Carotenoid quantification

Following electrotreatment, *H. pluvialis* cells were collected by centrifugation at 1,952×*g* for 10 min (Combi R515; Hanil Science Co., Gimpo, Korea). The collected cells were washed twice with distilled water, freeze-dried using a lyophilizer (FD8508; IlShinBioBase Co., Daejeon, Korea) for 48 h, and stored at −22 °C until further analysis.

The cellular contents of astaxanthin (AXT) and its intermediates (i.e., canthaxanthin, zeaxanthin, and β-carotene) in *H. pluvialis* were quantified using high-performance liquid chromatography (HPLC) (1260 Infinity Series, Agilent Technologies, Santa Clara, CA, USA) equipped with a diode-array detector and a YMC carotenoid column (C30, 5 μm, 250×4.6 mm; YMC Co., Kyoto, Japan). To extract ketocarotenoid pigments, approximately 2 mg of lyophilized *H. pluvialis* cells was mixed with 1.0 g of glass beads (1.5 mm diameter; Daihan Scientific, Gangwon, Korea) and 1 mL of extraction solution (dichloromethane/methanol [1:1, v/v] containing 0.025 M NaOH). The algal cells were mechanically disrupted using a FastPrep-24 bead-beater (6 m/s for 30 s and 3 cycles; MP Biomedicals, Irvine, CA, USA). The algal extract was then incubated in the dark for 2 h at 4 °C to allow the saponification reaction. Subsequently, the extract was filtered through a 0.20 μm polytetrafluoroethylene-hydrophobic (PTFE-O) disposable syringe filter (Biofact Co., Daejeon, Korea), and analyzed using HPLC.

The HPLC mobile phase comprised solvent A (methanol/acetonitrile/distilled water [84/14/2, v/v/v]) and solvent B (100% dichloromethane). The ratio of solvent A was sequentially changed from 100 to 80, 70, 50, and 45%, and then back to 100% at 5 min intervals during the 30 min analysis. The flow rate was maintained at 1.0 mL/min at 30 °C. The sample injection volume was 20 μL, and the chromatographic peak intensity was measured at a wavelength of 474 nm. Standards for AXT and other carotenoids were

obtained from Dr. Ehrenstorfer GmbH (Augsburg, Germany) and Sigma-Aldrich (St. Louis, MO, USA), respectively.