

Application of natural astaxanthin from *Haematococcus Pluvialis*

Tao Chen

demp1e1999@163.com

School of Energy and Engineering, Chongqing University

Qiang Liao

School of Energy and Engineering, Chongqing University

Yun Huang

School of Energy and Engineering, Chongqing University

Xun Zhu

School of Energy and Engineering, Chongqing University

Research Article

Keywords: *Haematococcus Pluvialis*, Natural astaxanthin, Application

Posted Date: September 10th, 2024

DOI: <https://doi.org/10.21203/rs.3.rs-4846175/v1>

License:   This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Additional Declarations: No competing interests reported.

Application of natural astaxanthin from *Haematococcus Pluvialis*

Tao Chen^{1,2} Qiang Liao¹* Yun Huang¹ and Xun Zhu¹

1、 School of Energy and Engineering, Chongqing University, Chongqing 400044, China

2、 Kunming Biogenic Co.,Ltd, Kunming 650117,China

*corresponding author: (Qiang Liao , professor,doctoral supervisor,new and renewable energy utilization,microbial energy conversion,and energy conservation and emission reduction, lqzx@cqu.edu.cn)

Construction of the Yunnan Astaxanthin Industrial Technology Research and Development Platform, it is founding by The National Science and Technology Department's 2021 High-end Foreign Expert Introduction Program Project (G2021039004L) funding

Abstract

Haematococcus Pluvialis, a freshwater microalgae, is renowned as a significant source of natural astaxanthin, an essential biological resource. This unicellular organism is known to contain over 5% astaxanthin, making it one of the most abundant sources. Astaxanthin levels in *H. Pluvialis* are elevated under growth conditions stressors such as high salinity, temperature, and light exposure. It is intriguing to observe that all astaxanthin molecules within the microalgae exhibit in a left-handed molecular configuration (3S, 3'S). This paper presented a comprehensive review on *H. Pluvialis*, focusing on its culture technology, astaxanthin extraction process, and derivative products. The aim is to facilitate and enhance the broader and more in-depth utilization of natural astaxanthin.

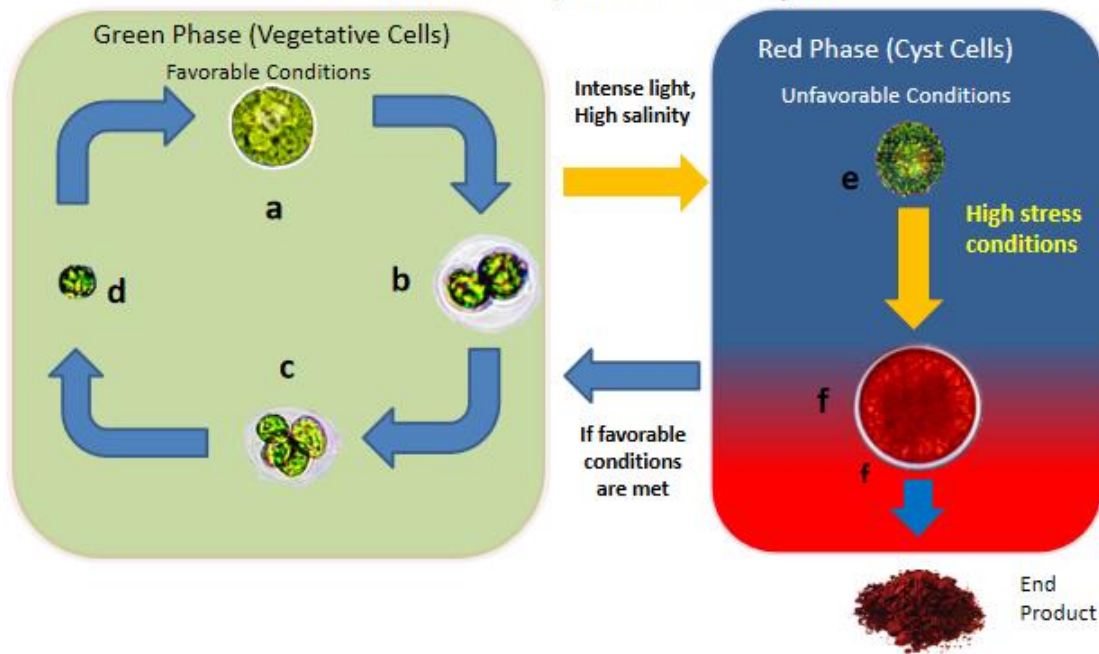
Keywords: *Haematococcus Pluvialis*; Natural astaxanthin; Application

Astaxanthin, a derivative of oxygenated β -carotene, exhibits a chemical structure analogous to that of β -carotene, with a chemical formula $C_{40}H_{52}O_4$ and a relative molecular mass of 596.86 [1]. The chemical structure of astaxanthin is characterized by four isoprenoid units, which are interconnected via a network of conjugated double bonds, culminating in a cyclic hexaene structure. Each isoprenoid unit is linked to the two ends of the ring, constituting the carbon skeleton of astaxanthin [2,3]. The astaxanthin molecule features aromatic rings at both terminus of the carbon skeleton, each substituted with a hydrogen group and a ketone group. *Haematococcus pluvialis*, a unicellular green alga classified under *Chlorophyta*, *Chlorophyceae*, *Volvocales*, *Haematococcaceae*, *Haematococcus*, and *Pluvialis*[4,5]. The paper presented research findings on the culture technology, astaxanthin extraction process, and derivative products of *H. Pluvialis*. The objective is to catalyze a more expansive and profound integration of naturally occurring astaxanthin into various applications.

