Microalgae nutrient requirements for enhanced lutein production

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ABSTRACT

Microalgae has growing importance in the feed, food, and pharmaceutical industries, with impressive contents of protein, carbohydrates, and other interesting bioproducts. In particular, carotenoids derived from microalgae are gaining attention due to their nutraceutical properties and high productivity, a result of the fast growth rate of these microorganisms. Microalgae already are an important source of the carotenoids astaxanthin and β-carotene. Lutein as a microalgae product is emerging but promising. While several studies have proved the capacity of specific microorganisms to produce large amounts of astaxanthin or β-carotene, there isn't yet a specific strain recognized as a superior producer of lutein. As a primary pigment, lutein can be obtained proportionally to biomass production – apparently, with no benefit of "stressing" induction phases. Therefore, optimizing cultivation conditions for selected strains is the main requisite for lutein production. Based on that, this document brings a compilation and analysis of recent research about the nutritional composition of the media and the key ingredients that may affect lutein production by microalgae.

Keywords: Natural pigments. Antioxidant. Chlamydomonas. Muriellopsis. Retina.

1 INTRODUCTION

Based on the European Food Safety Authority (EFSA, 2010), lutein is recognized as a nutritionally important carotenoid pigment in the human diet. The expected daily intake advocated is 1 mg kg^{-1} of lutein per body weight. The consumed lutein and its isomers zeaxanthin and meso-zeaxanthin play crucial roles in the health of the human eye's macula, preventing and aiding in the recovery of Age-Related Macular Degeneration (ARMD), a leading cause of vision loss in industrialized countries. The pigment is commonly consumed from various natural sources, especially green leafy vegetables and green algae. Lutein is a crucial component of the light-harvesting protein complex, associated with photosystem II in photosynthetic organisms. Classified as a xanthophyll, lutein acts as part of the antenna complex that captures energy capture for photosystem II reaction centers in the chloroplast [1].

Lutein can be found in higher concentrations in green leafy vegetables such as spinach and kale, which have lutein contents of 1.1 mg g^{-1} and 1.0 mg g^{-1}, respectively, on a dry basis. Microalgae can reach up to 17 mg Lutein g^{-1} dry biomass, making them a good production source. Research already exists on industrial microalgae strains capable of high lutein productivity. Additionally, some microalgae are already produced for direct consumption (e.g., *Spirulina* sp. and *Chlorella* sp.) or for extract valuable fractions (e.g., astaxanthin from *Haematococcus* sp. and β-carotene from *Dunaliella* sp.). However, there are no related microalgae that accumulate lutein as a secondary carotenoid, unlike the examples above with astaxanthin and β-carotene. As a primary carotenoid, lutein production occurs in parallel with biomass growth under well-optimized conditions [2].

In terms of cultivation, various advancements have been proposed for microalgae biomass and lutein production: light configurations, abiotic stress, and medium optimization. Among these, medium manipulation seems to be more feasible, as high biomass production directly impacts the final lutein production (g L^{-1}). Moreover, optimized media can create the most adequate environment for microalgae metabolism, including photosynthesis [1]. Stress factors such as nutritional deprivation, as is performed with *Haematococcus*, and salinity increase with *Dunaliella*, usually do not work for the accumulation of lutein. As previously mentioned, the decrease in biomass health results in this pigment depletion. Similarly, light strategies may cause stress at high value of irradiance levels, and insufficient light will result in low biomass production. The color of light applied to the cultivation seems to be effective for lutein production, which can be explained by the protective of the carotenoids in the photosynthetic system [3]. In this work, a brief review of microalgae lutein and the importance of nutritional compounds in the media that can affect the final production is presented.

2 REVISION

Similar to other organisms, microalgae require several components to grow. Those needs can be determined throught laboratory scale experiments for culture optimization. For specific bioproduct production, the most common strategy is a two-phase culture system: first phase of biomass growth, and second phase where accumulation of the pigments may occur [4] For lutein production, this strategy is not recommended, as mentioned previously, no strains that are capable of secondary lutein accumulation have been reported until now. According to Fu et al. [3], the research is unclear about the capacity of studied algae on lutein increase after abiotic stress. For most types of strains, stress leads to the end of biomass or cellular growth.

In terms of nutrients, the basis of culture medium contains nitrogen, phosphorus, calcium, potassium, magnesium, microelements, and vitamins. Although autotrophic, they grow well on heterotrophic and mixotrophic conditions. The media must be balanced in terms of the proportion of N to P. Significant biomass production can only occur after population increases, which means protein and cell wall synthesis, nucleic acid replication, and energy carrier production. Several minerals are also needed for microalgal growth: from Mg which is essential in chlorophyll, to potassium and sodium for osmoregulation and maintenance of cell potential, to trace elements that are cofactors for enzymes. All this will require nitrogen and phosphorus. As microalgae can grow in various types of synthetic media, mixtures with residues, and even with organic carbon sources, studies have been conducted to enhance lutein production by algae [4]. Microelements seem to impact biomass and lutein production but are less explored in comparison to macronutrients (i.e., C: N:P ratios). In Table 1, related strains and the media studied are presented for lutein production.

