



DEEP EUTECTIC SOLVENTS FOR ASTAXANTHIN EXTRACTION FROM *Haematococcus pluvialis*: RECENT ADVANCES

Henrique Vechio¹
André Bellin Mariano²
Rafael Bruno Vieira³
Fernando Augusto Pedersen Voll⁴

ABSTRACT

Objective: This study evaluates recent advances in deep eutectic solvent (DES) systems for astaxanthin extraction from *Haematococcus pluvialis*, comparing their efficiency and limitations.

Theoretical Framework: The review synthesizes knowledge on *H. pluvialis* biology and astaxanthin biosynthesis, limitations of physical/chemical extraction methods, and DES design principles for algal biorefining.

Method: A systematic literature review was conducted in Web of Science databases using search terms aligned with the research objectives. Selected articles were analyzed according to inclusion/exclusion criteria to compose the discussion.

Results and Discussion: Recent DES innovations have transformed astaxanthin extraction from *H. pluvialis*, achieving 83-99.6% yields under mild conditions (25-50°C). Hydrophobic DES (thymol-oleic acid) demonstrated dual extraction-stabilization functions, while IL-DES hybrids enabled selective fractionation. Aqueous DES-ATPS systems reached 99.6% efficiency through targeted cell wall disruption, and acidic DES (menthol-acetic acid) combined 91.3% recovery with extended extract stability.

Research Implications: The reviewed advances position DES as sustainable alternatives for industrial astaxanthin production, offering integrated extraction-stabilization processes and biorefinery potential through biomass valorization. GRAS-compliant formulations address safety concerns, though future work should optimize solvent viscosity.

Originality/Value: This is the first comparative analysis of DES systems for *H. pluvialis* biorefining, providing design guidelines for industrial implementation.

Keywords: Astaxanthin, *Haematococcus pluvialis*, Deep Eutectic Solvents, Green Extraction, Microalgae Biorefinery.

SOLVENTES EUTÉTICOS PROFUNDOS PARA EXTRAÇÃO DE ASTAXANTINA DE *Haematococcus pluvialis*: AVANÇOS RECENTES

RESUMO

Objetivo: Realizar uma revisão bibliográfica sobre os avanços recentes do uso de solventes eutéticos profundos (DES) para extração de astaxantina de *H. pluvialis*, comparando eficiência e limitações.

Referencial Teórico: Sintetiza a biologia de *H. pluvialis* e sua biossíntese de astaxantina, as limitações dos métodos convencionais, e os fundamentos dos DES para aplicação na biorrefinaria.

¹ Universidade Federal do Paraná, Curitiba, Paraná, Brazil. E-mail: henriquevechio@ufpr.br

² Universidade Federal do Paraná, Curitiba, Paraná, Brazil. E-mail: andrebmariano@ufpr.br

³ Universidade Federal de Uberlândia, Uberlândia, Minas Gerais, Brazil. E-mail: rafael.bruno@ufu.br

⁴ Universidade Federal do Paraná, Curitiba, Paraná, Brazil. E-mail: fernando_voll@ufpr.br



Método: Foi realizado uma revisão sistemática na base de dados do Web of Science usando palavras-chave alinhadas ao resultado desse trabalho. Os artigos selecionados foram analisados de acordo com os critérios de inclusão e exclusão para compor a discussão.

Resultados e Discussão: O uso inédito de DES alcançou 83-99,6% de recuperação de astaxantina sob condições brandas (25-50°C). DES hidrofóbicos (ácido oleico-timol) atuaram como extrator e estabilizante simultaneamente, enquanto sistemas híbridos IL-DES permitiram fracionamento seletivo. O sistema aquoso DES-ATPS atingiu 99,6% de eficiência via degradação controlada da parede celular, e os DES ácidos (mentol-ácido acético) resultaram em 91,3% de recuperação com estabilidade prolongada.

Implicações da Pesquisa: Os avanços levantados sugerem que os DES podem ser alternativas eficientes e sustentáveis para aplicação produção industrial de astaxantina, oferecendo processos integrados de extração-estabilização. Sua característica GRAS atende os requisitos de segurança, porém estudos futuros devem otimizar sua viscosidade.

Originalidade/Valor: Primeira análise comparativa do uso de DES para biorrefinaria de *H. pluvialis*, oferecendo diretrizes para escala industrial.