Morphological alterations in the process of astaxanthin accumulation in *H. Pluvialis*

In the life cycle of *H. Pluvialis*, distinct cellular morphologies are observed: microscopic cells, swimming cells, immobile cells, and immobile spores. The microscopic cells, measuring less than 10 μm in diameter, are flagellated and capable of rapid movement, representing the initial phase of the life cycle [6]. Subsequently, the mobile cells, 20-50 μm in diameter, emerge as larger, elliptical or pear-shaped cells with two flagella, typically exhibiting a green hue. These mobile cells, referred to as green trophoblasts, thrive in optimal conditions. However, when faced with unfavorable environmental factors like bright light, high temperatures, high salinity, and nutrient deficiencies (e.g., nitrogen and phosphorus), the motile cells undergo a transformation. In response to these stressful conditions, the cells increase in size, shed their flagella, and metamorphose into spherical, immobile cells [7]. As the living environmental degradation persists, these immobile cells subsequently develop thick cell walls and transition into immobile spores. Characterized by their bright or deep red pigmentation, these spores possess the capacity to synthesize and accumulate astaxanthin [8].

Haematococcus pluvialis Life Cycle



Biological activity of astaxanthin

Antioxidant activity

The astaxanthin molecule possesses a unique structure featuring active electron effects. These effects enable astaxanthin to either donate electrons to free radicals or attract the unpaired electrons of free radicals. Consequently, the stabilized free radicals revert to a non-reactive state, thereby mitigating cellular damage [9].

Astaxanthin molecule structure incorporates a series of conjugated double bonds, hydroxyl groups, and unsaturated ketone groups. Notably, the hydroxyl and ketone groups are positioned at the termini of the conjugated double bond chain, resulting in the formation of an α -hydroxy ketone [10,11]. These structural components exhibit active electronic effects, rendering astaxanthin particularly effective at neutralizing highly oxidative, single-linear reactive oxygen species and other free radicals in the environment [12].

Astaxanthin's superior antioxidant capabilities are evident, it exhibits 800 times the free radical scavenging efficacy of Coenzyme Q10, surpasses Vitamin C by a factor of 6,000, and outperforms Vitamin E by 500-fold. Owing to these remarkable properties, astaxanthin is referred to as Super Vitamin E [13].

77 **Enhancement of immune function**

78 Investigators at the Medical and Healthcare Research Center of Kanazawa University
79 conducted a study wherein mice were administered a diet high in cholesterol leading to
80 NAFLD for a duration of three months. The study compared these mice with another group
81 received a diet high in cholesterol supplemented with astaxanthin[14,15]. The findings
82 indicated that the group supplemented with astaxanthin experienced a notable reduction in
83 liver fat deposition, by approximately 50 percent, and a decreased susceptibility to develop
84 fatty liver in comparison to the non-supplemented group [16]. Moreover, the study revealed a
85 significant inhibition of lipid peroxidation, which arises from lipid aggregation, by over
86 eighty percent. The researchers posited that the transition from fatty liver to non-alcoholic
87 steatohepatitis might be mitigated through the modulation of inflammatory macrophages into
88 anti-inflammatory macrophages, thereby reducing inflammation[17].

89 **Heart health**

90 Elevated levels of LDL cholesterol in the blood are correlated with an increased risk
91 of atherosclerosis. LDL is not fully oxidized in plasma, but its oxidation is considered a
92 contributing factor to the formation of atherosclerosis plaque [18]. Astaxanthin
93 supplementation is believed to increase plasma concentrations of HDL, thereby potentially
94 enhancing the balance of both LDL and HDL cholesterol concentrations in plasma, which
95 may subsequently foster cardiovascular health[19,20].

96 Additionally, astaxanthin exerts an influence on aortic vascular function by
97 modulating both endothelium-dependent and endothelium-independent vasodilation[21].
98 The endothelium-dependent effects of astaxanthin may be attributed to its antioxidant
99 properties, indicating that astaxanthin acts as a scavenger of superoxide radicals to
100 prevent the degradation of nitric oxide (NO), a crucial molecule with vasodilatory
101 properties. This action prolongs the lifespan of NO, resulting in a vasodilatory effect [23].

102 **Protection of vision**

103 Two prevalent etiologies of visual impairment and blindness are AMD and age-related
104 cataracts. Both conditions are associated with light-induced oxidative stress within the ocular
105 tissues [24,25]. Astaxanthin has demonstrated the capacity to cross the blood-brain barrier
106 and accumulate in the mammalian retina. This selective accumulation of astaxanthin in the
107 mammalian retina provides effective protection against UV irradiation and oxidative stress in
108 retinal cells[26,27].

109 **Coloring effects**

110 Li et al. discovered that the optimal pigment deposition in various fish body tissues,
111 including the body, scales, fins, and skin, was attained at a dosage of 200 mg/kg of
112 astaxanthin by the fifteenth day of supplementation. The study also revealed that the pigment
113 deposition was heterogeneous across different tissues, suggesting tissue-specific uptake and
114 deposition profiles of astaxanthin [28].

115 The efficacy of astaxanthin in coloration is dosage-dependent; however, excessive
116 levels of astaxanthin result in its metabolism being excreted from the body, preventing
117 further metabolic impacts [29,30].