Table 1 Related media, strains, and lutein content on biomass.

Microalgae	Medium	Important compounds	Biomass production ($g\ L^{-1}$)	Lutein content ($mg\ g^{-1}$)	Lutein productivity ($mg\ L^{-1}\ day^{-1}$)	Reference
Chlorella sorokiniana MR-16	BG11	CO_2	8,3	5	*2,2	
Chlorella sorokiniana MB-1-M12	BG11 + glucose	Heterotrophic	10,8	4,84	6,96	
Chlorella sorokiniana Kh12	Benjerinck + acetate	Mixotrophic (CO_2 + acetate)	4,61	14,3	0,451	3
Chlorella protothecoides CS-41	BBM	Mixotrophic (CO_2)	31,2	4,44	*8,77	
		Heterotrophic (only glucose)	46,9	4,92	22,67	
Murielopsis sp.		CO_2	5,37	5,55	*2,48	
Chlorella minutissima	BBM	Normal	*0,64	2,67	0,228	2
	Optimized BBM	Microelement Cu and Mn	*1,75	5,58	0,655	
Chlorella vulgaris	BBM	N, P, K, Ca, pH, temperature, salinity, and light optimization	Nd	*88,0	Nd	5
Chlorella protothecoides UTEX 205	Modified Bristol's medium	Containing 15 g L^{-1} reducing sugar	6,59	0,175	*0,096	6
	Fruit and vegetable juices	Containing 15 g L^{-1} reducing sugar	7,28	204,98	*124,35	
Tetradesmus almeriensis	Freshwater		4,0	1.154		
	Primary wastewater		8,6	0.732	Nd	7
	Secondary wastewater		7,2	0,943		
	Diluted pig slurry		6,2	1,290		
Scenedesmus almeriensis	Mann & Myers medium + CO_2	3% CO_2 injection (without re-usage of medium)	3,7	5,71	*1,76	8
Haematococcus pluvialis	Optimized BBM	Without Vn, I, Bo, and Zn.	$3,77.\ 10^5$ cells mL^{-1}	*3,16	*672,92	9
	Green phase			4,18		
	Red phase			4,20		10

* Values were calculated from article data (i.e., content in % to mg g^{-1} or productivity expected on cultivation time). Nd: not described.

It must be highlighted that green microalgae are rich in lutein. This group, Chlorophyceae, includes various species such as *Chlorella* sp., *Scenedesmus* sp., *Neochloris* sp., *Muriellopsis* sp., *Tetradesmus* sp., *Chlamydomonas* sp., *Dunaliella* sp. and *Haematococcus* sp. Interestingly, the last two algae, Dunaliella and Haematococcus, which produce orange β-carotene and red astaxanthin, are green during the vegetative phase. This characteristic makes them valuable for research focused on lutein production. In *Chlorella's* sp. lutein content is around 5 to 14 mg g^{-1} and productivity at 2.2 to 124 mg L^{-1} day^{-1} is conducted with inorganic or added organic carbon sources. Similarly, *Haematococcus* sp. contains 4,2 mg g^{-1} of lutein in the biomass. Unlike other species, *Haematococcus* does not seem to lose lutein during the red phase, making this alga as an interesting source of both astaxanthin and lutein, counterbalancing the low growth rate of [10]. In some studies, with *C. minutissima* [2] and *C. vulgaris* [5], medium optimization strategies had a great impact on biomass and lutein production, in contrast to cultivation supplemented with organic sources, as optimization leads to an economical medium without addition of more compounds. In particular, *C. protothecoides* growing on expired juice residue [6] highlights the importance of microalgae on residual/effluent valorization. In figure 2 a graphical comparison of some microalgae mentioned are presented for better clarity.

