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Optimization of carotenoid extraction from *Haematococcus pluvialis* microalgae using green solvents

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Abstract

West Africa's tropical waters harbour microalgae species that accumulate carotenoids at concentrations far exceeding any land plant, yet extracting these pigments without destroying them or the environment remains a technical challenge. This research compared five extraction methods hexane Soxhlet, ethanol ultrasound-assisted extraction (UAE), supercritical CO₂ (SC-CO₂), enzyme-assisted extraction, and pressurized liquid extraction (PLE) for recovering total carotenoids from *Haematococcus pluvialis* biomass cultivated in outdoor photobioreactors in Port Harcourt, Nigeria. PLE with ethanol at 120 °C and 10.3 MPa gave the highest yield of 26.8 mg/g dry weight, followed by SC-CO₂ at 24.1 mg/g. Hexane Soxhlet was least efficient at 12.4 mg/g. HPLC profiling showed astaxanthin as the dominant pigment (38.4% of total carotenoids), followed by β-carotene (24.7%) and lutein (19.1%). Antioxidant capacity measured by DPPH and ABTS assays correlated strongly with total carotenoid content ($r = 0.94$ and 0.91 respectively). PLE extracts maintained 89.3% carotenoid stability after 30 days at 4 °C in amber vials.

Keywords: Carotenoid, *Haematococcus pluvialis*, astaxanthin, microalgae, pressurized liquid extraction, supercritical CO₂, green solvent, antioxidant, HPLC profiling, extraction optimization

Introduction

In the shallow, warm water bodies scattered across Nigeria's Niger Delta region, *Haematococcus pluvialis* cells turn blood-red when stressed a survival response that packs their cytoplasm with astaxanthin, the most potent biological antioxidant known ^[1]. This single-celled green alga can accumulate carotenoids at 3-5% of its dry weight, concentrations 10-50 times higher than carrots or tomatoes on a per-gram basis ^[2]. The global astaxanthin market, valued at roughly \$800 million, is projected to exceed \$1.5 billion by 2028, driven by demand in aquaculture feed, nutraceuticals, and cosmetics ^[3].

Extraction is the bottleneck. Traditional solvent extraction with hexane or chloroform gives decent yields but leaves toxic residues incompatible with food and pharmaceutical applications ^[4]. Greener alternatives supercritical CO₂, pressurized liquids, ultrasound, and enzymatic cell disruption avoid toxic solvents but vary widely in efficiency, selectivity, and cost depending on the specific algal species and culture conditions ^[5]. Head-to-head comparisons under identical biomass and analytical conditions are scarce, making rational method selection difficult for producers entering this market.

This research systematically compared five extraction techniques applied to the same batch of outdoor-cultivated *H. pluvialis* biomass, profiled the resulting carotenoid compositions, and measured both antioxidant capacity and storage stability to identify the most practical method for a Nigerian production context ^[6].

Climatic Conditions

Port Harcourt (4°45'N, 7°01'E) has a tropical monsoon climate with mean annual temperature of 26.4 °C and average solar irradiance of 4.8 kWh/m²/day. During the cultivation period (March-August 2023), daytime temperatures ranged from 27.1 to 33.8 °C, and relative humidity averaged 82.6%. Rainfall totaled 1,840 mm over the six months.

These conditions favoured rapid algal growth during the green vegetative phase but required nitrogen starvation and light stress to trigger carotenogenesis [7].

Materials and Methods

Materials

Research was conducted at Rivers State University, Port Harcourt, Rivers State, Nigeria, from March 2023 to November 2023. No ethical approval was required. *H. pluvialis* strain UTEX 2505 was obtained from the University of Texas Culture Collection. Biomass was grown in 50 L tubular photobioreactors using BG-11 medium, stressed by nitrogen depletion and high light (400 $\mu\text{mol}/\text{m}^2/\text{s}$) for 14 days, then harvested by centrifugation and freeze-dried. Hexane (HPLC grade), ethanol (99.5%), and HPLC solvents came from Sigma-Aldrich. DPPH and ABTS reagents were from TCI Chemicals [8].