118 **Cultivation system of the red algae *H. Pluvialis***

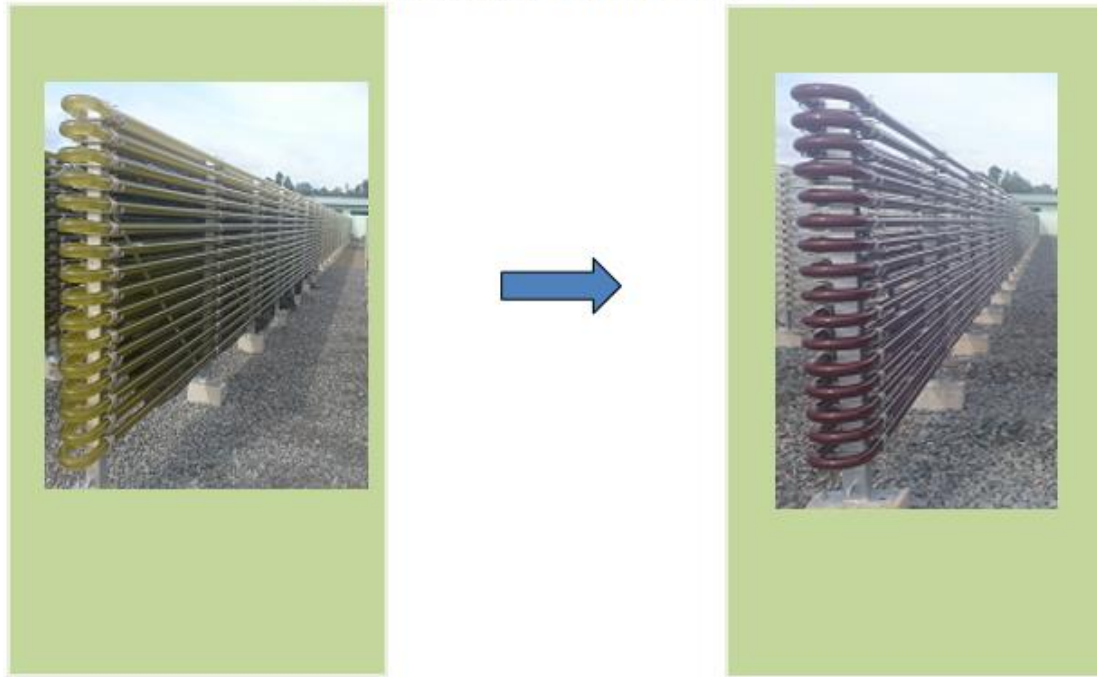
119 In modern red algae culture facilities, the culture systems are broadly categorized into two
120 types: open systems and closed systems. The open system, commonly known as a runway or
121 open tank, and the closed system, also referred to as a pipeline photobioreactor, each offer
122 unique advantages and limitations [31,32].

123 **Open culture system**

124 Open culture systems represent the simplest and most prevalent form of microalgae
125 cultivation, typically exemplified by the raceway tank design. A runway pool, with a water
126 depth typically ranging from 20 to 30 centimeters, is a commonly used facility for cultivation
127 [33]. The dimensions of length and width can be customized based on specific requirements.
128 During the cultivation process, the flow rate of the culture liquid is typically maintained at 25-
129 30 cm/s. The mixing process is frequently facilitated by a waterwheel-type aeration pump,
130 which is employed to regulate parameters such as flow, velocity, and vortex. Thus, the runway
131 tank is recognized for its relatively low cost and favorable scalability [35,36]. However, the
132 open system is also associated with challenges such as unstable culture conditions,
133 susceptibility to contamination, volatile water quality, and difficulty in controlling water
134 temperature. These factors contribute to a limited adoption of this technology as the primary
135 cultivation system by companies. Notably, Cyanotech Company in Hawaii, USA, and overseas
136 companies, along with Jingzhou Natural Astaxanthin Co. Ltd. in Hubei Province and Yunnan
137 Green A Biological Engineering Co., Ltd. In China, are among the limited organizations that
138 choose to utilize runway tank systems for microalgae cultivation[37].

139

Glass Pipe Culture- From green to red



Confined photobioreactors

Pipeline photobioreactor is considered highly suitable for large-scale open-air cultivation of *H. Pluvialis*. Several enterprises in China that have embraced this technology for cultivating *H. Pluvialis*, including Algatechnologies in Israel and Kunming Biogenic Co., Ltd. Kunming Biogenic Co., Ltd. was founded in 2004 as the first company in China to utilize outdoor closed photobioreactor culture technology. The company has played a crucial role in advancing the Yunnan astaxanthin industry and has a long-standing expertise of 20 years in cultivating astaxanthin extracted from *H. Pluvialis* [38].

153 Additionally, Kunming Biogenic Co., Ltd. boasts over 20 independent intellectual
154 property rights and has received recognition from national, provincial, and municipal
155 government departments as a national high-tech enterprise. The company has been
156 designated as a national high-tech enterprise and has received recognition as a science and
157 technology-based small and medium-sized enterprise at both the national and provincial
158 levels. Additional honors include the title of Yunnan Provincial Intellectual Property
159 Advantageous Enterprise, a small giant enterprise specializing in science and technology
160 within Yunnan Province, and a key contributor to the Yunnan (Kunming) Toru Numasawa
161 Microalgae Expert Workstation[39].

