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CHARACTERISTICS AND CHROMATOGRAPHIC SEPARATION OF ASTAXANTHIN AND ITS ESTERS FROM THE MICROALGA Haematococcus pluvialis

by

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A thesis submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy at The University of Hong Kong

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DECLARATION

I declare that this thesis represents my own work, except where due acknowledgment is made, and that it has not been previously included in a thesis, dissertation or report submitted to this University or to any other institution for a degree, diploma or other qualification.

Signed J. P. Yrraw

Jian-Ping Yuan



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Reversed-phase high performance liquid chromatographic methods were developed for the separation, identification, and determination of astaxanthin esters and the isomers of astaxanthin in the unsaponified and saponified pigment extracts from the microalga *Haematococcus pluvialis*. HPLC was conducted on a Waters liquid chromatograph equipped with two 510 pumps and a 996 photodiode array detector set at 250–700 nm. Beckman Ultrasphere C_{18} column and Waters Symmetry C_{18} column were used to separate carotenoids and chlorophylls in pigments extracts. The mobile phase consisted of methanol, dichloromethane, acetonitrile, and water.

The hydrolysis of astaxanthin esters and the degradation of astaxanthin during saponification of the pigment extract were investigated. Different concentrations of NaOH in methanol were used for the saponification under nitrogen in darkness. The concentration of NaOH was important for promoting the hydrolysis of astaxanthin esters and minimizing the degradation of astaxanthin during saponification. A high temperature should be avoided to minimize the degradation of astaxanthin.

The purification method including extraction, saponification, and separation was established for preparing purified *trans*-astaxanthin from a high-yielding



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