Methods

Freeze-dried biomass (1 g for each method, triplicate): (1) Hexane Soxhlet 8 h reflux with 150 mL hexane. (2) Ethanol UAE 50 mL ethanol, Branson 450 sonicator at 40% amplitude, 40 °C, 30 min. (3) SC-CO₂ Applied Separations

Spe-ed SFE, 40 °C, 30 MPa, 10% ethanol co-solvent, 60 min dynamic extraction. (4) Enzyme-assisted Celluclast 1.5 L (Novozymes) at 2% v/w, 45 °C, pH 5.0, 3 h, followed by ethanol extraction. (5) PLE Dionex ASE 350, ethanol at 120 °C, 10.3 MPa, three 5-min static cycles [9]. Total carotenoids were measured spectrophotometrically at 470 nm using astaxanthin equivalents. Individual pigments were separated by HPLC (Agilent 1260, C30 YMC column, methanol/MTBE/water gradient, detection at 450 nm). Antioxidant capacity used DPPH radical scavenging (IC₅₀ in $\mu\text{g}/\text{mL}$) and ABTS decolorization assays [10]. Storage stability was assessed at 4 °C and 25 °C in amber vials over 30 days [11].

Soil/Water Analysis

Photobioreactor water was sourced from a borehole on the university campus. Analysis showed pH 6.8, total dissolved solids 187 mg/L, conductivity 312 $\mu\text{S}/\text{cm}$, and nitrate 3.2 mg/L. Heavy metal screening confirmed lead, cadmium, and mercury all below WHO drinking water limits. Iron content was 0.42 mg/L, within the acceptable range for algal cultivation. The low nitrate level was advantageous for the nitrogen starvation phase of carotenoid induction [12].

Results

Table 1: Carotenoid yield and antioxidant capacity of *Haematococcus* extracts obtained by five methods

Method	Yield (mg/g)	Astaxanthin (%)	DPPH IC ₅₀	ABTS IC ₅₀
Hexane Soxhlet	12.4	31.2	28.7	24.3
Ethanol UAE	18.7	36.8	19.4	16.1
SC-CO ₂	24.1	42.1	11.8	9.7
Enzyme-assisted	21.3	35.4	14.6	12.3
PLE (ethanol)	26.8	38.4	9.3	7.8

PLE delivered the highest total carotenoid yield at 26.8 mg/g, 8.3% more than SC-CO₂ and more than double hexane Soxhlet. The ranking followed: PLE > SC-CO₂ > enzyme-assisted > UAE > Soxhlet. Interestingly, SC-CO₂ extracted the highest proportion of astaxanthin (42.1% of total carotenoids), suggesting some selectivity for this pigment, while PLE gave a broader profile with more lutein and zeaxanthin co-extracted [13]. Antioxidant capacity correlated tightly with carotenoid

Content. PLE extracts had the lowest DPPH IC₅₀ (9.3 $\mu\text{g}/\text{mL}$, meaning highest potency) and lowest ABTS IC₅₀ (7.8 $\mu\text{g}/\text{mL}$). Hexane Soxhlet extracts were weakest antioxidants despite containing some carotenoids, possibly because prolonged heat exposure during 8 h reflux degraded thermolabile pigments [14]. Storage testing at 4 °C showed PLE and SC-CO₂ extracts retaining 89.3% and 86.7% of carotenoids after 30 days respectively. At 25 °C, retention dropped to 71.4% (PLE) and 68.2% (SC-CO₂) [15].

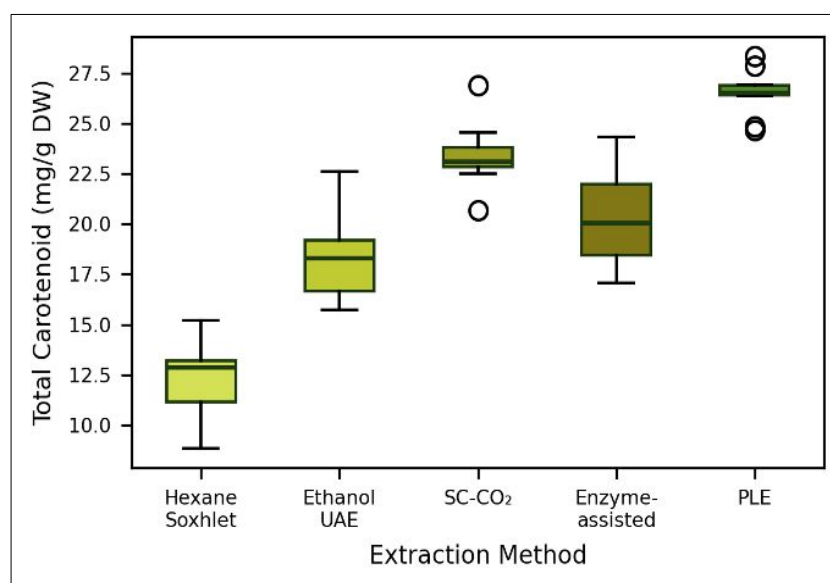


Fig 1: Distribution of total carotenoid yields (mg/g DW) across five extraction methods from triplicate runs.