162 **Factors affecting the culture process of *Haematococcus Pluvialis***

163 **Temperature**

164 Scientific literatures have indicated that rain-grown red algae exhibit optimal growth at
165 temperatures within the range of 14 to 15°C. However, further studies suggest that their
166 optimum growth occurs at approximately 25-28°C [40,41]. Elevated temperatures exceeding
167 28°C, however, have been found to impede the reproduction of red algae cells. Despite the
168 hindrance to growth at high temperatures, it has been observed that astaxanthin accumulation
169 is enhanced. Specifically, at 30°C, the levels of astaxanthin can triple compared to that at
170 20°C [42]. Consequently, the consensus is that the growth and development of rain-grown red
171 algae cells are most favorable within the temperature range of 20-28°C. Nevertheless,
172 temperatures exceeding 28°C lead to diminished reproduction rates of algal cells, causing a
173 transition from swimming cells to stationary spores [43,44].

174 **Light intensity**

175 During the cultivation of *R. amylovora*, light is identified as the primary factor
176 responsible for astaxanthin accumulation [46]. Appropriate light conditions are essential for
177 astaxanthin synthesis, yet excessive light intensity can lead to photoinhibition, photodamage,
178 and even apoptosis of algal cells. High light intensity is found to be detrimental to the
179 biomass proliferation of *H. Pluvialis*,, whereas it promotes astaxanthin synthesis[47,48].
180 Different research experiments have reported varying results on the optimal light intensity for
181 astaxanthin accumulation, with some studies suggesting 50-60 mol quanta/(m²-s) as the
182 optimum for growth and others proposing 30 μE(m²-s) for *H. Pluvialis* cultivation [49]. Red
183 light has been shown to favor the reproduction of *H. Pluvialis*, while blue light is beneficial
184 for the synthesis of astaxanthin by this microorganism [50].

186 *H. Pluvialis* shows optimal growth at a neutral or weakly alkaline pH level of $7.8 \pm$
187 0.10. Current research practice employ various culture media, involving BBM, HGZ, MCM,
188 and BG11. for the cultivation of this microalga species. However, a common challenge
189 observed during the cultivation process is the natural increase in pH levels of the media over
190 time, which tends to rise rapidly. Consequently, it becomes essential to maintain the pH
191 stability by employing food-grade CO₂ for periodic adjustments [51,52].

192 **Nutrients such as nitrogen and phosphorus**

193 Moderate regulation of nitrogen and phosphorus content is crucial for astaxanthin
194 synthesis and accumulation in microalgae [53,54]. Studies have indicated that an increase in
195 nitrogen and phosphorus sources has adverse effects on the growth of *H. Pluvialis*, yet it is
196 beneficial for astaxanthin accumulation. Specifically, an increased ratio of nitrogen and
197 phosphorus can significantly diminish the growth rate of *H. pluvialis*, leading to decreased
198 reproduction rates [55,56]. However, this unfavorable growth condition leads to higher
199 astaxanthin content in the microalgae. Consequently, balancing the levels of nitrogen and
200 phosphorus is essential to optimize astaxanthin production and accumulation in microalgae
201 [57].

202 **Application of astaxanthin**

203 Astaxanthin is a versatile compound with applications across various industries, including
204 healthcare, skincare, pet care, aquaculture, poultry farming, as well as in food additives and
205 other sectors.

206 **Raw materials**

207 Astaxanthin encompasses multiple forms based on its processing methods, each
208 tailored for specific applications in various industries. Astaxanthin unbroken algae powder
209 is characterized by its dark red appearance and a slight fishy odor reminiscent of seaweed,
210 however, insoluble in water, suitable for pressed tablet candy and hard capsules.
211 Astaxanthin wall-broken algae powder, similar dark red and subtle seaweed scent, exhibits
212 solubility in water. Consequently, it finds its place in feed formulations for aquatic,
213 poultry, and animal protection purposes. In addition, astaxanthin oil, obtained through
214 supercritical CO₂ technology sans solvent, takes the form of a deep red viscous liquid with
215 a hint of seaweed odor. Being soluble in organic solvents like oils and fats, it is ideal for

softgel capsules and cosmetics. Furthermore, Astaxanthin microcapsule powder utilizes natural astaxanthin oil extracted from rainbow red algae. Through emulsification and spray drying processes, this technology yields water-soluble microcapsules suitable for a wide array of applications including pressed tablet confectionery, solid beverages, food additives, hard capsules, and cosmetics.

Overall, by harnessing these diverse forms of astaxanthin, industries can create products catering to consumer needs across various sectors, demonstrating the versatility and applicability of this compound in different settings [62,58].

Foodstuffs

Astaxanthin-enriched yogurt is a popular product that retains the original flavor of yogurt. Other forms such as capsules, candies, solid and liquid beverages are also prevalent in the market [59].

Prominent global brands, such as Gossypol, Zizi, etc., have expanded a series of skincare products like moisturizing creams, anti-wrinkle eye creams, and face masks to incorporate extracts derived from rain-grown red algae [63,65]. Domestic manufacturers are pioneering the use of natural astaxanthin as raw materials for producing a variety of commodities including astaxanthin water sensation masks, eye patches, and cleansing soap. This trend underscores the burgeoning integration of astaxanthin into various consumer products in the beauty and wellness industry [66,67].

