Poster code: M29

Carotenoid profile of *Tetraselmis striata* grown under optimal cultivation conditions in a lab pilot-scale raceway pond

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Introduction

Microalgae are recognized as a valuable natural source of bioactive compounds, such as proteins, lipids, pigments and vitamins, for the aquaculture industry and their global market is projected to reach approximately 53.43 billion USD by 2026. Among the few high-value added products derived from microalgae, carotenoids have made a real impact on the global economy. Several carotenoids have been identified in microalgae, such as b-carotene, astaxanthin and lutein, which have the most significant market potential, although violaxanthin, antheraxanthin, zeaxanthin and neoxanthin are also commonly found.

Objective: The objective of this study was to evaluate the carotenoid profile of the microalgae *Tetraselmis striata* cultivated in laboratory and lab pilot-scale production, under different pH, temperature and photoperiod, to determine the optimum conditions for cell growth and carotenoid production.

Material and methods

- **Standard solutions** (astaxanthin, lutein, zeaxanthin, canthaxanthin, echinenone, lycopene, b-cryptoxanthin, b-carotene)
- **Internal standard** (trans-8'-apo-beta-Caroten-8'-al)

➢ **Carotenoid extraction**

- Centrifugation of wet microalgae biomass (7,000rpm/ 5°C/ 5mins)
- Freeze-dry (3 days)
- Weight 15 mg microalgae +38.50 μL internal standard (10 ppm)
- Vortex for 30s
- +450μL MeOH/H_2O (1:1)
- Shake for 1 hour
- Extraction with chloroform until colorless
- Evaporation

Saponification protocol

- Reconstitution in 4 ml Petroleum ether/Diethyl ether (1:1)
- +4 ml KOH 10% in MEOH
- Overnight stirring
- +4 ml ultrapure H_2O
- Extraction with P. ether / D. ether until colorless
- Evaporation
- Reconstitution in 1 ml MeOH/$CHCl_3$ (9:1)
- 0.45μm PVDF syringe filter
- Injection in UPLC-QTOF

Non-saponification protocol

- Reconstitution in 1 ml MeOH/$CHCl_3$ (9:1)
- 0.45μm PVDF syringe filter
- Injection in UPLC-QTOF

Figure 1. Freeze-dried *T. striata* biomass

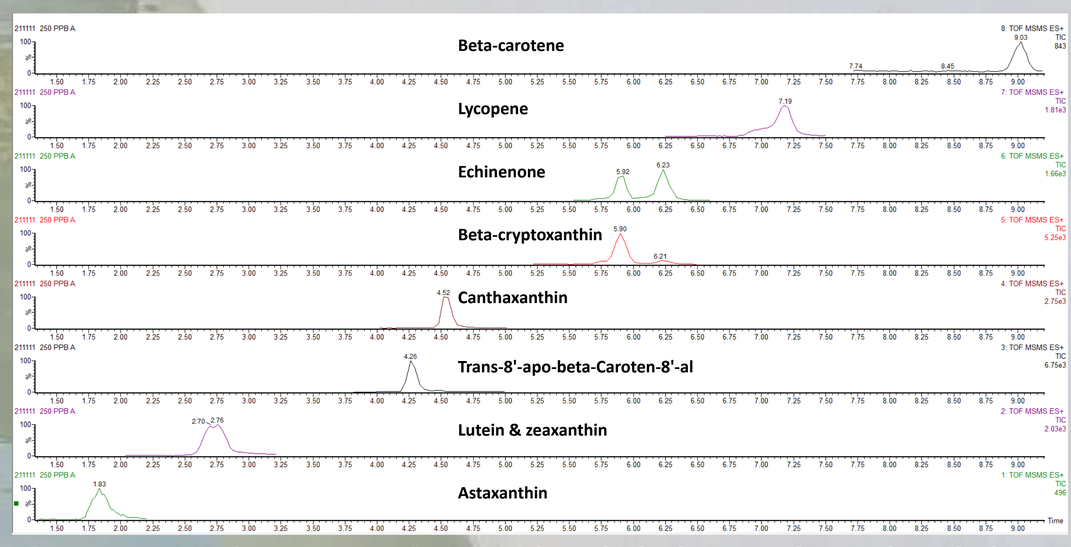

Figure 2. LC-MS chromatogram of carotenoid standard solutions and internal standard