Palavras-chave: Astaxantina, *Haematococcus pluvialis*, Solventes Eutéticos Profundos, Extração Verde, Biorrefinaria de Microalgas.

DISOLVENTES EUTÉTICOS PROFUNDOS PARA LA EXTRACCIÓN DE ASTAXANTINA DE HAEMATOCOCCUS PLUVIALIS: AVANCES RECIENTES

RESUMEN

Objetivo: Realizar una revisión bibliográfica sobre los avances recientes en el uso de disolventes eutécticos profundos (DES) para la extracción de astaxantina de *H. pluvialis*, comparando su eficiencia y limitaciones.

Marco teórico: Resumir la biología de *H. pluvialis* y su biosíntesis de astaxantina, las limitaciones de los métodos convencionales y los fundamentos del DES para su aplicación en biorrefinería.

Método: Se realizó una revisión sistemática en la base de datos Web of Science utilizando palabras clave alineadas con los resultados de este trabajo. Los artículos seleccionados se analizaron según los criterios de inclusión y exclusión para elaborar la discusión.

Resultados y discusión: El novedoso uso del DES logró una recuperación de astaxantina del 83-99,6 % en condiciones suaves (25-50 °C). El DES hidrofóbico (ácido oleico-timol) actuó como extractante y estabilizador simultáneamente, mientras que los sistemas híbridos IL-DES permitieron el fraccionamiento selectivo. El sistema acuoso DES-ATPS alcanzó una eficiencia del 99,6 % mediante la degradación controlada de la pared celular, y el DES ácido (mentol-ácido acético) resultó en una recuperación del 91,3 % con una estabilidad prolongada.

Implicaciones de la investigación: Los avances obtenidos sugieren que el DES puede ser una alternativa eficiente y sostenible para la producción industrial de astaxantina, ofreciendo procesos integrados de extracción y estabilización. Su característica GRAS cumple con los requisitos de seguridad, pero estudios futuros deberían optimizar su viscosidad.

Originalidad/Valor: Primer análisis comparativo del uso de DES para la biorrefinería de *H. pluvialis*, que ofrece directrices para la escala industrial.

Palabras clave: Astaxantina, *Haematococcus pluvialis*, Disolventes Eutécticos Profundos, Extracción Ecológica, Biorrefinería de Microalgas.

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1 INTRODUCTION

Astaxanthin, a potent red xanthophyll carotenoid, demonstrates antioxidant activity up to 100-fold stronger than other carotenoids, with applications spanning nutrition, cosmetics, and biomedicine (Park et al., 2018). Its unique photoprotective and oxidative stress modulation properties have spurred research into therapeutic uses, including neuroprotection and anti-inflammatory effects (Chang & Xiong, 2020; Fakhri et al., 2019). While synthetic astaxanthin dominates the market (95% share) due to lower cost (~1000 USD/kg), concerns over safety and bioavailability are driving demand for natural alternatives (6000–7150 USD/kg), despite the price disparity (B. Kim et al., 2022; Zhou et al., 2023).

The microalga *Haematococcus pluvialis* is the most efficient natural producer of astaxanthin, accumulating up to 4% of its dry weight under stress conditions such as nutrient deprivation or high light intensity (Patel et al., 2022; Wayama et al., 2013). Stress triggers a morphological shift from motile cells to dormant cysts, encased in a remarkably resilient cell wall that hinders astaxanthin extraction (Boussiba & Vonshak, 1991; Hagen et al., 2002).

Physical disruption methods, including bead milling (Su et al., 2023) and high-pressure homogenization (Koopmann et al., 2022), achieve high efficiency but demand substantial energy and costly infrastructure. Alternative approaches—such as pulsed electric fields (Liu et al., 2018) or enzymatic treatments (Zhao et al., 2019)—are less energy-intensive but suffer from lower yields or scalability issues. Chemical methods using acids (Vechio et al., 2021) or ionic liquids (L. Zhang et al., 2024) offer energy-efficient alternatives, though their potential toxicity raises concerns for food and feed applications (B. Kim et al., 2022). Thus, optimizing extraction remains a critical challenge for commercial astaxanthin production.