Feed additive

A collaborative study on rainbow trout by Guangdong Ocean University and Kunming Biogenic Co., Ltd. revealed noteworthy benefits of astaxanthin as a feed additive. The study demonstrated improvements in seven key aspects pertaining to rainbow trout, including growth performance, intestinal development, intestinal digestive and absorptive enzymes, intestinal morphology, antioxidant ability, immune ability, and muscle quality.

Future prospects

As of 2022, more than 20 enterprises in Yunnan Province have engaged in the cultivation of rainbow red algae and extraction of astaxanthin, with some advancing towards engineering and industrialization initiatives [68] China declared in September 2020 its commitment to achieving carbon peaking by 2030 and carbon neutrality by 2060. To reach this goal, the foremost CO₂ emitting industries, namely power generation, cement production, iron and steel manufacturing, need to implement various carbon sequestration measures. Microalgae

cultivation presents a viable avenue for transforming CO₂ into organic matter for carbon sequestration purposes [69,72]. This approach not only aids in lowering CO₂ emissions but also brings significant economic advantages , attracting more participants to the microalgae carbon sequestration sector. Currently, red algae cultivation remains largely in the agricultural stage, susceptible to weather conditions. To address this limitation, the integration of indoor culture technology is essential to achieve automation and non-stop operability in the cultivation process [70,71].

References:

- [1] Cassamo U. Mussagy, Kiki A. Kurnia, Ana Cláudia Dias, et al (2022) . An eco-friendly approach for the recovery of astaxanthin and β -carotene from *Phaffia rhodozyma* biomass using bio-based solvents. *Bioresource Technology*. 345:126555-126555.
- [2] Jinpeng Sun, Xuehong Wei, Xiao Cong, et al (2023) . Expression of fatty acid related gene promotes astaxanthin heterologous production in *Chlamydomonas reinhardtii*. *Frontiers in Nutrition*. 10 (0):0-1
- [3] Zhixian Li, Zhuqing Dai, Enjuan Shi, et al (2023) . Study on the interaction between β -carotene and gut microflora using an in vitro fermentation model. *Food Science and Human Wellness*. 12 (4):1369-1378.
- [4] Anagha Nair, Ashok Kumar Ahirwar, Shikha Singh, et al. (2023) Astaxanthin as a King of Ketocarotenoids: Structure, Synthesis, Accumulation, Bioavailability and Antioxidant Properties. *Marine Drugs*. 21 (3):176-176.
- [5] Enrique A. Iturriaga, M. I. Alvarez, Arturo P. Eslava, et al (2018) . Expression Vectors and Gene Fusions for the Directed Modification of the Carotenoid Biosynthesis Pathway in *Mucor circinelloides*. *Methods in molecular biology*. ;0 (0):239-256.
- Chen Sun, Shigang Cui, Yongli Zhang, et al (2022) . Research on Image Classification Algorithm of *Haematococcus Pluvialis* Cells. 2022 IEEE 6th Advanced Information Technology, Electronic and Automation Control Conference (IAEAC). 0 (0):0-0.
- [6] Shigang Cui, Chen Sun, Yongli Zhang, et al (2022) . Classification of *Haematococcus pluvialis* cells based on Bayesian Classifier. 2022 34th Chinese Control and Decision Conference (CCDC). ;0 (0):0-0.
- [7] Cui S, Cai X, Zhang Y, et al. Research on pH Value Control System of *Haematococcus Pluvialis* Algae Based on Fuzzy PID Algorithm[C]//Hubei Zhongke Institute of Geology and Environment Technology. Proceedings of the 2nd International Conference on Artificial Intelligence and Computer Science (AICS 2020). Key Laboratory of Information Sensing and Intelligent Control of Tianjin; Tianjin University of Technology and Education;

284 [8] Zhengli Hua,Xuedi Zhang,Xue Zhao, et al (2023) . Hepatic-targeted delivery of astaxanthin for enhanced scavenging free radical scavenge and preventing mitochondrial depolarization. Food Chemistry. ;406 (0):135036-135036.

285

286

287 [9] Nuo Heng,Shan Gao,Chaoqin Yu, et al (2021) . Dietary supplementation with natural astaxanthin from *Haematococcus pluvialis* improves antioxidant enzyme activity, free radical scavenging ability, and gene expression of antioxidant enzymes in laying hens. Poultry Science. ;100 (5):101045-101045

288

289

290

291 [10] Janina Dose,Seiichi Matsugo,Haruka Yokokawa, et al (2016) . Free Radical Scavenging and Cellular Antioxidant Properties of Astaxanthin. International Journal of Molecular Sciences. ;17 (1):103-103.

292

293

294 [11] Zhe Jia,Ruihua Song,Yan Xu, et al (2022) . Astaxanthin absorption modulated antioxidant enzyme activity and targeted specific metabolic pathways in rats. Journal of the Science of Food and Agriculture.102 (15):7003-7016.

295

296

297 [12] Violeta Rodriguez-Ruiz,José Ángel Salatti-Dorado,Abolfazl Barzegari, et al (2018) . Astaxanthin-Loaded Nanostructured Lipid Carriers for Preservation of Antioxidant Activity. Molecules. ;23 (10):2601-2601.

298

299

300 [13] Haziq Kamal,Samia Heneidak,Gihan El Shoubaky, et al (2023) . Evaluation of Antioxidant and Free Radical Scavenging Activities of Some Abundant Marine Macroalgae, Egypt.. Bulletin of Pharmaceutical Sciences. ;0 (0):0-0.

