

LUTEIN FROM *CHLORELLA VULGARIS*: A SOURCE FOR THE TREATMENT OF AGE-RELATED MACULAR DEGRADATION (AMD): - A REVIEW

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Abstract

Lutein, a natural xanthophyll pigment found in green plants and microalgae like *Chlorella vulgaris*, has the formula $C_{40}H_{56}O_2$ and has high antioxidant effects. *Chlorella vulgaris* produced reactive oxygen species (ROS), which exposed to stress conditions like strong light, deflection in nutrition, salt and high temperature. To neutralize the reactive oxygen species, lutein play a vital role. It is synthesized in chloroplasts via the Methylerythritol phosphate (MEP) route, preserves the photosynthetic system and promotes cell stability. In humans, it accumulates in the retinal macula, where it filters damaging blue light and promotes visual health. To extract lutein effectively, the algal cell wall must be disrupted. Using sophisticated techniques like ultrasound, microwave, enzyme-assisted, or supercritical CO_2 help to separate lutein from *Chlorella vulgaris*. For the purification of lutein use of liquid-liquid partitioning chromatography and counter-current chromatography. The other chromatography like HSCCC, HPLC, and CPC recommended for industrial scale purpose. A Lutein molecules have significant biological, industrial, and therapeutic applications, notably in increasing visual function and treating age-related macular degeneration.

Key word: - Lutein, *Chlorella vulgaris*, AMD, MPOD

Introduction

Chlorella vulgaris is unicellular algae, belonging to division Chlorophyta. It contains pigments like xanthophyll and carotenoid. *Chlorella vulgaris* synthesized the lutein molecule naturally (**Plate A fig.no.1**) [1]. Lutein is essential for prevention of photo-system damage and protects plant cells from light damage [2]. *Chlorella vulgaris* generates increased lutein in response to environmental stresses such as high light intensity, nitrogen deficiency, salinity, and temperature fluctuations [3]. For self-defence, the algae produces more carotenoid, notably lutein, which acts as a potent antioxidant [4]. The stress-induced lutein synthesis serves as a preventive survival strategy in *Chlorella vulgaris* [4]. Lutein is produced in chloroplasts via the Methylerythritol phosphate (MEP) pathway. Due to the presence of oxygen atoms, in its structure, it is classified as a xanthophyll. Lutein appears as a yellow to orange colour due to its ability to absorb blue light [5]. In the visible spectrum, its absorption is 445 nm [2, 5]. The Chemical formula of lutein is $C_{40}H_{56}O_2$, with a molecular weight of **568.87 g/mol** [5]. Lutein is tetraterpenoid molecule having a 40-carbon backbone. Its structure consists of a long polyene chain with conjugated double bonds [6]. This conjugated system is responsible for its high antioxidant activity. Lutein has two cyclic end groups: one β -ionone and one ϵ -ionone ring [7].

It also has two hydroxyl (-OH) functional groups at certain carbon positions. Lutein hydroxyl groups make it more polar than other carotenoids (**Plate A fig.no.2**). Lutein molecule occurs in both Trans and cis-isomers, with all-trans being the most stable [8].

Lutein is susceptible to heat, light, and oxygen, which can all cause deterioration. It is insoluble in water due to its lipo-philicity [7, 8]. It is more dissolves in nonpolar solvents such as hexane and chloroform [9]. It is also dissolve in polar solvents like ethanol and acetone.

In human, Lutein was found in macula of human eyes, help to prevent excess of light. Lutein protects the retina by blocking blue light and reducing oxidative damage [10]. It acts as an antioxidant in the macula, work to maintain healthy photoreceptor. Lutein help in the curing of age-related macular degeneration, and reduce the risk of serious vision loss (**Plate A fig.no.3**) [1, 2, 11].

Methods and Material

1. Extraction (Plate A fig.no.4)

Lutein is extracted from *Chlorella vulgaris*, using simple approach which involves, solvent extraction using acetone or ethanol, which is inexpensive and appropriate for laboratory-scale work and easily available [1, 3]. The use of food-grade ethanol in combination with bead milling or homogenization can enhance safely extraction of Lutein molecule [4, 5].

For Lutein purity, saponification process is used with alcoholic KOH [6, 12]. The Ultrasound-assisted extraction improves efficiency by breaking down cell walls via cavitation, it required less time and help to save solvent consumption and easily extract entire Lutein [7, 13]. The microwave-assisted extraction is also used to speed up the process by quickly heating intracellular moisture, resulting in faster pigment release which were associated Lutein molecule [8].