Among chemical methods, deep eutectic solvents (DESs) have emerged as particularly effective for this process, as demonstrated in recent studies (W. Cheng et al., 2025; Nemani et al., 2024). However, a systematic comparison of these advances remains unexplored. Therefore, this work aims to present a review evaluating the latest DES-based extraction techniques, assessing their efficiency and biocompatibility to identify optimal strategies for astaxanthin recovery from *H. pluvialis*.

2 THEORETICAL FRAMEWORK

This section establishes the foundational concepts critical to understanding astaxanthin extraction from *Haematococcus pluvialis*. First, we outline the fundamentals of astaxanthin



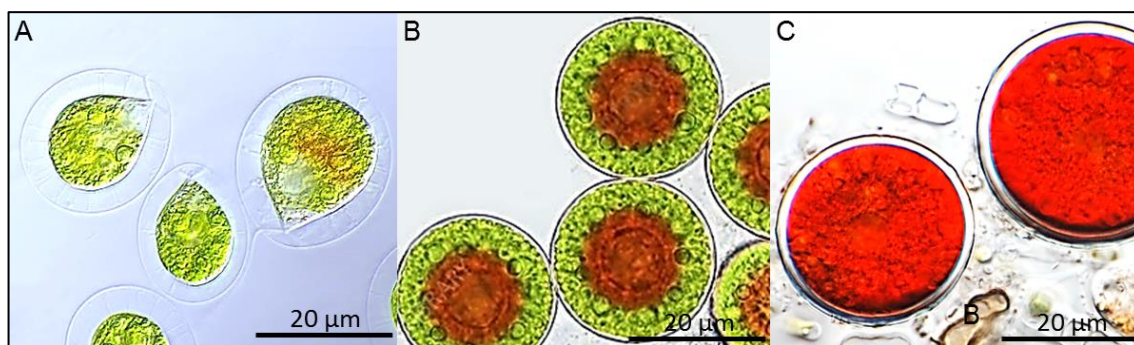
biosynthesis, including its stress-induced accumulation. Next, we examine conventional extraction methods, highlighting their limitations in efficiency, scalability, and environmental impact. We then introduce deep eutectic solvents (DES), explaining their green chemistry principles and tunable properties that address these limitations.

2.1 *H. pluvialis* BIOLOGY AND ASTAXANTHIN BIOSYNTHESIS

Astaxanthin is synthesized by various microorganisms including the microalgae *Haematococcus pluvialis*, *Chlorella zofingiensis*, and *Chlorococcum* sp., the yeasts *Xanthophyllomyces dendrorhous* and *Candida utilis*, and bacteria such as *Agrobacterium aurantiacum* (Ambati et al., 2014). This pigment enters the food chain through plankton, crustaceans (e.g., krill), and fish (e.g., salmon) (Higuera-Ciapara et al., 2006; Rao et al., 2013). Among these organisms, *H. pluvialis* stands out for its exceptional astaxanthin content (1-4% of dry weight), surpassing even *X. dendrorhous* (~2%) (Boussiba & Vonshak, 1991).

Figure 1

Microscopic image of *H. Pluvialis* cells in different life cycle stages. (a) flagellated cells; (b) non-motile intermediate cells; (c) hematocysts

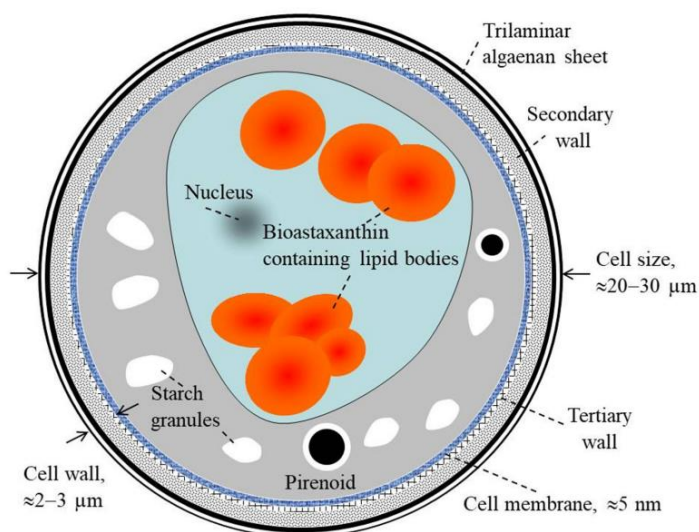


Source: Adapted from Fox et al. (2011).