301

302

303 [14] Qiang Fan,Zhan Chen,Yougui Wu, et al (2021) . Study on the Enhancement of Immune Function of Astaxanthin from *Haematococcus pluvialis*. Foods. ;10 (8):1847-1847.

304

305 [15] Janina Dose,Seiichi Matsugo,Haruka Yokokawa, et al (2016) . Free Radical Scavenging and Cellular Antioxidant Properties of Astaxanthin. International Journal of Molecular Sciences. ;17 (1):103-103.

306

307

308 [16] Nuo Heng,Shan Gao,Chaoqin Yu, et al (2021) . Dietary supplementation with natural astaxanthin from *Haematococcus pluvialis* improves antioxidant enzyme activity, free radical scavenging ability, and gene expression of antioxidant enzymes in laying hens. Poultry Science. ;100 (5):101045-101045.

309

310

311

312 [17] Takao Kato,Takatoshi Kasai,Shoichiro Yatsu, et al (2017) . Effects of 3-month Astaxa
313 nthin Supplementation on Sleepiness, Physical Activity and Health Related Quality-of-life
314 in Heart Failure Patients with Sleep-disordered Breathing. Journal of Cardiac Failure.23 (
315 10):S53-S53.

316 [18] Sayaki Ishiwata,Takao Kato,Takatoshi Kasai, et al (2021) . Changes in self-reported
317 physical activity and health-related quality of life following 3-month astaxanthin supple
318 mentation in patients with heart failure: results from a pilot study. Annals of palliative me
319 dicine. 10 (2):1396-1403.

320 [19] Michela Cecchin,Stefano Cazzaniga,Flavio Martini, et al (2022) . Astaxanthin and eic
321 osapentaenoic acid production by S4, a new mutant strain of *Nannochloropsis gaditana*.
322 Microbial Cell Factories. 21 (1):0-0.

323 [20] Saikat Mitra,Abdur Rauf,Abu Montakim Tareq, et al (2021) . Potential health benefit
324 s of carotenoid lutein: An updated review. Food and Chemical Toxicology. 154 (0):112328
325 -112328.

326 [21] Eiji Yamashita (2015) . Let astaxanthin be thy medicine. PharmaNutrition. ;3 (4):115-
327 122.

328 [22] Dalal Asker,Tarek S. Awad,Teruhiko Beppu, et al (2012) . A Novel Radio-Tolerant As
329 taxanthin-Producing Bacterium Reveals a New Astaxanthin Derivative: Astaxanthin Dirha
330 mnoside. Methods in molecular biology. ;0 (0):61-97.

331 [23] Sasmita Biswal (2014) . Oxidative stress and astaxanthin: The novel supernutrient c
332 arotenoid. International Journal of Health & Allied Sciences. ;3 (3):147-147.

333 [24] Joy Udensi,Ekaterina Loskutova,James Loughman, et al (2022) . Quantitative Raman
334 Analysis of Carotenoid Protein Complexes in Aqueous Solution. Molecules. 27 (15):4724-
335 4724.

336 [25] Khunjamayum Romapati Devi,S. Rubavathi,G Ayyappadasan. (2020) Optimization
337 of Physical Parameters of Astaxanthin Production from *Haematococcus pluvialis*. Zenodo
338 (CERN European Organization for Nuclear Research). ;0 (0):0-0.

339 [26] Yuanhao Chen,Hong Du,Honghao Liang, et al (2023) . Enhanced Carotenoid Produ
340 ction in *Chlamydomonas reinhardtii* by Overexpression of Endogenous and Exogenous B
341 eta-Carotene Ketolase (BKT) Genes. *International Journal of Molecular Sciences*. ;24 (14):
342 11382-11382.

343 [27] Jiqing Liu,Chen Bao,Wangting Li (2022) . Modification and performance analysis of
344 pigment red 122 by fumed silica. *Molecular Crystals and Liquid Crystals*. 742 (1):119-132

345 [28] M. Manimegalai,Giridharan Bupesh,M. Mirunalini, et al (2010) . Color Enhancement
346 Studies on *Etroplus Maculatus* using Astaxanthin and B-Carotene. *International Journal o*
347 *n Environmental Sciences*. ;1 (3):403-418.

348 [29] Ying Lyu,Jinfeng Bi,Qinqin Chen, et al (2022) . Color enhancement mechanisms ana
349 lysis of freeze-dried carrots treated by ultrasound-assisted osmosis (ascorbic acid-CaCl₂)
350 dehydration. *Food Chemistry*. ;381 (0):132255-132255.

351 [30] Jialin Sun,Zihao Wei,Changhu Xue (2021) . Recent research advances in astaxanthin
352 delivery systems: Fabrication technologies, comparisons and applications. *Critical Revie*
353 *ws in Food Science and Nutrition*. ;63 (19):3497-3518.

354 [31] Wa Iba,. Sumarno,. Ruslaini, et al (2023) . Price Analysis of Aquafeed Containing Mi
355 croalgae (*Dunaliella salina* (Dunal) Teodoresco 1905 Meal for Color Enhancement in Shri
356 mp. *KnE Social Sciences*. ;0 (0):0-0.

357 [32] Bao Yu Zhang,Ya Hong Geng,Zhong Kui Li, et al (2009) . Production of astaxanthin
358 from *Haematococcus* in open pond by two-stage growth one-step process. *Aquaculture*.
359 ;295 (3-4):275-281.

