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# Eco-friendly and energy-saving strategy for high-yield astaxanthin recovery from wet *Haematococcus pluvialis*

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## 1. Introduction

Recently, *Haematococcus pluvialis* (*H. pluvialis*) has garnered considerable attention because it is a natural enriched source of a red colored carotenoid, viz. astaxanthin (3,3'-dihydroxy-*β*,*β*'-carotene-4,4'-dione, $C_{40}H_{52}O_4$), which has a strong potent antioxidant activity.[1] Numerous beneficial biological activities associated with astaxanthin, including anti-inflammatory, anti-cancer, and anti-aging effects alongside preventing age-related dieases and promoting immune responses, are belived to be related to its antioxidant activity.[2] Although astaxanthin synthesized from petrochemical resources is avaiable, synthetic astaxanthin is considered unsitable for human consumption owing to toxicity concerns.[3] Owing to the increasing demand for natural astaxanthin and its high market value in the nutraceutical, food, and pharmaceutical industries, developing effective and efficient technologies to produce high-purity natural astaxanthin is crucial. However, the formation of a highly robust 1.8–2.2-μm-thick multilayered cell wall structure in the red cyst cells of *H. pluvialis* renders extracting a high yield of astaxanthin difficult.[4] This is because astaxanthin is present inside lipid droplets in the cytoplasm of the cells. Therefore, the cell wall disruption of *H. pluvialis* constitues the major technical challenge in astaxanthin extraction. To date, several cell disruption and solvent extraction methods have been proposed to extract astaxanthin from *H. pluvialis*, including chemical, physical and biological-based technologies.[4] Although high-yield of astaxanthin recovery (87–100%) could be achieved by the previously proposed techniques, high energy consumption for mechanical pretreatment steps of grinding and high-pressure homogenization, a long operation time, remarkably high-pressure, and an addition of chemical modifier to increase the solubility of astaxanthin in supercritical $CO_2$ are major drawbacks for use in a practical scale. In most of the proposed extraction techniques, drying the wet *H. pluvialis* is a prerequisite to cell wall disruption to prevent astaxanthin degradation and increase the extraction yield.[5] Commonly used techniques to dehydrate wet *H. pluvialis* constitute spray drying and lyophilization (freeze-drying), which both require high-energy inputs. There is a growing concern associated industrial-scale production; for instance, drying wet microalgal biomass prior to extraction can account for up to 25% energy consumption of the overall extraction process.[6] Therefore, the high-cost and energy-intensive drying step constitutes the major bottleneck in recoverying astaxanthin and developing profitable and sustainable microalgal biorefineries.

Since about two decades ago, subcritical dimethyl ether (subDME) has been utilized on the extraction technologies in removing water, lipid/oil, proteins, pigments and specific ingredients derived from various natural resources owing to its unique properties such as the high misibility of water in DME, strong abilitity for extracting organic compounds, environmental friendliness, safety, and good compressibility.[7] Yet, there has been proposed only one study on the subDME extraction of lipid and astaxanthin directly from wet *H. pluvialis* under a specified condition at 25 °C and 0.59 MPa.[8] Due to the limits of information by the previous study, more researches should be performed to get insight into dewatering and extraction mechanism of chemical species from wet *H. pluvialis* using subDME. In this study, we demostrate a new integrated green and energy efficient extraction technique for the recovery of high-yield and pure astaxathin from wet encyst *H. pluvialis* with subDME and sequential dimethyl sulfoxide (DMSO) extraction of the residual solid generated from the subDME extraction.

## 2. Materials and Methods

A wild-type strain of *H. pluvialis* (NIES-44), acquired from the National Institute for Environmental Studies (Tsukuba, Japan), were cultivated photoautotrophically in an outdoor culture system using thin-film photobioreactors.[9] The wet *H. pluvialis* cyst cells were directly used as feedstock on the subDME extraction without any pretreatment such as drying and grinding. The water content of the wet *H. pluvialis*, obtained using an oven drying method at 70 °C for 24 h in vacuum, was 76.7 ± 0.5 wt%.