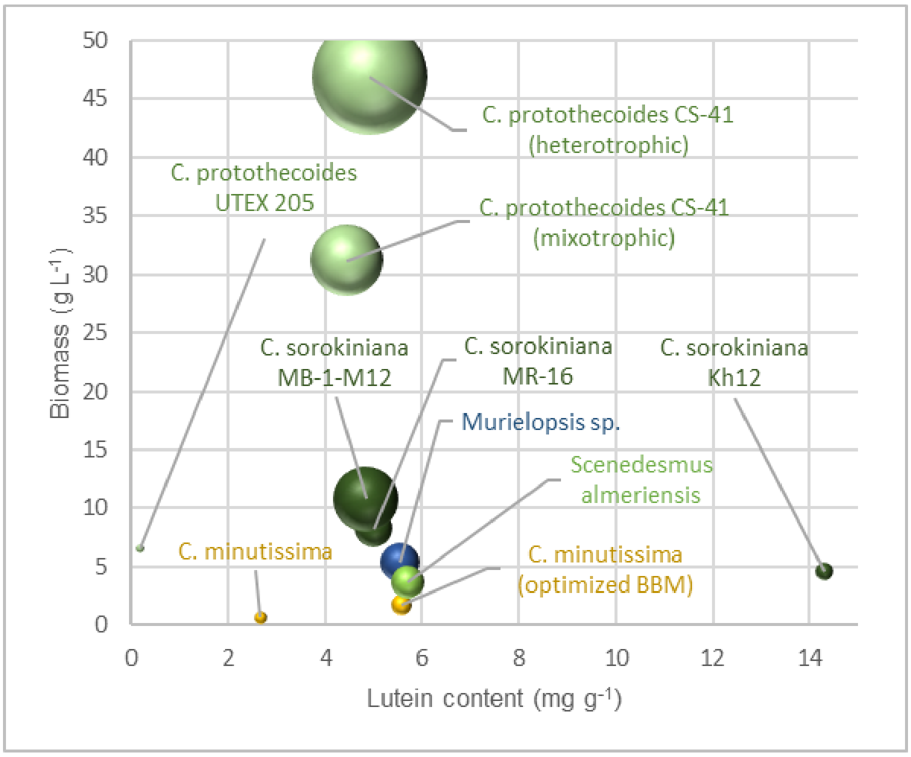

Figure 1 Lutein productivity (mg L^{-1} day^{-1}) represented by buble size. Color differs microagae by same species group.

3 CONCLUSION

Lutein is gaining importance as a nutraceutical and pharmaceutical for ocular diseases. Lutein is poised to join other microalgal carotenoids on the market, such as β-carotene from *Dunaliella* sp. and astaxanthin from *Haematococcus* sp. Highly productive strains such as those of *Chlorella* and *Muriellopsis*, have already been isolated and can be used to develop efficient industrial processes. However, unlike secondary carotenoids, lutein production does not increase under stress conditions. It can however, be modulated by increasing biomass production, which requires culture media optimization as the primary pathway for process intensification. By media manipulation and wastewater treatment valorization, microalgae can be cultivated at lower costs.

REFERENCES

1. IWAMOTO, H. *et al.* Lutein from Microalgae: An Industrial Perspective of Its Production, Downstream Processing, and Market. *Fermentation* **10**, 106 (2024).
2. DINESHKUMAR, R., DHANARAJAN, G., DASH, S. K. & SEN, R. An advanced hybrid medium optimization strategy for the enhanced productivity of lutein in Chlorella minutissima. *Algal Res* **7**, 24–32 (2015).
3. FU, Y. *et al.* Lutein production from microalgae: A review. *Bioresour Technol* **376**, 128875 (2023).
4. DE CARVALHO, J. C., SYDNEY, E. B., ASSÚ TESSARI, L. F. & SOCCOL, C. R. Culture media for mass production of microalgae. in *Biomass, Biofuels, Biochemicals: Biofuels from Algae, Second Edition* 33–50 (Elsevier, 2018). doi:10.1016/B978-0-444-64192-2.00002-0.
5. YADAV, S., BANSAL, S., CHAITHRA, M. L. & SIBI, G. Assessment of optimal growth conditions for specific carotenoids production by chlorella vulgaris. *Journal of Applied and Natural Science* **12**, 550–555 (2020).
6. IBARRURI, J., MANSO, M. & CEBRIÁN, M. Enhancement of Biomass and Protein Production of Chlorella protothecoides in Heterotrophic Cultivation Using Expired Juices as Alternative Source of Nutrients for an Added-Value Biorefinery Scheme. *Fermentation* **9**, (2023).
7. VILLARÓ-COS, S. *et al.* Composition of microalgae produced using different types of water and nutrient sources. *Algal Res* **78**, (2024).
8. MOLINO, A. *et al.* Enhancing Biomass and Lutein Production From Scenedesmus almeriensis: Effect of Carbon Dioxide Concentration and Culture Medium Reuse. *Front Plant Sci* **11**, (2020).
9. FÁBREGAS, J., DOMÍNGUEZ, A., REGUEIRO, M., MASEDA, A. & OTERO, A. Optimization of culture medium for the continuous cultivation of the microalga Haematococcus pluvialis. *Appl Microbiol Biotechnol* **53**, 530–535 (2000).
10. HU, Z., LI, Y., SOMMERFELD, M., CHEN, F. & HU, Q. Enhanced protection against oxidative stress in an astaxanthin-overproduction Haematococcus mutant (Chlorophyceae). *Eur J Phycol* **43**, 365–376 (2008).

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