360 [33] Mark Seger,Fakhriyya Mammadova,Melany Villegas-Valencia, et al. (2023) Enginee
361 red ketocarotenoid biosynthesis in the polyextremophilic red microalga *Cyanidioschyzon*
362 *merolae* 10D. *bioRxiv* (Cold Spring Harbor Laboratory). ;0 (0):0-0.

363 [34] Mohd Jahir Khan,Richard Gordon,Sunita Varjani, et al (2022) . Employing newly dev
364 eloped plastic bubble wrap technique for biofuel production from diatoms cultivated in
365 discarded plastic waste. *Science of The Total Environment*. ;823 (0):153667-153667.

366 [35] Michael A. Borowitzka (1999) . Commercial production of microalgae: ponds, tanks,
367 and fermenters. Progress in Industrial Microbiology. ;0 (0):313-321.

368 [36] Joseph Levy,Emili Bosin,Bianca Feldman, et al (1995) . Lycopene is a more potent in
369 hibitor of human cancer cell proliferation than either α -carotene or β -carotene. Nutrition
370 and Cancer. ;24 (3):257-266.

371 [37] Jiří Masojídek,Giuseppe Torzillo (2008) . Mass Cultivation of Freshwater Microalgae.
372 Elsevier eBooks. ;0 (0):2226-2235.

373 [38] Michael A. Borowitzka (1999) . Commercial production of microalgae: ponds, tanks,
374 tubes and fermenters. Journal of Biotechnology. ;70 (1-3):313-321.

375 [39] Lamei Li,Zhu Chen,Adolf Acheampong, et al (2022) . Low-temperature plasma pro
376 motes growth of Haematococcus pluvialis and accumulation of astaxanthin by regulating
377 histone H3 lysine 4 tri-methylation. Bioresource Technology. ;343 (0):126095-126095.

378 [40] Lili Huang,Rong Hu,Yike Xie, et al (2023) . High light and temperature enhancing as
379 taxanthin production in Microcystis aeruginosa—Providing a potential thought for contr
380 olling cyanobacterial blooms through resource utilization. Journal of water process engin
381 eering. ;56 (0):104318-104318.

382 [41] Gamze Turan (2023) . Haematococcus Cultivation for Astaxanthin Production. null. ;
383 0 (0):59-68.

384 [42] Jianing Pu (2022) . Development of stable microencapsulated astaxanthin powders
385 using extracted astaxanthin from crawfish and shrimp byproducts. null. ;0 (0):0-0.

386 [43] Zhu Chen,Lamei Li,Zhang Hong, et al (2020) . Stimulation of biomass and astaxanth
387 in accumulation in Haematococcus pluvialis using low-temperature plasma (LTP). Bioreso
388 urce Technology Reports. ;9 (0):100385-100385.

389 [44] Feng Liang,Minggang Cai,Yanqi Wu, et al (2022) . Effects of Nitrogen and Light Inte
390 nsity on the Astaxanthin Accumulation in Motile Cells of Haematococcus pluvialis. Fronti
391 ers in Marine Science. ;9 (0):0-0.

- [45] T.V. Samovich,R. G. Goncharik,Elizaveta I. Pechenkina, et al (2020) . Astaxanthin accumulation in *Haematococcus pluvialis* cells induced by nitrogen deficiency and high light intensity. *Žurnal Beloruskogo gosudarstvennogo universiteta*. ;0 (3):37-45.
- [46] Wenwen Zhang,Xuefei Zhou,Yalei Zhang, et al (2018) . Enhancing astaxanthin accumulation in *Haematococcus pluvialis* by coupled light intensity and nitrogen starvation in column photobioreactors. *Journal of Microbiology and Biotechnology*. ;28 (12):2019-2028.
- [47] Ria Amelia,Wulan Rahmani Akmal,Eko Agus Suyono (2023) . Enhancement of Astaxanthin Content in Mixed Culture of *Dunaliella* sp. and *Azospirillum* sp. under Light Intensity Treatment. *Jurnal Ilmiah Perikanan dan Kelautan*. ;15 (2):430-437.
- [48] Lu Liu,Kezhen Ying,Kebi Wu, et al (2022) . Promoting the Growth of *Haematococcus lacustris* under High Light Intensity through the Combination of Light/Dark Cycle and Light Color. *Journal of Marine Science and Engineering*. ;10 (7):839-839.
- [49] Ha Eun Yang,Byung Sun Yu,Sang Jun Sim (2023) . Enhanced astaxanthin production of *Haematococcus pluvialis* strains induced salt and high light resistance with gamma irradiation. *Bioresource Technology*. ;372 (0):128651-128651.
- [50] Bryan Ashley Goyudianto,Catarina Meliana,Debby Muliani, et al (2021) . The Stability of Phycocyanin, Phycoerythrin, and Astaxanthin from Algae Towards Temperature, pH, Light, and Oxygen as a Commercial Natural Food Colorant. *null*. ;3 (2):28-42.
- [51] Rongrong Lv,Kai Li,Feng Chen, et al (2022) . Buffering culture solution significantly improves astaxanthin production efficiency of mixotrophic *Haematococcus pluvialis*. *Bioresource Technology*. ;354 (0):127175-127175.
- [52] Hanyi Shi,Xuejuan Deng,Xiaoyu Ji, et al (2023) . Sources, dynamics in vivo, and application of astaxanthin and lutein in laying hens: A review. *Animal Nutrition*. ;13 (0):324-333.
- [53] Cassamo U. Mussagy,Pedro G.P. Silva,Camila F. Amantino, et al (2022) . Production of natural astaxanthin by *Phaffia rhodozyma* and its potential application in textile dyeing. *Biochemical Engineering Journal*.;187 (0):108658-108658.