The Supercritical CO₂ extraction provides pure lutein without harmful solvent residues. The modern studies expand that ethanol work as co-solvent to extraction lutein more effectively [14]. Enzyme-assisted extraction employs cellulase or similar enzymes to breakdown the algal cell wall and easily separation Lutein from cell and help maintain stable structure of lutein [15].

2. Separation or purification

There are many purification technique which are as follows, (**Plate A fig.no.4**)

Liquid-liquid partition chromatography is a simple and cost-effective approach that can achieve 85-90% recovery and purity levels of Lutein [1]. High-speed counter-current chromatography (HSCCC) effectively separates lutein, about 95-98% purity in a single pass of column [2]. High-performance counter-current chromatography (HPCCC) enhances scalability while maintaining excellent purity and recovery [1, 2]. The Gel permeation chromatography is occasionally used as a polishing step to eliminate small contaminants and easily obtaining Lutein [5].

Flash column chromatography on silica gel achieves extremely high purity (up to 99%), but cost effective [5]. Centrifugal partition chromatography (CPC) is a new liquid-liquid chromatography widely used in industrial applications [6].

Result

Chlorella vulgaris is a single cell, non-motile green algae. Now a day *Chlorella vulgaris* is widely used for commercial purpose in agriculture and industries. Due to high availability of *Chlorella vulgaris* help to use in Lutein extraction, purification and it commercial uses. One of application of Lutein is protects against age-related macular degeneration (AMD) in human eyes. Lutein accumulates in the macula, the core area of the retina crucial for clear vision. Lutein contributes to the filtering of damaging blue light entering

the eye by producing macular pigment. This filtering activity minimizes photo oxidative damage to retinal cells. Oxidative stress is a crucial component in the development of AMD, and lutein is a powerful antioxidant. It neutralizes reactive oxygen species and prevents photoreceptor degradation. Higher lutein consumption enhances macular pigment optical density (MPOD).

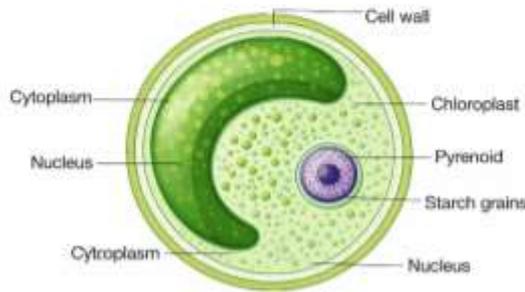
Higher MPOD levels correlate with improved visual performance and contrast sensitivity. Lutein also work in anti-inflammatory effects, which aid to decrease retinal damage. It stabilizes cell membranes and lowers lipid peroxidation in ocular tissues.

Long-term supplementation may slow the progression from intermediate to advanced AMD. Individuals with greater blood lutein levels had a decreased chance of serious vision loss. Lutein is regarded as safe and well tolerated for long-term usage. It is frequently advised as part of nutritional therapy for AMD treatment. So it is necessary to maintain Lutein in the dietary supplements.

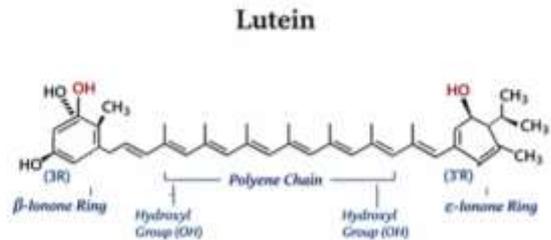
Conclusion

Chlorella vulgaris is the source of Lutein production at industrial scale level. Lutein have many application in biological use, commercial, and medicinal purpose. It promotes retinal health by filtering blue light and decrease the oxidative damage associated with age-related macular degeneration (AMD). With additional supplement of Lutein in diet help to improve the eye vision.

Plate No. A



Chlorella vulgaris
 Fig.No. 1



Molecular Formula: $C_{40}H_{56}O_2$
 Molecular Weight: 568.87 g/mol

Fig.No.2

Age-Related Macular Degeneration (AMD)

Normal Eye

AMD Eye

Progression of AMD

Early AMD		Intermediate AMD		Late AMD	
Drusen yellow deposits		Pigment Changes		Dry AMD Geographic Atrophy	Wet AMD Choroidal Neovascularization

Vision Changes with AMD

Blurry Central Vision

Dark or "Blind Spot"