The freshwater microalga *H. pluvialis* (Chlorophyta, Chlorophyceae) undergoes dramatic morphological changes under stress: motile biflagellate cells transform into non-motile cysts (hematocysts) with thick, trilayered cell walls (Han et al., 2013). This encystment involves four stages: (i) protoplast rounding and flagella loss, (ii) primary wall formation, (iii) trilaminar layer deposition, and (iv) secondary wall thickening via polysaccharide accumulation (Hagen et al., 2002). The mature cyst wall (~16% of dry mass) consists mainly of non-cellulosic carbohydrates (70%, predominantly mannose), with minor cellulose (3%), protein (6%), and acetolysis-resistant materials (3%) (Hagen et al., 2002).

**Figure 2**

Description of *H. pluvialis* cell structure at the resistant cyst stage.



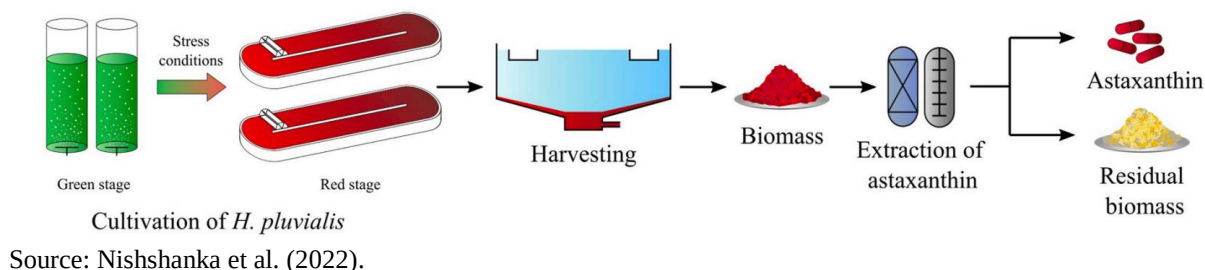
Source: Gherabli et al. (2023).

The wall's extreme resistance was initially attributed to sporopollenin (Burczyk, 1986), but later studies identified algaenan, a recalcitrant biopolymer in green algae (Montsant et al., 2001). However, algaenan alone cannot explain the wall's robustness - its molecular arrangement and cross-linking with polysaccharides likely contribute, though this hypothesis requires validation (Montsant et al., 2001). This structural complexity poses significant challenges for astaxanthin extraction, necessitating efficient cell disruption strategies.

The industrial production of astaxanthin from *H. pluvialis* follows a well-established biphasic cultivation strategy (Fábregas et al., 2001). The initial green stage utilizes closed photobioreactors to maximize biomass production under optimal growth conditions while preventing contamination of motile cells (Butler et al., 2017). This is followed by a red stage where stress conditions (nutrient limitation, high light) induce astaxanthin accumulation, typically conducted in open ponds for cost efficiency (Olaizola, 2000). The hybrid system - combining controlled PBRs for growth with open ponds for pigment induction - has been successfully implemented at commercial scales (25,000L systems) and represents the current industry standard (Li et al., 2011; Liyanaarachchi et al., 2021).

**Figure 3**

*Two-stage cultivation of *H. pluvialis* in closed photobioreactors and open raceway ponds with subsequent harvesting and astaxanthin extraction.*



2.2 ASTAXANTHIN EXTRACTION METHODS FROM *H. pluvialis*

The robust trilaminar cell wall of *Haematococcus pluvialis* cysts, characterized by its algaenan-polysaccharide composite structure, necessitates sophisticated disruption strategies for efficient astaxanthin recovery. Current methodologies universally employ a sequential approach involving primary cell wall compromise followed by non-polar solvent extraction, with selection criteria heavily dependent on energy inputs, scalability constraints, and final product stability requirements.

Direct solvent extraction represents the most elementary approach, relying solely on solvent penetration without mechanical or chemical pretreatment. Acetone-based systems have demonstrated particularly poor efficacy, with recovery rates stagnating at 14-21% even after prolonged 16-24 hour exposures at ambient temperature, as documented by Mendes-Pinto et al. (2001) and Sarada et al. (2006). Paradoxically, elevated temperatures to 50°C resulted in drastic yield reductions to merely 1% after 20 minutes, suggesting significant thermal degradation pathways. More promising results emerged from ethyl acetate systems, where Samorì et al. (2019) reported remarkable 88% recovery within 30 minutes, though these findings remain controversial given the solvent's established mass transfer limitations in aqueous biomass matrices. Supercritical CO₂ extraction has shown intermediate efficacy, achieving 81% yield through two sequential 4-hour cycles, as demonstrated by Kwan et al. (2018), though the prerequisite biomass dehydration imposes substantial cost penalties of \$15-20/kg.