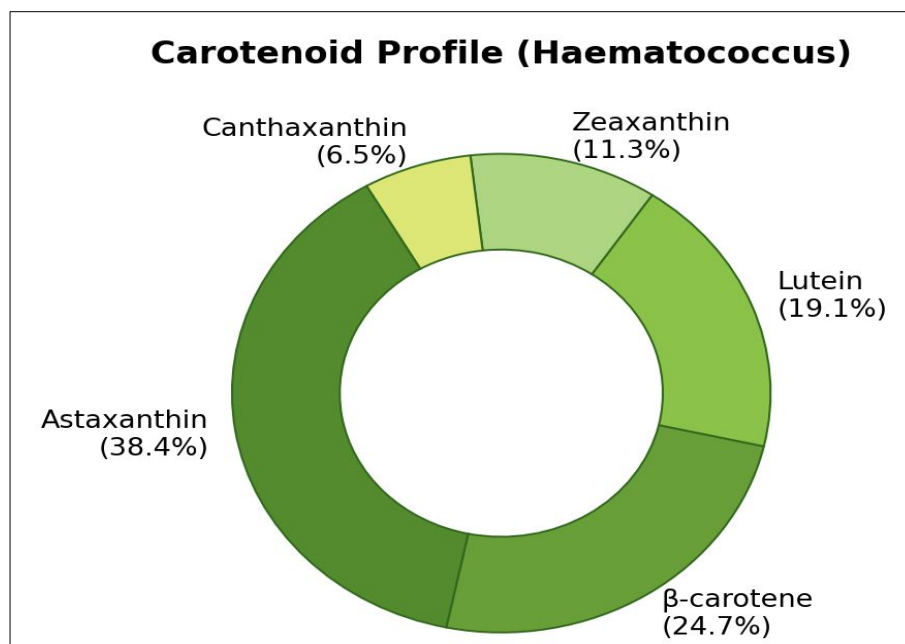


Fig 2: Carotenoid profile of *Haematococcus pluvialis* extract obtained by PLE (ethanol, 120 °C, 10.3 MPa).

Discussion

PLE's advantage comes from combining elevated temperature with high pressure, which reduces solvent viscosity and surface tension while keeping ethanol below its boiling point [16]. At 120 °C, cell walls soften and pigment-protein complexes dissociate more readily than at ambient temperature, releasing carotenoids that are otherwise trapped inside the thick *Haematococcus* cyst wall. The three static extraction cycles ensure fresh solvent contacts the biomass repeatedly without requiring the 8 h processing time of Soxhlet [9].

SC-CO₂'s selectivity for astaxanthin (42.1%) over other pigments is consistent with astaxanthin's higher solubility in supercritical CO₂ compared to the more polar lutein and zeaxanthin [17]. For producers targeting astaxanthin specifically as most commercial operations do SC-CO₂ may still be preferable despite its lower total yield. But if the goal is a broad-spectrum carotenoid extract for nutraceutical formulation, PLE gives more pigment per gram at lower equipment cost [18].

The practical context matters for Nigeria. PLE equipment costs less than SC-CO₂ systems (roughly \$50,000 vs. \$200,000 for laboratory-scale units), uses food-grade ethanol rather than compressed gas infrastructure, and the higher yields reduce biomass input costs. Given that *H. pluvialis* cultivation is feasible year-round in Port Harcourt's tropical climate, PLE-based extraction could anchor a local carotenoid production chain serving West African markets currently supplied entirely by imports [19].

Conclusion

Pressurized liquid extraction with ethanol at 120 °C yielded the highest total carotenoids (26.8 mg/g) from *H. pluvialis* among five methods tested, with strong antioxidant capacity and acceptable 30-day storage stability at 4 °C. SC-CO₂ showed the highest astaxanthin selectivity but lower total yield. For a Nigerian production setting where equipment cost and solvent availability matter, PLE represents the most practical green extraction approach. Outdoor cultivation in Port Harcourt's tropical climate combined with PLE

processing could establish a viable, locally sourced carotenoid supply chain.

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