentrations and cognitive performance in the oldest old from the georgia centenarian study," *Journal of Aging Research*, vol. (2013); Article ID 951786, 13 pages.
- Van Leeuwen R, Boekhoorn S, Vingerling JR, Witteman JC, Klaver CC, Hofman. Dietary Intake of antioxidants and risk of age-related macular degeneration. *JAMA* (2005); 294:3101–3107.
- Shun-Fa Yang, Joan E. Roberts, Qing-huai Liu, Jijing Pang, and Tadeusz Sarna. Zeaxanthin and Lutein in the Management of Eye Diseases. *J Ophthalmol* (2016); 4915916.
- Busaba Tharasena and Siriporn Lawan Content of Beta-Carotene, Xanthophyll, Lutein and Zeaxanthin in Vegetables of Thai side Dish. *International Conference on Nutrition and Food Sciences*. (2012); vol. 39, IACSIT Press, Singapore.
- Markus Dachtler, Tobias Glaser, Konrad Kohler, and Klaus Albert Combined HPLC-MS and HPLC-NMR On-Line Coupling for the Separation and Determination of Lutein and Zeaxanthin Stereoisomers in Spinach and in Retina. *Anal. Chem.* (2001); 73, 667-674.
- Hart DJ, Scott KJ. Development and evaluation of an HPLC method for the analysis of carotenoids in foods, and the measurement of the carotenoid content of vegetables and fruits commonly consumed in the UK. *Food Chem* (1995); 54: 101-111.
- Marisiddaiah Raju, Sadineni Varakumar, Rangaswamy Lakshminarayana, Thirumalai Parthasarathy Krishnakantha, Vallikannan Baskaran. Carotenoid composition and vitamin A activity of medicinally important green leafy vegetables, *Food Chemistry* 101 (2007) 1598–1605.
- Joanne M. Holden, carotenoid content of U.S foods : An Update of the Database. *Journal of food composition and Analysis*. (1999), vol 12, issue 3, September, pages 169-196.