420 [54] Gannoru Kankanamalage Sanuji Hasara Nishshanka,Vinoj Chamilka Liyanaarachchi,M
421 alith Premaratne, et al (2021) . Wastewater-based microalgal biorefineries for the produ
422 ction of astaxanthin and co-products: Current status, challenges and future perspectives.
423 Bioresource Technology. ;342 (0):126018-126018.

424 [55] Lanqing Huang,Donghui Li,Yu Ma, et al (2022) . Dietary fatty acid-mediated protein
425 encapsulation simultaneously improving the water-solubility, storage stability, and oral
426 absorption of astaxanthin. Food Hydrocolloids. ;123 (0):107152-107152.

427 [56] Jing Gao,Chunli Fang,Yongzi Lin, et al (2020) . Enhanced extraction of astaxanthin u
428 sing aqueous biphasic systems composed of ionic liquids and potassium phosphate. Foo
429 d Chemistry. ;309 (0):125672-125672.

430 [57] Juan Huang,Xuan Feng,Shuo Zhang, et al (2022) . Preparation and characterization
431 of astaxanthin-loaded microcapsules and its application in effervescent tablets. Journal o
432 f the Science of Food and Agriculture. ;103 (3):1421-1431.

433 [58] Lucio Rodríguez-Sifuentes,Jolanta E. Marszalek,Gerardo Hernández-Carbajal, et al (2
434 021) . Importance of Downstream Processing of Natural Astaxanthin for Pharmaceutical
435 Application. Frontiers in chemical engineering. ;2 (0):0-0.

436 [59] Bayan Yousef AL-Tarifi,Azizah Mahmood,Suvik Assaw, et al (2020) . Application of
437 Astaxanthin and its Lipid Stability in Bakery Product. Current research in nutrition and fo
438 od science. ;8 (3):962-974.

439 [60] Ting Zhou,Xiaofei Wang,Yun Ju, et al (2018) . Stability application and research of a
440 staxanthin integrated into food. IOP Conference Series: Materials Science and Engineerin
441 g. ;394 (0):022007-022007.

442 [61] Xin-Yuan Cong,Huizhen Zhang (2019) . Recent Progress in Sources, Biological Activ
443 ity and Application of Astaxanthin. Zenodo (CERN European Organization for Nuclear Re
444 search). ;0 (0):0-0.

445 [62] Abiola Folakemi Olaniran,Joshua Opeyemi Folorunsho,Bolanle Adenike Akinsanola, e
446 t al (2023) . Application of Astaxanthin and Carotenoids Derived from Algae for the Pro

duction of Nutraceuticals, Pharmaceuticals, Additives, Food Supplement and Feed. null.;0
(0):95-124.

[63] Jing-Xian Lai,Wanping Liu,Jie Bu, et al (2023) . Enhancement of astaxanthin product
ion from food waste by *Phaffia rhodozyma* screened by flow cytometry and feed applicat
ion potential. *Biotechnology and Applied Biochemistry*. ;0 (0):0-0.

[64] Balaji Subramanian,Marie-Hélène Thibault,Yahia Djaoued, et al (2015) . Chromatog
raphic, NMR and vibrational spectroscopic investigations of astaxanthin esters: applicatio
n to "Astaxanthin-rich shrimp oil" obtained from processing of Nordic shrimps. *Analys
t*. ;140 (21):7423-7433.

[65] Bin Sheng,Fei Fan,Jianke Huang, et al (2018) . Investigation on models for light dist
ribution of *Haematococcus pluvialis* during astaxanthin accumulation stage with an appli
cation case. *Algal Research-Biomass Biofuels and Bioproducts*. ;33 (0):182-189.

[66] Xiaoting Yan,Yuan Zhang,Mei Yang, et al (2021) . An accurate, rapid, and sensitive
method for simultaneous determination of four typical heterocyclic amines in roasted po
rk patties: Application in the study of inhibitory effects of astaxanthin. *Journal of Separati
on Science*. ;44 (9):1833-1842.

[67] Patrizia Casella,Dino Musmarra,Salvatore Dimatteo, et al (2020) . Purification of Ast
axanthin from Microalgae by Using Commercial Activated Carbon. *Chemical engineering
transactions*. ;79 (0):295-300.

[68] Thomas A. Kwan,Sarah E. Kwan,Jordan Peccia, et al (2018) . Selectively biorefining a
staxanthin and triacylglycerol co-products from microalgae with supercritical carbon dio
xide extraction. *Bioresource Technology*. ;269 (0):81-88.

[69] David Christian,Jun Zhang,Alicia J. Sawdon, et al (2018) . Enhanced astaxanthin acc
umulation in *Haematococcus pluvialis* using high carbon dioxide concentration and light
illumination. *Bioresource Technology*. ;256 (0):548-551.

[70] Gamze Turan (2023) . *Haematococcus* Cultivation for Astaxanthin Production. null. ;
0 (0):59-68.

474 [71] Giuseppe Di Sanzo, Sanjeet Mehariya, Maria Martino, et al (2018) . Supercritical Car
475 bon Dioxide Extraction of Astaxanthin, Lutein, and Fatty Acids from Haematococcus pluvi
476 alis Microalgae. Marine Drugs.;16 (9):334-334.
477