Table 1. Chromatographic conditions of Acquity UPLC H-Class

UPLC system	UPLC H-Class (Waters, USA)
Analytical column	C18 BEH column (50 mm, 2.1 mm, 1.7 mm)
Mobile phase	Gradient elution A: 0.1% aqueous formic acid B: acetonitrile (0.1% formic acid)
Injection volume	4 μL
Flow rate	0.4 - 0.6 ml/min
Column temperature	32°C
Sample temperature	15°C

Figure 3. Acquity UPLC H-Class UPLC coupled with Q-TOF Premier mass spectrometer

Figure 4. Pilot-scale raceway pond (culture volume 40 L)

Results

✓ **The targeted carotenoids were detected in all the samples except lycopene**

✓ **Combination of a rich carotenoid profile and optimum biomass productivity of *T. striata cultivated in* laboratory scale was observed at**

- **25°C**
- **constant illumination (56 μmol m^{-2} s^{-1} intensity Photosynthetic Photon Flux Density)**
- **pH=8**

✓ **Up-scale cultivation in 40 L capacity paddlewheel stainless steel raceway pond under optimum conditions presented:**

- **b-carotene (7,063.4 – 9,835.1 mg/kg dry biomass)**
- **lutein & zeaxanthin (1,214.6 - 1,692.8 mg/kg dry biomass)**
- **echinenone (190.9 - 205.7 mg/kg dry biomass)**
- **b-cryptoxanthin (33.7 - 40.7 mg/kg dry biomass)**
- **astaxanthin (33.5 – 46.5 mg/kg dry biomass)**
- **canthaxanthin (1.96 - 2.12 mg/kg dry biomass)**

Table 2. Biomass productivity (calculated in mg biomass $L^{-1}d^{-1}$) and carotenoid profile of *T. striata* cultivated in lab and lab pilot-scale 40 L raceway pond under different pH, temperature and photoperiod conditions. Carotenoid concentrations are in mg/kg of dry microalgae biomass.

Parameters	pH		Temperature			Photoperiod			Lab- pilot-scaled culture	
pH	pH=7	pH=8	pH=8	pH=8	pH=8	pH=8	pH=8	pH=8	pH=8	pH=8
Photoperiod	24h/0h	24h/0h	24h/0h	24h/0h	24h/0h	12h/12h	18h/6h	20h/4h	24/0h	24/0h
Temperature	T = 24-27	T = 24-27	T= 19±2	T= 25±1	T= 28±1	T= 25±1	T= 25±1	T= 25±1	T = 24-27	T = 24-27
Cultivation Scale (Volume)	Lab (4 L)	Lab (4 L)	Lab (4 L)	Lab (4 L)	Lab (4 L)	Lab (4 L)	Lab (4 L)	Lab (4 L)	Pilot (40 L)	Pilot (40 L)
Astaxanthin	324.15	130.03	336.81	284.42	428.69	31.91	109.91	74.58	46.46	33.49
Lutein & Zeaxanthin	1,454.92	916.37	270.42	2,026.54	401.68	32.92	12.46	17.44	1,214.60	1,692.78
Canthaxanthin	19.66	20.56	14.46	43.54	16.52	12.83	3.63	7.62	1.96	2.12
b-Cryptoxanthin	24.56	66.65	21.46	29.09	39.73	56.11	61.14	28.69	33.73	40.72
Echinenone	497.32	238.98	227.55	403.64	340.37	200.83	395.96	281.75	205.68	190.94
Lycopene	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
b-Carotene	5,575.81	5,359.9	4,905.19	3,355.85	8,151.77	8,639.20	7,644.58	12,395.39	9,835.11	7,063.36
Biomass Productivity	60.10	79.80	69.30	93.70	61.50	30.00	36.30	46.30	92.50	72.50

Conclusions

Microalgae *T. striata* can be considered as a potential natural source of carotenoid compounds, especially due to its high concentration in b-carotene, lutein & zeaxanthin, when cultivated at 25°C, constant illumination and pH=8.

Acknowledgements

The project "Large-scale cultivation of microalgae and utilization of the biomass, was produced as an alternative raw material in fish feed-AlgaFeed4Fish" (MIS 5045858, FK 80916), was co-funded by European (European Regional Development Fund) and National Resources (General Secretariat for Research and Innovation), in the context of the Operational Program "Competitiveness, Entrepreneurship and Innovation (EPANEK, NSRF 2014-2020), specifically through the Action "Special Actions" - "Aquaculture" - "Industrial Materials" - "Open Innovation in Culture".

Co-financed by Greece and the European Union