Physical disruption techniques currently dominate industrial-scale operations due to their reliability and high extraction efficiencies. Bead milling and high-pressure homogenization consistently deliver 91-98% recovery rates by generating sufficient shear forces to mechanically fracture the cyst wall, as quantified by Di Sanzo et al. (2018).



Hydrothermal processing has demonstrated peak theoretical yields of 99% (X. Cheng et al., 2017), though the extreme 200°C operational conditions induce problematic isomerization of esterified astaxanthin to its less stable free form, compromising both product stability and bioavailability. Alternative approaches including ultrasonication and pulsed electric fields show more modest 60-75% efficiencies (Zhao et al., 2016), while also presenting substantial scale-up challenges. The critical limitation across all physical methods remains their prohibitive energy intensity, with industrial homogenizers consuming 50-100 kWh per ton of processed biomass (D. Y. Kim et al., 2016), alongside stringent requirements for oxygen-free processing environments to prevent oxidative degradation.

Chemical and biological methods present intriguing alternatives with distinct advantages and constraints. Enzymatic pretreatment using glucanase cocktails has achieved 79% recovery within 30 minutes (Burkert et al., 2015), though enzyme costs remain economically restrictive at production scale. Ionic liquid systems have shown exceptional 99% efficiency using [Bmim]HSO₄ (Choi et al., 2019), but face unresolved challenges regarding solvent recovery and potential ecotoxicity. Acid hydrolysis optimization studies have revealed a critical operational window, with Sarada et al. (2006) demonstrating 96% recovery using 2M HCl at 70°C for 10 minutes - conditions mild enough to preserve astaxanthin esterification while sufficiently compromising wall integrity. Emerging methods employing deep eutectic solvents (Cheng et al., 2025) show particular promise due to their tunable properties and moderate energy requirements, though comprehensive toxicological assessments remain pending.

The selection matrix for extraction technologies ultimately presents complex trade-offs between efficiency, operational costs, and product quality. While physical methods deliver uncompromised yields, their energy demands and capital costs prove prohibitive for many applications. Chemical alternatives offer more sustainable energy profiles but introduce concerns regarding solvent residues and biomolecule integrity. This technological landscape has consequently driven growing interest in hybrid approaches that combine moderate-intensity physical disruption with selective solvent systems, aiming to balance throughput with sustainability considerations - a paradigm that will likely dominate future optimization efforts.



2.3 DEEP EUTECTIC SOLVENTS (DES): THEORY AND GREEN CHEMISTRY PRINCIPLES

Deep Eutectic Solvents (DES) represent a novel class of designer solvents formed through the self-organization of hydrogen bond acceptors (HBAs; e.g., choline chloride, betaine) and hydrogen bond donors (HBDs; e.g., amides, alcohols, carboxylic acids). This unique interaction creates mixtures with depressed eutectic points, exhibiting significantly lower melting temperatures than their individual components (Pontes et al., 2017). Initially conceptualized as sustainable alternatives to ionic liquids (ILs), DES share key advantages including negligible volatility and tunable physicochemical properties, while offering superior green chemistry credentials through their typically non-toxic, biodegradable constituents (Dai et al., 2013).

DES systems are broadly classified into hydrophilic and hydrophobic variants, each with distinct applications in bioactive compound recovery. Hydrophilic DES, typically composed of choline chloride paired with diols or carboxylic acids, primarily function as cell wall disruptants that enhance subsequent extraction efficiency (Zhang et al., 2014). In contrast, hydrophobic DES - particularly terpene-based systems like menthol-myristic acid mixtures - directly solubilize non-polar compounds such as astaxanthin, achieving exceptional recovery rates from complex matrices (Rodrigues et al., 2020). These terpene-fatty acid combinations offer additional advantages as Therapeutic DES (TheDES), where both solvent components contribute bioactive properties including anti-inflammatory and antimicrobial effects (Silva et al., 2018).