Distorted & Wavy Vision

Fig.No.3

Purification Methods of Lutein from *Chlorella vulgaris*

Lab Scale: Simple & Economical Industrial Scale: High Purity & Efficient

Fig.No.4

References

1. Bernstein, Paul S., et al. "Lutein, Zeaxanthin, and Meso-Zeaxanthin: The Basic and Clinical Science Underlying Carotenoid-Based Nutritional Interventions against Ocular Disease." *Progress in Retinal and Eye Research*, vol. 50, 2016, pp. 34–66. <https://doi.org/10.1016/j.preteyeres.2015.10.003>.
2. Bone, Richard A., John T. Landrum, and Susan L. Tarsis. "Preliminary Identification of the Human Macular Pigment." *Vision Research*, vol. 25, no. 11, 1985, pp. 1531–1535. [https://doi.org/10.1016/0042-6989\(85\)90123-4](https://doi.org/10.1016/0042-6989(85)90123-4).
3. Krinsky, Norman I., John T. Landrum, and Richard A. Bone. "Biologic Mechanisms of the Protective Role of Lutein and Zeaxanthin in the Eye." *Annual Review of Nutrition*, vol. 23, 2003, pp. 171–201. <https://doi.org/10.1146/annurev.nutr.23.011702.073307>.
4. Ma, L., et al. "Lutein and Zeaxanthin Supplementation Improves Visual Performance in Patients with Age-Related Macular Degeneration." *Ophthalmology*, vol. 119, no. 11, 2012, pp. 2290–2297. <https://doi.org/10.1016/j.ophtha.2012.06.002>.
5. Age-Related Eye Disease Study 2 (AREDS2) Research Group. "Lutein + Zeaxanthin and Omega-3 Fatty Acids for Age-Related Macular Degeneration." *JAMA*, vol. 309, no. 19, 2013, pp. 2005–2015. <https://doi.org/10.1001/jama.2013.4997>.
6. Del Campo, Juan A., et al. "Outdoor Cultivation of Microalgae for Carotenoid Production: Current State and Perspectives." *Applied Microbiology and Biotechnology*, vol. 74, no. 6, 2007, pp. 1163–1174. <https://doi.org/10.1007/s00253-007-0844-9>.
7. Gong, Meng, and Amarjeet Bassi. "Carotenoids from Microalgae: A Review of Recent Developments." *Biotechnology Advances*, vol. 34, no. 8, 2016, pp. 1396–1412. <https://doi.org/10.1016/j.biotechadv.2016.10.005>.
8. Lin, J. H., D. J. Lee, and J. S. Chang. "Lutein Production from Microalgae: Biosynthesis, Extraction, and Purification." *Bioresource Technology*, vol. 184, 2015, pp. 421–428. <https://doi.org/10.1016/j.biortech.2014.09.052>.
9. Ribeiro, H. S., and H. Schubert. "Aqueous Emulsions for Lutein Encapsulation." *Journal of Agricultural and Food Chemistry*, vol. 51, no. 24, 2003, pp. 7118–7123. <https://doi.org/10.1021/jf0347030>.
10. Zhao, L., G. Chen, and X. Hu. "Supercritical CO₂ Extraction of Carotenoids from Microalgae: Optimization and Purification." *Journal of Supercritical Fluids*, vol. 112, 2016, pp. 97–104. <https://doi.org/10.1016/j.supflu.2016.02.019>.
11. Chandanshive, V. R., and P. B. Cholke. "Assessment of Fresh Water Algal Diversity of Jawhar, Dist-Palghar (MS), India." *Ecology, Environment and Conservation*, vol. 28, Oct. Suppl. Issue, 2022, pp. S306–S310. <https://doi.org/10.53550/EEC.2022.v28i06s.052>.
12. Mercadante, A., et al. *Carotenoids Handbook*. Edited by G. Britton, S. Liaaen-Jensen, and H. Pfander, Springer Science & Business Media, 2004.
13. Akuffo, K., et al. "Sustained Supplementation and Monitored Response with Differing Carotenoid Formulations in Early Age-Related Macular Degeneration." *Eye*, vol. 29, 2015, pp. 902–912.
14. Shyam, R., et al. "RPE65 Has an Additional Function as the Lutein to Meso-Zeaxanthin Isomerase in the Vertebrate Eye." *Proceedings of the National Academy of Sciences of the United States of America*, vol. 114, 2017, pp. 10882–10887.

15. Karadas, F., et al. "Tissue-Specific Distribution of Carotenoids and Vitamin E in Tissues of Newly Hatched Chicks from Various Avian Species." *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, 2005.