A schematic diagram of the apparatus used for the subDME

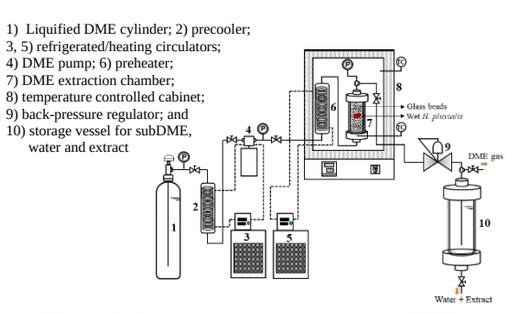


**Figure 1.** Schematic diagram of the subDME extraction apparatus.

extraction is shown in Figure 1. In order to identify the efficiency of subDME for the extraction of water and chemical species from the wet *H. pluvialis* and to gain insight into the extraction mechanism, subDME extraction was performed at the various temperature (25–55 °C), pressure (1–4 MPa) and time (30–150 min). Subsequently, DMSO extraction was performed at 60 °C for 10 min for the *H. pluvialis* that was collected after the subDME extraction (hereafter denoted as DME-RS), and then the DMSO extracts were subjected to enzymatic hydrolysis with cholesterol esterase solution in Tris-HCl buffer for 60 min at 37 °C in a thermostatic shaking incubator to measure astaxanthin in the DME-RS. Fatty acids in the DME liquid extracts and DME-RS were measured after in-situ transesterifialtion according to the NREL standard menthods using an Agilent GC-FID installed with DB-Fast FAME column and quantified with 37 components of FAME standard (Sigma-Aldrich, USA). In addition, DMSO extraction on the freeze-dried and ball-milling of the freeze-dried *H. pluvious* at 200 rpm and 30 min were performed. The chemical species were characterized and used as reference samples in this study.

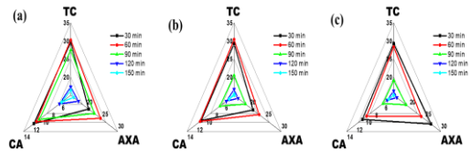


**Figure 2.** Effect of subDME extraction temperature and time on the contents of total carotenoids, astaxanthin, and chalorphyll a (mg/g) in the residual solids at 35 °C (a), 45 °C (b), and 55 °C (c) at 2 MPa. TC, total carotenoids; AXA, astaxanthin; and CA, chlorophyll *a*.

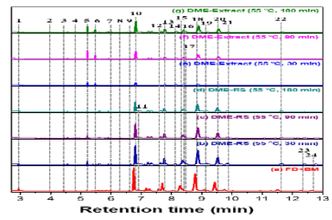


**Figure 3.** GC-FID chromatogras of atty acids components in the freeze-dry plus ball milled H. pluvialis (a) and various DME-RS and DME extarcts obtained by subDME extraction process.

## 3. Results and discussion

This study demonstrated that subDME can remove 94–98 wt% of water present in the wet *H. pluvialis* in short times (<30 min) at all extraction temperatures studied. The recovery yield of DME-RS were ranged from 35 to 55 wt% depending on the intensity of extraction temperature and time, which is relatively higher than that of dry biomass obtained by freeze-drying of the wet *H. pluvialis* for 48 h (23.4 ± 0.3 wt%).

Furthermore, there are no significant changes of total carotenoids contents in the DME-RS during the extraction times between 30 and 60 min for all the extraction temperatures (Figure 2). However, the total carotenoids contents were sharply decreased with the extraction time increased from 60 to 120 min, particularly at high temperatures of 45–55 °C, and then a slightly decreased or no change when the time was further increased to 150 min. A similar trend was observed for the chlorophyll *a* in the DME-RS samples. Interestingly, the amount of astaxanthin increased when the extraction time increased from 30 to 60 min at 35–45 °C, whereas that decreased at the high extraction temperature of 55 °C. The amount of total astaxanthin was observed for the DME-RS obtained by extraction at 55 °C for 30 min (26.93 ± 1.65 mg/g), which was higher than in the DMSO extract solution derived from freeze-dry *H. pluvialis* without grinding (19.74 ± 0.2 mg/g). As results, approximately 84.3 wt%, 76.4 wt%, and 92.0 wt% of total carotenoids, chlorophyll *a*, and astaxthin contents in the DME-RS obtained by subDME extraction at 55 °C and 30 min based on their contents in the freeze-dry plus ball milled *H. pluvioalis*.

We observed that the recovery of total carotenoids is strongly related with the extraction of lipid from the wet *H. pluvialis* under the studied conditions. For example, as the longer extraction time at 55 °C, lipid extraction incrersed (Figure 3). The main fatty acids in the feedstock were palmitic (C16 :0, peak #10), stearic acid (C18 :0, peak #14), oleic (C18 :1 cis n9, peak #16), linoleic acid (C18 :2 cis n6, peak #18), and α-linolenic (C18 :3n3, peak #20). The total fatty acids contents decreased from 26.9 to 13.2 wt% when the extraction time increased from 30 to 180 min in the DME-RS, while that increased from 12.4 wt% to 35.1 wt% in the liquid extracts collected from the DME extraction, which are valuable components for the biodiesel production.

## 4. Conclusions

A new eco-friendly and energy efficient technique for the recovery of high astaxanthin from wet *H. pluvialus* using subDME followed by DMSO extraction was demostrated. The results of this study can imply to establish a profitable microalgae biorefinery in a practical scale.

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