Natural Deep Eutectic Solvents (NaDES) constitute a specialized subclass where all components originate from biological sources. These multimolecular networks maintain the core DES characteristics of low volatility and designer functionality while offering enhanced biocompatibility for food and pharmaceutical applications (Choi et al., 2019). The "natural" designation reflects both their biological origin and their remarkable capacity to dissolve water-insoluble metabolites through tailored hydrogen bonding networks. NaDES demonstrate particular promise for circular extraction processes, where solvent polarity can be reversibly modulated between hydrophilic and hydrophobic states through the addition of switchable agents like Jeffamine D-230 or supercritical CO₂ - enabling near-quantitative recovery of diverse biomolecules (Sed et al., 2018).

Despite these advantages, practical challenges persist. High viscosity remains a primary limitation for industrial implementation, typically addressed through temperature modulation



or controlled water addition (10-30% v/v). However, excessive water (>50%) disrupts the hydrogen bonding network, while elevated temperatures risk degrading thermolabile target compounds (Dai et al., 2015). Strategic formulation is therefore essential to balance solvent viscosity, polarity, and extraction specificity for particular applications. Ongoing advances in hydrophobic NaDES design, particularly those incorporating medium-chain fatty acids and monoterpenes, show exceptional promise for astaxanthin recovery by combining high extraction efficiency with inherent biocompatibility (Osch et al., 2019).

3 METHODOLOGY

This review employed a systematic literature search protocol to identify relevant studies on deep eutectic solvent (DES)-mediated astaxanthin extraction from *Haematococcus pluvialis*. The Web of Science Core Collection database served as the primary resource, utilizing a targeted search strategy encompassing peer-reviewed articles and book chapters.

The search query combined the following key terms:

("deep eutectic solvent" OR "DES") AND ("astaxanthin" OR "carotenoid") AND ("*Haematococcus pluvialis*" OR "*H. pluvialis*") AND ("extract" OR "recov" OR "isolat*"). No publication date restrictions were applied to ensure comprehensive coverage of the evolving DES technology scenario.

4 RESULTS AND DISCUSSIONS

Pitacco et al., (2022) demonstrated that hydrophobic deep eutectic solvents (DES) could overcome key limitations in astaxanthin recovery from *Haematococcus pluvialis*. Their investigation of three novel DES formulations - thymol-oleic acid (TAO), DL-menthol-oleic acid (MAO), and geraniol-oleic acid (GAO) - revealed distinct advantages over conventional solvents. When applied to freeze-dried biomass, all three DES achieved approximately 60% astaxanthin recovery within six hours, significantly outperforming oleic acid alone (41% recovery). Extended extraction periods further differentiated the systems, with TAO reaching 83% recovery after 24 hours - a 1.4-fold improvement over equivalent non-eutectic mixtures. This enhanced performance was attributed to reduced viscosity and potential interactions between the terpene components and astaxanthin's conjugated system.

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investigation of three novel DES formulations - thymol-oleic acid (TAO), DL-menthol-oleic acid (MAO), and geraniol-oleic acid (GAO) - revealed distinct advantages over conventional solvents.

Table 1

Results of DES-based systems for astaxanthin extraction from H. pluvialis, highlighting recovery yields (%) under optimized conditions for dry and wet biomass.

Biomass state	DES	Condition	R_{ast}	Reference
dry	DL-menthol-oleic acid	24 h, 25 °C	74%	Pitacco et al. (2022)
dry	thymol-oleic acid	24 h, 25 °C	83%	Pitacco et al. (2022)
dry	geraniol-oleic acid	24 h, 25 °C	66%	Pitacco et al. (2022)
wet	DL-menthol-oleic acid	48 h, 25 °C	56%	Pitacco et al. (2022)
wet	thymol-oleic acid	48 h, 25 °C	58%	Pitacco et al. (2022)
wet	geraniol-oleic acid	48 h, 25 °C	68%	Pitacco et al. (2022)
wet	choline chloride-fructose + [P ₄₄₄₈]Cl	3 h, 35 °C	81%	Zhang et al. (2024)
wet	choline chloride-urea + K ₂ HPO ₄	1 h, 50 °C	99%	Nemani et al. (2024)
wet	choline chloride-glucose + K ₂ HPO ₄	1 h, 50 °C	99%	Nemani et al. (2024)
wet	DL-menthol-acetic acid	UA 300 W, 0.5 h, 25 °C	91%	Cheng et al. (2025)
wet	DL-menthol-propionic acid	UA 300 W, 0.5 h, 25 °C	80%	Cheng et al. (2025)
wet	DL-menthol-valeric acid	UA 300 W, 0.5 h, 25 °C	71%	Cheng et al. (2025)
wet	DL-menthol-octanoic acid	UA 300 W, 0.5 h, 25 °C	70%	Cheng et al. (2025)
wet	lauric acid-decanoic acid	UA 300 W, 0.5 h, 25 °C	73%	Cheng et al. (2025)
wet	lauric acid-nonanoic acid	UA 300 W, 0.5 h, 25 °C	72%	Cheng et al. (2025)
wet	decanoic acid-myristic acid	UA 300 W, 0.5 h, 25 °C	71%	Cheng et al. (2025)

R_{ast} = astaxanthin recovery; [P₄₄₄₈]Cl = Tributyl-octylphosphine chloride; UA = ultrasound-assisted.

When applied to freeze-dried biomass, all three DES achieved approximately 60% astaxanthin recovery within six hours, significantly outperforming oleic acid alone (41% recovery). Extended extraction periods further differentiated the systems, with TAO reaching 83% recovery after 24 hours - a 1.4-fold improvement over equivalent non-eutectic mixtures. This enhanced performance was attributed to reduced viscosity and potential interactions between the terpene components and astaxanthin's conjugated system.

The study's most industrially relevant finding involved successful direct extraction from wet algal cultures, bypassing energy-intensive dewatering steps. While all DES systems showed slower kinetics in aqueous environments (30% recovery in six hours versus 60% from dried biomass), they ultimately achieved 56-68% recovery after 48 hours. However, this aqueous extraction came with significant trade-offs: all DES formulations completely eliminated algal viability within four hours of contact, as evidenced by photosynthetic activity measurements. NMR analysis revealed this cytotoxicity stemmed from terpene component leaching into the aqueous phase, with geraniol showing particular toxicity despite its superior extraction efficiency.



A critical advancement emerged in the examination of astaxanthin stability within DES matrices. Under light exposure, TAO demonstrated remarkable protective capabilities, retaining 40% of initial astaxanthin content after 13.5 hours - a fourfold improvement over unprotected controls. This stabilization effect correlated with TAO's exceptional antioxidant activity, measured at thirty times greater than MAO systems. The authors attributed this to thymol's known singlet oxygen quenching capacity and synergistic interactions with astaxanthin monoesters within the extract. Such findings position TAO not merely as an extraction medium, but as a multifunctional formulation capable of both recovery and stabilization of this labile carotenoid.

The study's conclusions highlight TAO's dual promise as both an efficient extractant and stabilizer for astaxanthin. Its GRAS-component-based formulation addresses biocompatibility concerns while demonstrating tangible improvements over conventional methods in both recovery efficiency (83% from dried biomass) and product stability (40% retention after prolonged light exposure). These advances suggest potential for simplified, continuous production systems that integrate extraction and stabilization into a single unit operation - a significant stride toward economically viable algal astaxanthin production.

To improve astaxanthin recovery from wet biomass, Zhang et al. (2024) combined ionic liquids (tributyl-octylphosphine chloride, or $[P_{4448}][Cl]$) and DESs (choline chloride-D-fructose, or $[ChCl][Fru]$) to form aqueous biphasic systems. This system demonstrated remarkable operational advantages under optimized conditions (35% $[P_{4448}][Cl]$, 25% $[ChCl][Fru]$, 1:20 biomass-to-solvent ratio, 260 rpm, 35°C, 3 h), achieving 81.27% of astaxanthin recovery. Compared to organic solvent, the $[P_{4448}][Cl]$ - $[ChCl][Fru]$ ABS had high stability, making it a potential replacement for astaxanthin extraction solvent. In contrast to the oil solution method, enzymatic hydrolysis method, supercritical fluid method, microemulsion method, and IL-salt ABS extraction method reported in the literature, the $[P_{4448}][Cl]$ - $[ChCl][Fru]$ ABS possessed the qualities of high extraction rate and stability, low cost, recyclability, operation easily and environmental friendliness. It was not only played the polar properties of IL, but also achieved the selective separation of astaxanthin, making it an ideal solvent for astaxanthin extraction.

Nemani et al. (2024) demonstrated unprecedented astaxanthin recovery rates (99.6%) from *H. pluvialis* using a choline chloride-urea deep eutectic solvent (DES) combined with dipotassium hydrogen phosphate in an aqueous two-phase system (ATPS). This marked a significant improvement over conventional methods, at mild operational conditions (50°C, pH 7.5). The DES-ATPS approach proved particularly effective in disrupting the trilaminar cyst



wall structure while preserving astaxanthin stability, as confirmed by UV-Vis and FTIR analyses showing no carotenoid degradation products.

The exceptional performance stems from the system's dual mechanism of action. Microscopic observations by Nemani et al. (2024) revealed sequential cell wall degradation: (1) initial rupture of the outer algaenan layer through hydrophobic interactions with the DES phase, (2) hydrolysis of the middle cellulose-mannan matrix via hydrogen bond disruption (DES chloride anions acting as hydrogen bond acceptors to cellulose hydroxyl groups), and (3) complete solubilization of inner wall components through phosphate-mediated polarity gradients. This contrasted sharply with ionic liquid treatments reported by Liu et al. (2018), where only superficial pitting occurred without full wall penetration.

Notably, the 35% DES / 30% K_2HPO_4 formulation created an ideal microenvironment for maintaining astaxanthin stability during extraction. The urea component served dual roles - as hydrogen bond disruptor for cell wall polymers and as stabilizer for released astaxanthin molecules through interactions with their conjugated polyene chains. This combination of high extraction efficiency (99.64% recovery) and product protection represents a significant advance over prior methods requiring organic solvents or extreme temperatures.

Cheng et al. (2025) achieved a breakthrough in astaxanthin extraction using acidic deep eutectic solvents (DES), with the DL-menthol:acetic acid (MAA) system demonstrating 91.25% recovery efficiency (18.25 mg/g yield) under optimized ultrasonic conditions (300 W, 30 min, 1:20 g/mL solid-liquid ratio). This performance surpassed conventional methods, showing 1.3-fold and 1.5-fold improvements over ionic liquid $[P_{4448}]Cl$ (69.91%) and ethanol (59.34%) extractions, respectively. The MAA system also exhibited exceptional operational stability, maintaining >90% efficiency through three reuse cycles and preserving astaxanthin integrity (72.94-day half-life) – doubling the stability achieved with $[P_{4448}]Cl$.

The superior extraction capability originated from MAA's unique physicochemical properties. Quantum chemical calculations revealed that the DES's balanced polarity (high π -value, low β -value) enabled simultaneous cell wall penetration through hydrogen bonding with cellulose and van der Waals interactions with algaenan layers. Ultrasonic irradiation enhanced this process by accelerating solvent permeation while preventing thermal degradation. Notably, the extracted astaxanthin demonstrated excellent storage stability but showed moderately reduced antioxidant activity (15-20% lower DPPH/ROS scavenging versus $[P_{4448}]Cl$ extracts), suggesting potential interactions between acetic acid and astaxanthin's conjugated system.

A key secondary benefit emerged in downstream processing: enzymatic hydrolysis of MAA-treated residues yielded 0.12 g/g fermentable sugars, enabling integrated biorefinery



approaches. This dual functionality positions acidic DES as a versatile tool for complete microalgal biomass valorization, addressing both extraction efficiency and circular economy objectives.

5 CONCLUSION

Recent breakthroughs in DES-based extraction have established new benchmarks for *Haematococcus pluvialis* astaxanthin recovery (91-99%), while addressing key industrial challenges. Pitacco's hydrophobic DES (83% recovery) demonstrated dual extraction-stabilization capabilities, while Zheng's IL-DES system enabled selective fractionation. Nemani's aqueous DES-ATPS reached 99.6% efficiency through targeted cell wall disruption, and Cheng's acidic DES combined high recovery (91%) with exceptional extract stability. These systems share critical advantages: mild operational conditions (35–50°C), reduced energy inputs, and compliance with green chemistry principles.

Moving forward, research must focus on scaling these technologies while addressing viscosity challenges and cytotoxicity in wet extraction. The progress reviewed here positions DES as a cornerstone for sustainable astaxanthin production, merging efficiency with environmental responsibility. Future work should validate these systems at pilot scale and explore their applicability to other high-value algal metabolites.

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